Merus

Phase 2 study of MCLA-128-based combinations in metastatic breast cancer (MBC): MCLA-128/trastuzumab/chemotherapy in HER2-positive MBC and MCLA-128/endocrine therapy in estrogen receptor positive and low HER2 expression MBC

STUDY CODE:	**MCLA-128-CL02**
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IND:	**131752**
SPONSOR:	**Merus N.V., Yalelaan 62, 3584 CM Utrecht, The Netherlands**
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SPONSOR SIGNATURE PAGE

Phase 2 study of MCLA-128-based combinations in metastatic breast cancer (MBC): MCLA-128/trastuzumab/chemotherapy in HER2-positive MBC and MCLA-128/endocrine therapy in estrogen receptor positive and low HER2 expression MBC

MCLA-128-CL02, Version 4.0, 30 April 2021

I, the undersigned, have read this protocol and agree that it contains all the necessary information required for the conduct of the study.

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Electronic signature page is attached

PRINCIPAL INVESTIGATOR SIGNATURE PAGE

I confirm that I have read the study protocol MCLA-128-CL02, entitled "Phase 2 study of MCLA-128-based combinations in metastatic breast cancer (MBC): MCLA-128/trastuzumab/chemotherapy in HER2-positive MBC and MCLA-128/endocrine therapy in estrogen receptor positive and low HER2 expression MBC", Version 4.0, 30 April 2021.

The information it contains is consistent with the current risk-benefit evaluation of the investigational product.

I understand it, and I agree to conduct the study according to this protocol, GCP principles as described in CPMP/ICH/135/95 and 21 CFR Parts 50, 54, 56, and 312, and applicable local requirements, and will comply with the protocol requirements, subject to scientific, ethical and safety considerations.

I understand that I must keep confidential the information contained in the study documents that I have been or will be provided with.

I understand that, should the decision be made by the Sponsor to terminate prematurely or suspend the study at any time for any reason, such a decision will be communicated to me in writing. Should I decide to withdraw from participating in the study I will communicate immediately such a decision in writing to the Sponsor.

Furthermore, by the present, I am committed to enroll the first patient in this study within a month.

NAME & QUALIFICATION: _____

INSTITUTION & LOCATION: _____

_____

_____

Signature _____

Date _____

SUMMARY OF PROPOSED CHANGES AND RATIONALE

Section / Page	Throughout the document Version Number and Version Date.
Rationale	Administrative changes to clearly document new version.

Section / Page	Section in Protocol Synopsis: Study duration
Rationale	The initial anticipated "Completion of the data analysis" date of October 2019 has been exceeded due to a handful of patients who still benefit from the study drug. Protocol Version 5.0 provides a new expected date.

Section / Page	Section 3.2: Study duration
Rationale	Update to reduce the study procedures. Since the Development International Birth Date of 13 January 2015, until the cutoff date 12 January 2022, a total of 363 cancer patients had been treated with MCLA-128 in 2 Clinical Trials. So far, the safety profile including identified (IRRs) and potential risks (Diarrhea and decreased cardiac ejection fraction) associated with MCLA-128 is considered acceptable with the implementation of appropriate risk management measures including directed exclusion criteria, premedication, symptomatic treatment, and targeted monitoring. With this consideration, the Sponsor proposes for the remaining patients who have been on treatment for at least 24 months in this study, to reduce the study procedures in order to decrease their burden, but to offer the opportunity to continually derive benefit from the Study drug. The Sponsor will continue to monitor Safety and efficacy, either via an alternative "post-study or Expanded access" program or via the implementation of Protocol Version 5.0. This will allow an expedited analysis of study results, while maintaining an appropriate level of safety surveillance.

Section / Page	Summary of study procedures
Rationale	The replacement of columns Study treatment period, End of Treatment Visit and Follow-Up period, to reduce the study procedures for the patients remaining on the study. Patients transferring into the alternative program will follow a similar treatment plan.

Section / Page	Section 8.2: Laboratory Assessments
Rationale	Updated for clarity

Section / Page	Section 8.8.2: LVEF (by ECHO or MUGA) and 12-lead electrocardiogram (ECG) + Appendix 3
Rationale	Updated from every 4 cycles (3 months) to every 6 months due to the current Safety Profile and the focus on the appropriate level of safety surveillance.

Section / Page	Section 9.1: Tumor Assessments
Rationale	Updated from every 6 weeks to every 12 weeks to reduce the patient's burden, but still allows the possibility to have a Tumor Assessment done in between in case of symptomatic deterioration signs or signs of disease progression.

Section / Page	Efficacy assessments central review: Protocol synopsis and section 9.1.1.
Rationale	Enough independent central analysis of patient imaging has been completed and additional assessment of imaging for those patients currently on treatment for at least 24 months is no longer required. Therefore, central review of imaging by an independent radiologist(s) for all patients on-study is no longer applicable as of Protocol Version 5.0. The Sponsor relies on the local Investigator assessment and continued focus will be on the appropriate level of safety surveillance.

Section / Page	Protocol synopsis Biomarkers; section 11
Rationale	Enough biomarker data has been accumulated from all patients treated on the trial. Therefore the additional data from the few patients who have been on treatment for 24 months is not needed as per Protocol Version 5.0.

PROTOCOL SYNOPSIS

TITLE: Phase II study of MCLA-128-based combinations in metastatic breast cancer (MBC): MCLA-128/trastuzumab/chemotherapy in HER2-positive MBC and MCLA-128/endocrine therapy in estrogen receptor positive and low-HER2 expression MBC

INVESTIGATIONAL PRODUCT: MCLA-128

SPONSOR: Merus N.V., Yalelaan 62, 3584 CM Utrecht, The Netherlands

OBJECTIVES

Cohort 1 (HER2-positive/amplified MBC): MCLA-128 + trastuzumab ± vinorelbine

Primary objective:

- Evaluate efficacy of MCLA-128 combined with trastuzumab ± vinorelbine in terms of clinical benefit rate (CBR) at 24 weeks based on RECIST 1.1 (per investigator review) in HER2-positive/amplified MBC patients who have progressed on prior HER2-directed therapy that included trastuzumab, pertuzumab, and an HER2 antibody drug conjugate (ADC) in any sequence and in any setting

Secondary objectives:

- Evaluate CBR at 24 weeks based on RECIST 1.1 per central review
- Evaluate progression-free survival (PFS; per investigator and central review)
- Evaluate overall response rate (ORR) based on RECIST 1.1 (per investigator and central review)
- Evaluate duration of response (DoR) based on RECIST v1.1 (per investigator and central review)
- Evaluate overall survival (OS)
- Evaluate safety and tolerability of MCLA-128 in combination with trastuzumab ± vinorelbine
- Characterize pharmacokinetics (PK) of MCLA-128 in combination with trastuzumab ± vinorelbine
- Characterize immunogenicity of MCLA-128 in combination with trastuzumab

Exploratory objective:

- Evaluate potential correlations between biomarkers in tumor or blood samples and antitumor activity (including HER2, HER3, HER2:HER3 dimers, heregulin and other potential biomarkers)

Cohort 2 (estrogen receptor [ER]-positive/low HER2 expression MBC): MCLA-128 + endocrine therapy

Primary objective:

- Evaluate efficacy of MCLA-128 combined with endocrine therapy in terms of CBR at 24 weeks based on RECIST 1.1 (per investigator review) in ER-positive and low HER2 expression MBC patients who have previously progressed on the same endocrine therapy

Secondary objectives:

- Evaluate CBR at 24 weeks based on RECIST 1.1 per central review
- Evaluate PFS (per investigator and central review)
- Evaluate ORR based on RECIST 1.1 (per investigator and central review)
- Evaluate DoR based on RECIST 1.1 (per investigator and central review)
- Evaluate OS
- Evaluate safety and tolerability of MCLA-128 combined with endocrine therapy
- Characterize PK of MCLA-128 combined with endocrine therapy
- Characterize immunogenicity of MCLA-128 combined with endocrine therapy

Exploratory objective:

- Evaluate potential correlations between biomarkers in tumor or blood samples and antitumor activity (including HER2, HER3, HER2:HER3 dimers, heregulin and other potential biomarkers).

STUDY DESIGN

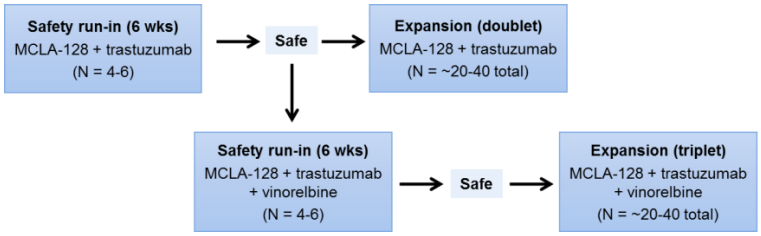
A phase 2, open-label, multicenter international study will be performed to evaluate the efficacy of MCLA-128-based combinations in two metastatic breast cancer (MBC) populations, HER2-positive/amplified (Cohort 1) and ER-positive/low HER2 expression (Cohort 2). Three combination treatments will be evaluated, two in Cohort 1 and one in Cohort 2, in 20-30 sites in 7 countries in Europe and the USA.

Cohort 1: Patients with HER2-positive/amplified MBC, having confirmed HER2 overexpression by immunohistochemistry (IHC) 3+ or IHC 2+ combined with positive fluorescence *in situ* hybridization (FISH), who have been treated with up to a maximum of 5 lines of HER2-directed therapy in the metastatic setting and have progressed on the most recent line as per RECIST v1.1, are eligible. Patients must have been treated previously with trastuzumab, pertuzumab and an HER2 ADC in any sequence and in any setting. For enrollment, HER2 status will be based on medical records, and eligibility will be confirmed subsequently as soon as possible, by central lab review. Patients found to be ineligible retrospectively will not be evaluable for the primary objective and may be replaced. Documented imaging proof of disease progression on the last prior line of therapy should be made available when possible.

Initially MCLA-128 will be administered with trastuzumab (doublet combination). Safety will be reviewed by an Independent Data Monitoring Committee (IDMC). After the safety of the doublet has been assessed, MCLA-128 + trastuzumab + vinorelbine (triplet combination) will be evaluated in parallel with the doublet combination (see figure below).

The doublet and triplet Cohort 1 combinations will both be evaluated in two steps with an initial safety run-in in 4 to 6 patients who will be reviewed by the IDMC, followed by a cohort efficacy expansion, as described below. The triplet combination go/no-go decision will be made by the IDMC after evaluation of the doublet safety run-in patients. If the triplet combination is considered safe, the expansion of the doublet and triplet combinations will be performed in parallel. Patients with be included with a 3:1 ratio for triplet or doublet respectively, favoring the triplet combination and taking into account previous exposure to vinorelbine.

Study design for Cohort 1 combination therapies (doublet and triplet)



Safety run-in: After 4-6 patients have received at least 2 complete cycles (6 weeks) of MCLA-128 + trastuzumab, a safety review will be performed by the IDMC. If the doublet combination is considered safe, the safety run-in for the triplet combination will be initiated in the next 4 to 6 successive eligible patients. Safety of the triplet will be evaluated by the IDMC after these 4-6 patients have received at least 2 complete cycles (6 weeks) of MCLA-128 + trastuzumab + vinorelbine.

Based on the observed safety in the first 4-6 patients (adverse events [AEs], serious adverse events [SAEs], relationship to study drug, and other clinically relevant parameters [e.g. laboratory parameters], available PK, immunogenicity, and cytokine data) the IDMC, investigators and Sponsor will decide on a potential additional run-in period for each combination (i.e. doublet and triplet).

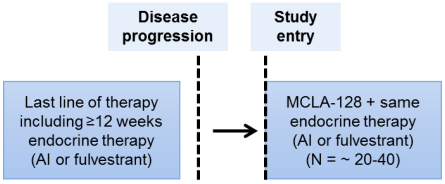
Expansion: After the safety run-in, each Cohort 1 combination therapy considered tolerable by the IDMC will be expanded to a total of up to 40 patients evaluable for efficacy.

If the doublet combination regimen is not tolerated, Cohort 1 will be closed. If the triplet combination is not well tolerated but the doublet is acceptable, the doublet expansion will be continued.

Cohort 2: Patients with ER-positive and low HER2 expression MBC (IHC 1+, or IHC 2+ combined with negative FISH) who have radiologic or photographic evidence of disease progression on the last line of prior endocrine therapy (administered for at least 12 weeks) that included an aromatase inhibitor or fulvestrant. Patients who have received up to 3 prior endocrine therapies in the metastatic setting and have progressed on a cyclin-dependent kinase inhibitor (in any line) are eligible. For enrollment, HER2 and HR status and radiologic/photographic documentation of prior progression will be based on medical records. Eligibility will be confirmed as soon as possible for HER2/HR status by central lab review and for prior disease progression by central imaging review. Patients found to be ineligible retrospectively will not be evaluable for the primary objective and may be replaced.

MCLA-128 will be administered in combination with the same previous endocrine therapy on which progressive disease is radiologically/photographically documented. A total of up to 40 patients evaluable for efficacy will be included.

Study design for Cohort 2 combination therapies



An Independent Data Monitoring Committee (IDMC), composed of at least 2 physicians expert in the domain of early clinical development in MBC, will review safety and efficacy throughout the study and decide on the addition of extra patients in the expansion parts, opening of the triple combination cohorts, and any *ad hoc* safety decisions. The principal investigators, the Sponsor's medical expert(s) and other representatives may be called upon to assist as observers.

STUDY POPULATION

Inclusion criteria

Patients must fulfill all of the following requirements to enter the study:

1. Signed informed consent before initiation of any study procedures.
2. Women with histologically or cytologically confirmed breast cancer with evidence of metastatic or locally advanced disease not amenable to any local therapy with curative intent:

   2.1 Cohort 1 (MCLA-128 + trastuzumab ± vinorelbine)

   a. Documented HER2 overexpression/amplification, defined as immunohistochemistry (IHC) 3+ positive, or IHC 2+ combined with positive fluorescence *in situ* hybridization (FISH), based on local analysis on the most recent tumor biopsy (preferably metastatic, otherwise primary), either fresh or archival collected within 24 months before screening.
   b. Documented disease progression (by investigator assessment) on up to a maximum of 5 lines of HER2-directed therapy administered in the metastatic setting, and have progressed on the most recent line. Trastuzumab, pertuzumab and an HER2 antibody drug conjugate (e.g. T-DM1) must have been previously administered in any sequence and in any setting.

   2.2 Cohort 2 (MCLA-128 + endocrine therapy)

   a. Documented hormone receptor positive status (estrogen receptor positive [ER+] and/or progesterone receptor positive [PR+]), defined as ≥ 1% positive stained cells by local standards, based on local analysis on the most recent tumor biopsy.
   b. Documented low-level HER2 expression, defined as IHC HER2 1+, or IHC HER2 2+ combined with negative FISH, based on local analysis on a fresh tumor biopsy or an archival biopsy collected within 24 months before screening (preferably metastatic, otherwise primary).
   c. No more than 3 lines of prior endocrine therapy (aromatase inhibitor or fulvestrant) for metastatic disease, with radiologic or photographic evidence of disease progression on the last line, after at least 12 weeks of therapy.
   d. Progression on a cyclin-dependent kinase inhibitor.
   e. No more than two previous chemotherapy regimens for advanced/metastatic disease.

   Note: Pre/peri-menopausal women can be enrolled if amenable to be treated with the LHRH agonist goserelin. Such patients must have commenced treatment with goserelin or an alternative LHRH agonist at least 4 weeks prior to study entry, and patients who received an alternative LHRH agonist prior to study entry must switch to goserelin for the duration of the trial.
3. In Cohort 1, patients must have at least one lesion with measurable disease as defined by RECIST version 1.1. In Cohort 2, patients must have at least one lesion with measurable disease as defined by RECIST version 1.1. Patients with bone-only disease are eligible, even in the absence of measurable

disease. Patients with bone-only disease must have lytic or mixed lesions (lytic + sclerotic). For Cohort 2, imaging documenting progression on the last line of hormone therapy must be available for central review.

4. Age ≥ 18 years at signature of informed consent.
5. Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1.
6. Life expectancy of ≥ 12 weeks, as per investigator.
7. Left ventricular ejection fraction (LVEF) ≥ 50% by echocardiogram (ECHO) or multiple gated acquisition scan (MUGA).
8. Adequate organ function:
    a. Absolute neutrophil count (ANC) ≥ 1.5 X 10^9/L
    b. Hemoglobin ≥ 9 g/dL
    c. Platelets ≥ 100 x 10^9/L
    d. Serum calcium within normal ranges (or corrected with supplements)
    e. Alanine aminotransferase (ALT), aspartate aminotransferase (AST) ≤ 2.5 x upper limit of normal (ULN) and total bilirubin ≤ 1.5 x ULN (in cases of liver involvement, ALT/AST ≤ 5 x ULN and total bilirubin within normal ranges will be allowed)
    f. Serum creatinine ≤ 1.5 x ULN or creatinine clearance ≥ 60 mL/min calculated according to the Cockroft and Gault formula or MDRD formula for patients aged > 65 years (Appendix 19.2)
    g. Serum albumin > 3.0 g/dL

Exclusion Criteria

The presence of any of the following criteria excludes a patient from participating in the study:

1. Central nervous system metastases that are untreated or symptomatic, or require radiation, surgery, or continued steroid therapy to control symptoms within 14 days of study entry.
2. Known leptomeningeal involvement.
3. Advanced/metastatic, symptomatic, visceral spread, with a risk of life-threatening complications in the short term (including patients with massive uncontrolled effusions [pleural, pericardial, peritoneal], pulmonary lymphangitis, and over 50% liver involvement).
4. Participation in another interventional clinical trial or treatment with any investigational drug within 4 weeks prior to study entry.
5. Any systemic anticancer therapy within 3 weeks of the first dose of study treatment. For cytotoxic agents that have major delayed toxicity, e.g. mitomycin C and nitrosoureas, or anticancer immunotherapies, a washout period of 6 weeks is required. For patients in Cohort 2, this does not apply to the most recently received hormone therapy.
6. Major surgery or radiotherapy within 3 weeks of the first dose of study treatment. Patients who received prior radiotherapy to ≥ 25% of bone marrow are not eligible, irrespective of when it was received.
7. Persistent grade > 1 clinically significant toxicities related to prior antineoplastic therapies (except for alopecia); stable sensory neuropathy ≤ grade 1 NCI-CTCAE v. 4.03 is allowed.
8. History of hypersensitivity reaction or any toxicity attributed to trastuzumab, murine proteins, or any of the excipients that warranted permanent cessation of these agents (applicable for Cohort 1 only).
9. Previous exposure to vinorelbine (applicable for Cohort 1 triplet combination only).
10. Exposure to the following cumulative anthracycline doses:
    a. Doxorubicin or liposomal doxorubicin > 360 mg/m²
    b. Epirubicin > 720 mg/m²
    c. Mitoxantrone > 120 mg/m² and idarubicin > 90 mg/m²
    d. Other anthracycline at a dose equivalent to > 360 mg/m² doxorubicin
    e. For patients having received > 1 anthracycline, the cumulative dose must not exceed the equivalent of 360 mg/m² doxorubicin

11. Chronic use of high-dose oral corticosteroid therapy (>10 mg of prednisone equivalent a day).
12. Uncontrolled hypertension (systolic > 150 mmHg and/or diastolic > 100 mmHg) or unstable angina.
13. History of congestive heart failure of Class II-IV New York Heart Association (NYHA) criteria, or serious cardiac arrhythmia requiring treatment (except atrial fibrillation, paroxysmal supraventricular tachycardia).
14. History of myocardial infarction within 6 months of study entry.
15. History of prior or concomitant malignancies (other than excised non-melanoma skin cancer or cured *in situ* cervical carcinoma) within 3 years of study entry.
16. Current dyspnea at rest of any origin, or other diseases requiring continuous oxygen therapy.
17. Current serious illness or medical conditions including, but not limited to uncontrolled active infection, clinically significant pulmonary, metabolic or psychiatric disorders.
18. Known HIV, HBV, or HCV infection.
19. Pregnant or lactating women; women of childbearing potential must use effective contraception methods (patient and/or partner, e.g., surgical sterilization, a reliable barrier method) prior to study entry, for the duration of study participation, and for 7 months after the last dose of MCLA-128/trastuzumab. See Section 8.10.
20. Patients with only non-measurable lesions other than bone metastasis (e.g. pleural effusion, ascites or other visceral locations).
21. Patients with bone-only disease with blastic-only metastasis.

INVESTIGATIONAL AND COMPANION THERAPIES

MCLA-128: 750 mg intravenous flat dose over 2 hours, Day 1 every 3 weeks (q3w).

Premedication with paracetamol/acetaminophen, antihistamines and corticosteroids (as per standard practices) is mandatory for every MCLA-128 infusion.

Trastuzumab: 8 mg/kg intravenous loading dose over 90 minutes on Day 1 Cycle 1, then from Cycle 2, 6 mg/kg will be administered intravenously over 30-90 minutes, on Day 1 of each cycle, q3w. For safety run-in patients, trastuzumab administration will be delayed to Day 2 in Cycle 1.

Vinorelbine: 25 mg/m² intravenously over 10 minutes, Days 1 and 8, every 3 weeks. For safety run-in patients, vinorelbine administration will be delayed to Days 2 and 9 in Cycle 1.

Endocrine therapy: Patients will receive the same dose and regimen as that administered under the last line of endocrine therapy prior to study entry on which the patient progressed.

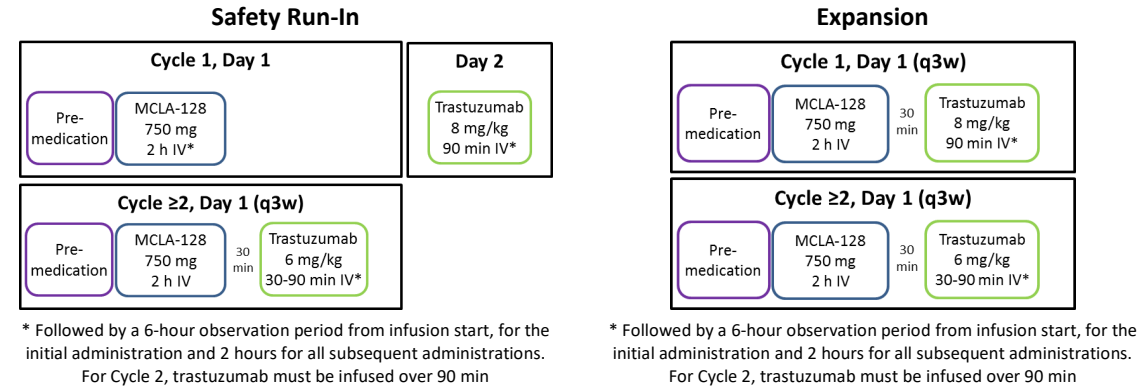
TREATMENT REGIMENS

For all combinations a cycle is considered 3 weeks (including Cohort 2 which may include q4w fulvestrant dosing). A 6-hour observation period will be implemented following infusion start for the initial MCLA-128 and/or trastuzumab administration, and 2 hours for all subsequent administrations.

Cohort 1 doublet combination:

- Safety run-in (4-6 patients): for Cycle 1, MCLA-128 will be administered on Day 1, and trastuzumab on Day 2. From Cycle 2, trastuzumab will be administered on Day 1, 30 minutes after the completion of the MCLA-128 administration.
- Expansion: for all cycles, MCLA-128 will be administered on Day 1 followed by trastuzumab 30-minutes after the end of the MCLA-128 infusion.

Cohort 1 Doublet treatment administration



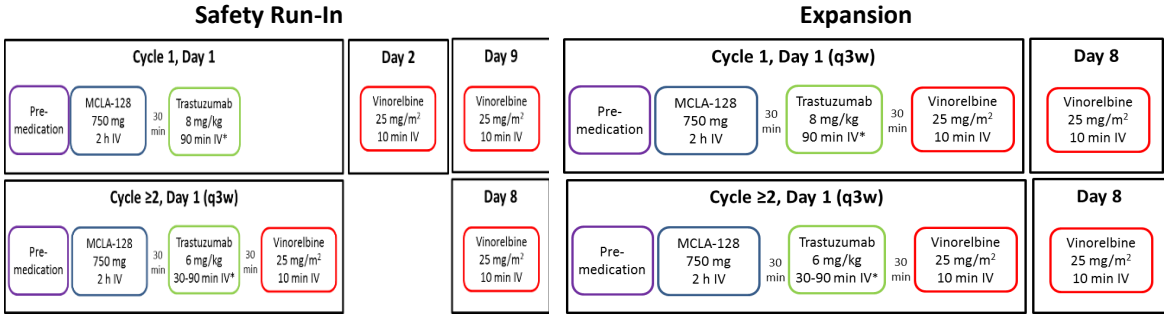
* Followed by a 6-hour observation period from infusion start, for the initial administration and 2 hours for all subsequent administrations. For Cycle 2, trastuzumab must be infused over 90 min

* Followed by a 6-hour observation period from infusion start, for the initial administration and 2 hours for all subsequent administrations. For Cycle 2, trastuzumab must be infused over 90 min

Cohort 1 triplet combination:

- Safety run-in (4-6 patients): for Cycle 1, MCLA-128 will be administered on Day 1 followed 30 minutes later by trastuzumab, and vinorelbine will be administered on Days 2 and 9. From Cycle 2, vinorelbine will be administered on Day 1, 30 minutes after trastuzumab, and on Day 8.
- Expansion: for all cycles, MCLA-128 will be administered on Day 1 followed 30 minutes later by trastuzumab, followed by vinorelbine 30 minutes after the end of the trastuzumab infusion.

Cohort 1 Triplet treatment administration



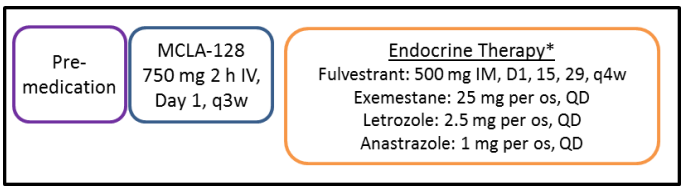
* Followed by a 6-hour observation period from infusion start, for the initial administration and 2 hours for all subsequent administrations. For Cycle 2, trastuzumab must be infused over 90 min

* Followed by a 6-hour observation period from infusion start, for the initial administration and 2 hours for all subsequent administrations. For Cycle 2, trastuzumab must be infused over 90 min

For both the doublet and the triplet combinations, if an individual patient does not tolerate all drugs on the same day, the safety run-in Cycle 1 dosing schedule will be maintained for that patient.

Cohort 2: All patients will receive MCLA-128 administration on Day 1 q3w. For endocrine therapy patients will receive the same dose and regimen as that administered under the last line of endocrine therapy prior to study entry and on which the patient progressed. Fulvestrant will be administered on Days 1, 15, 29 and once every 28 days thereafter, or aromatase inhibitor therapy (letrozole, anastrazole and exemestane) will be administered daily from Day 1.

Treatment administration for Cohort 2 (all cycles)



A 6-hour observation period will be implemented following infusion start for the initial MCLA-128 administration, and 2 hours for all subsequent administrations.

* Same endocrine therapy under which the patient progressed prior to study entry. Can be administered before, during, or immediately after the MCLA-128 infusion.

Treatment assignment: for Cohort 1, if the triplet combination is considered safe, the expansion of the doublet and triplet combinations will be performed in parallel. Patients with be included with a 3:1 ratio for triplet or doublet respectively, favoring the triplet combination and taking into account previous exposure to vinorelbine; for Cohort 2, no specific treatment assignment is required.

Treatment adaptation

- No dose reductions are permitted for MCLA-128, trastuzumab, or endocrine therapy agents.
- The vinorelbine dose will be decreased or interrupted in cases of decreased neutrophil counts or elevated bilirubin levels, according to the SPC, and discontinued if grade ≥ 2 neurotoxicity (NCI-CTCAE v. 4.03) occurs.
- MCLA-128 infusion will be interrupted in the event of an infusion-related reaction (IRR) and must be stopped definitively for severe IRRs. For mild to moderate events the infusion can be resumed at a 50% infusion rate and infusion duration extended to 4 hours.
- MCLA-128 and trastuzumab administration can be delayed for a maximum of 6 weeks between infusions to manage AEs, specifically for clinically significant LVEF decreases, signs of congestive heart failure or persistent grade 2 or grade 3-4 diarrhea.
- Hormone therapy drugs will be administered according to the SPC of each drug.

Treatment duration

Study treatment will be administered until confirmed progressive disease (as per RECIST 1.1), unacceptable toxicity, withdrawal of consent, patient non-compliance, investigator decision (e.g. clinical deterioration), treatment interruption > 6 consecutive weeks, withdrawal of any study drug. Patients will be followed up for safety for at least 35 ± 5 days following the last study drug administration and until recovery/stabilization of related toxicities, and for disease progression and survival status for 12 months.

PROPHYLACTIC AND CONCOMITANT MEDICATION

Permitted

- Administration of paracetamol/acetaminophen, antihistamines and corticosteroids is mandatory with every MCLA-128 administration. In the event of an IRR or hypersensitivity, the patient will be managed according to local clinical practice, as clinically indicated.
- All medication necessary for the wellbeing of the patient and which is not expected to interfere with evaluation of the study drug, including supportive treatment of symptoms and AEs or standard treatment of concomitant conditions may be given at the investigator's discretion.
- Goserelin in pre/peri-menopausal women who started an LHRH agonist >4 weeks prior to study entry.

Prohibited

- Concomitant chronic oral corticosteroids (>10 mg/day prednisone equivalent), TNF-alpha inhibitors, anti-T-cell antibodies (due to risk of immunosuppression).
- Any investigational drugs during the study or 4 weeks prior to the first dose of study treatment.
- Systemic anticancer therapy (other than the last endocrine therapy in Cohort 2) or yellow fever vaccine (Cohort 1) during the study or within 3 weeks of the first dose of study treatment.

SAFETY / TOLERABILITY ASSESSMENTS

- AEs (CTCAE version 4.03), SAEs
- Lab parameters: hematology, biochemistry, coagulation, urinalysis, cytokines
- ECG, MUGA/ECHO
- Medical history, vital signs, performance status and physical exam
- Concomitant medications
- Dose modifications (reductions, interruptions, delays), discontinuation due to toxicity

EFFICACY ASSESSMENTS

Tumor assessment will be based on CT/MRI with contrast per RECIST 1.1, every 6 weeks after treatment start. With the implementation of Protocol version 5.0 this will be every 12 weeks. Objective responses

must be confirmed at least 4 weeks after first observation. Central review of imaging by an independent radiologist(s) will be performed for all patients (screening and on-study).

Enough independent central analysis of patient imaging has been completed and additional assessment of imaging for those patients currently on treatment for at least 24 months is no longer required. Therefore, central review of imaging by an independent radiologist(s) for all patients on-study is no longer applicable as of Protocol Version 5.0.

Bone scans will be performed as clinically indicated for patients with bone metastases at baseline or suspected lesions on study.

Tumor markers (CA15-3, CEA, CA27-29) will be assessed on Day 1 every cycle.

BIOMARKERS

Candidate exploratory biomarkers will be evaluated in tumor tissue (screening, optional after 12 weeks and End of Treatment [EOT]) and blood (pre-dose on Day 1 every 4 cycles and EOT).

Enough biomarker data has been accumulated from all patients treated on the trial. Therefore the additional data from the few patients who have been on treatment for 24 months is not needed as per Protocol Version 5.0.

Tumor: HER2, HER3, HER2:HER3 dimerization, downstream signaling proteins (eg PIK3CA), heregulin, phosphorylation of HER2, HER3 and proteins in the MAPK and AKT signaling pathway, expression of inhibitors such as PTEN, mutations in cancer-related genes including HER2 and HER3 signaling, heregulin-gene fusions.

Blood: Fcγ receptor polymorphism, plasma circulating tumor DNA mutations, exploratory serum biomarkers (e.g. soluble HER2, heregulin).

PHARMACOKINETICS

PK sampling is no longer applicable as of Protocol Version 4.0.

For Protocol Versions 1.0, 2.0 and 3.0, blood samples will be collected to measure serum MCLA-128 and trastuzumab exposure. No PK sampling will be performed for vinorelbine, fulvestrant or aromatase inhibitors.

PK sampling will be performed at the following time points:

<u>Cohort 1 doublet and triplet combinations: MCLA-128</u>

- Cycle 1: Day 1, pre-dose, EOI, and at 2, 4, and 22 hours post EOI, then at any time on Day 8 (or Day 9 for safety run-in triplet patients)
- Cycle 2: Day 1, pre-dose, EOI (run-in and expansion), and at 2, 4, and 22 hours post EOI, then at any time on Day 8 (run-in only)
- Cycles 3 and 5: Day 1, pre-dose and EOI
- Every 4 cycles thereafter: pre-dose

<u>Cohort 1 doublet combination: trastuzumab</u>

- Cycle 1 Day 1: pre-dose and EOI (expansion only)
- Cycle 1 Day 2: pre-dose and EOI (run-in only)
- Cycle 2 Day 1: pre-dose and EOI (run-in and expansion)

<u>Cohort 1 triplet combination: trastuzumab</u>

- Cycles 1 and 2, Day 1: pre-dose and EOI (run-in and expansion)

<u>Cohort 2 MCLA-128/endocrine therapy combination: MCLA-128</u>

- Cycle 1: Day 1, pre-dose, EOI, and at 2, 4, and 22 hours post EOI, then at any time on Day 8;
- Cycle 2, 3, 5: Day 1, pre-dose, EOI
- Every 4 cycles thereafter: pre-dose

IMMUNOGENICITY

Anti-MCLA-128 antibody sampling is no longer applicable as of Protocol Version 4.0.

For Protocol Versions 1.0, 2.0 and 3.0, blood samples (5 mL) will be collected in all patients to assess serum titers of anti-MCLA-128 antibodies pre-dose on Day 1 pre-dose for Cycles 1, 3, 5, every 4 cycles thereafter, and EOT.

CYTOKINES

Blood samples will be collected to analyze a serum cytokine panel (TNFα, IFNγ, IL-1β, IL-6, IL-8, IL-10) in the safety run-in patients of Cohort 1 only as follows (no cytokine evaluation is planned in Cohort 2):

Cohort 1 doublet combination (run-in only):

- Cycle 1: Day 1, pre-dose, 2, 4, and 22 hours post end of infusion (EOI) of MCLA-128
- Cycle 1: Day 2, pre-dose, 2 hours post-EOI of trastuzumab
- Cycle 2: Day 1, pre-dose, 2, 4, and 22 hours post-EOI of MCLA-128

Cohort 1 triplet combination (run-in only):

- Cycles 1 and 2: Day 1, pre-dose, 2, 4, and 22 hours post-EOI of MCLA-128

STATISTICAL CONSIDERATIONS

Sample size

Cohort 1 safety run-in: 4 to 6 evaluable patients in the safety run-in will have power to detect an AE with a true incidence of 33% is 80 to 90%.

Cohort 1 efficacy expansion: 40 evaluable patients in the doublet or triplet combination will have adequate precision to exclude 30% (lower limit of 90% CI > 30%). The threshold for the CBR rate at 24 weeks is defined based on the assumption that PFS follows an exponential distribution with a median of 5 months (clinically relevant) and 3.5 months (not clinically relevant).

Cohort 2: 40 evaluable patients with observed CBR of at least 45% will provide enough precision to exclude 30% (lower limit of 90% CI>30%). The threshold for CBR at 24 weeks is defined based on the assumption that PFS follows an exponential distribution with a median of 5 months (clinically relevant) and 3.5 months (not clinically relevant).

The final number of patients will depend on the safety and efficacy outcomes during the study. Up to ~130 patients are anticipated, allowing for a total of 40 patients in each of the three planned combination regimens and a ~10% rate of non-evaluable patients.

Definitions

All efficacy endpoints will be defined and analyzed based on tumor assessment by RECIST 1.1.

CBR: the proportion of patients with a best overall response of CR, PR or SD ≥ 24 weeks.

ORR: the proportion of patients with best overall response of CR or PR.

PFS: the time from treatment start until radiologic progression or death due to any cause.

PFS ratio: the ratio of PFS with the previous regimen to PFS on study treatment.

DoR: the time from response (CR or PR) until progression or death due to underlying cancer.

OS: the time from treatment start until death due to any cause.

Endpoints

Primary

Cohorts 1 and 2: CBR per investigator radiologic review at 24 weeks

Key secondary

Cohort 1: CBR at 24 weeks per central review, and ORR, PFS, and DoR per investigator and central review

Cohort 2: CBR at 24 weeks per central review, and PFS per investigator and central review

Other secondary (both cohorts):

Safety: Incidence, severity and relationship of AEs, laboratory abnormalities, SAEs, ECG and LVEF measurements and vital signs

Tolerability: discontinuations due to AEs, dose modifications due to AEs, immunogenicity, and cytokine assessments

Other efficacy: DoR (Cohort 2), PFS ratio (Cohort 2), ORR (Cohort 2), and OS (Cohorts 1 and 2)

Pharmacokinetics: C_{max}, C_{0h}, AUC, CL, V_{ss}, t_{max} and $t_{1/2}$ for MCLA-128, and C_{EOI} and C_{0h} for trastuzumab.

Analysis populations

Treated population: patients who receive at least one dose of MCLA-128.

Evaluable for efficacy: patients who receive at least 2 complete cycles (6 weeks) of treatment and have undergone baseline assessment and one on-study tumor assessment, or who discontinue early due to disease progression.

Analyses

Patient disposition and demographics will be analyzed in the treated population, efficacy will be analyzed in the evaluable for efficacy population, and safety will be analyzed in the treated population.

Quantitative variables will be summarized using descriptive statistics. Continuous variables will be presented as N, mean and/or median, standard deviation, range. Categorical variables will be presented using frequencies and percentage.

Criteria for success primary endpoint for Cohorts 1 and 2: A median PFS of 5 months is assumed as relevant, with the activity threshold for CBR at 24 weeks set to 45%.

CBR and ORR will be summarized with accompanying 90% exact binomial CI.

For PFS, OS and DoR the survival function will be estimated using the Kaplan-Meier product limit method; probability estimates and 90% CI will be provided at specified time points; median duration and 90% CI will also be provided. DoR will be estimated for responders only. The number and proportion of any patients with a PFS ratio ≥ 1.3 will be tabulated for Cohort 2 with 90% exact CI.

AEs will be tabulated by the Medical Dictionary for Regulatory Activities (MedDRA®) preferred term and by organ class according to incidence and severity. Severity of AEs will be based on CTCAE 4.03.

PK, immunogenicity, cytokines and biomarkers will be analyzed centrally and reported separately.

STUDY DURATION

First patient in: November 2017

Completion of data analyses: June 2024

Summary of study procedures for all patients

	Protocol Section	Screening	STUDY TREATMENT PERIOD 1 Cycle = 21 days		STUDY TREATMENT PERIOD AS PER PROTOCOL VERSION 5.0	END OF TREATMENT VISIT (EOT)	FOLLOW-UP PERIOD	END OF TREATMENT VISIT (EOT) & FOLLOW-UP VISIT AS PER PROTOCOL VERSION 5.0
STUDY PROCEDURES (all patients)		**≤28 days before treatment**	**Day 1 of each cycle**	**As indicated on-study**	**1 Cycle = 21 days**	**35±5 days from last dosing**	**Every 3 months**	**35±5 days from last dosing**
General Assessments								
Informed consent	15.3	X						
Patient enrollment form		X						
Eligibility criteria	4	X						
Demographics, medical history	-	X						
Diagnosis and prior treatment	-	X						
Baseline symptoms and complaints	8	X						
Concomitant medication	6.2		Continuous		X			
Laboratory Tests								
Serum pregnancy test (other than for patients with proven menopause or surgically sterile)	8.10	X ≤7 days before treatment				X		X
Urine pregnancy test (a serum test must be carried out to confirm a positive result)	8.10		X		X		Up to 7 months after last MCLA-128/trastuzumab treatment	
Urinalysis (dipstick)	8.2	X	As clinically indicated			X		
Tumor Assessments								
CT/MRI scans of chest, abdomen, pelvis, and brain	9.1	X		X every 6 weeks after start of	X every 4 cycles (12 weeks). In	If previous imaging	For patients who discontinue for reasons other	For patients who discontinue for

	Protocol Section	Screening	STUDY TREATMENT PERIOD 1 Cycle = 21 days		STUDY TREATMENT PERIOD AS PER PROTOCOL VERSION 5.0	END OF TREATMENT VISIT (EOT)	FOLLOW-UP PERIOD	END OF TREATMENT VISIT (EOT) & FOLLOW-UP VISIT AS PER PROTOCOL VERSION 5.0
STUDY PROCEDURES (all patients)		**≤28 days before treatment**	**Day 1 of each cycle**	**As indicated on-study**	**1 Cycle = 21 days**	**35±5 days from last dosing**	**Every 3 months**	**35±5 days from last dosing**
any clinically indicated sites of disease and bone lesions as appropriate throughout treatment; clinical evaluation of superficial disease * Follow-up brain scans should only be conducted if lesions are present at baseline				study treatment until progression	case of symptomatic deterioration signs or signs of PD in between the assessments an unscheduled assessment must be performed.	assessment is >6 weeks prior	than progression, as clinically indicated	reasons other than progression, as clinically indicated
Radionuclide bone scan, whole body	9.1	X as close as possible and ≤9 weeks before starting study treatment		As clinically indicated to assess baseline or suspected new bone metastases	As clinically indicated to assess baseline o suspected new bone metastases		As clinically indicated to assess suspected new bone metastases for patients who discontinue for reasons other than progression	As clinically indicated to assess suspected new bone metastases for patients who discontinue for reasons other than progression

Tumor markers (CA15-3, CEA, CA27-29) (as far as possible, measures should be made in the same laboratory)	9.2	X within 7 days before treatment	X			X	For patients with elevated levels at screening who discontinue for reasons other than progression	For patients with elevated levels at screening who discontinue for reasons other than progression.
Other Clinical Assessments								
Adverse events	8		Continuous		X		Related cardiac events up to 1 year	X
Serious adverse events	8		Continuous		X		Suspected only, up to 1 year	X
LVEF (by ECHO or MUGA) and 12-lead electrocardiogram (ECG)	8.8.2	X As close as possible to and within 4 weeks prior to Day 1 Cycle 1.		X Pre-dose Day 1, every 4 cycles (C5, C9, etc) unless performed <7 days, and as clinically indicated	LVEF/ECG every 6 months and at any time during the study if clinically indicated.	X if >6 weeks since last assessment	All patients will undergo LVEF/ECG every 6 months for 1 year after the last MCLA-128 dose. Patients who discontinue due to decrease in LVEF and/or possible CHF should continue LVEF assessments as clinically indicated (≤18 weeks between assessments), until initiation of a new anticancer therapy, LVEF values return to ≥50% or for 1 year, whichever is first.	X
Pharmacokinetics	10			Protocol Versions 1.0,				

				2.0 and 3.0 only: Cycles 1, 2, 3, 5, then every 4 cycles				
Immunogenicity assessment	13			Protocol Versions 1.0, 2.0 and 3.0 only: Pre-dose Day 1 Cycles 1, 3, 5 then every 4 cycles (C9, C13, etc)		Protocol Versions 1.0, 2.0 and 3.0 only: X		
Fresh tumor OR archival sample within 24 months prior to screening (for HER2 status, exploratory biomarkers, and HR status as appropriate)*	11.1.1	X		Optional sample 12 weeks after start of study treatment		Optional (preferably from site of progression)		Optional (preferably from site of progression)
Liquid biopsy (for exploratory biomarkers)	11.1.2			Pre-dose Day 1 Cycle 1, then every 4 cycles (C5, C9, etc)		X		
Other investigations as clinically indicated	-	X	X	X	x	X	X	X

***For Cohort 2 patients with bone-only disease, primary tumor tissue will be used (**unless it is determined that testing for HER2 expression in a bone biopsy is possible)

Summary of study procedures and timings specific to Cohort 1 patients (doublet and triplet combinations)

	Protocol Section	Screening	STUDY TREATMENT PERIOD 1 Cycle = 21 days	STUDY TREATMENT PERIOD AS PER PROTOCOL VERSION 5.0	END OF TREATMENT VISIT	END OF TREATMENT VISIT (EOT) & FOLLOW-UP VISIT AS PER PROTOCOL VERSION 5.0
STUDY PROCEDURES (Cohort 1)		**≤7 days before treatment**	**Days 1, 2, 8 and 9 of each cycle as specified**	**1 Cycle = 21 days**	**35±5 days from last dosing**	**35±5 days from last dosing**
Clinical Assessments						
Physical examination, ECOG PS, height (screening only) and weight, vital signs	Appendix 19.1	X	**Doublet:** Days 1 and 8 of Cycle 1 and Day 1 of each subsequent cycle **Triplet safety run-in:** Days 1,2 and 9 of Cycle 1 and Days 1 and 8 of each subsequent cycle **Triplet expansion:** Days 1 and 8 of each cycle		X	X
Cytokine panel	12		**Doublet safety run-in:** Days 1 and 2 of Cycle 1 and Day 1 of Cycle 2 **Triplet safety run-in**: Day 1 of Cycles 1 and 2			
Laboratory Assessments						
Complete blood count (CBC)	8.2	X	**Doublet:** Days 1 and 8 of Cycle 1 and Day 1 of each subsequent cycle **Triplet safety run-in:** Days 1 and 9 of Cycle 1 and Days 1 and 8 of each subsequent cycle **Triplet expansion:** Days 1 and 8 of each cycle	Days 1 and 8 of each cycle	X	X
Serum chemistries	8.2	X	**Doublet:** Days 1 and 8 of Cycle 1 and Day 1 of each subsequent cycle **Triplet safety run-in:** Days 1 and 9 of Cycle 1 and Days 1 and 8 of each subsequent cycle **Triplet expansion:** Days 1 and 8 of each cycle	Days 1 and 8 of each cycle	X	X
Study Treatment						
MCLA-128 premedication (immediately prior to MCLA-128)	5.3.1.1		Day 1 of each cycle	X		
MCLA-128 (intravenous)	5.3.1.1		Day 1 of each cycle	X		
Trastuzumab (intravenous; 30 min after MCLA-128 when given on the same day)	5.3.1.2		**Doublet safety run-in:** Day 2 of Cycle 1 and Day 1 of each subsequent cycle	X		

	Protocol Section	Screening	STUDY TREATMENT PERIOD 1 Cycle = 21 days	STUDY TREATMENT PERIOD AS PER PROTOCOL VERSION 5.0	END OF TREATMENT VISIT	END OF TREATMENT VISIT (EOT) & FOLLOW-UP VISIT AS PER PROTOCOL VERSION 5.0
STUDY PROCEDURES (Cohort 1)		**≤7 days before treatment**	**Days 1, 2, 8 and 9 of each cycle as specified**	**1 Cycle = 21 days**	**35±5 days from last dosing**	**35±5 days from last dosing**
			Doublet expansion and all triplet: Day 1 of each cycle			
Vinorelbine (intravenous; 30 min after trastuzumab when given on the same day)	5.3.1.3		**Triplet safety run-in:** Days 2 and 9 of Cycle 1, then Days 1 and 8 of each subsequent cycle **Triplet expansion:** Days 1 and 8 of each cycle	X		

Summary of study procedures and timings specific to Cohort 2 patients (MCLA-128 + endocrine therapy combination)

	Protocol Section	Screening	STUDY TREATMENT PERIOD 1 Cycle = 21 days		STUDY TREATMENT PERIOD AS PER PROTOCOL VERSION 5.0	END OF TREATMENT VISIT	END OF TREATMENT VISIT (EOT) & FOLLOW-UP VISIT AS PER PROTOCOL VERSION 5.0
STUDY PROCEDURES (Cohort 2)		**≤7 days before treatment**	**Day 1 of each cycle**	**Day 8 of Cycle 1 only**	**1 Cycle = 21 days**	**35±5 days from last dosing**	**35±5 days from last dosing**
Clinical Assessments							
Physical examination, ECOG PS, height (screening only) and weight, vital signs	Appendix 19.1	X	X	X		X	X
DEXA scan (bone mineral density)	-	X Up to 6 months prior to Day 1 Cycle1	X Every 12 months since last evaluation		X Every 12 months		
Laboratory Assessments							
Complete blood count (CBC)	8.2	X	X	X	Day 1 of each cycle	X	X
Serum chemistries	8.2	X	X	X	Day 1 of each cycle	X	X
Study Treatment							
MCLA-128 premedication (immediately prior to MCLA-128)	5.3.1.1		X		X		
MCLA-128 (intravenous)	5.3.1.1		X		X		
Endocrine therapy (as per last prior line immediately before study entry)	5.3.1.4		Same dose and regimen on which the patient most recently progressed		X		

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ABBREVIATIONS

ADA	Anti-Drug Antibody
ADCC	Antibody-Dependent Cell-Mediated Cytotoxicity
AE	Adverse Event
ALT	Alanine Transaminase
AST	Aspartate Transaminase
AUC	Area Under The Curve
CBR	Clinical Benefit Rate
CEOI	Concentration at the end of the infusion
CHF	Congestive Heart Failure
CHO	Chinese Hamster Ovary
CL	Clearance
C_{max}	Maximum Drug Concentration
CR	Complete Response
CRA	Clinical Research Associate
CRO	Clinical Research Organization
CTCAE	Common Terminology Criteria For Adverse Events
ctDNA	Circulating Tumor DNA
DEXA	Dual-Energy X-ray Absorptiometry
$EC_{20/50}$	Concentration Corresponding to 20%/50% of Maximum Effect
ECG	Electrocardiogram
ECHO	Echocardiogram
ECOG	Eastern Cooperative Oncology Group
EGF	Epidermal Growth Factor
EGFR	Epidermal Growth Factor Receptor
ELISA	Enzyme-Linked Immunosorbent Assay
EOI	End of Infusion
EOT	End of Treatment
ER	Estrogen Receptor
ET	Endocrine Therapy
Fab	Fragment Antigen-Binding
Fc	Fragment Crystallizable
FDA	Food And Drug Administration
FFPE	Formalin-Fixed Paraffin-Embedded
HER	Human Epidermal Growth Factor Receptor
FISH	Fluorescence *In Situ* Hybridization
HR	Hazard Ratio

HRG	Heregulin
IC_{50}	Half Maximal Inhibitory Concentration
ICH	International Conference On Harmonization
IDMC	Independent Data Monitoring Committee
IFN	Interferon
Ig	Immunoglobulin
IEC	Independent Ethics Committee
IHC	Immunohistochemistry
IRB	Independent Review Board
IL	Interleukin
IM	Intramuscular
IRR	Infusion Related Reactions
IV	Intravenous
K_D	Dissociation Constant
LDH	Lactate Dehydrogenase
LVEF	Left Ventricular Ejection Fraction
MBC	Metastatic Breast Cancer
MTD	Maximum Tolerated Dose
MUGA	Multiple Gated Acquisition
NFKB	Nuclear Factor Kappa
ORR	Overall Response Rate
OS	Overall Survival
OTD	Oncology Therapeutic Development
PBMC	Peripheral Blood Mononuclear Cell
PD	Progressive Disease
PFS	Progression-Free Survival
PK	Pharmacokinetics
PO	Per Os
PR	Partial Response
PT	Preferred Term
QD	Once Daily
q3w	Once Every 3 Weeks
QTc	Corrected QT Interval
RP2D	Recommended Phase 2 Dose
RBC	Red Blood Cell
RECIST	Response Evaluation Criteria In Solid Tumors Guidelines
RTK	Receptor Tyrosine Kinase

SAE	Serious Adverse Event
SD	Stable Disease
SOC	System, Organ, Class
SPC	Summary of Product Characteristics
SUSAR	Suspected Unexpected Serious Adverse Reaction
$t_{1/2}$	Terminal half-Life
T_{max}	Time Of Observed Maximum Serum Drug Concentration
TNBC	Triple Negative Breast Cancer
TNF	Tumor Necrosis Factor
V	Apparent Volume Of Distribution
V_{ss}	Volume Of Distribution At Steady State
WBC	White Blood Cell

1 BACKGROUND

1.1 Metastatic Breast Cancer and Current Therapeutic Options

Breast cancer is a global disease, with a yearly incidence of over 1.67 million patients worldwide reported in 2012, accounting for over 25% of all cancers in women and ranking as the fifth cause of cancer death in women (522,000 deaths) (IARC/WHO). In the United States, over 250,000 women are expected to develop the disease in 2017, with over 40,010 deaths (Siegel et al., 2017).

Approximately 5-10% of breast cancer patients are diagnosed with metastasis at initial presentation, and overall 20% will eventually develop metastasis (Chang et al., 2003). The development of metastases dramatically worsens the prognosis. About 90% of breast cancer mortality is due to metastases that are resistant to adjuvant therapies (Gonzalez-Angulo et al., 2007). Metastatic breast cancers (MBC) represent a heterogeneous population with a diverse clinical course. Median survival ranges from 15 to 27 months in stage IV breast cancer despite more aggressive disease management and recent more effective therapeutic agents (Andre et al., 2004; Giordano et al., 2004; Dawood et al., 2008), and overall survival (OS) rates vary significantly. This highly unpredictable clinical behavior reflects the biologic heterogeneity of the disease, which is classified based on the presence or the absence of hormone receptors for estrogen and/or progesterone, and the expression/amplification status of the human epidermal growth factor receptor 2 (HER2) protein/oncogene (Hammond et al., 2010; Wolff et al., 2013).

The primary goals of systemic treatment for MBC are prolongation of survival, alleviation of symptoms, and maintenance or improvement in quality of life, despite the toxicity associated with treatment (Osoba, 1995; Geels et al., 2000; Stockler et al., 2000). Systemic treatments for MBC include chemotherapy, endocrine therapy (ET), biological targeted therapy, and supportive therapy. For patients with advanced MBC, the choice of therapy is based on considerations related to patient characteristics and comorbidities, disease status, prior treatments, and biological characteristics of the tumor (NCCN, 2017.; Bernard-Marty et al., 2004).

1.1.1 HER2-Positive Breast Cancer

Approximately 15% to 20% of breast cancer tumors overexpress HER2 (Slamon et al., 1987; Witton et al., 2003), a member of the HER superfamily composed of tyrosine kinase receptors regulating proliferation and survival of epithelial cells. The family includes four receptors: HER1 (epidermal growth factor receptor [EGFR]), HER2 [neu, C-erbB2], HER3 and HER4. HER2 is considered an orphan receptor as it has no known ligand, while the three other HER receptors have known ligands and form either homodimers or heterodimers upon ligand binding. HER2 can heterodimerize with any of the other receptors and is considered the preferred dimerization partner. Dimerization results in autophosphorylation of tyrosine residues within the receptor cytoplasmic domain and initiates signal transduction via the PI3K/AKT and RAS/MAPK pathways (Browne et al., 2009; Castaneda et al., 2010).

HER2 overexpression/amplification in breast cancers is associated with a more aggressive clinical phenotype and historically portends poor prognosis (Slamon et al., 1987). Although HER2-overexpression identifies patients who are likely to respond to therapy with trastuzumab, not all these patients retain benefit from treatment. Approximately 15% of patients relapse after therapy, indicating the presence of *de novo* or acquired resistance (Nahta and Esteva, 2006). Several resistance mechanisms have been proposed including (1) altered receptor-antibody interaction, (2) activation of downstream pathways via increased signaling from other HER family members or other receptors, or (3) constitutive activation of downstream elements.

1.1.1.1 Anti-HER2 Antibodies in HER2-Positive MBC

The humanized anti-HER2 antibody trastuzumab (Herceptin®) improves response rate, PFS and OS when added to chemotherapy for advanced HER2-positive breast cancers (Slamon et al., 2001). In early stage HER2-positive breast cancer, several pivotal trials have shown improvements in disease-free survival and OS, and trastuzumab/chemotherapy combination has now become standard therapy for most such cases (O'Sullivan et al., 2015). Nonetheless approximately 30–50% of patients with naïve HER2-positive MBC do not achieve an objective response in the first-line setting indicating *de novo* resistance to trastuzumab, with both time to progression (TTP) and OS being short (7–17 months and 22–38 months, respectively) (Slamon et al., 2001; Baselga et al., 2012b; Nielsen et al., 2013; Swain et al., 2013). This has motivated the development of alternative approaches to block HER2 signaling.

Multiple lines of research have highlighted a central role for HER3 signaling as a key mechanism for *de novo* and acquired resistance to HER2 therapies in several tumor types leading to the development of compounds aimed at disrupting signaling through HER2:HER3 heterodimerization, the mechanism by which HER3 elicits signaling (Jiang et al., 2012).

Pertuzumab is a fully humanized monoclonal antibody that binds to a different epitope of the HER2 extracellular domain (subdomain II) than trastuzumab (subdomain IV), presumably preventing HER2 from dimerizing with other HER family members (EGFR, HER3 and HER4), notably HER3. In 2012, results from the phase 3 CLEOPATRA study (Clinical Evaluation of Pertuzumab and Trastuzumab) led to FDA approval for pertuzumab in combination with trastuzumab and docetaxel for the treatment of patients with HER2-positive MBC who have not received prior anti-HER2 or chemotherapy for metastatic disease. The primary endpoint of centrally confirmed PFS was significantly longer with the addition of pertuzumab, improved by 6.3 months from a median of 12.4 to 18.5 months (hazard ratio [HR] of 0.62 for progression or death, 95% CI 0.51–0.75, p <0.001) (Baselga et al., 2012b). OS was also markedly prolonged with the addition of pertuzumab, with 15.7-month increase from 40.8 to 56.5 months (HR 0.68, 95% CI 0.56–0.84, p <0.001) (Swain et al., 2015), confirming that this triple drug combination is a promising approach.

1.1.1.2 T-DM1 in HER2-Positive MBC

Ado-trastuzumab emtansine (T-DM1) is a novel antibody drug conjugate composed of trastuzumab linked to emtansine (DM1; a derivative of maytansine), a highly potent anti-microtubule cytotoxic agent. T-DM1 was granted FDA approval in 2013 for HER2-positive MBC progressing after trastuzumab and taxane therapy in the metastatic setting, or following early relapse on adjuvant trastuzumab-based therapy. Approval was based on the EMILIA trial, a randomized, open-label, international phase 3 trial comparing T-DM1 with lapatinib plus capecitabine in patients with HER2-positive MBC previously treated with trastuzumab and a taxane (Verma et al., 2012). Median PFS was significantly prolonged with T-DM1 compared with lapatinib plus capecitabine (9.6 versus 6.4 months; HR for progression or death from any cause 0.65, 95% CI 0.55–0.75, p <0.001). Median OS was also significantly improved with T-DM1 (30.9 versus 25.1 months; HR for death from any cause 0.68, 95% CI 0.55–0.85, p <0.001) and ORR was higher with T-DM1 (43.6% versus 30.8%, p <0.001). Based on this, T-DM1 is now the standard of care as second-line therapy for HER2-positive MBC. A second randomized, open-label, international phase 3 trial (TH3RESA) provided supportive data (Krop et al., 2017). For patients progressing on trastuzumab-based therapy and T-DM1, treatment options include trastuzumab with another chemotherapy agent, or with combined capecitabine and lapatinib.

1.1.2 Hormone Receptor Positive Breast Cancer

Approximately 75% of breast cancers are considered estrogen and/or progesterone receptor positive (Nadji et al., 2005; Rugo, 2008). Such tumors are associated with better survival than those with low or no estrogen receptor expression (Yu et al., 2012). The hormone estrogen (17ß-estradiol E2) represents the primary stimulant in the growth and development of these tumors (Johnston and Dowsett, 2003). Thus, deprivation of estrogen signaling via ET has become the mainstay of treatment for early and metastatic estrogen receptor positive (ER+) disease in the absence of visceral crisis.

1.1.2.1 Endocrine Therapy

A variety of ET options are available, including oophorectomy, gonadotropin-releasing hormone analogs, selective estrogen receptor modulators (SERMs; tamoxifen), selective estrogen receptor degraders (SERDs; fulvestrant) and aromatase inhibitors (AIs; letrozole, anastrozole and exemestane) (Buzdar and Hortobagyi, 1998; Lumachi et al., 2015). AIs block estrogen production by inhibiting aromatases in both peripheral tissues and the cancer, and are only effective for postmenopausal women in whom the major source of estrogen comes from the conversion of androgens to estrogens via aromatase enzyme activity in peripheral tissues such as adipose tissue and the breast. Tamoxifen was the first selective estrogen receptor modulator used clinically and is still widely prescribed in pre-menopausal women.

The use of hormone therapies targeting and downregulating estrogen receptor signaling is a mainstay of treatment for patients with ER+ breast cancers, and has substantially reduced the relapse rate for early-stage breast cancer (Early Breast Cancer Trialists' Collaborative Group (EBCTCG), 2005). However about one-third of all hormone receptor-positive/HER2-negative patients diagnosed with early stage disease, experience disease recurrence (Baselga et al., 2012b), and in this setting single-agent AIs or tamoxifen show limited clinical benefit (Klijn et al., 2000; Baselga et al., 2012a). Resistance manifests as either relapse after treatment of primary hormone receptor-positive breast cancer or as disease progression in the metastatic setting (Larionov and Miller, 2009; Dalmau et al., 2014).

ET resistance is complex and heterogeneous and may differ from patient to patient, between primary and acquired resistance and even between ET types. A wide range of distinct mechanisms implicated in ET resistance in breast cancer have been described including 1) ER mutations in the ligand-binding domain (Li et al., 2013; Merenbakh-Lamin et al., 2013; Segal and Dowsett, 2014), epigenetic changes (Parl, 2003; Fan et al., 2006) or reduced expression (Martínez-Galán et al., 2014), 2) crosstalk between the ER and EGFR/HER2 involving the PI3K-Akt-mTOR and MAPK pathways, with HER2 overexpression conferring ET resistance, even in the presence of hormone receptors (Lupien et al., 2010) with crosstalk resulting in estrogen receptor phosphorylation and activation (Campbell et al., 2001; Yamnik et al., 2009), 3) NFKB signaling and inflammation including overexpression in ET-resistant breast tumors (Gu et al., 2002; deGraffenried et al., 2004a; Zhu et al., 2006; Morrison et al., 2014), 4) cancer stem cell development may be resistant to ET due to low estrogen receptor expression, 5) prolonged estrogen deprivation can lead to estrogen hypersensitivity (Herynk et al., 2010), 6) epithelial-mesenchymal transition with loss of estrogen receptor expression, and 7) inhibition of phagocytosis can re-sensitize resistant cells to tamoxifen (Clarke, 2011).

1.1.2.2 CDK4/6 Therapy

A more recent strategy in treating ER+ breast cancer is to target cyclin-dependent kinases 4 and 6 (CDK4/6), a key pathway involved in regulation of the G1/S transition of the cell cycle. Preclinical

models suggest a particular role for CDK4/6 inhibition in ER+ breast cancer cells, including estrogen-sensitive and estrogen-resistant models. Cyclin D1 amplification is a common event in ER+ breast cancer, identified in 58% of hormone receptor-positive/HER2-negative breast cancer (Cancer Genome Atlas Network, 2012). Antiestrogen-induced growth arrest in ER+ breast cancer cells is accompanied by decreased cyclin D1 expression, whereas the emergence of ET resistance is associated with persistent cyclin D1 expression and retinoblastoma (Rb) phosphorylation (Watts et al., 1995; Thangavel et al., 2011). Evidence of a continued role for CDK4/6 inhibition in ET-resistant ER+ cells comes from *in vitro* evaluation of the CDK4/6 inhibitor palbociclib in a panel of molecularly characterized breast cancer cell lines that demonstrated the most activity in ER+ cancers including those with conditioned estrogen resistance (Finn et al., 2009).

Anti-CDK4/6 agents inhibit the phosphorylation of the Rb tumor suppressor, which promotes Rb-E2F binding and prevents E2F-mediated oncogenic transcription. Compelling preclinical data indicate the efficacy of palbociclib in ER+ breast cancer cell lines (Fry et al., 2004; Toogood et al., 2005; Dean et al., 2010). The phase 3 PALOMA-2 trial confirmed 10-month improved PFS in women with ER+ MBC treated with first-line letrozole plus palbociclib versus letrozole alone (24.8 vs 14.5 months, HR, 0.58; P <.000001) (Finn et al., 2016), i.e., in women who had not received ET for metastatic disease. The phase 3 PALOMA-3 trial demonstrated 5-month improved PFS in women with ER+ MBC who had progressed despite ET for metastatic disease, treated with palbociclib plus fulvestrant, versus fulvestrant alone (9.5 vs 4.6 months; HR, 0.46; P <.0001) (Cristofanilli et al., 2016). Thus palbociclib plus letrozole is the preferred first-line therapy in women with ER+ MBC, and palbociclib plus fulvestrant is effective therapy in patients with ER+ MBC not previously treated with palbociclib who have progressed on a nonsteroidal AI.

The success of palbociclib spurred the development of other CDK4/6 inhibitors including ribociclib (Hortobagyi et al., 2016), which is now FDA approved in combination with fulvestrant, and abemaciclib, which has been granted an FDA breakthrough therapy designation, while FDA approval will be requested for abemaciclib in combination with fulvestrant, based on the MONARCH-2 study (Sledge et al., 2017).

1.1.2.3 mTOR Inhibition

The PI3K-Akt-mTOR pathway is a key intracellular signaling pathway playing a significant role in cell growth and proliferation, and is implicated in resistance to ET, HER2-directed therapy, and cytotoxic chemotherapy. Furthermore, mTOR inhibitors such as everolimus synergized with letrozole in preclinical models (Mayer, 2013) and mTOR was described as a mechanism facilitating escape of long-term estrogen deprivation (deGraffenried et al., 2004b). The addition of mTOR inhibitors to ET has been investigated in phase 2 and 3 trials, including patients with ER+/HER2-negative MBC.

The phase 3 trial BOLERO-2 enrolled 724 patients with ER+ advanced breast cancer with recurrence during or within 12 months after adjuvant non-steroidal AIs or progression during or within 1 month after AI treatment (Baselga et al., 2012a). Patients were randomized to receive everolimus combined with exemestane versus exemestane plus placebo. The pre-planned interim analysis showed that median PFS was significantly better for everolimus plus exemestane compared to the control arm, for both investigator analyses (6.9 months versus 2.8 months; HR = 0.43, 95% CI 0.35-0.54, p <0.001) and central analyses (10.6 versus 4.1 months, HR = 0.36, 95% CI 0.27 to 0.47, p <0.001). After a median 18-month follow-up, the addition of everolimus to exemestane was confirmed to significantly improve patient outcome over exemestane alone (median PFS 7.8 versus 3.2 months, HR = 0.45, 95% CI 0.38

to 0.54, p <0.0001) (Yardley et al., 2013). Everolimus in combination with exemestane is approved by both the FDA and the EMA for hormone receptor-positive/HER2-negative advanced MBC patients after failure of a non-steroidal AI.

1.2 Development of MCLA-128

1.2.1 Description of MCLA-128

MCLA-128 is a bispecific humanized full length IgG1 antibody with enhanced antibody-dependent cell-mediated cytotoxicity (ADCC) activity targeting the receptor tyrosine kinases HER2 and HER3. It is produced by recombinant expression in the Chinese hamster ovary cell line, CHO-DG44.

Molecular formula: $C_{6479}H_{9997}N_{1725}O_{2024}S_{45}$

Molecular mass: 145.9 kDa

Chemical name: Not yet issued

INN: Not yet issued

MCLA-128 is composed of two identical common light chains and two different heavy chains each containing a different fragment antigen-binding. The Fc regions of both heavy chains have been engineered in a complementary manner using CH3 engineering, to ensure efficient heterodimerization and bispecific antibody formation and to enhance ADCC activity following a reduction of fucosylation. MCLA-128 has a 1331 amino acid sequence containing four disulfide bonds and a molecular weight of 145.9 kDa.

MCLA-128 acts via two independent mechanisms of action: 1) inhibition of HER3 pathway signaling by disruption of HER2:HER3 heterodimerization and 2) elimination of tumor cells via enhanced ADCC, and employs a 'dock and block' mechanism. Based on X-ray crystal structure, MCLA-128 docks to the HER2 domain I which orientates the HER3 binding arm to block the HER3 domain III (putative HRG domain), thus blocking oncogenic signaling via the HER2:HER3 heterodimer (Maussang-Detaille et al.). MCLA-128 does not compete with trastuzumab for binding to HER2 since it is binding to a different domain.

MCLA-128 is formulated as a clear liquid solution for infusion at a target concentration of 20 mg/mL in 25 mM histidine, 220 mM trehalose and 0.16 mM polysorbate 20 at pH 6.0 in clear type I glass vials with an extractable volume of 5 mL or 18.8 mL.

1.2.2 Preclinical Studies

1.2.2.1 Pharmacology

In vitro pharmacodynamic studies showed the mean K_D values for MCLA-128 of 3.2 and 2.0 nM in BT-474 and SK-BR-3 cells (HER2-amplified breast cancer cell lines also expressing HER3), respectively, and which were in the same range as for trastuzumab (3.7 and 1.3 nM respectively). Although K_D values of the individual HER2 and HER3 Fabs of MCLA-128 showed a higher affinity for the HER3 Fab compared to HER2 Fab, the overall affinity of MCLA-128 was closest to the HER2 Fab and correlates with the overexpression of HER2 in the cell lines. MCLA-128 potently inhibited heregulin (HRG)-induced proliferation and morphologic changes of HER2-overexpressing breast cancer cell lines SK-BR-3 and BT-474. MCLA-128 has been shown to inhibit HRG-mediated proliferation in breast and gastric cell lines, at a notably lower half-maximal inhibitory concentration (IC_{50}=35 pM) than combined

trastuzumab and pertuzumab, and fully inhibited HRG (3.75 nM)-induced HER2:HER3 dimerization with a higher potency than the anti-HER2 pertuzumab plus trastuzumab antibody combination. HRG binds to HER3, resulting in its dimerization with HER2 and subsequent induction of intracellular signaling (Peess et al., 2015). MCLA-128 selectively inhibits the formation of HRG-induced HER2:HER3 dimers.

In vivo, MCLA-128 was evaluated in a xenograft murine model with the breast cancer cell line JIMT-1 leading to tumor volume reduction after 29 days with 60% survival (with four weekly doses of 25 or 2.5 mg/kg MCLA-128), while no animals treated with trastuzumab and pertuzumab experienced a decrease in tumor volume and none survived. Both HER2:HER3 heterodimerization and HER3:PI3K formation was significantly inhibited in MCLA-128-treated tumor tissue.

Breast cancer brain metastases are known to use an HRG-dependent survival mechanism (Momeny et al., 2015). In an orthotopic ST1360B murine brain metastatic breast cancer model, all mice treated with MCLA-128 (25 mg/kg twice weekly) survived for 21 days, compared to all vehicle animals which were sacrificed.

1.2.2.2 Pharmacokinetics

The cynomolgus monkey was selected as the non-clinical toxicology species due to cross reactivity of the two Fab fragments in MCLA-128 towards both human and monkey HER2 and HER3, and lack of cross-reactivity of the HER2 binding arm to rodent HER2.

Following a single intravenous dose of MCLA-128 in cynomolgus monkeys, systemic exposure was dose proportional across the range 10-100 mg/kg, with terminal elimination half-lives in the range 73-210 hours. Half-life tended to increase with increasing dosage, suggesting a possible contribution from target-mediated clearance at lower dosages, with endogenous IgG clearance mechanisms being more predominant at higher dosages. The calculated volumes of distribution were in the range 68.3-121 mL/kg, slightly higher than the plasma volume in monkeys, suggesting limited distribution outside the plasma compartment.

Exposure following the first dose in the repeated dose toxicity study (five doses of MCLA-128, once weekly for 5 weeks) was dose-proportional and there was no sex difference in PK parameters. Most animals were continuously exposed to MCLA-128 during the treatment period, with a 3-fold accumulation (based on AUC) observed in females at 10 mg/kg/week, no accumulation at 30 mg/kg/week, and only slight accumulation (1.4-1.7 fold) in low-dose males (10 mg/kg/week) and animals at 100 mg/kg/week. Few animals showed evidence of loss of exposure towards the end of the treatment period, which correlated with the presence of ADA.

1.2.2.3 Toxicology

MCLA-128 was well tolerated following single and repeated dose (up to five weekly doses) intravenous administration in cynomolgus monkeys, with no treatment-related clinical, laboratory or pathological findings up to the highest dose tested (100 mg/kg).

There was no evidence of cardiotoxicity or effects on thyroid function. The 1-month repeated dose toxicity study in cynomolgus monkeys, at IV doses up to 100 mg/kg, showed that MCLA-128 had no effect on the central or peripheral nervous systems, ECG intervals, blood pressure, or respiration rate. There was some evidence of an immune response to MCLA-128 in a few treated animals in the repeated dose toxicity study, but all treated animals were exposed to MCLA-128 for all of or the

majority of the treatment period, such that the formation of ADA in isolated animals was not considered to have compromised the safety evaluation. There were no treatment-related changes to hematologic, chemistry or urinalysis parameters. Overall, all changes observed were procedural in origin, or related to an immune response to MCLA-128 in individual monkeys in the treatment groups. There was no evidence of target organ toxicity in the clinical pathology investigations or terminal pathology data.

MCLA-128 formulations showed good local tolerability, with no evidence of local irritation at injection sites in the cynomolgus monkey toxicity studies, and no hemolytic potential or plasma incompatibility in an *in vitro* hemocompatibility study conducted in human blood. A tissue cross-reactivity study conducted with human tissues showed no evidence of any non-specific or 'off-target' binding that was likely to be of clinical concern, most non-epithelial binding being cytoplasmic in nature, and thus unlikely to be relevant to the *in vivo* administration.

There was no evidence of cytokine release after infusion of MCLA-128 in cynomolgus monkeys at doses up to 100 mg/kg. Since monkeys are known to be poor predictors of cytokine release in humans, *in vitro* cytokine release studies were performed. *In vitro* cytokine release studies conducted using whole human blood from healthy donors showed a very low potential for MCLA-128 to induce cytokine release, compared to reference antibodies such as Campath®. In this test system, both MCLA-128 and trastuzumab showed little or no cytokine release However, since trastuzumab is known to induce a certain incidence of infusion reactions in clinical use, and there are likely to be low levels of HER2/HER3 expression in healthy donor blood (as used in the *in vitro* cytokine release assays), further cytokine release testing was performed as part of the ADCC effector function assays conducted with HER2 and HER3 expressing cell lines. In these studies, cytokines were determined in supernatants collected following co-incubation of human effector cells (PBMCs) with the HER2 and HER3 expressing SK-BR-3 cell line, with or without addition of MCLA-128 (and also trastuzumab as a comparator antibody). Based on the EC_{20} and EC_{50} values for the four cytokines that were significantly released upon incubation with the therapeutic antibodies (i.e. IFNγ, IL-6, IL-8 and TNFα), it can be concluded that MCLA-128 was 2.2 to 7.1 times more potent in inducing cytokine release than trastuzumab.

Genotoxicity and carcinogenicity studies are not considered appropriate for this class of biological product.

1.2.3 MCLA-128 Clinical Experience

At the time of protocol writing, MCLA-128 was under evaluation in an ongoing first-in-human phase 1/2 study in adult patients with advanced solid tumors (MCLA-128-CL01). A summary of this open-label dose-escalation study of single agent MCLA-128 followed by cohort expansion at the recommended phase 2 dose (RP2D) is summarized here. See the Investigator Brochure for further details.

The primary objective of the dose escalation part of the study was to determine the maximum tolerated dose (MTD) in patients with advanced or metastatic epithelial tumors relapsed or refractory to at least 2 prior regimens of standard treatment or without any standard treatment available. Secondary objectives included safety/tolerability, PK, immunogenicity and efficacy.

The phase 1 part of the study is complete with 9 dose levels investigated with MCLA-128 administered at 40, 80, and 160 mg in cohorts of 1 patient, then 240, 360, 480, 600, 750, and 900 mg (flat dose) once every 3 weeks (q3w) administered over 60-120 minutes intravenously (IV) in cohorts of 3 to 6

patients. No dose limiting toxicities were experienced up to and including 900 mg in the phase 1 part. The Data Review Committee selected 750 mg q3w as the RP2D as a 120-minute IV infusion based on the cumulative safety profile, available PK data and a PK simulation study.

At the initiation of the current MCLA-128-CL02 study, safety, tolerability and efficacy were under evaluation at the RP2D in the phase 2 part in selected solid tumor expansion cohorts with advanced or metastatic relapsed or refractory epithelial HER2-expressing tumors; to date, cohorts have been opened for metastatic breast, gastric/gastroesophageal, ovarian, colorectal (cohort closed prematurely), non-small cell lung cancer and endometrial cancers.

1.2.3.1 Patients and Treatment

As of 15 February 2017, a total of 47 patients were enrolled in the study, 28 in the phase 1 part and 19 in the phase 2 part.

In the phase 1 part, median age was 60 years (range 25-83), 61% of patients were male, and ECOG PS was 0/1 in 43%/57% of patients. The main tumor types were colorectal (8 patients), gastric/gastroesophageal (6 patients), and breast (3 patients). Patients had a median of 2 metastatic sites (0-14) and had received a median of 3 lines of prior metastatic therapy (range 1-7).

Among the 19 patients enrolled in the phase 2 part (3 not yet treated at the data cut-off), ten had breast cancer, 6 gastric/gastroesophageal, 2 ovarian, and 1 colorectal cancer. Median age was 54 years (range 33-74), 32% of patients were male, and ECOG PS was 0/1 in 17%/83% of patients. Patients had a median of 2.5 metastatic sites (range 1-4) and had received a median of 5.5 prior lines of metastatic therapy (2-18). All 10 enrolled breast cancer patients had received 3-4 prior lines of anti-HER2 therapies.

A median of 2 cycles (range 1-28) were administered to the phase 1 patients and as of 15 Feb 2017, of the 19 enrolled phase 2 patients, 16 had been treated, receiving a median of 3 cycles (range 1-12), while the 9 treated MBC patients receiving a median of 5 cycles (range 2-12).

1.2.3.2 Safety

In the phase 1 part, the most frequent related adverse events (AEs) were infusion-related reactions (IRRs; 10 patients, 36%), and gastrointestinal toxicity, notably nausea (8 patients, 29%), vomiting (7 patients, 25%), diarrhea (5 patients, 18%), and stomatitis (4 patients, 14%). Asthenia/fatigue were reported in 4/2 patients (14%/7%) and skin disorders (rash/dermatitis/fissures/nail disorder) in 6 patients (21%). All related AEs were grade 1-2 other than one grade 3 event (suspected related IRR).

In the phase 2 part, the most frequent AEs were IRRs (4 patients, 25%), diarrhea (3 patients, 19%), and fatigue (2 patients, 13%). All but one were grade 1-2, with 1 patient experiencing a grade 4 suspected related hypersensitivity reaction (allergy) which had a fatal outcome. This 71-year-old gastric carcinoma patient experienced a grade 4 hypersensitivity reaction (life-threatening). At baseline she had 56% LVEF and severe aortic valve and mitral valve stenosis with no clinical manifestations. On the first day of treatment she was premedicated with dexamethasone (5 mg), polaramine (5 mg) and paracetamol (1 g). After administration of 60 mL of MCLA-128 (out of 250 mL total) she presented with a grade 4 allergic reaction, and subsequent cardiorespiratory arrest, and died 9 hours after treatment initiation. The investigator reported that the allergic reaction was definitely related to the study drug. The patient had no history of hypersensitivity, allergic or anaphylactic reactions. As per

the investigator, the allergic reaction was the driver of the causality of the death however the patient's comorbidities, notably severe aortic stenosis likely played a major contributing role.

Key aspects of the MCLA-128 safety profile are:

- IRRs, which were analyzed according to all preferred terms considered by the investigator as manifestations of IRRs occurring within 24 hours of the MCLA-128 infusion (including notably IRR, hypersensitivity, nausea, vomiting, pyrexia, etc.) were reported in 14 phase 1 patients (50%) and 5 phase 2 patients (31%). All but 2 IRRs were grade 1-2 and resolved completely with symptomatic treatment.
- No suspected cardiac clinical AEs or clinically significant LVEF declines occurred; overall 5/27 (19%) evaluable patients had grade 2 LVEF decreases, none symptomatic or clinically significant (defined as LVEF decline > 10% from baseline and to a value of < 50%).
- No severe (grade 3-4) diarrhea or diarrhea requiring treatment discontinuation was observed.

1.2.3.3 Pharmacokinetics, Cytokines and Immunogenicity

In the 28 phase 1 patients C_{max} and area under the concentration versus time curve (AUC) increased with increasing dose. Terminal half-life ($t_{½}$) increased with increasing dose up to 360 mg, as is typically observed for antibodies that are cleared by target-mediated clearance, after which it did not increase significantly, giving an approximately dose-proportional increase of MCLA-128 exposure from 480 mg.

Among the 16 patients treated at the RP2D of 750 mg q3w (6 phase 1, 10 phase 2), mean C_{max} was 194 µg/mL, and $AUC_{0-\infty}$ was 22500 µg·h/mL, V_{ss} was 4.5 L (indicating that distribution is confined to the blood and interstitial fluids, typical of antibodies), CL was 37 mL/h, and $t_{½}$ was 101 h. After 21 days, median trough levels were 2.6 µg/mL, well above the MCLA-128 concentration that results in 50% receptor occupancy in vitro (K_D) (Alsina et al., 2017).

An interim population PK analysis on PK samples of 26 phase 1 patients covering all dose levels, showed PK data were best described by a two-compartment model with parallel linear and concentration-dependent (nonlinear) clearance from the central compartment. The nonlinear clearance reflects elimination processes of MCLA-128 after its saturating binding to the targets. The linear clearance represents the elimination of unbound MCLA-128. Estimated parameters were in accordance with parameters calculated by the non-compartmental analysis and with PK characteristics of therapeutic antibodies in general. The K_m value that determines the serum concentration at which a half maximal elimination rate is achieved was estimated at 0.290 µg/mL which is in the same range as the KD obtained for MCLA-128 on HER2-amplified BC cell lines.

Only 1 patient (160 mg MCLA-128) of 34 evaluable (from both phase 1 and 2 patients) had a positive anti-drug-antibody (ADA) titer at Day 36, with no apparent effect on PK. All other patients were ADA-negative throughout treatment.

At the RP2D, modest and transient cytokine elevations from baseline were observed 2 hours after the end of the infusion, mainly in IFNγ (0.4-3.4 fold), IL-8 (0.7-8.4 fold) and TNFα (0.8-11.6 fold) (Alsina et al., 2017).

1.2.3.4 Clinical Activity Including MBC Patients

Among the 28 evaluable phase 1 patients, 1 non-small cell lung cancer patient had a confirmed PR (360 mg, 18.0 months) and 3 patients had SD >3 months (breast, 3.6 months; colorectal, 7.3 months;

gastroesophageal junction, 13.4+ months).

Sixteen phase 2 patients were evaluable, 9 MBC and 7 with other tumors. Antitumor activity was seen in 6 MBC patients (1 PR and 5 SD), and 2 other tumor types (2 SD including a gastric patient with 7+ cycles).

Evaluation of efficacy in the overall MBC cohort at potentially active MCLA-128 doses (9 phase 2 treated at 750 mg, 2 phase 2 treated at 480 mg [1 phase 1 patient treated at 40 mg was not included for efficacy evaluation]), 1 patient had PR and 7 had SD (4 lasting ≥ 5 months), giving a clinical benefit rate (CBR) in MBC patients of 64% (7/11 patients). All these patients were heavily pretreated, all with 2-5 anti-HER therapy lines (Alsina et al., 2017).

1.3 Rationale for the Current Study

Breast cancer management has a significant need in the MBC setting for new agents with novel mechanisms of action and non-overlapping toxicity, which can be combined with established breast cancer treatments to overcome resistance for different patient populations.

1.3.1 Preclinical Studies for MCLA-128 + Trastuzumab in HER2-Positive MBC

There is a strong rationale to combine trastuzumab with MCLA-128 for the treatment of MBC. Trastuzumab is effective in blocking constitutive ligand-independent signaling through the HER2:HER3 heterodimer in the context of HER2 overexpression. However, it has very poor growth inhibition activity in tumors and cell lines where HER2:HER3 signaling is driven by HER3 ligand dependency (Wehrman et al., 2006; Junttila et al., 2009). Preclinical and clinical evidence points to increased HER3 expression and/or HER3 ligand upregulation as the main drivers leading to trastuzumab resistance (Sergina et al., 2007; Ocana et al., 2013). The bispecific antibody MCLA-128 docks HER2 present on the cancer cells to subsequently lock HER3 in an inactive confirmation. This results in potent inhibition of the HRG ligand-induced HER2:HER3 dimer formation and may overcome the resistance mechanism observed with trastuzumab.

Preclinical studies showed that the combination of MCLA-128 and trastuzumab was more potent than single-agent MCLA-128 to block HRG-induced tumor proliferation, supporting the addition of MCLA-128 to a trastuzumab-containing regimen in the clinic. In addition, the bispecific anti-HER2xHER3 antibody MCLA-128 presents enhanced ADCC. The monovalent interaction of the antibody to HER2 results in a greater density of antibody per tumor cell. In turn, this will result in greater recruitment of immune effector cells and, combined with ADCC, superior tumor cell killing activity.

In light of the distinct mechanisms of action for MCLA-128 and trastuzumab, these preclinical data suggest that administration of MCLA-128 and trastuzumab in HER2-positive patients has the potential for additive or synergistic activity in this setting.

1.3.2 Preclinical Studies for MCLA-128 + Endocrine Therapy in Hormone Receptor-Positive/HER2-Negative MBC

Bidirectional crosstalk between ER and HER2/3 has been hypothesized to underlie ET resistance via their activation (Houston et al., 1999; Thrane et al., 2013). The HER2:HER3 heterodimer activates PI3K/AKT and MAPK downstream signaling cascades but can also induce ER phosphorylation independent of estrogen (Pietras et al., 1995; Curley et al., 2015) which may reduce the effectiveness of endocrine therapies (Arpino et al., 2008). On the other hand, HER2 may be preferentially upregulated by estrogen deprivation, while tamoxifen induces expression of EGFR and HER2 (García-

Becerra et al., 2012). The nature of the HER2/ERα cross-talk seems to be conditioned by the context of endocrine resistance; in a series of paired biopsies from patients relapsing on tamoxifen evaluated in an *in vivo* model, conversion from HER2-negative to HER2-positive was rare, however <20% of tamoxifen-resistant patients relapsed with adaptive upregulation in HER2 and either suppressed or enhanced ERα expression and signaling (Gutierrez et al., 2005).

Taken together, these data support a combined ER/HER2-targeted approach in patients with acquired ET resistance in ERα-positive/HER2-negative breast cancer. The EGF30008 trial comparing letrozole alone versus in combination with lapatinib in hormone receptor-positive MBC suggests this may be translated into the clinic, as although the addition of lapatinib did not confer benefit in the overall hormone receptor-positive/HER2-negative group, there was a trend toward a benefit in the endocrine-resistant hormone receptor-positive/HER2-negative subset (Johnston et al., 2009).

A recent preclinical study took this concept a step further, demonstrating direct cross-talk between HER2/HER3 and ER with phosphorylation of ER only observed in cells expressing both HER2 and ERα or HER3 and ERα (Collins et al., 2017). The authors used an *in vivo* mouse model of ER+/HER2-low (HER2 IHC 1+ or 2+ without gene amplification) human breast cancer to evaluate a triple therapy targeting HER2 (pertuzumab), HER3 (lumretuzumab) and the ER (fulvestrant), resulting in long-lasting tumor regression.

Preclinical studies support that adding MCLA-128 to hormonal treatment could delay or reverse resistance to therapy in ER+ HER2- (HER2 IHC 1+ or 2+ by HercepTest™) breast cancer. MCLA-128 with anti-ER drugs (tamoxifen and fulvestrant) combined with the AI letrozole was evaluated in two *in vivo* xenograft models of ER+ HER2-low breast cancer. All hormone therapies reduced tumor growth, while MCLA-128 single agent had no efficacy. However, when MCLA-128 was added to the hormone therapy regimen, it led to a further decreased in tumor growth. Mechanistically, HER2 expression was increased with fulvestrant and tamoxifen, which is supported by published studies (Osipo et al., 2007; García-Becerra et al., 2012). When MCLA-128 was added to anti-ER drugs, this increase in HER2 could be reversed. Most importantly, a proximity ligation assay showed that fulvestrant treatment upregulated the formation of HER2:HER3 dimers and enhanced activation of the Akt signaling pathway, which could be inhibited by adding MCLA-128. Together, these data show that the efficacy of hormonal therapies may be limited due to activation of the HER2/HER3 signaling axis, which could be reversed by MCLA-128.

While the *in vivo* tumor models tested responded to hormonal therapy, there is also evidence that in the context of ET-resistant tumors, the inhibition of HER3 by MCLA-128 could be therapeutically beneficial, as suggested by Collins et al. (2017). The MCF-7 cell line engineered to respond to AIs was initially sensitive to letrozole, but tumors subsequently became resistant (Curley et al., 2015). The addition of an anti-HER3 antibody at the start of treatment was more effective than hormone therapy alone, as observed for MCLA-128. Importantly, the combination of the anti-HER3 with hormone therapy after resistance restored antitumor activity. This was not the case when the anti-HER3 was given as single agent, demonstrating that hormone receptor and HER3 inhibition work in synergy before and after the occurrence of HT resistance.

In patients with ER-positive/low HER2 expression MBC, based on this strong preclinical evidence, a rescue approach will be used to evaluate the effect of the addition of MCLA-128 on the natural history of the disease when treated with ET.

1.3.3 Rationale for MCLA-128 Combination Therapy Study Design

The study will be carried out in two distinct patient populations, HER2-positive/amplified MBC who will be treated with MCLA-128 and trastuzumab ± chemotherapy (Cohort 1), evaluated as doublet and triplet combinations, and ER-positive/low HER2 expression MBC patients who will be treated with MCLA-128 combined with the same ET that each patient progressed on immediately prior to study entry (Cohort 2).

The RP2D of 750 mg MCLA-128 IV, over 2 hours, q3w, was established in the phase 1/2 dose escalation study, a dose shown to be well tolerated. Mandatory premedication with all MCLA-128 infusions has been implemented to mitigate IRRs, and the infusion duration can be extended from 2 to 4 hours to further improve infusion tolerance. MCLA-128 is very well tolerated with few and mainly mild to moderate toxicities and potential toxicity associated with the proposed combination agents is expected to be manageable; the IRRs reported with MCLA-128 at the RP2D were almost all grade 1 or 2 and were managed with medication, while the introduction of mandatory premedication improved management of this toxicity. Clinical cardiac toxicity has not been reported, and gastrointestinal toxicity is grade 1 or 2.

The study design allows for cautious verification of the safety of combining two anti-HER2 monoclonal antibodies in Cohort 1, at multiple levels. The initial administration of the doublet agents, MCLA-128 and trastuzumab will be on consecutive days. Consecutive administration but on the same day is anticipated for subsequent cycles if the combination is considered well tolerated. Subsequent evaluation of administration of the two antibodies from treatment initiation will be investigated in patients enrolled later, to support a practical administration regimen in the long-term. The same principle will be applied for assessment of the triplet combination, with the antibody doublet administration initially, followed by chemotherapy the next day. In addition, the doublet and triplet combinations themselves will be evaluated sequentially to ensure safety. The implementation of a safety run-in evaluation in a small group of patients for each combination will provide safety data before committing to continuing full recruitment.

2 STUDY OBJECTIVES

2.1 Cohort 1: MCLA-128 + Trastuzumab ± Vinorelbine in HER2-Positive/Amplified MBC

Primary objective:

- Evaluate efficacy of MCLA-128 combined with trastuzumab ± vinorelbine in terms of clinical benefit rate (CBR) at 24 weeks based on RECIST 1.1 (per investigator review) in HER2-positive/amplified MBC patients who have progressed on prior HER2-directed therapy that included trastuzumab, pertuzumab, and an HER2 antibody drug conjugate (ADC) in any sequence and in any setting.

Secondary objectives:

- Evaluate CBR at 24 weeks based on RECIST 1.1 per central review
- Evaluate progression-free survival (PFS; per investigator and central review)
- Evaluate overall response rate (ORR) based on RECIST 1.1 (per investigator and central review)
- Evaluate duration of response (DoR) based on RECIST v1.1 (per investigator and central review)
- Evaluate overall survival (OS)
- Evaluate safety and tolerability of MCLA-128 in combination with trastuzumab ± vinorelbine
- Characterize pharmacokinetics (PK) of MCLA-128 in combination with trastuzumab ± vinorelbine
- Characterize immunogenicity of MCLA-128 in combination with trastuzumab

Exploratory objective:

- Evaluate potential correlations between biomarkers in tumor or blood samples and antitumor activity (including HER2, HER3, HER2:HER3 dimers, heregulin and other potential biomarkers)

2.2 Cohort 2: MCLA-128 + Endocrine Therapy in Estrogen Receptor [ER]-Positive/Low HER2 Expression MBC

Primary objective:

- Evaluate efficacy of MCLA-128 combined with endocrine therapy in terms of CBR at 24 weeks based on RECIST 1.1 (per investigator review) in ER-positive and low HER2 expression MBC patients who have previously progressed on the same endocrine therapy

Secondary objectives:

- Evaluate CBR at 24 weeks based on RECIST 1.1 per central review
- Evaluate PFS (per investigator and central review)
- Evaluate ORR based on RECIST 1.1 (per investigator and central review)
- Evaluate DoR based on RECIST 1.1 (per investigator and central review)
- Evaluate OS
- Evaluate safety and tolerability of MCLA-128 combined with endocrine therapy
- Characterize PK of MCLA-128 combined with endocrine therapy
- Characterize immunogenicity of MCLA-128 combined with endocrine therapy

Exploratory objective:

- Evaluate potential correlations between biomarkers in tumor or blood samples and antitumor activity (including HER2, HER3, HER2:HER3 dimers, heregulin and other potential biomarkers)

3 STUDY DESIGN AND DURATION

3.1 Study Design

This is a phase 2, open-label, multicenter international study to evaluate the efficacy of MCLA-128-based combinations in two metastatic breast cancer (MBC) populations, HER2-positive/amplified (Cohort 1) and ER-positive/low HER2 expression (Cohort 2).

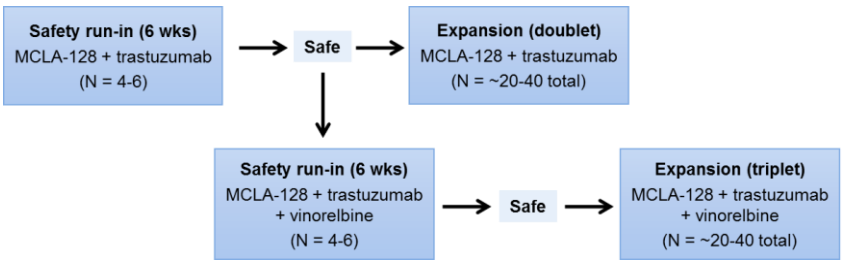
Three combination treatments will be evaluated, two in Cohort 1 and one in Cohort 2. A total of up to 120 evaluable patients will be included in approximately 20-30 centers in 7 countries in Europe and the USA.

Cohort 1: Patients with HER2-positive/amplified MBC, having confirmed HER2 overexpression by immunohistochemistry (IHC) 3+ or IHC 2+ combined with positive fluorescence *in situ* hybridization (FISH), who have been treated with up to a maximum of 5 lines of HER2-directed therapy in the metastatic setting and have progressed on the most recent line as per RECIST v1.1, are eligible. Patients must have been treated previously with trastuzumab, pertuzumab and an HER2 ADC in any sequence and in any setting. For enrollment, HER2 status will be based on medical records, and eligibility will be confirmed subsequently as soon as possible, by central lab review. Patients found to be ineligible retrospectively will not be evaluable for the primary objective and may be replaced. Documented imaging proof of disease progression on the last prior line of therapy should be made available when possible.

Initially MCLA-128 will be administered with trastuzumab (doublet combination). Safety will be reviewed by an Independent Data Monitoring Committee (IDMC). After the safety of the doublet has been assessed, MCLA-128 + trastuzumab + vinorelbine (triplet combination) will be evaluated in parallel with the doublet combination (see Figure 3-1 below).

The doublet and triplet Cohort 1 combinations will both be evaluated in two steps with an initial safety run-in in 4 to 6 patients who will be reviewed by the IDMC, followed by a cohort efficacy expansion, as described below. The triplet combination go/no-go decision will be made by the IDMC after evaluation of the doublet safety run-in patients. If the triplet combination is considered safe, the expansion of the doublet and triplet combinations will be performed in parallel. Patients with be included with a 3:1 ratio for triplet or doublet respectively, favoring the triplet combination and taking into account previous exposure to vinorelbine.

Figure 3-1: Study design for Cohort 1 combination therapies (doublet and triplet)



Safety run-in: After 4-6 patients have received at least 2 complete cycles (6 weeks) of MCLA-128 + trastuzumab, a safety review will be performed by the IDMC. If the doublet combination is considered safe, the safety run-in for the triplet combination will be initiated in the next 4 to 6 successive eligible patients. Safety of the triplet will be evaluated by the IDMC after these 4-6 patients have received at least 2 complete cycles (6 weeks) of MCLA-128 + trastuzumab + vinorelbine.

Based on the observed safety in the first 4-6 patients (adverse events [AEs], serious adverse events [SAEs], relationship to study drug, and other clinically relevant parameters [e.g. laboratory parameters], available PK, immunogenicity, and cytokine data) the IDMC, investigators and Sponsor will decide on a potential additional run-in period for each combination (i.e. doublet and triplet).

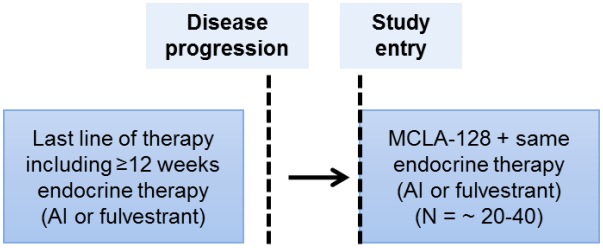
Expansion: After the safety run-in, each Cohort 1 combination therapy considered tolerable by the IDMC will be expanded to a total of up to 40 patients evaluable for efficacy.

If the doublet combination regimen is not tolerated, Cohort 1 will be closed. If the triplet combination is not well tolerated but the doublet is acceptable, the doublet expansion will be continued.

Cohort 2: Patients with ER-positive and low HER2 expression MBC (IHC 1+, or IHC 2+ combined with negative FISH) who have radiologic or photographic evidence of disease progression on the last line of prior endocrine therapy (administered for at least 12 weeks) that included an aromatase inhibitor or fulvestrant. Patients who have received up to 3 prior endocrine therapies in the metastatic setting and have progressed on a cyclin-dependent kinase inhibitor (in any line) are eligible. For enrollment, HER2 and HR status and radiologic/photographic documentation of prior progression will be based on medical records. Eligibility will be confirmed as soon as possible for HER2/HR status by central lab review and for prior disease progression by central imaging review. Patients found to be ineligible retrospectively will not be evaluable for the primary objective and may be replaced.

MCLA-128 will be administered in combination with the same previous endocrine therapy on which progressive disease is radiologically/photographically documented. A total of up to 40 patients evaluable for efficacy will be included.

Figure 3-2: Study design for Cohort 2 combination therapies



The IDMC will meet to review safety and efficacy data at select time points during the study (e.g. completion of safety run in, completion of enrollment), according to an IDMC Charter (see section 8.12).

Imaging for all patients will be reviewed centrally to confirm on-study disease assessments for analysis of efficacy endpoints.

All patients will receive MCLA-128 at 750 mg IV over 2 hours, Day 1, q3w, with 3-week cycles until disease progression or unacceptable toxicity (see also section 5.4). Trastuzumab and vinorelbine will be administered according to standard doses and regimens, and endocrine therapy will be administered as the same dose and regimen of the last line of endocrine therapy received prior to study entry on which the patient progressed.

3.2 Study Duration

Recruitment in this study has been closed and there remain a few patients on treatment who have all been on treatment for at least 24 months at the time of Protocol Version 5.0 implementation.

The implementation of Protocol Version 5.0, allows as per Investigator discretion, patients who have been treated for at least 2 years and continue to benefit from the study drug, to roll over in an alternative program e.g. Post-study or Expanded Access Use. Patients who will continue in this protocol, will only be followed for local efficacy, related adverse events, all related Serious Adverse Events and LVEF. After treatment withdrawal patients should attend the combined EOT and Follow-Up visit 35 ± 5 days after the last treatment administration.

The study will be closed after the last patient on study has been followed-up for 35 ± 5 days after the last treatment administration or after the last patient is rolled over into an alternative program.

Protocol Version 5.0 is applicable for the few patients who currently remain on treatment. Any patients who discontinued their treatment and are in Follow-Up at the time of amendment, will be assessed as per the previous Protocol Version 4.0.

The Sponsor has the right to prematurely terminate or suspend the entire study or close a given study site at any time for any reason. Such a decision will be communicated to the investigator(s) in writing.

4 SELECTION OF PATIENTS

4.1 Number of Patients

Across the two population cohorts, in total up to approximately 120 evaluable patients are planned to be accrued in this study. The final sample size will depend on the safety and efficacy encountered in each of the three treatment combinations.

4.2 Inclusion Criteria

Patients must fulfill all of the following requirements to enter the study:

1. Signed informed consent before initiation of any study procedures.
2. Women with histologically or cytologically confirmed breast cancer with evidence of metastatic or locally advanced disease not amenable to any local therapy with curative intent:

   2.1 Cohort 1 (MCLA-128 + trastuzumab ± vinorelbine)

   a. Documented HER2 overexpression/amplification, defined as immunohistochemistry (IHC) 3+ positive, or IHC 2+ combined with positive fluorescence *in situ* hybridization (FISH), based on local analysis on the most recent tumor biopsy (preferably metastatic, otherwise primary), either fresh or archival collected within 24 months before screening.
   b. Documented disease progression (by investigator assessment) on up to a maximum of 5 lines of HER2-directed therapy administered in the metastatic setting and have progressed on the most recent line. Trastuzumab, pertuzumab and an HER2 antibody drug conjugate (e.g. T-DM1) must have been previously administered in any sequence and in any setting.

   2.2 Cohort 2 (MCLA-128 + endocrine therapy)

   a. Documented hormone receptor positive status (estrogen receptor positive [ER+] and/or progesterone receptor positive [PR+]), defined as ≥ 1% positive stained cells by local standards, based on local analysis on the most recent tumor biopsy.

b. Documented low-level HER2 expression, defined as IHC HER2 1+, or IHC HER2 2+ combined with negative FISH, based on local analysis on a fresh tumor biopsy or an archival biopsy collected within 24 months before screening (preferably metastatic, otherwise primary).
c. No more than 3 lines of prior endocrine therapy (aromatase inhibitor or fulvestrant) for metastatic disease, with radiologic or photographic evidence of disease progression on the last line, after at least 12 weeks of therapy.
d. Progression on a cyclin-dependent kinase inhibitor.
e. No more than two previous chemotherapy regimens for advanced/metastatic disease.

Note: Pre/peri-menopausal women can be enrolled if amenable to be treated with the LHRH agonist goserelin. Such patients must have commenced treatment with goserelin or an alternative LHRH agonist at least 4 weeks prior to study entry, and patients who received an alternative LHRH agonist prior to study entry must switch to goserelin for the duration of the trial.

3. In Cohort 1, patients must have at least one lesion with measurable disease as defined by RECIST version 1.1. In Cohort 2, patients must have at least one lesion with measurable disease as defined by RECIST version 1.1. Patients with bone-only disease are eligible, even in the absence of measurable disease. Patients with bone-only disease must have lytic or mixed lesions (lytic + sclerotic). For Cohort 2, imaging documenting progression on the last line of hormone therapy must be available for central review.
4. Age ≥ 18 years at signature of informed consent.
5. Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1.
6. Life expectancy of ≥ 12 weeks, as per investigator.
7. Left ventricular ejection fraction (LVEF) ≥ 50% by echocardiogram (ECHO) or multiple gated acquisition scan (MUGA).
8. Adequate organ function:
    a. Absolute neutrophil count (ANC) ≥ 1.5 X 10^9/L
    b. Hemoglobin ≥ 9 g/dL
    c. Platelets ≥ 100 x 10^9/L
    d. Serum calcium within normal ranges (or corrected with supplements)
    e. Alanine aminotransferase (ALT), aspartate aminotransferase (AST) ≤ 2.5 x upper limit of normal (ULN) and total bilirubin ≤ 1.5 x ULN (in cases of liver involvement, ALT/AST ≤ 5 x ULN and total bilirubin within normal ranges will be allowed)
    f. Serum creatinine ≤ 1.5 x ULN or creatinine clearance ≥ 60 mL/min calculated according to the Cockroft and Gault formula or MDRD formula for patients aged > 65 years (Appendix 19.2)
    g. Serum albumin > 3.0 g/dL

Note: For both cohorts, patients will be enrolled based on their HER2 status, HR status (Cohort 2 only), and disease progression on the prior line of therapy, as reported in their medical records. Evaluability for the primary endpoint will be confirmed by central review after enrolment. Any patient found to be ineligible retrospectively will not be evaluable for the primary objective and may be replaced.

4.3 Exclusion Criteria

The presence of any of the following criteria excludes a patient from participating in the study:

1. Central nervous system metastases that are untreated or symptomatic, or require radiation, surgery, or continued steroid therapy to control symptoms within 14 days of study entry.
2. Known leptomeningeal involvement.

3. Advanced/metastatic, symptomatic, visceral spread, with a risk of life-threatening complications in the short term (including patients with massive uncontrolled effusions [pleural, pericardial, peritoneal], pulmonary lymphangitis, and over 50% liver involvement).
4. Participation in another interventional clinical trial or treatment with any investigational drug within 4 weeks prior to study entry.
5. Any systemic anticancer therapy within 3 weeks of the first dose of study treatment. For cytotoxic agents that have major delayed toxicity, e.g. mitomycin C and nitrosoureas, or anticancer immunotherapies, a washout period of 6 weeks is required. For patients in Cohort 2, this does not apply to the most recently received hormone therapy.
6. Major surgery or radiotherapy within 3 weeks of the first dose of study treatment. Patients who received prior radiotherapy to ≥ 25% of bone marrow are not eligible, irrespective of when it was received.
7. Persistent grade > 1 clinically significant toxicities related to prior antineoplastic therapies (except for alopecia); stable sensory neuropathy ≤ grade 1 NCI-CTCAE v. 4.03 is allowed.
8. History of hypersensitivity reaction or any toxicity attributed to trastuzumab, murine proteins, or any of the excipients that warranted permanent cessation of these agents (applicable for Cohort 1 only).
9. Previous exposure to vinorelbine (applicable for Cohort 1 triplet combination only)
10. Exposure to the following cumulative anthracycline doses:
    a. Doxorubicin or liposomal doxorubicin > 360 mg/m²
    b. Epirubicin > 720 mg/m²
    c. Mitoxantrone > 120 mg/m² and idarubicin > 90 mg/m²
    d. Other anthracycline at a dose equivalent to > 360 mg/m² doxorubicin
    e. For patients having received > 1 anthracycline, the cumulative dose must not exceed the equivalent of 360 mg/m² doxorubicin
11. Chronic use of high-dose oral corticosteroid therapy (>10 mg of prednisone equivalent a day).
12. Uncontrolled hypertension (systolic > 150 mmHg and/or diastolic > 100 mmHg) or unstable angina.
13. History of congestive heart failure of Class II-IV New York Heart Association (NYHA) criteria, or serious cardiac arrhythmia requiring treatment (except atrial fibrillation, paroxysmal supraventricular tachycardia).
14. History of myocardial infarction within 6 months of study entry.
15. History of prior or concomitant malignancies (other than excised non-melanoma skin cancer or cured *in situ* cervical carcinoma) within 3 years of study entry.
16. Current dyspnea at rest of any origin, or other diseases requiring continuous oxygen therapy.
17. Current serious illness or medical conditions including, but not limited to uncontrolled active infection, clinically significant pulmonary, metabolic or psychiatric disorders.
18. Known HIV, HBV, or HCV infection.
19. Pregnant or lactating women; women of childbearing potential must use effective contraception methods (patient and/or partner, e.g., surgical sterilization, a reliable barrier method) prior to study entry, for the duration of study participation, and for 7 months after the last dose of MCLA-128/trastuzumab. See Section 8.10.
20. Patients with only non-measurable lesions other than bone metastasis (e.g. pleural effusion, ascites or other visceral locations).
21. Patients with bone-only disease with blastic-only metastasis.

5 STUDY TREATMENT

MCLA-128 is an investigational medicinal product (IMP), and the companion drugs trastuzumab, vinorelbine and each endocrine therapy are considered non-IMP.

5.1 Formulations and Storage

5.1.1 MCLA-128

MCLA-128 is formulated as a clear liquid solution for infusion at a target concentration of 20 mg/mL in 25 mM histidine, 220 mM trehalose and 0.16 mM polysorbate 20 at a pH of 6.0, in Type I clear glass vials with an extractable volume of 5 mL or 18.8 mL. MCLA-128 is manufactured by Boehringer Ingelheim Pharma GmbH & Co KG, Biberach, Germany on behalf of Merus NV. Table 5-1 provides details on composition of MCLA-128.

Table 5-1: Composition of MCLA-128 for Intravenous Infusion, 20 mg/mL

Active Component	MCLA-128 bispecific humanized full length IgG1 antibody, 20 mg/mL
Excipients	25 mM histidine, 220 mM trehalose and 0.16 mM polysorbate 20, pH 6.0
Vehicle	Water for Injection
Stability	A shelf-life of 36 months has been established. After preparation of study drug, diluted MCLA-128 is stable for up to 12 hours at room temperature (time from preparation until end of infusion) or for up to 24 hours at 2-8°C and a subsequent 4 hours at room temperature.
Storage & Handling	2-8°C in the dark, no special storage or handling instructions are required. Infusion tubes do not need to be covered for light exposure.

5.1.2 Trastuzumab

Trastuzumab will be supplied centrally as a lyophilized powder in single-dose vials (150 mg, to be reconstituted with 7.2 mL of Sterile Water for Injection) for IV administration. Details of the excipients, stability, storage and handling can be found in the most recent local SPC (see Appendix 19.5).

5.1.3 Vinorelbine

Vinorelbine will be supplied centrally as single-use vials of 1 or 5 mL (10 mg/1 mL or 50 mg/5 mL) for IV administration. Details of the excipients, stability, storage and handling can be found in the most recent local SPC (see Appendix 19.5).

5.1.4 Endocrine Therapy

Endocrine therapy will be provided locally or centrally depending on local requirements. Details of the excipients, stability, storage and handling can be found in the appropriate local SPC (see Appendix 19.5).

5.2 Preparation for Administration

5.2.1 MCLA-128

MCLA-128 is prepared for administration according to the instructions in the Pharmacy Manual for this study. In short, the required volume of formulated MCLA-128 to be injected (37.5 mL) is first removed from the saline bag (containing 250 mL) using a sterile syringe and a sterile 18-22G needle. The same volume of MCLA-128 (37.5 mL to give a 750 mg flat dose) is then added to the saline bag using a sterile syringe and an 18-22G needle. Upon mixing, the saline infusion bag can be kept at room

temperature for up to 12 hours (time from preparation until end of infusion). Alternatively, the saline infusion bag containing MCLA-128 can be kept at 2-8°C for up to 24 hours prior to the start of infusion and a subsequent 4 hours at room temperature.

If the MCLA-128-containing saline infusion bag has been stored at 2-8°C, it should be allowed to reach room temperature (approximately 30 minutes prior to administration). A DEHP-free PVC infusion set containing an in-line 0.2 µm filter must be used to connect to the IV catheter. Flush the infusion line with NaCl 0.9%. The priming volume depends on the infusion line. The entire content of the infusion bag must be administered. The infusion rate should be adjusted accordingly to ensure the infusion is complete in approximately 120 minutes. After the end of the infusion, the infusion line must be flushed with NaCl 0.9% at the same rate to ensure the dosing is complete.

5.2.2 Companion Study Drugs

For trastuzumab, vinorelbine and endocrine therapies, refer to the most recent local SPC for drug preparation (see Appendix 19.5).

5.3 Study Drugs Administration

5.3.1 Study Drugs

5.3.1.1 MCLA-128 and Premedication

Premedication with paracetamol/acetaminophen, antihistamines, and corticosteroids is mandatory immediately before every MCLA-128 administration. A recommended regimen is provided below:

- Paracetamol/ acetaminophen 1000 mg PO or IV
- Dexchlorpheniramine 5 mg IV (or equivalent by PO or IV)
- Dexamethasone 10 mg IV (or equivalent by PO or IV)

The investigator can use equivalent drugs (e.g. prednisone instead of dexamethasone) and doses, in accordance with local standard practices.

A flat dose of 750 mg MCLA-128 will be infused intravenously over 2 hours, on Day 1 every 3 weeks. The infusion duration may be extended up to ~4 hours where considered appropriate to avoid or reduce the incidence or severity of IRRs.

5.3.1.2 Trastuzumab

Trastuzumab will be administered as an 8 mg/kg IV loading dose over 90 minutes for Cycle 1, then 6 mg/kg for subsequent cycles. For Cycle 2, the trastuzumab administration should be maintained at 90 min, and over 30-90 minutes thereafter. The trastuzumab dose should be recalculated if the patient's body weight changes by more than ± 10% from baseline. When trastuzumab is administered on the same day as MCLA-128, the infusion is initiated 30 minutes after the end of the MCLA-128 infusion. Refer also to the most recent local SPC (see Appendix 19.5).

5.3.1.3 Vinorelbine

Vinorelbine will be administered as an IV dose of 25 mg/m² over 10 minutes. A maximum body surface area of 2 m² should be used. When vinorelbine is administered on the same day as MCLA-128 and trastuzumab, the infusion is initiated 30 minutes after the end of the trastuzumab infusion.

Anti-emetic medication should be given according to local standard practices. Refer also to the most recent local SPC (see Appendix 19.5)

5.3.1.4 Endocrine therapies

For endocrine therapy, patients will receive the same dose and regimen as that administered as the last line of endocrine therapy prior to study entry on which the patient progressed.

Standard endocrine therapy doses and regimens are (refer also to the most recent local SPC; see Appendix 19.5):

- Fulvestrant 500 mg, administered intramuscularly into the buttocks slowly (1-2 minutes per injection) as two 5 mL injections, one in each buttock, on Days 1, 15, 29, and once every 28 days thereafter. Day 15 treatment will not be administered in patients with <40 days since the last fulvestrant administration prior to study entry.
- Exemestane 25 mg per os, administered once daily (QD), after food.
- Letrozole 2.5 mg per os, administered QD.
- Anastrazole 1 mg per os, administered QD.

5.3.2 Combination Treatment Schedules

Three combination treatment regimens are planned, two in Cohort 1 and one in Cohort 2:

- **Cohort 1 HER2-positive/amplified:** an MCLA-128 + trastuzumab (doublet combination) will be evaluated initially, followed by an MCLA-128 + trastuzumab + vinorelbine (triplet combination) after safety of the doublet has been established and which will be evaluated in parallel with the doublet.
- **Cohort 2 hormone receptor-positive/low HER2 expression:** MCLA-128 + endocrine therapy combination.

For all combinations, a treatment cycle is 21 days (3 weeks), including Cohort 2 which may include q4w fulvestrant dosing. Day 1 of the subsequent cycle will be on Day 22 or after recovery from any AEs associated with the previous cycle.

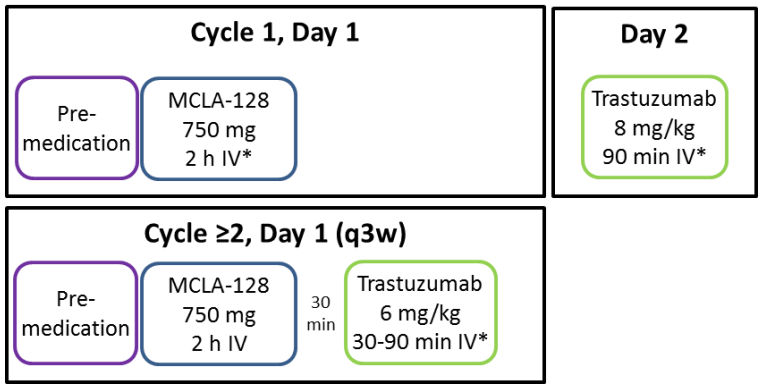
A 6-hour observation period will be implemented following infusion start for the initial MCLA-128 and/or trastuzumab administration, and 2 hours for all subsequent administrations (i.e. when a single antibody - MCLA-128 or trastuzumab - is administered on a given day, or after trastuzumab when they are administered on the same day).

5.3.2.1 Cohort 1: MCLA + Trastuzumab Doublet

Safety run-in patients

For Cycle 1, 750 mg MCLA-128 will be administered on Day 1 over 2 hours. An 8 mg/kg loading dose of trastuzumab will be administered on Day 2 of Cycle 1 over 90 minutes, 24 ± 2 hours after the MCLA-128 infusion. If administration of both agents is well tolerated in Cycle 1, they can be given sequentially on Day 1 from Cycle 2, with 750 mg MCLA-128 administered first followed 30 minutes later by 6 mg/kg maintenance dose of trastuzumab. In Cycle 2, trastuzumab will be administered over 90 minutes. For subsequent cycles it will be administered at 6 mg/kg over 30-90 minutes.

Figure 5-1: Doublet combination treatment administration in safety run-in patients

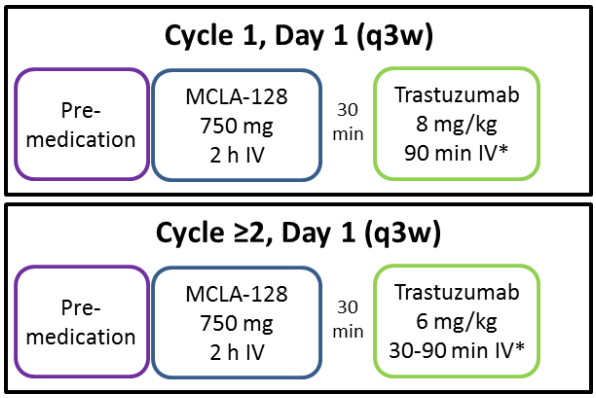


* Followed by a 6-hour observation period;
For Cycle 2, trastuzumab must be infused over 90 min.

Expansion patients

Once the safety run-in period has been completed and if the doublet combination is considered safe by the IDMC, all patients treated thereafter with the doublet will receive both drugs on Day 1 for all cycles, with 750 mg MCLA-128 over 2 hours for all cycles, and an 8 mg/kg loading dose of trastuzumab over 90 minutes for Cycle 1, and 6 mg/kg over 30-90 minutes for subsequent cycles (90 minutes for Cycle 2), with a 30-minute interval between infusions.

Figure 5-2: Doublet combination treatment administration in expansion patients



* Followed by a 6-hour observation period from infusion start, for the initial administration and 2 hours for all subsequent administrations
For Cycle 2, trastuzumab must be infused over 90 min.

If any patient does not tolerate both drugs on the same day, the safety run-in Cycle 1 dosing schedule will be followed (MCLA-128 on Day 1 and trastuzumab on Day 2) for that patient only after discussion with the Sponsor.

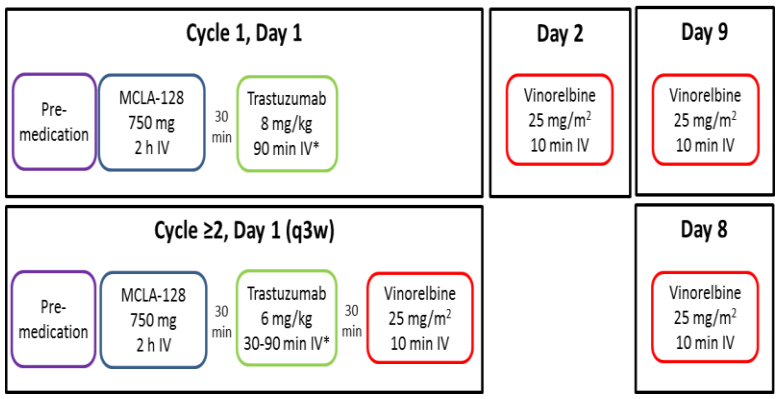
5.3.2.2 Cohort 1: MCLA + Trastuzumab + Vinorelbine Triplet

Safety run-in patients

For Cycle 1, patients will receive MCLA-128 and trastuzumab on the same day, with 750 mg MCLA-128 over 2 hours followed 30 minutes later by an 8 mg/kg IV loading dose of trastuzumab over 90 minutes. Vinorelbine 25 mg/m² (up to maximum of 2 m^2) IV over 10 minutes will be administered on Days 2 (24 ± 2 hours after the MCLA-128 infusion) and Day 9 of Cycle 1.

If administration of all three agents is well tolerated in Cycle 1, they can all be given sequentially on Day 1 from Cycle 2, with 750 mg MCLA-128 administered first followed 30 minutes later by 6 mg/kg trastuzumab infused over 30-90 minutes (90 minutes for Cycle 2), followed 30 minutes later by vinorelbine. The second vinorelbine administration will be administered on Day 8.

Figure 5-3: Triplet combination treatment administration in safety run-in patients

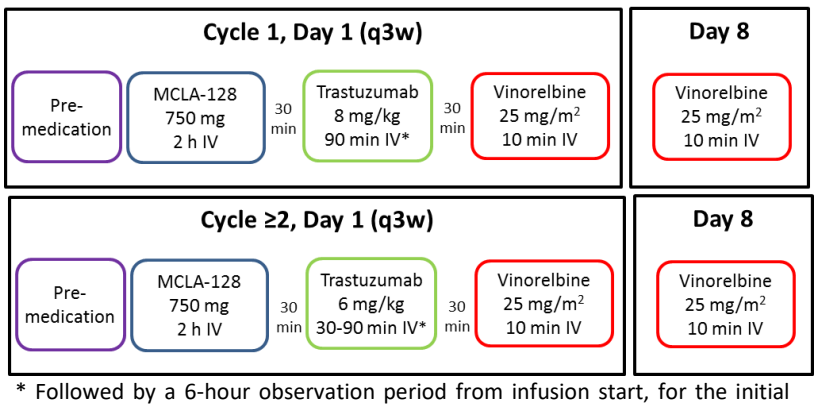


* Followed by a 6-hour observation period from infusion start, for the initial administration and 2 hours for all subsequent administrations
For Cycle 2, trastuzumab must be infused over 90 min.

Expansion patients

Once the safety run-in period has been completed and if the triplet combination is considered safe by the IDMC, all patients treated thereafter with the triplet will receive all three drugs on Day 1 for all cycles (750 mg MCLA-128 for all cycles, an 8 mg/kg IV loading dose of trastuzumab over 90 minutes for Cycle 1, and 6 mg/kg over 30-90 minutes for subsequent cycles (90 minutes for Cycle 2), and 25 mg/m² vinorelbine, with a 30-minute interval between infusions). The second vinorelbine administration will be administered on Day 8 for all cycles.

Figure 5-4: Triplet combination treatment administration in expansion patients



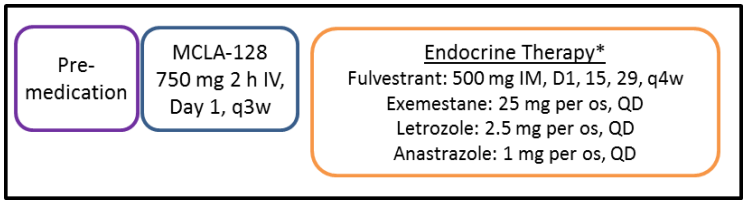
* Followed by a 6-hour observation period from infusion start, for the initial administration and 2 hours for all subsequent administrations
For Cycle 2, trastuzumab must be infused over 90 min.

If a given patient does not tolerate all three drugs on the same day, the safety run-in Cycle 1 dosing schedule will be followed (MCLA-128/trastuzumab on Day 1 and vinorelbine on Days 2 and 9) for that patient only.

5.3.2.3 Cohort 2: MCLA + Endocrine Therapy

For all cycles, MCLA-128 will be given on Day 1 over 2 hours, along with the same dose and regimen of prior endocrine therapy on which the patient had progressed prior to study entry.

Figure 5-5: Cohort 2 treatment administration in all patients



A 6-hour observation period will be implemented following infusion start for the initial MCLA-128 administration, and 2 hours for all subsequent administrations

* Same endocrine therapy on which the patient progressed prior to study entry. Can be administered before, during, or immediately after the MCLA-128 infusion.

5.3.3 Dose Adaptation

5.3.3.1 MCLA-128 and Trastuzumab

Actions to take in the event of toxicity related to MCLA-128 and/or trastuzumab are summarized below. See Section 8.8 for additional details of AEs of special interest (IRRs, cardiotoxicity and diarrhea).

Interruptions: MCLA-128 and trastuzumab infusions must be interrupted in the event of an IRR. IRRs include the preferred term (PT) "IRR" and any other PTs occurring within 24 hours of the MCLA-128 or trastuzumab infusion that the investigator judges as a sign or symptom of an IRR (e.g., nausea, vomiting, abdominal pain, headache, hypotension, pyrexia, tremor, and hypersensitivity).

The infusion should be stopped immediately, and the patient should receive symptomatic treatment according to the investigator's or treating physician's judgment.

For non-severe IRRs, the MCLA-128 or trastuzumab infusion can be re-started upon clinical improvement of symptoms, at 50% of the infusion rate prior to the reaction and the infusion duration can be increased to 4 hours, to administer the totality of the dose.

For severe IRRs (CTCAE grade 4, life-threatening), MCLA-128 or trastuzumab infusion should be stopped immediately and all study treatment discontinued definitively.

For IRRs considered to be due to an IgE-mediated anaphylactic reaction, the MCLA-128 or trastuzumab infusion should be interrupted and not resumed, irrespective of the severity of the reaction, and is contraindicated for subsequent cycles.

Delays: Planned MCLA-128 and trastuzumab administration can be delayed for up to 1 cycle (i.e., a maximum 6-week interval between infusions), in the event of the following:

- Cardiotoxicity: In the event of LVEF < 50% with a decline of ≥ 10 ejection fraction points or LVEF ≤ 44%, antibody administration will be delayed for 3 weeks and another LVEF evaluation performed. In the absence of improvement or if the patient has a confirmed documented LVEF

decrease to a value ≤ 44% or clinical signs and symptoms suggesting congestive heart failure, both antibodies will be withdrawn. See Appendix 19.3 for the algorithm for treatment management in the event of cardiotoxicity.

- Diarrhea: In the event of persistent grade 2 (> 48 hours) or grade 3-4 diarrhea, administration of MCLA-128 and trastuzumab should be delayed (for a maximum of 6 weeks between infusions).

Reductions: Dose reductions are not permitted for MCLA-128 or trastuzumab.

Incomplete trastuzumab loading dose:

- If the patient receives < 50% of the Cycle 1 loading dose, the remainder should be administered before Day 22, preferably within the first week. Thereafter, the usual maintenance dose should be administered 3 weeks after the first interrupted dose, as scheduled; e.g., if a patient received up to 50% of the scheduled loading dose, the remaining dose should be administered, preferably in the first week, then regular maintenance doses (6 mg/kg of trastuzumab) from Day 22, as scheduled.
- If the patient receives between 50-75% of the Cycle 1 loading dose, the remainder should be administered before Day 22, preferably within the first 2 weeks of Cycle 1; e.g., if ~60% of the scheduled loading dose is administered, the remaining 40% should be administered preferably within 2 weeks, then regular maintenance doses (6 mg/kg of trastuzumab) from Day 22, as scheduled.
- If the patient receives ≥ 75% of the Cycle 1 loading dose, additional loading is probably not necessary, however, the remainder of the loading dose may be given at the investigator's discretion. In this case, the remainder may be given at any time before the next scheduled dose or the patient may be given an additional loading dose on Day 22.

If, after receiving an incomplete loading dose on Day 1, the patient cannot attend the site until Day 22, a second loading dose should be given on Day 22. However, every effort should be made to give the remainder of the dose prior to Day 22.

5.3.3.2 Vinorelbine

Dose reductions and delays are required in the event of toxicity as follows:

Hematologic Toxicity:

Hold or decrease the vinorelbine dose in patients with decreased neutrophil counts using the following schema.

Neutrophils on Day of Treatment (cells/mm^3)	Percentage of Starting Dose
≥ 1,500 (≥ grade 1)	100%
< 1,500 (grade 2)	Do not administer. Repeat neutrophil count in 1 week. If treatment is delayed due to neutrophil count < 1,500 cells/mm^3 for ≥3 consecutive weeks, discontinue vinorelbine

Note : For patients who experience fever and/or sepsis while neutrophil count is < 1,500 or had 2 consecutive weekly doses held due to neutropenia, subsequent doses should be:

> 1,500 (≥ grade 1)	75%
< 1,500 (grade 2)	Do not administer. Repeat neutrophil count in 1 week.

Vinorelbine should be held if a patient experiences a reduction in platelets to <100,000 cells/mm^3 and counts repeated 1 week later. If treatment is delayed due to platelets to <100,000 cells/mm^3 for ≥3 consecutive weeks, discontinue vinorelbine.

Hepatic Impairment/Toxicity: Reduce vinorelbine dose in patients with elevated serum total bilirubin concentration according to the following schema.

Serum total bilirubin concentration (mg/dL)	Percentage of Starting Dose
≤ 2.0	100%
2.1 to 3.0	50%
> 3.0	25%

Concurrent Hematologic Toxicity and Hepatic Impairment: Administer the lower doses based on the corresponding starting dose of vinorelbine determined from the above schemas.

Neurologic Toxicity: Discontinue vinorelbine for NCI-CTCAE v. 4.03 ≥ grade 2 peripheral neuropathy or autonomic neuropathy causing constipation.

5.3.3.3 Endocrine Therapies

Treatment adaptations for endocrine therapies will be as per the SPC. Note that for fulvestrant:

Delays: In cases of fulvestrant-related toxicity as per investigator's judgement, the injection can be delayed for up to 7 days, after which the planned monthly injection should be skipped. Fulvestrant should not be administered if the platelet count is < 50,000/mm^3.

Reductions: Dose reductions are not permitted.

5.3.3.4 Supportive Care Guidelines for Study Treatment Toxicity

Toxicity will be managed by the investigator according to the local standard of care. Supportive treatment must be reported in the concomitant medication section of the eCRF.

5.4 Treatment Withdrawal

Study treatment will be administered until any of the following occurs, at which point it will be definitively discontinued, except in cases of perceived benefit and in agreement with the Sponsor:

- Confirmed progressive disease (as per RECIST)
- Unacceptable toxicity (including a confirmed documented LVEF decrease to a value <40% or clinical signs and symptoms suggesting congestive heart failure)
- Withdrawal of consent
- Patient non-compliance
- Investigator decision (e.g. clinical deterioration; in the rare event that a patient discontinues due to global deterioration of health status considered related to the underlying disease but without objective evidence of disease progression at that time, it should be reported as "symptomatic deterioration" – all efforts should be made to document objective progression)
- Treatment interruption > 6 consecutive weeks
- Withdrawal of any of the study drugs (MCLA-128, trastuzumab, vinorelbine or endocrine

- therapy)

In all cases, the reason for and date of study treatment discontinuation must be recorded in the eCRF and documented in the patient's medical records. As far as possible, there should be only one reason for treatment discontinuation. If several reasons apply (e.g. concomitant progressive disease and toxicity) the primary reason must be reported.

As far as possible, all examinations scheduled for the final study day must be performed for all patients who receive any study medication but who do not complete the study according to protocol.

The investigator must make every effort to contact patients lost to follow-up, especially when a patient is treated in another non-study center.

5.5 Packaging and Labeling

5.5.1 MCLA-128

MCLA-128 is provided as single-use glass vials of MCLA-128 for intravenous administration. All vials and secondary packaging are labelled for the purpose of the clinical trial in accordance with applicable regulatory requirements. Labels are prepared in the local language(s) of the countries involved.

5.5.2 Companion Study Drugs

Commercial preparations of trastuzumab, vinorelbine and fulvestrant, exemestane, letrozole, and anastrazole will be used. Trastuzumab and vinorelbine which are centrally supplied by Merus will include a label (in the local language) specifying they are for the purpose of the clinical trial, in accordance with applicable local regulatory requirements.

5.6 Supplies and Accountability

The study drugs supplied by Merus must be used only as directed in the protocol and as detailed in the Pharmacy Manual. They must only be dispensed to patients enrolled in this study and must be kept in a locked area with restricted access and stored and handled in accordance with the manufacturer’s instructions and temperature traceability.

Sufficient vials of MCLA-128, trastuzumab and vinorelbine will be supplied to the Pharmacy at each Investigational site. If a vial is broken or unusable, it should be replaced. Although the Sponsor need not be notified immediately in these cases, documentation of the use and/or loss of any vial must be recorded by the pharmacist on the medication accountability form.

It is the Investigator/Institution’s responsibility to establish a system for handling study medication to ensure that:

- Deliveries of study medication are correctly received by a responsible person and recorded;
- Study medication is handled and stored safely and properly as stated on the label, in this protocol document and in the Pharmacy Manual;
- Study medication is only dispensed to study patients in accordance with this protocol, the local prescribing information (as appropriate) and the Pharmacy Manual;
- Used vials of study medication are destroyed by the investigational site according to local procedures and regulations;

- Any unused study medication should be destroyed or returned to Merus or their designee in liaison with the study monitor.

The pharmacist of the investigational center must inventory and acknowledge receipt of all shipments of study medication and accurately document the delivery, use, destruction or return of used, partially used or unused vials of MCLA-128, trastuzumab and vinorelbine, including dates, quantities, patient numbers, batch numbers or other identification numbers. Records must be accurate and kept up to date. Throughout the study, delivery records for all study medications must be reconciled with records of usage and any returned stock. Records of usage should include the identification of the patient to whom the study medication was dispensed and the quantity and date of dispensing. Any discrepancies must be accounted for and documented. Certificates of delivery and return must be signed by the responsible pharmacist, with copies retained in the Pharmacy File. Unused study medication will be returned after written approval or request by the Sponsor.

The Sponsor's study monitor will periodically check the supplies of study medication held by the pharmacist to verify accountability of all study medication used. The Sponsor's study monitor will verify that a final report of drug accountability to the unit dose level is prepared and maintained in the Pharmacy Study File.

Complete instructions and contact information for ordering clinical supplies will be supplied in the Investigator File.

5.7 Compliance

Administration of MCLA-128, trastuzumab and vinorelbine will be supervised by the investigator or designee. Any delegation of this responsibility must follow Section 15.2. The hormone therapy given to patients in Cohort 2 should be administered as per local standard of care.

The dose, date and start and stop time of administration of all study drugs (MCLA-128, trastuzumab, vinorelbine, and endocrine therapy), any interruptions, and any immediate reactions at the time of infusion must be reported in the eCRF.

In Cohort 2, to be considered compliant for hormone therapy, patients must receive at least 75% of the planned hormone dose according to the planned regimen.

6 PRIOR AND CONCOMITANT ILLNESSES AND TREATMENTS

6.1 Medical Conditions

Additional illnesses, independent of the treated condition, present at the time informed consent is obtained or within 4 weeks prior to first study treatment administration must be documented in the eCRF. Relevant past illnesses (including other cancers) must be documented in the eCRF.

Medical conditions first occurring or detected during the study or worsening of a concomitant illness during the study, are to be considered TEAEs and must be documented in the adverse events section of the eCRF.

6.2 Medications

All treatments taken by patients at study entry or within 4 weeks prior to initiating treatment or at any time during the study, along with all prescription, non-prescription or over-the-counter

medications, including herbal remedies, dietary and nutritional supplements and complementary and alternative therapies, are considered concomitant medications and must be documented in the eCRF.

Permitted

- Systemic corticosteroids will be used as a part of a mandatory premedication regimen before each MCLA-128 administration (see Section 5.3.1.1).
- In cases of hypersensitivity or IRRs, paracetamol/acetaminophen, diphenhydramine, chlorpheniramine, or other antihistamines can be used according to local clinical practice, as clinically indicated.
- Any medication considered necessary for the patient's safety and wellbeing may be given at the discretion of the Investigator.
- Supportive treatment of symptoms and AEs or standard treatment of concomitant conditions, including aspirin, transfusion support, G-CSF, antibiotics, inhaled steroids (for asthma), antiemetics, antidiarrheals (e.g. loperamide), and bisphosphonates (according to their product license and routine clinical practice).
- Pre/peri-menopausal women who started treatment with goserelin or an alternative LHRH agonist at least 4 weeks prior to study entry may continue to receive goserelin, and patients who received an alternative LHRH agonist prior to study entry must switch to goserelin for the duration of the trial.
- Concurrent radiation treatment will be permitted during this study for symptom control without evidence of progression.

Prohibited concomitant medications (risk of immunosuppression)

- Chronic oral corticosteroid therapy > 10 mg/day prednisone equivalent excluding inhaled and topical steroids.
- Tumor necrosis factor (TNF)-alpha inhibitors.
- Anti-T cell antibodies.

Prohibited medications (other)

- Any investigational drug during the study or within 4 weeks prior to the first dose of study treatment.
- Any other anticancer therapy (other than the last endocrine therapy in Cohort 2) during the study or within 3 weeks of the first dose of study treatment. For cytotoxic agents that have major delayed toxicity, e.g. mitomycin C and nitrosoureas, or anticancer immunotherapies, a washout period of 6 weeks is required.
- Initiation of herbal remedies for cancer treatment. Herbal remedies initiated prior to study entry and continuing during the study are permitted and must be reported in the eCRF.
- Major surgery or radiotherapy within 3 weeks prior to the first dose of study treatment.
- Prior radiotherapy to ≥25% of bone marrow.
- Yellow fever vaccine while on therapy (Cohort 1) or within 3 weeks prior to the first dose of study treatment.

7 STUDY PROCEDURES AND SCHEDULE OF ASSESSMENTS

Screening, enrollment and on-study assessments to perform by visit are detailed below. Tables of assessments by visit and by cohort are provided for all patients, and for Cohort 1 and Cohort 2 specific assessments.

Further details of assessments and analyses for safety, efficacy, PK, biomarkers, cytokines, and immunogenicity are provided in Sections 8, 9, 10, 11, 12, and 13, respectively.

Table 7-1: Study procedures for all patients

	Protocol Section	Screening	STUDY TREATMENT PERIOD 1 Cycle = 21 days		STUDY TREATMENT PERIOD AS PER PROTOCOL VERSION 5.0	END OF TREATMENT VISIT (EOT)	FOLLOW-UP PERIOD	END OF TREATMENT VISIT (EOT) & FOLLOW-UP VISIT AS PER PROTOCOL VERSION 5.0
STUDY PROCEDURES (all patients)		**≤28 days before treatment**	**Day 1 of each cycle**	**As indicated on-study**	**1 Cycle = 21 days**	**35±5 days from last dosing**	**Every 3 months**	**35±5 days from last dosing**
General Assessments								
Informed consent	15.3	X						
Patient enrollment form		X						
Eligibility criteria	4	X						
Demographics, medical history	-	X						
Diagnosis and prior treatment	-	X						
Baseline symptoms and complaints	8	X						
Concomitant medication	6.2		Continuous		X			
Laboratory Tests								
Serum pregnancy test (other than for patients with proven menopause or surgically sterile)	8.10	X ≤7 days before treatment				X		X
Urine pregnancy test (a serum test must be carried out to confirm a positive result)	8.10		X		X		Up to 7 months after last MCLA-128/trastuzumab treatment	
Urinalysis (dipstick)	8.2	X	As clinically indicated			X		
Tumor Assessments								
CT/MRI scans of chest, abdomen, pelvis, and brain	9.1	X		X every 6 weeks after start of	X every 4 cycles (12 weeks). In	If previous imaging	For patients who discontinue for reasons other	For patients who discontinue for

	Protocol Section	Screening	STUDY TREATMENT PERIOD 1 Cycle = 21 days		STUDY TREATMENT PERIOD AS PER PROTOCOL VERSION 5.0	END OF TREATMENT VISIT (EOT)	FOLLOW-UP PERIOD	END OF TREATMENT VISIT (EOT) & FOLLOW-UP VISIT AS PER PROTOCOL VERSION 5.0
STUDY PROCEDURES (all patients)		**≤28 days before treatment**	**Day 1 of each cycle**	**As indicated on-study**	**1 Cycle = 21 days**	**35±5 days from last dosing**	**Every 3 months**	**35±5 days from last dosing**
any clinically indicated sites of disease and bone lesions as appropriate throughout treatment; clinical evaluation of superficial disease * Follow-up brain scans should only be conducted if lesions are present at baseline				study treatment until progression	case of symptomatic deterioration signs or signs of PD in between the assessments an unscheduled assessment must be performed.	assessment is >6 weeks prior	than progression, as clinically indicated	reasons other than progression, as clinically indicated
Radionuclide bone scan, whole body	9.1	X as close as possible and ≤9 weeks before starting study treatment		As clinically indicated to assess baseline or suspected new bone metastases	As clinically indicated to assess baseline o suspected new bone metastases		As clinically indicated to assess suspected new bone metastases for patients who discontinue for reasons other than progression	As clinically indicated to assess suspected new bone metastases for patients who discontinue for reasons other than progression

Tumor markers (CA15-3, CEA, CA27-29) (as far as possible, measures should be made in the same laboratory)	9.2	X within 7 days before treatment	X			X	For patients with elevated levels at screening who discontinue for reasons other than progression	For patients with elevated levels at screening who discontinue for reasons other than progression.
Other Clinical Assessments								
Adverse events	8		Continuous		X		Related cardiac events up to 1 year	X
Serious adverse events	8		Continuous		X		Suspected only, up to 1 year	X
LVEF (by ECHO or MUGA) and 12-lead electrocardiogram (ECG)	8.8.2	X As close as possible to and within 4 weeks prior to Day 1 Cycle 1.		X Pre-dose Day 1, every 4 cycles (C5, C9, etc) unless performed <7 days, and as clinically indicated	LVEF/ECG every 6 months and at any time during the study if clinically indicated.	X if >6 weeks since last assessment	All patients will undergo LVEF/ECG every 6 months for 1 year after the last MCLA-128 dose. Patients who discontinue due to decrease in LVEF and/or possible CHF should continue LVEF assessments as clinically indicated (≤18 weeks between assessments), until initiation of a new anticancer therapy, LVEF values return to ≥50% or for 1 year, whichever is first.	X
Pharmacokinetics	10			Protocol Versions 1.0,				

				2.0 and 3.0 only: Cycles 1, 2, 3, 5, then every 4 cycles				
Immunogenicity assessment	13			Protocol Versions 1.0, 2.0 and 3.0 only: Pre-dose Day 1 Cycles 1, 3, 5 then every 4 cycles (C9, C13, etc)		Protocol Versions 1.0, 2.0 and 3.0 only: X		
Fresh tumor OR archival sample within 24 months prior to screening (for HER2 status, exploratory biomarkers, and HR status as appropriate)*	11.1.1	X		Optional sample 12 weeks after start of study treatment		Optional (preferably from site of progression)		Optional (preferably from site of progression)
Liquid biopsy (for exploratory biomarkers)	11.1.2			Pre-dose Day 1 Cycle 1, then every 4 cycles (C5, C9, etc)		X		
Other investigations as clinically indicated	-	X	X	X	x	X	X	X

***For Cohort 2 patients with bone-only disease, primary tumor tissue will be used (**unless it is determined that testing for HER2 expression in a bone bio

Table 7-2: Study procedures and timings specific to Cohort 1 patients (doublet and triplet combinations)

	Protocol Section	Screening	STUDY TREATMENT PERIOD 1 Cycle = 21 days	STUDY TREATMENT PERIOD AS PER PROTOCOL VERSION 5.0	END OF TREATMENT VISIT	END OF TREATMENT VISIT (EOT) & FOLLOW-UP VISIT AS PER PROTOCOL VERSION 5.0
STUDY PROCEDURES (Cohort 1)		**≤7 days before treatment**	**Days 1, 2, 8 and 9 of each cycle as specified**	**1 Cycle = 21 days**	**35±5 days from last dosing**	**35±5 days from last dosing**
Clinical Assessments						
Physical examination, ECOG PS, height (screening only) and weight, vital signs	Appendix 19.1	X	**Doublet:** Days 1 and 8 of Cycle 1 and Day 1 of each subsequent cycle **Triplet safety run-in:** Days 1,2 and 9 of Cycle 1 and Days 1 and 8 of each subsequent cycle **Triplet expansion:** Days 1 and 8 of each cycle		X	X
Cytokine panel	12		**Doublet safety run-in:** Days 1 and 2 of Cycle 1 and Day 1 of Cycle 2 **Triplet safety run-in**: Day 1 of Cycles 1 and 2			
Laboratory Assessments						
Complete blood count (CBC)	8.2	X	**Doublet:** Days 1 and 8 of Cycle 1 and Day 1 of each subsequent cycle **Triplet safety run-in:** Days 1 and 9 of Cycle 1 and Days 1 and 8 of each subsequent cycle **Triplet expansion:** Days 1 and 8 of each cycle	Days 1 and 8 of each cycle	X	X
Serum chemistries	8.2	X	**Doublet:** Days 1 and 8 of Cycle 1 and Day 1 of each subsequent cycle **Triplet safety run-in:** Days 1 and 9 of Cycle 1 and Days 1 and 8 of each subsequent cycle **Triplet expansion:** Days 1 and 8 of each cycle	Days 1 and 8 of each cycle	X	X
Study Treatment						
MCLA-128 premedication (immediately prior to MCLA-128)	5.3.1.1		Day 1 of each cycle	X		
MCLA-128 (intravenous)	5.3.1.1		Day 1 of each cycle	X		
Trastuzumab (intravenous; 30 min after MCLA-128 when given on the same day)	5.3.1.2		**Doublet safety run-in:** Day 2 of Cycle 1 and Day 1 of each subsequent cycle	X		

	Protocol Section	Screening	STUDY TREATMENT PERIOD 1 Cycle = 21 days	STUDY TREATMENT PERIOD AS PER PROTOCOL VERSION 5.0	END OF TREATMENT VISIT	END OF TREATMENT VISIT (EOT) & FOLLOW-UP VISIT AS PER PROTOCOL VERSION 5.0
STUDY PROCEDURES (Cohort 1)		**≤7 days before treatment**	**Days 1, 2, 8 and 9 of each cycle as specified**	**1 Cycle = 21 days**	**35±5 days from last dosing**	**35±5 days from last dosing**
			Doublet expansion and all triplet: Day 1 of each cycle			
Vinorelbine (intravenous; 30 min after trastuzumab when given on the same day)	5.3.1.3		**Triplet safety run-in:** Days 2 and 9 of Cycle 1, then Days 1 and 8 of each subsequent cycle **Triplet expansion:** Days 1 and 8 of each cycle	X		

7-3: Study procedures and timings specific to Cohort 2 patients (MCLA-128 + endocrine therapy combination)

	Protocol Section	Screening	STUDY TREATMENT PERIOD 1 Cycle = 21 days		STUDY TREATMENT PERIOD AS PER PROTOCOL VERSION 5.0	END OF TREATMENT VISIT	END OF TREATMENT VISIT (EOT) & FOLLOW-UP VISIT AS PER PROTOCOL VERSION 5.0
STUDY PROCEDURES (Cohort 2)		**≤7 days before treatment**	**Day 1 of each cycle**	**Day 8 of Cycle 1 only**	**1 Cycle = 21 days**	**35±5 days from last dosing**	**35±5 days from last dosing**
Clinical Assessments							
Physical examination, ECOG PS, height (screening only) and weight, vital signs	Appendix 19.1	X	X	X		X	X
DEXA scan (bone mineral density)	-	X Up to 6 months prior to Day 1 Cycle1	X Every 12 months since last evaluation		X Every 12 months		
Laboratory Assessments							
Complete blood count (CBC)	8.2	X	X	X	Day 1 of each cycle	X	X
Serum chemistries	8.2	X	X	X	Day 1 of each cycle	X	X
Study Treatment							

	Protocol Section	Screening	STUDY TREATMENT PERIOD 1 Cycle = 21 days		STUDY TREATMENT PERIOD AS PER PROTOCOL VERSION 5.0	END OF TREATMENT VISIT	END OF TREATMENT VISIT (EOT) & FOLLOW-UP VISIT AS PER PROTOCOL VERSION 5.0
STUDY PROCEDURES (Cohort 2)		**≤7 days before treatment**	**Day 1 of each cycle**	**Day 8 of Cycle 1 only**	**1 Cycle = 21 days**	**35±5 days from last dosing**	**35±5 days from last dosing**
MCLA-128 premedication (immediately prior to MCLA-128)	5.3.1.1		X		X		
MCLA-128 (intravenous)	5.3.1.1		X		X		
Endocrine therapy (as per last prior line immediately before study entry)	5.3.1.4		Same dose and regimen on which the patient most recently progressed		X		

7.1 Screening, Eligibility and Enrolment Procedures

Each candidate patient will be examined before starting the study to determine eligibility for participation (as per Table 7-1, Table 7-2 and **Error! Reference source not found.**). This must be done within 28 days prior to the first study treatment administration.

All patients must provide written informed consent prior to any specific study procedures being performed.

A centralized patient enrolment process will be used to track patient screening and planned timing of Cycle 1, Day 1. The investigator (or designee) must verify that the patient meets eligibility criteria and has signed the Informed Consent Form, then request patient inclusion by faxing/e-mailing the Patient Enrolment Request Form (provided separately) to the Sponsor's Medical expert (or designee) as specified on the form.

Patients must be approved for enrolment by the Sponsor prior to treatment start. For Cohort 1, eligible patients will be allocated centrally by the Sponsor to the doublet or triplet regimen (when both expansion phases are running in parallel) as follows. After completion of the evaluation of the doublet safety run-in patients, if the doublet combination is considered toxic, all inclusions in Cohort 1 will be stopped. If the doublet is considered safe, the next 4 to 6 eligible successive patients will be treated with the triplet combination. If the triplet combination is considered toxic in these patients, further exploration of the triplet combination will be stopped, and only the doublet combination will be evaluated for efficacy. If both the doublet and triplet combinations are considered safe, they will be expanded for efficacy evaluation in parallel. Eligible patients will be allocated centrally by the Sponsor to one of the two cohorts using a 3:1 ratio for the triplet or doublet respectively and taking into account previous exposure to vinorelbine. For Cohort 2, no treatment assignment is required.

After validation of eligibility (as per Request Form, the enrolment form signed by the Sponsor's Medical Monitor or designee will be returned to the investigator within 24 hours (on working days), with or without inclusion agreement. For patients not included, the reason for inclusion refusal will be specified.

Patients who signed an Informed Consent Form but who failed to start treatment will be considered a screen failure and reported on the screening log in the Investigator Site File. Screen failures will not be reported in the eCRF. If the patient is screened and not enrolled, only SAEs suspected to be caused by study procedures should be reported.

Patients who are enrolled but are not treated will be reported in the eCRF. eCRF pages for demographic information, informed consent, inclusion/exclusion and the "leading question" on the AE (to confirm if any AEs occurred during screening) must also be completed for screen failure patients. No other data will be entered into the clinical database for these patients unless they experienced an SAE during the screening phase.

For included patients, the patient cohort and combination therapy arm allocated will be specified on the form, along with a unique patient number. Included patients will only receive a unique patient number when treatment is about to be started, which must be within 7 days after enrolment.

The unique patient number will consist of 6 digits (2 for the center, 1 for the cohort, and 3 for the patient by consecutive increasing order of inclusion; XX-X-XXX). Once a number has been assigned, it must not be used again.

7.2 Evaluations On-Study

On-study evaluations will be performed as per the tables of assessments (Table 7-1, Table 7-2, and **Error! Reference source not found.**). A ± 3 day window is permitted for visit timing with the exception of the first follow-up tumor assessment at Day 43 which must only have a +3 day window. All safety assessments should occur prior to dosing on each scheduled dosing day and the results should be available prior to deciding to dose on that day. With the implementation of Protocol Version 5.0 imaging assessments should occur every ~~6~~ 12 weeks and their timing should not be influenced by dose delays or interruptions. Tumor biopsies for biomarker collection should coincide with the imaging assessment schedule.

7.3 End of Treatment Visit and Follow-Up

A visit will be performed 35 days (± 5 days) after the last study drug intake as per Table 7-1, Table 7-2, and **Error! Reference source not found.**.

After the last treatment administration, patients will be followed up for safety for 30 days and then until resolution of any TEAEs for which a relationship to study drug cannot definitely be excluded (or categorized as sequelae).

In addition, patients will be followed 35 ± 5 days after the last treatment intake, as per Table 7-1, Table 7-2, and **Error! Reference source not found.**, as follows:

- Patients who discontinue treatment for reasons other than disease progression will be followed-up for tumor assessments (imaging and tumor markers), disease progression, start of a new anticancer therapy, or 35 ± 5 days after the last treatment intake, whichever is earlier.
- Patients who discontinue treatment due to a drop in LVEF will have MUGA/ultrasound as clinically indicated (≤18 weeks between assessments) until initiation of a new anticancer therapy, resolution of LVEF to ≥50%, or 35 ± 5 days after the last treatment intake, whichever is earlier.
- All patients will undergo LVEF 35 ± 5 days after the last MCLA-128 dose.
- All patients will be followed up for survival status, related cardiac events and suspected related SAEs for up to 35 ± 5 days after the last treatment intake.

8 SAFETY ASSESSMENTS

In emergency situations, the investigator should immediately contact a Sponsor representative at the telephone number or email address given on the title page of this protocol.

Patients will be monitored for signs and symptoms of AEs throughout the study by a qualified oncologist, with experience in clinical research. All AEs will be reported in the eCRF, including seriousness, NCI-CTCAE v. 4.03 grade severity, causal relationship to the study medication, and action taken. The first concern will be the safety of the study participant.

Incidence, severity and relationship of AEs and laboratory abnormalities, SAEs, discontinuations and dose adaptations due to AEs will be recorded.

8.1 Clinical Safety

Clinical and physical examinations, as well as vital signs evaluation, will be performed. Patient data will be analyzed for evidence of cumulative toxicity with repeated cycles of therapy. AEs and signs and symptoms of disease observed by the investigator (preferably by the same physician for a same

patient) or reported by patients will be recorded and graded according to NCI-CTCAE v. 4.03. All events, including those considered not related to the study drug, must be reported in the eCRF.

[https://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf]

If symptomatic clinical deterioration occurs while progressive disease is evident from the patient's clinical symptoms but is not supported by tumor measurements or the investigator elects not to perform further disease assessments, the determination of clinical progression is based on symptomatic deterioration. These determinations should be a rare exception as every effort should be made to document the objective progression of underlying malignancy with imaging. If there is any uncertainty about an AE being due only to the disease under study, it should be reported as an AE or SAE.

8.2 Laboratory Measurements

Clinical laboratory tests will be performed at local laboratories. Laboratory test results will be recorded on the laboratory results section of the eCRF. In addition, any laboratory result abnormality fulfilling the criteria for an AE or SAE should be reported as such. Any clinically significant treatment-emergent laboratory abnormality (accompanied with clinical symptoms, requiring a change in study medication [reduction, interruption, discontinuation] or requiring a change in concomitant therapy) should be recorded as a single AE in the eCRF.

Table 8-1: Hematology and chemistry sampling

Sample	Time Point	Analytes
Complete blood count (hematology) Chemistry blood sample	Screening; Cycle 1 Day 1 and Day 8, and Day 1 of every subsequent cycle unless there is an ongoing ≥ grade 2 AE, then perform as clinically indicated until resolution or return to baseline, and End of Treatment. NOTE: For patients in the Cohort 1 triplet treatment arm, hematology and chemistry samples will be collected every cycle at Day 8	CBC: Hemoglobin, RBC, WBC and differential, and platelet counts Chemistry: Na, K, Ca, Mg, P, Cl, HCO_3, creatinine, total protein, albumin, alkaline phosphatase, total bilirubin, AST, ALT, LDH NOTE: Collect additional analytes as clinically indicated

Dipstick urinalysis will be performed at screening and then as clinically indicated.

8.3 Definitions (21 CFR§312.32)

- **Adverse event (AE)** means any untoward medical occurrence associated with use of a drug in humans, whether or not considered drug related. The term **treatment emergent adverse event** [TEAE] covers any unfavorable and unintended sign, symptom, syndrome, or illness that develops or worsens during the period of observation after the first treatment administration in the clinical study. Surgical or other procedures themselves are not TEAEs. The condition for which the surgery/other procedure is required, is a TEAE, if it occurs or is detected during the study period. Planned surgical or other procedures planned/permitted by the study protocol and the condition(s) leading to these measures are not TEAEs, if the condition(s) was (were) known before the start of study treatment. In the latter case the condition should be reported as medical history.
- **Adverse drug reaction (ADR)** is an AE caused by the drug.

- **Suspected adverse reaction (SAR)** is any AE for which there is a reasonable possibility that the drug caused the AE. A "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the AE. SAR implies a lesser degree of certainty about causality than an ADR, and include:
  - A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure;
  - One or more occurrences of an event that is not commonly associated with drug exposure, but is otherwise uncommon in the population exposed to the drug (e.g., tendon rupture);
  - An aggregate analysis of specific events observed in a clinical trial (such as known consequences of the underlying disease or condition under investigation or other events that commonly occur in the study population independent of drug therapy) that indicates those events occur more frequently in the drug treatment group than in a concurrent or historical control group.
- **Serious adverse event (SAE)** or **serious suspected adverse reaction (SSAR)** is an event that, in the view of either the investigator or Sponsor, results in any of the following outcomes:
  - Death occurring on study or within 30 days after the last administration of study drug (regardless of relationship to study treatment) or within any interval if related to the study drug. Although death may occur as a result of the disease, all deaths occurring within 30 days of the last administration of study drug must be managed as SAEs and reported as such;
  - A life-threatening AE;
  - Inpatient hospitalization or prolongation of existing hospitalization;
  - A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions;
  - A congenital anomaly/birth defect.

In addition, medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in situations where none of the outcomes listed above occurred. Important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient's safety or may require intervention to prevent one of the outcomes listed in the definition above should also usually be considered serious. Examples of such events include:

- Overdose, even without complications (see Section 8.11)
- An AE requiring intensive treatment without hospitalization
- A diagnosis of a new cancer type during the course of a treatment
- A pregnancy diagnosed in a female patient treated with the study medication must be reported to the Sponsor immediately.

See Section 8.10 for SAE reporting for pregnancies. Only pregnancies with an outcome meeting any of the serious criteria defined above (i.e., congenital anomaly, stillbirth, neonatal death) should be reported as an SAE. For any pregnancy without an adverse outcome, the Investigator should complete the appropriate section of the SAE report form and state clearly that no AE was observed.

Hospitalizations for the following reasons should not be reported as SAEs:

- Routine treatment or monitoring of the studied indication, not associated with any deterioration in condition
- Elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since signing the informed consent

- Social reasons and respite care in the absence of any deterioration in the patient's general condition
- Treatment on an emergency outpatient basis that does not result in hospital admission and involves an event not fulfilling any of the definitions of an SAE given above.

Reporting disease progression as an SAE: Progression of the underlying malignancy is not reported as an AE if it is clearly consistent with suspected progression of the underlying cancer as defined by RECIST, or other criteria as determined by the protocol. Clinical symptoms of progression should be reported as AEs/SAEs if there is any uncertainty that they are due only to the progression of the underlying malignancy or they do not fit the expected pattern of progression for the disease under study. The PT "disease progression" should not be used.

In rare cases, progression may be evident in the patient's clinical symptoms but is not supported by the tumor measurements, or disease progression is so evident that the investigator elects not to perform further disease assessments. Every effort should be made to document the radiologic objective progression of the underlying malignancy.

- **Life-threatening AE or life-threatening SAR** is an AE that, in the view of either the investigator or Sponsor, places the patient at immediate risk of death. It does not include an AE or SAR that, had it occurred in a more severe form, might have caused death.
- **Unexpected AE or unexpected SAR** is an AE or SAR that is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed. For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the investigator brochure referred only to elevated hepatic enzymes or hepatitis. Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the investigator brochure listed only cerebral vascular accidents. "Unexpected", as used in this definition, also refers to AEs or SARs that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacologic properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

8.4 Documentation

All TEAEs occurring during the course of the study, whether or not considered related to the study drug or to underlying disease, must be individually recorded in the eCRF, including the nature of the event, date and time of onset (where appropriate), duration of effect, action taken, seriousness, severity, and relationship to study medication. Any consequent change to the dosage schedule or corrective therapy should be recorded.

Every attempt should be made to describe the TEAE in terms of a diagnosis. If a clear diagnosis has been made, individual signs and symptoms will not be recorded. If a clear diagnosis cannot be established, each sign and symptom must be recorded individually.

All patients who experience a TEAE, regardless of the relationship to study treatment, must be monitored to determine the outcome. The clinical course of the AE will be followed up even after the end of the period of observation for related TEAEs, until a satisfactory explanation is found or the investigator considers it medically justifiable to terminate follow-up. Should the AE result in death, a post-mortem examination should be considered as far as possible.

After last study treatment administration, patients will be observed for **at least 30 days** to document any late side effects and until resolution in cases of TEAEs for which a relationship to the study drugs cannot definitely be excluded. Patients will be followed up until resolution or attribution of sequelae for any treatment-related AEs.

AEs already recorded and designated as "continuing" should be reviewed at each subsequent assessment. If resolved, details are to be recorded in the eCRF. If an AE worsens in frequency of attacks/symptoms or in severity, a new record of the event must be started (i.e., distinct AE reports are required for differing frequencies and/or severity of the same event to enable comprehensive safety reports and later analysis).

Diagnostic and therapeutic non-invasive and invasive procedures, such as surgery, must be reported under "concomitant procedures" in the eCRF and not as an AE. The medical condition for which the procedure was performed must be reported.

8.5 Grade and Severity

All AEs, whether or not they are considered related to the study drug, must be graded using the NCI Common Toxicity Criteria for Adverse Events (CTCAE) version 4.03, which can be found at: http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf. The worst grade is to be documented.

AE grades usually reflect their severity, with grade 1 meaning "mild", grade 2 meaning "moderate", grade 3 meaning "severe", grade 4 meaning "life-threatening" and grade 5 meaning "fatal".

The term "severity" is used to describe the intensity of an AE; the event itself, however, may be of relatively minor clinical significance (e.g., 'severe' headache). This is not the same as "serious". Seriousness of AEs is based on the outcome/action of an AE and associated with events that pose a threat to a patient's life or functioning (see definition in Section 8.3 above).

Intensity of the AE will be evaluated using the following criteria:

- **Mild:** The patient is aware of the sign or symptom, but finds it easily tolerated. The event is of little concern to the patient and of little clinical significance. The event is not expected to have any effects on the patient's overall health or wellbeing.
- **Moderate:** The patient has discomfort enough to cause interference with or change in usual activities. The event is of some concern to the patient's health or wellbeing and may require medical intervention and/or close follow-up.
- **Severe:** The AE interferes considerably with the patient's usual activities. The event is of definite concern to the patient and/or poses substantial risk to the patient's health or wellbeing. The event is likely to require medical intervention and/or close follow-up and may be incapacitating or life-threatening. Hospitalization and treatment may be required.

8.6 Relationship to Study Treatment

All TEAEs occurring during treatment with an investigational agent may be related to the investigational agent. All TEAEs, regardless of the supposed relationship to study treatment, must therefore be collected. However, the investigator often has arguments to think that a TEAE is or is not related to the study treatment. Albeit subjective, and susceptible to be challenged, the assessment of relationship to study treatment is an important role of the investigator. Thus, the investigators should

decide, based on their knowledge and medical expertise, whether, in their opinion, an AE is "reasonably related" or "unlikely related" to the study treatment (or to study specific procedures).

8.7 SAE Reporting Requirements

SAEs and other AEs that fulfill a reason for expedited reporting to Pharmacovigilance (overdose and pregnancy) must be documented on the SAE Report Form at the time the SAE is detected. This form must be completed by the investigator and sent within 24 hours to the Sponsor's Pharmacovigilance representative as per the contact details provided on the form.

The SAE report form and guidelines for completing it are provided in the Investigator Study File. The Sponsor is responsible for ensuring that all legal reporting requirements are met.

The initial report must be as complete as possible, including details of the current illness and AE(s), and an assessment of the causal relationship between the event(s) and the study medication.

Information not available at the time of the initial report (e.g., an AE end date or laboratory values received after the report) must be documented on a follow-up SAE report form.

The IDMC will be provided with monthly listings of suspected SAEs. Investigators will be informed of serious unexpected suspected AEs as per the standard reporting requirements.

The Sponsor must immediately declare all SUSARs resulting in death or endangering the life of a patient, and a follow-up report must be provided within the following 8 days. All other SUSARs must be declared within 15 days and follow-up report must be provided within the following 8 days.

8.8 AEs of Special Interest

8.8.1 Infusion-related reactions

Patients will be monitored closely during the MCLA-128 infusion period for signs of IRRs. IRRs include the preferred term (PT) "IRR" and any other PTs occurring within 24 hours of the MCLA-128 or trastuzumab infusion that the investigator judges as a sign or symptom of an IRR (e.g., nausea, vomiting, abdominal pain, headache, hypotension, pyrexia, tremor, and hypersensitivity).

All patients must be pre-medicated with antihistamines, paracetamol/acetaminophen and corticosteroids before each MCLA-128 infusion. If an IRR develops during administration of MCLA-128, the infusion should be stopped immediately, receive symptomatic treatment according to the investigator's or treating physician's judgment (e.g. antipyretics, bronchodilators, oxygen, corticosteroids, and vasopressors, anti-IL-6 compounds), and the patient should be monitored until resolution of the symptoms and signs.

Upon complete resolution of non-severe IRRs, the infusion may be resumed at 50% of the infusion rate prior to the reaction and the infusion duration can be increased to 4 hours, to administer the totality of the dose. For severe IRRs (CTCAE grade 4, life-threatening), study treatment should be discontinued definitively.

For IRRs considered to be due to an IgE-mediated anaphylactic reaction, the MCLA-128 infusion should not be resumed, irrespective of the severity of the reaction, and is contraindicated for subsequent cycles.

Local irritation at the injection site should be treated according to routine treatment guidelines.

8.8.2 Cardiotoxicity

Cardiac function will be assessed by evaluating LVEF and 12-lead ECG. The LVEF assessment will be made preferably by echocardiogram (ECHO), although a multiple gated acquisition (MUGA) scan may be used if this method is standard practice at the investigational site. One method of evaluation is adequate and the same method should be used for each assessment for an individual patient.

With the implementation of Protocol Version 5.0, LVEF and ECG assessments will be performed every 6 months and at any time during the study if clinically indicated and at the End Of Treatment visit (EOT) & Follow-up visit

MCLA-128 will be discontinued in any patient with a confirmed documented LVEF decrease to a value <40%, and/or if clinical signs and symptoms suggesting congestive heart failure develop. These patients should continue LVEF assessments as clinically indicated (≤18 weeks between assessments), until LVEF values return to ≥50%, or for 1 year, whichever is first.

Appendix 19.3 provides the algorithm for performing any additional required assessments of LVEF, as well as the continuation or discontinuation of treatment with MCLA-128 and/or trastuzumab in study patients with asymptomatic decline in LVEF during study treatment.

All related cardiac events will be followed up for 35+5 days after treatment discontinuation.

8.8.3 Diarrhea

Diarrhea is common with anti-HER2 therapies including monoclonal antibodies. Patients must be advised to inform the medical team if they develop grade 1 or 2 diarrhea that does not resolve within 48 h, or diarrhea with fever. Patients experiencing diarrhea should be managed rapidly, and investigators should make all efforts to determine if it is drug-related or due to a gastrointestinal infection. If grade 1 or 2 (< 48 h) diarrhea occurs, symptomatic measures should be initiated (e.g. loperamide and hydration). If persistent grade 2 diarrhea (> 48 hours) or grade 3 occurs, patients must be evaluated in the clinic. Stool cultures should be performed and patients should be hospitalized and rehydrated with oral and IV fluids if necessary. Treatment delay is indicated until event resolution. Patients must be withdrawn if > 6 weeks between infusions.

8.9 Period of Observation

For the purposes of this study, the period of observation for collection of AEs extends from the start of treatment with the study drugs until 35 ± 5 days after the last study drug administration. Related AEs and SAEs that were ongoing at the time of the EOT visit must continue to be monitored during follow-up until resolution, stability (i.e. sequelae), change of causality from related to non-related, or initiation of further antitumor therapy.

If the investigator detects an SAE after the end of the period of observation, and considers the event possibly related to prior study treatment, he/she should contact the Sponsor to determine how the AE should be documented and reported.

All new related cardiac events and new suspected related SAEs will be followed up until resolution or stability or for 35 ± 5 days after the last treatment administration or until initiation of a new anticancer treatment, whichever occurs first.

In addition, all patients will be followed up for up to 35 ± 5 days after the last treatment administration or until initiation of a new anticancer treatment, whichever occurs first, for disease status (patients who have not progressed at end of treatment) and survival status.

8.10 Patients of Reproductive Potential

Pregnant or breast-feeding women are excluded from this study. Absence of pregnancy must be demonstrated by a serum test within 1 week prior to initiating treatment or any study procedure with a potential risk to the fetus and at the End of Treatment visit, and by urine testing on Day 1 of each cycle from Cycle 2, and every 3 months up to 7 months after MCLA-128/trastuzumab discontinuation unless there is proven menopause (>12 months of amenorrhea) or surgical/chemical castration.

Female patients must not become pregnant or start breast-feeding during the study, and women of childbearing potential (i.e. without proven menopause) must use medically effective contraception during the study and for 7 months after the last MCLA-128/trastuzumab intake.

In the event of a pregnancy during the course of this study, the Sponsor must be notified immediately and the patient should be immediately withdrawn from study. The investigator should counsel the patient, and discuss the risks of continuing with the pregnancy and the possible effects on the fetus. The pregnant patient should be followed during the entire course of the pregnancy and postpartum period.

Parental and neonatal outcomes must be recorded even if they are completely normal and without AEs. Offspring should be followed up for at least 8 weeks after delivery. Longer observation periods may be determined by the Sponsor if an adverse outcome of the pregnancy was observed.

Pregnancies occurring during the study up to 7 months after the final dose of MCLA-128/trastuzumab must also be reported to the Sponsor's Pharmacovigilance representative on the appropriate section of the SAE report form, according to SAE reporting requirements (see Section 8.7). Note that a pregnancy is only considered an SAE if the outcome meets any of the serious criteria defined in Section 8.3 (i.e., congenital anomaly, stillbirth, neonatal death). For any pregnancy without an adverse outcome, the Investigator should complete the appropriate section of the SAE report form and state clearly that no AE was observed.

The following non-hormonal methods of contraception are acceptable:

- True abstinence when this is in line with the preferred and usual lifestyle of the patient. [Periodic abstinence (e.g., calendar, ovulation, symptothermal post-ovulation methods) and withdrawal are not acceptable methods of contraception].
- Patients with a sole partner who is vasectomized (with appropriate post-vasectomy documentation of the absence of sperm in the ejaculate)

Or two of the following effective forms of contraception:

- Placement of intrauterine device (IUD) or intrauterine system (IUS). Consideration should be given to the type of device being used, as there are higher failure rates quoted for certain types, e.g. steel or copper wire. The risks (in terms of potential stimulation of hormone-responsive breast cancer by systemically absorbed hormones) and benefits (effective contraception) of hormone-releasing IUDs/IUSs should also be carefully considered for individual patients.
- Condom with spermicidal foam/gel/film/cream/suppository
- Occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam, gel, film, cream or suppository.

The use of barrier contraceptives should always be supplemented with the use of a spermicide. The following should be noted:

- Failure rates indicate that, when used alone, the diaphragm and condom are not highly effective forms of contraception. Therefore the use of additional spermicides does confer additional theoretical contraceptive protection.
- However, spermicides alone are ineffective at preventing pregnancy when the whole ejaculate is spilled. Therefore, spermicides are not a barrier method of contraception and should not be used alone.

It should be noted that two forms of effective contraception are required. A double barrier method is acceptable which is defined as condom and occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/suppository.

8.11 Misuse and Overdose

Drug misuse and drug overdose for any of the study drugs should be reported to the Sponsor's Pharmacovigilance representative on the appropriate section of the SAE report form, according to SAE reporting requirements (see Section 8.7), even if it does not result in an adverse outcome (in which case it should be clearly stated that no AEs were observed).

Overdose with MCLA-128 is defined as any dose administration where >10% over the correct dose amount is administered, whether or not associated with an AE. Symptoms of overdose with MCLA-128 are not yet known. In the case of accidental overdose, patients should be treated symptomatically. An antidote is not available. See the SPCs for companion drugs as to definition of overdose and action to take.

If the pharmacy discovers that an overdose has or may have been administered they must contact the Investigator and study coordinator.

8.12 Independent Data Monitoring Committee

An Independent Data Monitoring Committee (IDMC), composed of at least 2 physicians expert in the domain of early clinical development in MBC, will meet to review safety and efficacy data at select time points during the study (e.g. completion of safety run in, completion of enrollment), and decide on the addition of extra patients in the expansion parts in Cohort 1, opening of the triple combination cohort, and any *ad hoc* safety decisions. The principal investigators, the Sponsor's medical expert(s) and other representatives may be called upon to assist as observers.

The composition, functioning (meeting frequency, data received, etc.), and responsibilities of the IDMC will be detailed in the IDMC Charter (provided separately). All decisions by the IDMC and their rationale will be recorded in meeting minutes.

9 EFFICACY ASSESSMENTS

9.1 RECIST Tumor Measurement and Analysis

Efficacy will be measured in terms of tumor response according to RECIST criteria (version 1.1) (Eisenhauer et al., 2009)(Appendix 19.4).

With the implementation of Protocol version 5.0, tumor measurements will be done every 4 cycles (12 weeks) (chest, abdomen and pelvis CT scan) until progression; In case of symptomatic signs of PD in between the assessments an unscheduled assessment must be performed to confirm progression; and at the End of Treatment/Follow-up visit for patients who discontinue for reasons other than progression, as clinically indicated.

Full body bone scan will be performed at screening as clinically indicated to assess suspected bone metastases, and localized CT will be performed at screening for any bone lesions detected by the full body bone scan that are not otherwise seen on the chest, abdomen and pelvis CTs, and then repeated at the same frequency as the chest, abdomen and pelvis exams. A brain MRI or CT will be performed at screening, and if brain metastases are detected then at the same frequency as the chest, abdomen and pelvis exams using the same method performed at screening.

Note: Clinical lesions will only be considered measurable when they are superficial and ≥10 mm diameter as assessed using calipers (for example, skin nodules). Skin lesions must be documented by color photography, including a ruler to estimate the size of the lesion. If the skin lesion has a subcutaneous soft tissue component a localized CT should be performed if it is not visible on the chest abdomen and pelvis CT/MRI.

Table 9-1: Tumor imaging assessments

Body Location	Modality	Timing
Chest, abdomen & pelvis	CT	Screening, every 6 weeks after start of study treatment until disease progression per RECIST 1.1, death or withdrawal of consent, EOT (if most recent evaluation is > 6 weeks from EOT). For patients who discontinue for reasons other than progression, assessments should continue as clinically indicated per the investigator's discretion until eventual progression per RECIST 1.1 or for 1 year whichever occurs first.
Bone	Full body bone scan	As close as possible to and within 9 weeks before starting study treatment. If disease progression was documented after the last bone scan and the patient manifests increased bone pain and/or evidence of a change in the bone lesion with other imaging modalities, a more recent bone scan collected within 4 weeks will be required. Bone scans should be performed as clinically indicated to assess suspected new bone metastases on-study and during follow-up.
	Localized CT	Screening if bone metastases are detected and then at the same frequency as the chest, abdomen and pelvis exams
Brain	CT/MRI	Screening, and if brain metastases are detected then at the same frequency as the chest, abdomen and pelvis exams
Skin	Color photography	If skin lesions are present, at the same time points and frequency at the chest abdomen and pelvis CT
	Localized CT	Screening if subcutaneous soft tissue component is detected and then at the same frequency as the chest, abdomen and pelvis exams
Any other location	CT/MRI	As clinically indicated, any other areas of disease (i.e. neck) should be completed as appropriate

9.1.1 Central review

For Cohort 2, eligibility will be confirmed in terms of prior disease progression on or after the most recent line of therapy by central imaging review as soon as possible after enrolment, and it is mandatory that prior imaging be available. For Cohort 1, as far as possible, documented imaging proof of disease progression on the last prior line of therapy should be made available.

9.1.2 Imaging Technique

To ensure comparability, the baseline and subsequent tumor measurements to assess response should be performed using identical imaging techniques (i.e., preferably the same machine, contrast agent and standard volume of contrast agent, etc.).

9.1.3 Evaluation of Lesions

Table 9-2: Evaluation of response in target lesions

Complete Response (CR)	Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.
Partial Response (PR)	At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters.
Progression (PD)	At least a 20% increase in the sum of diameters of target lesions, taking as reference the *smallest sum on study* (this includes the baseline sum if that is the smallest on study). 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 is also considered progression)
Stable Disease (SD)	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

Table 9-3: Evaluation of non-target lesions

Complete Response (CR)	Disappearance of all non-target lesions. All lymph nodes must be non-pathological in size (<10 mm short axis)
Non-CR / Non-PD	Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.
Progression (PD)	Unequivocal progression of existing non-target lesions; see Section 4.3.4 of Eisenhauer et al., 2009 (Appendix 19.4) for further details. *Note:* the appearance of one or more new lesions is also considered progression. Unequivocal progression of existing non-target lesions, other than pleural effusions without cytological proof of neoplastic origin, in the opinion of the treating investigator (in this circumstance an explanation must be provided)[1].

[1]Although clear progression of non-target lesions only is exceptional, in such circumstances, the opinion of the treating physician should prevail and the progression status should be confirmed later by the Medical Monitor.

9.1.4 Definition of Best Overall Tumor Response

Overall response is calculated for each assessment time point as per Table 9-4.

Table 9-4: Overall response in patients with target (+/- non-target) disease

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

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; NE, not evaluable

The best overall response is determined once all data for a given patient are available and is defined as the best response recorded between the start and end of treatment, as described in Table 9-5.

Table 9-5: Best overall response

Overall response First time point	Overall response Subsequent time points	Best overall response
CR	CR	CR
CR	PR	SD, PD or PR[1]
CR	SD	SD provided minimum criteria for SD duration met, otherwise, PD
CR	PD	SD provided minimum criteria for SD duration met, otherwise, PD
CR	NE	SD provided minimum criteria for SD duration met, otherwise NE
PR	CR	PR
PR	PR	PR
PR	SD	SD
PR	PD	SD provided minimum criteria for SD duration met, otherwise, PD
PR	NE	SD provided minimum criteria for SD duration met, otherwise NE
NE	NE	NE

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; NE, not evaluable

1. If a CR is truly met at first time point, then any disease seen at a subsequent time point, even disease meeting PR criteria relative to baseline, makes the disease PD at that point (since disease must have reappeared after CR). Best response would depend on whether minimum duration for SD was met. However, sometimes CR may be claimed when subsequent scans suggest small lesions were likely still present and in fact the patient had PR, not CR at the first time point. Under these circumstances, the original CR should be changed to PR and the best response is PR.

In the rare event that a patient experiences global deterioration of health status considered related to the underlying malignant disease which requires discontinuation of treatment but without objective evidence of disease progression at that time, they should be reported as "symptomatic deterioration" as the cause of treatment discontinuation. Every effort should be made to document the objective progression even after discontinuation of treatment.

For equivocal findings of progression (e.g. very small and uncertain new lesions; cystic changes or necrosis in existing lesions), treatment may continue until the next scheduled assessment. If at the next scheduled assessment, progression is confirmed, the date of progression should be the earlier date when progression was suspected.

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.

Carcinomatous meningitis diagnosed by cytologic evaluation of cerebral spinal fluid will also be defined as progressive disease. Medical photography can be used to monitor chest wall recurrences of subcutaneous lesions.

In Cohort 2, in the absence of measurable disease at baseline, patients with bone-only lesions, lytic or mixed (lytic + sclerotic), will be allowed to enter the study.

The following will be considered disease progression among these patients:

- The appearance of one or more new lytic lesions in bone
- The appearance of one or more new lesions outside of bone
- Unequivocal progression of existing bone lesions

Note: Pathologic fracture, new compression fracture, or complications of bone metastases will not be considered as evidence of disease progression, unless one of the above-mentioned criteria is fulfilled.

9.2 Tumor Markers

The tumor markers CA15-3, CEA, and CA27-29 will be assessed on Day 1 of every cycle as part of routine laboratory analyses. As far as possible, measures should be made in the same laboratory. Evolution of each tumor marker will be followed throughout treatment.

10 PHARMACOKINETICS ASSESSMENTS

PK sampling is no longer applicable as of Protocol Version 4.0.

For Protocol Versions 1.0, 2.0 and 3.0, PK blood sampling (4 mL per sample) will be performed in all patients to measure serum MCLA-128. Trastuzumab will be measured in all Cohort 1 patients. No PK sampling will be performed for vinorelbine or endocrine therapy. PK blood sampling will be performed at the following time points:

Table 10-1: MCLA-128 PK blood sampling (Cohort 1, doublet and triplet)

Cycle	Day	Run in	Expansion	Scheduled time relative to MCLA-128 dose
1	1	X	X	Predose (0-2 h before)
1	1	X	X	EOI (0-10 min before EOI)
1	1	X	X	2 h (±15 min) after EOI
1	1	X	X	4 h (±30 min) after EOI
1	2	X	X	22 h (±2 h) after EOI
1	8	X*	X	Any time
2	1	X	X	Predose (0-2 h before)
2	1	X	X	EOI (0-10 min before EOI)
2	1	X		2 h (±15 min) after EOI
2	1	X		4 h (±30 min) after EOI
2	2	X		22 h (±2 h) after EOI
2	8	X		Any time
3	1	X	X	Predose (0-2 h before)
3	1	X	X	End of Infusion (0-10 min before EOI)
5	1	X	X	Predose (0-2 h before)
5	1	X	X	EOI (0-10 min before EOI)
Every 4 cycles (C9, C13, etc.)	1	X	X	Predose

* Cycle 1 Day 9 for patients treated in the triplet safety run-in combination;
EOI, end of infusion

Table 10-2: Trastuzumab PK blood sampling (Cohort 1)

Cycle	Day	Doublet		Triplet		Scheduled time relative to trastuzumab dose
		Run-in	Expansion	Run-in	Expansion	
1	1		X	X	X	Predose (0-2 h before)
1	1		X	X	X	EOI (0-10 min before EOI)
1	2	X				Predose (0-2 h before)
1	2	X				EOI (0-10 min before EOI)
2	1	X	X	X	X	Predose (0-2 h before)
2	1	X	X	X	X	EOI (0-10 min before EOI)

EOI, end of infusion

Table 10-3: MCLA-128 PK blood sampling (Cohort 2)

Cycle	Day	Scheduled time relative to MCLA-128 dose
1	1	Predose (0-2 h before)
1	1	EOI (0-10 min before EOI)
1	1	2 h (±15 min) after EOI
1	1	4 h (±30 min) after EOI
1	2	22 h (±2 h) after EOI
1	8	Any time
2	1	Predose (0-2 h before)
2	1	EOI (0-10 min before EOI)
3	1	Predose (0-2 h before)
3	1	EOI (0-10 min before EOI)
5	1	Predose (0-2 h before)
5	1	EOI (0-10 min before EOI)
Every 4 cycles (C9, C13, etc.)	1	Predose (0-2 h before)

EOI, end of infusion

Planned PK blood sampling times should be adhered to as closely as possible. It is essential that the actual time (i.e., infusion start and stop) be recorded accurately in the patient's records and the eCRF, along with the date of collection of each blood sample. Full instructions for sample preparation, storage and shipping are provided in the Central Laboratory Manual.

Serum samples must be sent as soon as possible to the central laboratory for analysis, particularly for the first 4-6 safety run-in patients treated in each combination arm, to allow for availability of results when reviewing safety data. If 2 patients in the same center are treated at the same time or a few days apart, samples of these 2 patients should be sent together.

Based on 5 treatment cycles, in total the following amounts of blood will be drawn per patient from a peripheral venous access for PK analysis (for each additional cycle, 1 additional 2-mL blood sample will be drawn):

- Cohort 1 doublet and triplet safety run-in patients: 20 blood samples of 4 mL each, i.e. approximately 80 mL of blood.
- Cohort 1 doublet and triplet expansion patients: 16 blood samples of 4 mL each, i.e. approximately 64 mL of blood.
- Cohort 2 patients: 13 blood samples of 4 mL each, i.e. approximately 52 mL of blood.

11 PHARMACODYNAMICS ASSESSMENTS

Sample collection for Biomarker analyses is no longer applicable as of Protocol Version 5.0.

11.1 Sample collection

Collection of tumor and blood samples for HER2 and HR status and biomarker analyses is detailed below. The time and date of collection of all samples (tumor and blood) will be recorded in the eCRF. Analyses will be performed centrally. Full instructions for tumor and liquid biopsy sample preparation, storage and shipping are provided in the Central Laboratory Manual, as well as guidelines for procedures to follow for any unused samples obtained from a patient who withdraws consent at any time during or after the study.

11.1.1 Tumor sample

A mandatory formalin-fixed paraffin-embedded (FFPE) tumor sample is required from all patients (both cohorts) at screening for HER2 status and exploratory biomarkers for all patients, and for HR status for Cohort 2. The tumor sample can be either a fresh sample or an archival sample collected within 24 months before screening. All efforts should be made to obtain a fresh tumor sample. The tumor sample should preferably be from a metastatic site, otherwise primary tissue is acceptable.

Due to the difficulty to assess HER2 expression using IHC and FISH in bone tissue, a primary FFPE tumor specimen will be used for biomarker analyses in the event that Cohort 2 patients have bone-only disease, unless it is determined that testing for HER2 expression in a bone biopsy is possible.

Table 11-1: Tumor biomarker sampling

Sample	Time Point
FFPE tumor sample (one sample per time point)	Screening: A fresh tumor sample OR an archival sample collected within 24 months before screening (preferably metastatic otherwise primary)*.
	12 weeks from the start of first study treatment (optional): Fresh tumor sample.
	EOT, within 35 days of last treatment (optional): Fresh tumor sample.

*For Cohort 2 patients with bone-only disease, primary tumor tissue will be used (unless it is determined that testing for HER2 expression in a bone biopsy is possible)

FFPE tissue blocks must have a tumor content > 40%. A block of adequate size (at least 5 mm deep core with a surface area of 2.5 mm^2) or a minimum of 30 unbacked glass slides, each containing a freshly cut unstained 4-5 µm section, is required (separate instructions will be provided for slide shipping).

Samples will be sent to the sponsor-designated central laboratories for assessment of HER2 and HR status and biomarkers.

For enrollment, HER2 (all patients) and HR status (Cohort 2) will be based on medical records and eligibility will be confirmed by central lab review after enrollment. Any patient found retrospectively to be ineligible for either of these parameters will not be evaluable for the primary objective and may be replaced.

11.1.2 Liquid Biopsy

For all patients (Cohorts 1 and 2), the following blood samples will be collected as follows.

Table 11-2: Liquid biopsy biomarker sampling

Peripheral blood sample	Time Point (all pre-dose)
1 x 6 mL and 2 x 10 mL	Day 1 and Day 85 (Day 1 Cycle 5) of study treatment
2 x 10 mL	Every 12 weeks (Day 1 Cycles 9, 13, 17, etc.) thereafter
2 x 10 mL	End of Treatment (within 35 ± 5days of ending treatment)

11.2 Analyses

11.2.1 Tumor HER2 and Hormone Receptor Status

HER2 and HR status for eligibility for study enrolment will be based on medical records. For all patients, eligibility will be confirmed centrally from the screening tumor biopsy after enrolment.

Cohort 1: HER2 amplification will be confirmed by IHC 3+ or IHC 2+ combined with positive FISH.

Cohort 2: ER-positive status and low HER2 expression will be confirmed by IHC 1+, or IHC 2+ combined with negative FISH.

11.2.2 Exploratory Biomarker Analyses

Exploratory biomarker and pharmacodynamic analyses will be performed on tumor tissue and blood (liquid biopsy) from the screening tumor biopsy and any optional on-study biopsies to assess potential correlations between evidence of activity and expression of HER2, and exploratory biomarkers such as HER3 and HER2:HER3, and other candidate biomarkers.

Identification of new markers correlating with disease activity and activity of treatment is evolving, the definitive list of analyses will be determined during the course of the study. The tumor tissue and blood samples collected under informed consent may be used to develop and validate assays and allow the generation of statistically meaningful biomarker data. Tumor tissue and blood samples remaining after the pre-defined biomarker assessments (e.g., aliquots of tumor cell RNA or DNA) may be used for re-testing, developing, and validating assays related to HER2-positive breast cancer or the prediction of response to MCLA-128, or further assessment of the defined marker panels. Specific patient research consent will be required for long-term storage of their samples and additional exploratory analysis.

11.2.2.1 Tumor Tissue Biomarkers

In addition to HER2 status by IHC and FISH, the following exploratory analyses will be performed if sufficient tumor sample is available:

- HER3 protein, HER2:HER3 dimerization, and downstream signaling proteins (eg PIK3CA) including HER3:pPIK3A binding will be assessed by an appropriate method such as proximity ligation assay (PLA).
- Heregulin mRNA expression (NRG1) will be evaluated using in situ hybridization or RT-PCR, and protein analysis will be performed if an appropriate method (such as IHC) is available.

If sufficient material is available the following exploratory analyses will be performed:

- pHER2, pHER3, pAKT and pERK1/2 (members of the MAPK and AKT signaling pathway) will be assessed using a method such as Reverse Phase Protein Array (RPPA) analysis or IHC and will be scored relative to total HER2, HER3, AKT and ERK respectively. Expression of inhibitors of proteins in MAPK and AKT signaling pathway such as PTEN will be assessed by IHC.
- Mutations in cancer-related genes including those associated with HER2 and HER3 signaling.
- Heregulin-gene fusions, if required and if a validated assay is available.

11.2.2.2 Liquid Biopsy Biomarkers

Exploratory analyses will include:

- Fcγ receptor polymorphism to determine ADCC activity by genotyping on PBMCs (Day 1 only).
- Plasma ctDNA mutation analysis in cancer-related genes including those associated with HER2 and HER3 signaling.

- Exploratory serum biomarkers such as soluble serum HER2 (ADVIA centaur) and heregulin (using research-based quantitative methods such as ELISA).

12 CYTOKINE ASSESSMENTS

Blood samples (5 mL) will be collected in Cohort 1 safety run-in patients only, to assess a serum cytokine panel (TNFα, IFNγ, IL-1β, IL-6, IL-8, IL-10) as follows:

Table 12-1: Cytokine blood sampling (Cohort 1 doublet safety run-in)

Cycle	Day	Scheduled time relative to MCLA-128 dose	Scheduled time relative to trastuzumab dose
1	1	Predose (0-2 h before)	
1	1	2 h after EOI	
1	1	4 h after EOI	
1	2	22 h after EOI*	Predose (0-2 h before)
1	2		2 h after trastuzumab EOI
2	1	Predose (0-2 h before)	
2	1	2 h after EOI	Immediately after EOI
2	1	4 h after EOI	2 h after EOI
2	2	22 h after EOI	20 h after EOI

* Corresponds to pre-dose trastuzumab ~24 h after MCLA-128 initiation and can be 0-2 h before the infusion.
EOI, end of infusion

Table 12-2: Cytokine blood sampling (Cohort 1 triplet safety run-in)

Cycle	Day	Scheduled time relative to MCLA-128 dose	Scheduled time relative to trastuzumab dose
1 & 2	1	Predose (0-2 h before)	
1 & 2	1	2 h after EOI	Immediately after EOI
1 & 2	1	4 h after EOI	2 h after EOI
1 & 2	2	22 h after EOI	20 h after EOI

EOI, end of infusion

Additional cytokine samples may be collected in the expansion part of the study as needed.

Full instructions for sample preparation, handling procedures, aliquoting of samples, storage and shipping of these serum samples will be provided in the Central Laboratory Manual for this study. Serum samples must be sent as soon as possible to the central laboratory for analysis, particularly for the first 4-6 safety run-in patients treated in each combination arm, to allow for availability of results when reviewing safety data. If 2 patients in the same center are treated at the same time or a few days apart, samples of these 2 patients should be sent together.

Cytokine results will be reported separately including per-patient listings with descriptive statistics.

13 IMMUNOGENICITY ASSESSMENTS

Anti-MCLA-128 antibody sampling is no longer applicable as of Protocol Version 4.0.

For Protocol Versions 1.0, 2.0, and 3.0 blood samples (5 mL) will be collected in all patients to assess serum titers of anti-MCLA-128 antibodies on Day 1 at pre-dose for each of Cycles 1, 3 and 5, and every 4 cycles thereafter (Cycle 9, Cycle 13 etc.), and at the End of Treatment Visit.

Full instructions for sample preparation, handling procedures, aliquoting of samples, storage and shipping of these serum samples will be provided in the Central Laboratory Manual for this study.

Immunogenicity results will be reported separately including per-patient listings with descriptive statistics.

14 STATISTICAL CONSIDERATIONS

14.1 Sample Size

Cohort 1 safety run-in: 4 to 6 evaluable patients in the safety run-in will have power to detect an AE with a true incidence of 33% is 80 to 90%.

Cohort 1 efficacy expansion: 40 evaluable patients with observed CBR of > 45% will provide adequate precision to exclude 30% (lower limit of 90% CI > 30%). The threshold for the CBR rate at 24 weeks is defined based on the assumption that PFS follows an exponential distribution with a median of 5 months (clinically relevant) and 3.5 months (not clinically relevant).

Cohort 2: 40 evaluable patients with observed CBR of at least 45% will provide enough precision to exclude 30% (lower limit of 90% CI > 30%). The threshold for CBR at 24 weeks is defined based on the assumption that PFS follows an exponential distribution with a median of 5 months (clinically relevant) and 3.5 months (not clinically relevant).

The final number of patients will depend on the safety and efficacy outcomes during the study. Up to ~130 patients are anticipated, allowing for a total of 40 patients in each of the three planned combination regimens and a ~10% rate of non-evaluable patients.

14.2 Study Endpoints

14.2.1 Definitions

All efficacy endpoints will be defined and analyzed based on tumor assessment by RECIST 1.1 (Eisenhauer et al., 2009) (Appendix 19.4). Carcinomatous meningitis diagnosed by cytologic evaluation of cerebral spinal fluid will also be defined as progressive disease. See also Section 9.1.

- **Clinical benefit rate (CBR):** the proportion of patients with a best overall response of CR, PR (confirmed a minimum of 4 weeks later) or SD ≥24 weeks, based on tumor assessments up to PD, death or the next anti-cancer therapy, whichever occurs earlier.
- **Overall response rate (ORR):** the proportion of patients with best overall response of CR or PR. Objective response is defined as a CR or PR (RECIST 1.1) on two consecutive occasions ≥4 weeks apart.
- **Progression-free survival (PFS):** the time from treatment start until first documented radiographic progression as determined by the investigator using current RECIST or death due to any cause, whichever occurs first.
- **PFS ratio (Cohort 2 only):** the ratio of PFS observed on the previous regimen to PFS recorded on study treatment.
- **Duration of Response (DoR):** the time from the date of initial confirmed response (CR or PR) until the date of progression or death due to any cause.
- **Overall Survival (OS):** the time from treatment start until death due to any cause.

14.2.2 Endpoints

Primary (Cohort 1 and 2)

- CBR per investigator radiologic review at 24 weeks

Key secondary

- Cohort 1: CBR at 24 weeks per central review, and ORR, PFS, and DoR per investigator and central review
- Cohort 2: CBR at 24 weeks per central review, and PFS per investigator and central review

Other secondary (Cohort 1 and 2)

- Safety: incidence, severity and relationship of AEs, laboratory abnormalities, SAEs, ECG and LVEF measurements, and vital signs
- Tolerability: discontinuations due to AEs, dose modifications due to AEs, immunogenicity, and cytokine assessments
- Other efficacy: DoR (Cohort 2), PFS ratio (Cohort 2), ORR (Cohort 2), and OS (Cohorts 1 and 2)
- Pharmacokinetics: serum C_{max}, C_{0h}, AUC, CL, V_{ss}, t_{max} and $t_{1/2}$ for MCLA-128 and serum C_{EOI} and C_{0h} for trastuzumab

14.3 Analysis Populations

Treated population: patients who receive at least one dose of MCLA-128.

Evaluable for efficacy: patients who receive at least 2 complete cycles (6 weeks) of treatment and have undergone baseline assessment and one on-study tumor assessment, or who discontinue early due to disease progression.

Note: For both cohorts, patients will be enrolled based on their HER2 status, HR status (Cohort 2 only), and disease progression on the prior line of therapy, as reported in their medical records. Evaluability for the primary endpoint will be confirmed by central review after enrolment. Any patient found to be ineligible retrospectively will not be evaluable for the primary objective and may be replaced. Non-eligible patients are:

- Cohort 1: patient is not HER2-positive/amplified (by central lab), and/or disease progression under the last line of therapy by imaging is not confirmed (by central imaging review).
- Cohort 2: is not low HER2 expression (by central lab), and/or disease progression under the last line of therapy by imaging is not confirmed (by central imaging review).

14.4 Statistical Analyses

Patient disposition and demographics will be analyzed in the treated population, efficacy will be analyzed in the evaluable for efficacy population, and safety will be analyzed in the treated population.

Quantitative variables will be summarized using descriptive statistics. Continuous variables will be presented as N, mean and/or median, standard deviation, range. Categorical variables will be presented using frequencies and percentage.

Results will be summarized by cohort and treatment (i.e., Cohort 1 doublet, Cohort 1 triplet and Cohort 2), according to the treatment patients actually receive.

Full details of analyses including handling of missing data, will be provided in the Statistical Analysis Plan.

14.4.1 Analysis of the Primary Efficacy Endpoint

Criteria for success:

Cohort 1 (doublet and triplet): To be eligible, patients must have progressed on a maximum of 5 lines of HER2-directed therapy in the metastatic setting; trastuzumab with pertuzumab and an HER2 antibody drug conjugate (e.g. T-DM1) in any sequence and in any setting must have been previously administered. Although the level of antitumor activity is not well defined for patients in the metastatic setting progressing on T-DM1, the current assumptions are based on the TH3RESA trial (Krop et al., 2014) in which patients had a median of 4 previous regimens for advanced disease including both trastuzumab-containing and lapatinib-containing therapies. In this trial the T-DM1 arm showed a median PFS of 6.2 months (95% CI, 5.59 - 6.87) while in the control arm (investigator choice, primarily trastuzumab-based regimen) PFS was 3.3 months (95% CI, 2.89 - 4.14). It is assumed here that a clinically relevant median PFS is 5 months, therefore the activity threshold for CBR at 24 weeks is set to 45%.

Cohort 2: Eligible patients must have progressed on a maximum of 3 lines of endocrine therapy in the metastatic setting and on a CDK4/6 inhibitor. There are no well-established data that can serve as a reference for this population. Our assumptions are based on the phase 3 BELLE-3 study in patients who had progressed on an aromatase inhibitor and everolimus which demonstrated median PFS of 3.9-4.3 months in the overall and PIK3CA-mutated populations treated with buparlisib (a PI3K inhibitor) and fulvestrant, and 1.8 months in the fulvestrant-only arm (Angelo et al., 2016). It is assumed here that a clinically relevant median PFS is 5 months, therefore the activity threshold for CBR at 24 weeks is set to 45%.

Observed CBR in both cohorts will be presented with accompanying 90% exact binomial confidence interval.

Patients with only non-measurable disease at baseline will be analyzed for best overall response and CBR, and will be considered as a SD if their response is a 'Non-CR/Non-PD'.

14.4.2 Analysis of Other Efficacy Endpoints

Observed ORR in both cohorts will be presented with accompanying 90% exact binomial confidence interval. Only patients with measurable disease will be included in analyses.

For PFS, OS and DoR the survival function will be estimated using the Kaplan-Meier product limit method; probability estimates and 90% CI will be provided at specified time points; median duration and 90% CI will also be provided. DoR will be estimated for responders only.

For patients in Cohort 2, the number and proportion of any patients with a PFS ratio ≥ 1.3 (Von Hoff et al., 2010) will be tabulated together with 90% exact CI, along with descriptive data of the PFS ratio for each of these patients.

For patients with elevated tumor markers (CA15-3, CEA, and CA27-29) at baseline, evolution over time will be evaluated.

14.4.3 Analysis of the Safety Endpoints

The safety of MCLA-128 in combination with trastuzumab ± vinorelbine (Cohort 1) or with ET (Cohort 2) will be assessed by AEs, ECG and LVEF measurements, laboratory test results, and vital signs.

Adverse events: AEs will be tabulated by the Medical Dictionary for Regulatory Activities (MedDRA®) preferred term (PT) and by system organ class (SOC) according to incidence and severity (based on

NCI-CTCAE v. 4.03). For each patient, the maximum severity of a given AE recorded will be used. AEs of special interest (including IRRs) will be tabulated. In addition, AEs leading to discontinuation of study treatment and SAEs will be summarized.

Cardiac safety: The number and percentage of patients with congestive heart failure (NCI-CTCAE v.4.03 grades 3, 4, and 5) and asymptomatic LVEF events (NCI-CTCAE grades 1 and 2, as per MUGA or ECHO) at any time during the study will be summarized.

The baseline LVEF value and the maximum absolute decrease (or minimum absolute increase if patients' post-baseline LVEF measures are all above the baseline value) in LVEF measures from baseline will be summarized. LVEF measurements and change in LVEF from baseline will be summarized by scheduled visits in graphical and tabular format.

For each ECHO/MUGA evaluation following the initiation of study drug, the number and percentage of patients with dose delay and/or infusion interruption and ECHO/MUGA repeated will be summarized. The change in LVEF at that time point will be summarized using descriptive statistics.

Laboratory data: Laboratory toxicities will be defined based on local laboratory normal ranges and CTCAE version 4.03. For parameters without CTCAE grade the tables will be based on normal ranges. Toxicities for select laboratory abnormalities will be tabulated according to incidence and severity, and shift tables for the worst post-baseline value will be presented. For each patient, the maximum severity of a given laboratory toxicity will be used.

Vital signs: Maximal changes in vital signs will be summarized in tabular form.

14.4.4 Analyses of PK, Immunogenicity, Cytokines and Biomarkers

PK, immunogenicity, cytokines and biomarkers will be analyzed centrally and results for each will be presented separately.

For pharmacokinetics, serum concentrations of MCLA-128 and trastuzumab will be measured using validated bioanalytical methods at all time points. Descriptive statistics will be used to summarize PK parameters. Mean values for C_{max}, C_{0h}, AUC, CL, V_{ss}, t_{max} and $t_{1/2}$ will be calculated from the individual serum concentration versus time profiles of MCLA-128. Mean values for C_{EOI} and C_{0h} will be calculated for trastuzumab. Additional PK parameters may be calculated as appropriate. A population PK analysis of MCLA-128 may be performed with the available PK data.

14.5 Interim Analysis

No formal interim analysis is planned.

15 ETHICAL AND LEGAL ASPECTS

15.1 Ethical Conduct

This clinical study will be conducted in compliance with the protocol, Good Clinical Practice (GCP), as defined in ICH Guidance E6: *Good Clinical Practice: Consolidated Guidance*, the Declaration of Helsinki, the European Union (EU) Clinical Trials Directive 2001/20/EC, the GCP Directive 2005/28/EC, and applicable national and local regulatory requirements.

15.2 Delegation of Investigator Duties

The investigator must ensure that all persons assisting with the trial are adequately qualified, informed about the protocol, any amendments to the protocol, the study treatments, and their trial-related duties and functions.

The investigator must maintain a list of sub-investigators and other appropriately qualified persons to whom he/she has delegated significant trial-related duties.

15.3 Patient Information and Informed Consent

All patients will be informed that participation is voluntary and that they can cease participation at any time without necessarily giving a reason and without losing the right to receive appropriate medical care for their condition. Before being enrolled in the clinical study, patients must give written informed consent to participate in the study.

A Patient Information Leaflet (PIL), including an Informed Consent Form (ICF) (provided separately) will be given to each patient screened in the study. This document contains all the information required by the ICH E6 Guideline for Good Clinical Practice and any additional elements required by local regulations. The master document (in English) is also translated into the national language(s) and in terms that are understandable to any patient. In addition to the document, the investigator should provide oral information and answer all of the patient's questions. Patients must have adequate time for reflection and to ask questions and should not sign the ICF when it is first given to them.

The patient's consent must be confirmed by their dated signature and the name and dated signature of the principal investigator or sub-investigator conducting the informed consent discussions.

A copy of the signed consent document must be given to the patient. The original signed consent document will be retained in the Investigator Study File.

The investigator will not undertake any measure specifically required for the clinical study until valid consent has been obtained.

15.4 Confidentiality

Patient names will not be supplied to the Sponsor. Only the patient number and initials will be recorded in the eCRF and provided to any vendors working on behalf of the Sponsor. If the patient name appears on any document (e.g., laboratory report), it must be eliminated on the copy of the document supplied to the Sponsor. Study data stored on a computer will be kept in accordance with local data protection laws. Patients will be informed that representatives of the Sponsor, independent ethics committee (IEC)/ institutional review board (IRB), or regulatory authorities may inspect their medical records to verify the information collected, and that all personal information made available for inspection will be handled in strictest confidence and in accordance with local data protection laws.

The investigator will maintain a patient identification list (patient number with the corresponding patient codes, names, and date of birth) to enable records to be identified, which must be maintained in strict confidence.

15.5 Approval of the Clinical Study Protocol and Amendments

Before the start of the study, the clinical study protocol, PIL/ICF, and any other appropriate documents will be submitted to the IEC/IRB and to the national Health Authorities, in accordance with local legal requirements.

Before the first patient is enrolled in the study, all ethical and legal requirements must be met.

The IEC/IRB and the authorities must be informed of all administrative changes and any important finding that could modify the risk of exposed patients. They also must be informed or their authorization obtained for all subsequent amended protocols, in accordance with local legal requirements.

The investigator must keep a record of all communication with the IEC/IRB and the Health Authorities.

15.6 Ongoing Information for IEC/ IRB and Health Authorities

The Sponsor must submit the following to all investigators, the IEC/IRB and Health Authorities:

- Information on serious or unexpected AEs from any investigational site, as soon as possible.
- Expedited safety reports according to regulations.
- Periodic reports on the study progress.

15.7 Study Closure

The study must be closed at the site on completion. Furthermore, the Sponsor or the investigator has the right to close a study site at any time. Study materials must be returned, disposed of or retained as directed by the Sponsor.

15.8 Record Retention

The Investigator is required to maintain copies of all essential study documentation, including the Investigator Study File, a disc containing all CRF data (including the full audit trail and all data queries), signed informed consent forms, and records for the receipt and disposition of study medication, for a period of at least 15 years after study completion, as specified by ICH GCP and longer if required by local or regulatory authorities. Beyond this period, the investigator must obtain approval in writing from the Sponsor before destruction of any records.

The documents to be retained include:

- Original signed ICFs for all patients
- Patient identification code list, screening log and enrollment log
- Composition of the IEC/IRB
- List of sub-investigators and other appropriately qualified persons to whom the investigator has delegated significant trial-related duties, together with their roles in the study and signatures
- Records of all communications between the investigator and the IEC/IRB, and between the investigator and Sponsor (or CRO)
- A disc containing all CRF data and of documentation of corrections on data correction forms (DCF) for all patients (i.e., audit trail)
- Investigational product accountability records
- Record of any body fluids or tissue samples retained
- All other source documents (patient medical records, hospital records, laboratory records, etc.)
- All other documents as listed in Section 8 of the ICH E6 Guideline for Good Clinical Practice

15.9 Liability and Insurance

Liability and insurance provisions for this study are provided separately. The Sponsor has taken out an insurance covering their civil responsibility. A copy of the country-specific insurance certificates will

be maintained in the Investigator Study File and Trial Master File. Details of the insurance will be made available to patients in the ICF.

15.10 Financial Disclosure

Before the start of the study, the investigator will disclose to the Sponsor any proprietary or financial interests he or she might hold in the investigational products or the Sponsor company as outlined in the financial disclosure form provided by the Sponsor. The investigator agrees to update this information in case of significant changes during the study or within one year of its completion. The investigator also agrees that, where required by law or regulation, the Sponsor may submit this financial information to domestic or foreign regulatory authorities in applications for marketing authorizations.

Similar information will be provided by each sub-investigator to whom the investigator delegates significant study related responsibilities.

16 STUDY MONITORING AND AUDITING

Monitoring and auditing procedures developed or endorsed by the Sponsor will be followed, in order to comply with GCP guidelines. The investigator/institution must provide direct access to the on-site study documentation and medical records.

16.1 Monitoring and Source Data Verification

Monitoring will be done by in-person visits by Sponsor representatives (study clinical research associate [CRA] and medical monitors) who will check the eCRFs for completeness and clarity, and crosscheck them with source documents. In addition to the monitoring visits, frequent communications (letter, telephone, e-mail and fax), by the study monitors will ensure that the investigation is conducted according to protocol design and regulatory requirements.

A file for each patient must be maintained by the investigator that includes the signed informed consent form and all source documentation related to that patient. The Investigator must ensure the completeness and the availability of source documents from which the information in the CRF was derived.

At each study monitoring visit, the Investigator or designee will make available all records pertaining to the study. To allow sufficient time to assemble the documentation, monitoring visits will be confirmed by the site monitor in advance of planned visits.

Study close-out will be performed by the study monitor upon study closure.

16.2 On-Site Audits/Inspections

An external auditor, appointed by the Sponsor or the EC/IRBs, as well as inspectors, appointed by domestic and foreign regulatory authorities, may request access to all source documents, CRFs, and other study documentation for on-site audits or inspections. Direct access to these documents must be guaranteed by the investigator, who must provide support at all times for these activities. Medical records and other study documents may be copied during audit or inspection provided that patient names are obliterated on the copies to ensure confidentiality. Prior notice will be given to any sites selected for auditing.

17 DOCUMENTATION AND USE OF STUDY FINDINGS

17.1 Documentation of Study Findings

All CRF data will be collected using an electronic Case Report Form (eCRF) within a fully validated and CFR 21 Part 11 compliant Electronic Data Capture (EDC) system. All data will be entered into the CRF by the designated and appropriately trained site personnel whose name, initials, position, and signature must be supplied to the Sponsor.

The investigator, or designated representative, should complete the eCRF pages as soon as possible after data are collected, preferably on the same day that a study patient is seen for an examination, treatment, or any other study procedure and at the latest before the next monitoring visit. An explanation should be given for all missing data.

Data will be source data verified and reviewed by the study site monitor(s) before data cleaning by Data Management is performed. A source data location list will be prepared prior to study start and will be filed in both the Trial Master File and the Investigator Study File and updated as necessary. All queries will be raised and resolved within the EDC system. During entry, data will be checked by programing and once saved into the database further programing checks will be performed. During the conduct of the study, all system users will have real-time access to the data; the level of access to the data and study privileges will depend on their role.

The completed eCRFs must be reviewed and signed by the investigator named in the clinical study protocol or by a designated co-investigator.

All errors detected after the monitoring visit will be queried using Data Correction Forms (DCFs). The Sponsor will answer the question and/or correct errors in the DCF duly signed and dated. Original DCFs will be collected by the Sponsor's study monitor, while a copy will be kept by the investigator and recorded in the Investigator Study File with the corresponding eCRFs.

A copy of the eCRF will be printed and filed in the Trial Master File. The Sponsor will retain the originals of all eCRFs. The investigator will retain a copy of all completed eCRF pages and DCFs.

17.2 Confidentiality, Use of Study Findings and Publication Policy

All information concerning the product as well as any matter concerning the operation of the Sponsor, such as clinical indications for the drug, its formula, methods of manufacture and other scientific data relating to it, that have been provided by the Sponsor and are unpublished, are confidential and must remain the sole property of the Sponsor. The investigator will agree to use the information only for the purposes of carrying out this study and for no other purpose unless prior written permission from the Sponsor is obtained.

Samples and/or data will be processed centrally and the results will be sent electronically to the Sponsor or designated CRO. Data that will be processed centrally includes central imaging review data (CT/MRI) and centrally analyzed laboratory data including PK and blood/tumor biomarker samples.

The Sponsor has full ownership of the original eCRFs completed as part of the study.

By signing the clinical study protocol, the investigator agrees that the results of the study may be used for the purposes of national and international registration, publication, and information for medical and pharmaceutical professionals. The authorities will be notified of the investigator's name, address, qualifications, and extent of involvement.

The Sponsor will ensure that a clinical study report on the study is prepared.

All materials, documents and information supplied by the Sponsor to the investigator, and all materials, documents and information prepared or developed in the course of the study to be performed under this protocol, shall be the sole and exclusive property of the Sponsor. Subject to obligations of confidentiality, the investigator reserves the right to publish only the results of the work performed pursuant to this protocol, provided, however, that the investigator provides an authorized representative of the Sponsor with a copy of any proposed publication for review and comment at least 45 days in advance of its submission for publication. In addition, if requested, the investigator will withhold publication an additional 90 days to allow for filing a patent application or taking such other measures as Sponsor deems appropriate to establish and preserve its proprietary rights.

It is agreed that, consistent with scientific standards, publication of the results of the study shall be made only as part of a publication of the results obtained by all sites performing the protocol.

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19 APPENDICES

19.1 Appendix 1: ECOG Performance Status

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

19.2 Appendix 2: Calculation of renal clearance

Cockroft &Gault formula: Patients aged < 65 years

Male = 1.25 x **weight (kg)** x (140-**age**) / serum **creatinine (µmol/L)**

Female = 1.04 x **weight (kg)** x (140-**age**) / serum **creatinine (µmol/L)**

MDRD (Modification of Diet in Renal Disease) formula: Patients aged > 65 years

Male = 186 x (serum **creatinine (µmol/L)** x 0,0113)$^{-1,154}$ x **age**$^{-0,203}$

x 1,21 in subjects with black skin

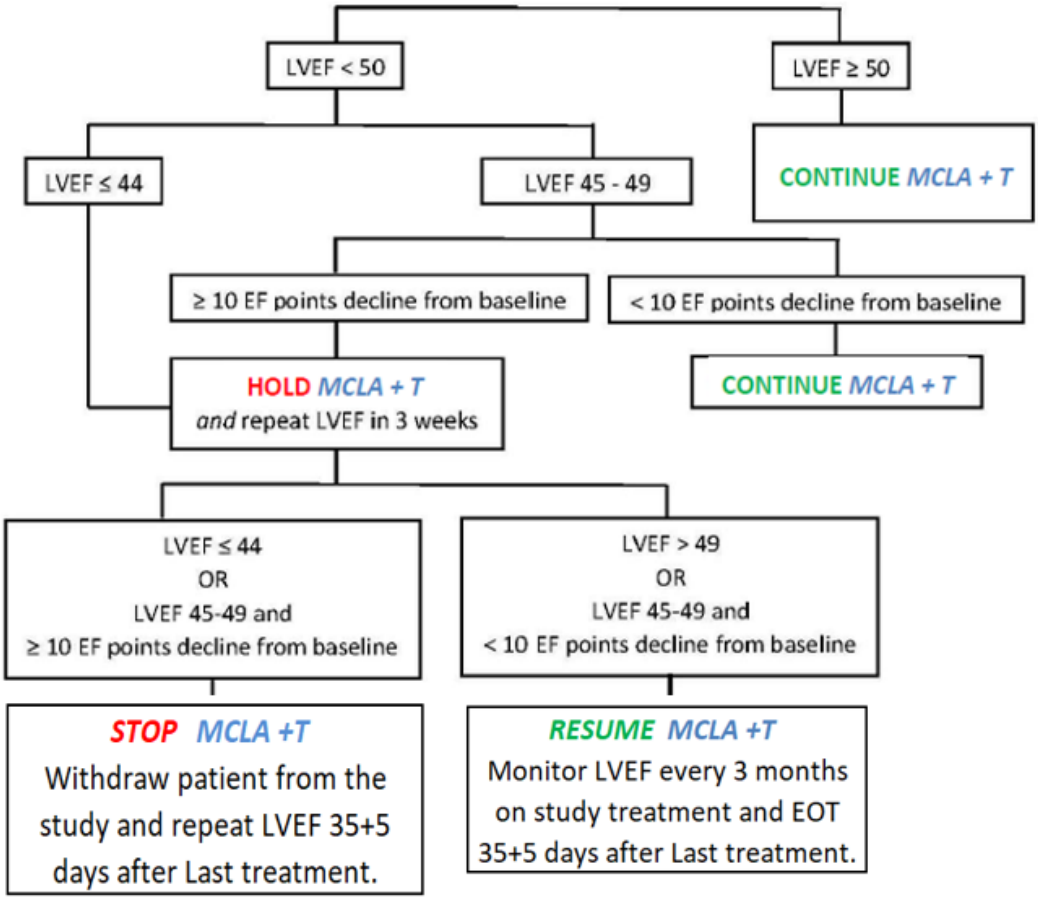
x 0.742 in female

Clearance can be calculated using tools available via the internet, e.g.

http://filfola.fr/medecine/cockroft_MDRD.html

or http://mdrd.com/

19.3 Appendix 3: Algorithm for MCLA-128 (MCLA) and trastuzumab (T) treatment with symptomatic LVEF decline



19.4 Appendix 4: New Response Evaluation Criteria in Solid Tumors - Revised RECIST Guidelines (Version 1.1)

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New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1)

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Guidelines

ABSTRACT

Background: Assessment of the change in tumour burden is an important feature of the clinical evaluation of cancer therapeutics: both tumour shrinkage (objective response) and disease progression are useful endpoints in clinical trials. Since RECIST was published in 2000, many investigators, cooperative groups, industry and government authorities have adopted these criteria in the assessment of treatment outcomes. However, a number of questions and issues have arisen which have led to the development of a revised RECIST guideline (version 1.1). Evidence for changes, summarised in separate papers in this special issue, has come from assessment of a large data warehouse (>6500 patients), simulation studies and literature reviews.

Highlights of revised RECIST 1.1: Major changes include: *Number of lesions to be assessed:* based on evidence from numerous trial databases merged into a data warehouse for analysis purposes, the number of lesions required to assess tumour burden for response determination has been reduced from a maximum of 10 to a maximum of five total (and from five to two per organ, maximum). *Assessment of pathological lymph nodes* is now incorporated: nodes with a short axis of ⩾15 mm are considered measurable and assessable as target lesions. The short axis measurement should be included in the sum of lesions in calculation of tumour response. Nodes that shrink to <10 mm short axis are considered normal. *Confirmation of response* is required for trials with response primary endpoint but is no longer required in randomised studies since the control arm serves as appropriate means of interpretation of data. *Disease progression* is clarified in several aspects: in addition to the previous definition of progression in target disease of 20% increase in sum, a 5 mm absolute increase is now required as well to guard against over calling PD when the total sum is very

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small. Furthermore, there is guidance offered on what constitutes 'unequivocal progression' of non-measurable/non-target disease, a source of confusion in the original RECIST guideline. Finally, a section on detection of new lesions, including the interpretation of FDG-PET scan assessment is included. *Imaging guidance*: the revised RECIST includes a new imaging appendix with updated recommendations on the optimal anatomical assessment of lesions.

Future work: A key question considered by the RECIST Working Group in developing RECIST 1.1 was whether it was appropriate to move from anatomic unidimensional assessment of tumour burden to either volumetric anatomical assessment or to functional assessment with PET or MRI. It was concluded that, at present, there is not sufficient standardisation or evidence to abandon anatomical assessment of tumour burden. The only exception to this is in the use of FDG-PET imaging as an adjunct to determination of progression. As is detailed in the final paper in this special issue, the use of these promising newer approaches requires appropriate clinical validation studies.

1. Background

1.1. History of RECIST criteria

Assessment of the change in tumour burden is an important feature of the clinical evaluation of cancer therapeutics. Both tumour shrinkage (objective response) and time to the development of disease progression are important endpoints in cancer clinical trials. The use of tumour regression as the endpoint for phase II trials screening new agents for evidence of anti-tumour effect is supported by years of evidence suggesting that, for many solid tumours, agents which produce tumour shrinkage in a proportion of patients have a reasonable (albeit imperfect) chance of subsequently demonstrating an improvement in overall survival or other time to event measures in randomised phase III studies (reviewed in [1–4]). At the current time objective response carries with it a body of evidence greater than for any other biomarker supporting its utility as a measure of promising treatment effect in phase II screening trials. Furthermore, at both the phase II and phase III stage of drug development, clinical trials in advanced disease settings are increasingly utilising time to progression (or progression-free survival) as an endpoint upon which efficacy conclusions are drawn, which is also based on anatomical measurement of tumour size.

However, both of these tumour endpoints, objective response and time to disease progression, are useful only if based on widely accepted and readily applied standard criteria based on anatomical tumour burden. In 1981 the World Health Organisation (WHO) first published tumour response criteria, mainly for use in trials where tumour response was the primary endpoint. The WHO criteria introduced the concept of an overall assessment of tumour burden by summing the products of bidimensional lesion measurements and determined response to therapy by evaluation of change from baseline while on treatment.[5] However, in the decades that followed their publication, cooperative groups and pharmaceutical companies that used the WHO criteria often 'modified' them to accommodate new technologies or to address areas that were unclear in the original document. This led to confusion in interpretation of trial results[6] and in fact, the application of varying response criteria was shown to lead to very different conclusions about the efficacy of the same regimen.[7] In response to these problems, an International Working Party was formed in the mid 1990s to standardise and simplify response criteria. New criteria, known as RECIST (Response Evaluation Criteria in Solid Tumours), were published in 2000.[8] Key features of the original RECIST include definitions of minimum size of measurable lesions, instructions on how many lesions to follow (up to 10; a maximum five per organ site), and the use of unidimensional, rather than bidimensional, measures for overall evaluation of tumour burden. These criteria have subsequently been widely adopted by academic institutions, cooperative groups, and industry for trials where the primary endpoints are objective response or progression. In addition, regulatory authorities accept RECIST as an appropriate guideline for these assessments.

1.2. Why update RECIST?

Since RECIST was published in 2000, many investigators have confirmed in prospective analyses the validity of substituting unidimensional for bidimensional (and even three-dimensional)-based criteria (reviewed in [9]). With rare exceptions (e.g. mesothelioma), the use of unidimensional criteria seems to perform well in solid tumour phase II studies.

However, a number of questions and issues have arisen which merit answers and further clarity. Amongst these are whether fewer than 10 lesions can be assessed without affecting the overall assigned response for patients (or the conclusion about activity in trials); how to apply RECIST in randomised phase III trials where progression, not response, is the primary endpoint particularly if not all patients have measurable disease; whether or how to utilise newer imaging technologies such as FDG-PET and MRI; how to handle assessment of lymph nodes; whether response confirmation is truly needed; and, not least, the applicability of RECIST in trials of targeted non-cytotoxic drugs. This revision of the RECIST guidelines includes updates that touch on all these points.

1.3. *Process of RECIST 1.1 development*

The RECIST Working Group, consisting of clinicians with expertise in early drug development from academic research organisations, government and industry, together with imaging specialists and statisticians, has met regularly to set the agenda for an update to RECIST, determine the evidence needed to justify the various changes made, and to review emerging evidence. A critical aspect of the revision process was to create a database of prospectively documented solid tumour measurement data obtained from industry and academic group trials. This database, assembled at the EORTC Data Centre under the leadership of Jan Bogaerts and Patrick Therasse (co-authors of this guideline), consists of >6500 patients with >18,000 target lesions and was utilised to investigate the impact of a variety of questions (e.g. number of target lesions required, the need for response confirmation, and lymph node measurement rules) on response and progression-free survival outcomes. The results of this work, which after evaluation by the RECIST Working Group led to most of the changes in this revised guideline, are reported in detail in a separate paper in this special issue.[10] Larry Schwartz and Robert Ford (also co-authors of this guideline) also provided key databases from which inferences have been made that inform these revisions.[11]

The publication of this revised guideline is believed to be timely since it incorporates changes to simplify, optimise and standardise the assessment of tumour burden in clinical trials. A summary of key changes is found in Appendix I. Because the fundamental approach to assessment remains grounded in the anatomical, rather than functional, assessment of disease, we have elected to name this version RECIST 1.1, rather than 2.0.

1.4. *What about volumetric or functional assessment?*

This raises the question, frequently posed, about whether it is 'time' to move from anatomic unidimensional assessment of tumour burden to either volumetric anatomical assessment *or* to functional assessment (e.g. dynamic contrast enhanced MRI or CT or (18)F-fluorodeoxyglucose positron emission tomographic (FDG-PET) techniques assessing tumour metabolism). As can be seen, the Working Group and particularly those involved in imaging research, did not believe that there is at present sufficient standardisation and widespread availability to recommend adoption of these alternative assessment methods. The only exception to this is in the use of FDG-PET imaging as an adjunct to determination of progression, as described later in this guideline. As detailed in paper in this special issue[12], we believe that the use of these promising newer approaches (which could either *add to* or *substitute for* anatomical assessment as described in RECIST) requires appropriate and rigorous clinical validation studies. This paper by Sargent et al. illustrates the type of data that will be needed to be able to define 'endpoints' for these modalities and how to determine where and when such criteria/modalities can be used to improve the reliability with which truly active new agents are identified and truly inactive new agents are discarded in comparison to RECIST criteria in phase II screening trials. The RECIST Working Group looks forward to such data emerging in the next few years to allow the appropriate changes to the next iteration of the RECIST criteria.

2. Purpose of this guideline

This guideline describes a standard approach to solid tumour measurement and definitions for objective assessment of change in tumour size for use in adult and paediatric cancer clinical trials. It is expected these criteria will be useful in all trials where objective response is the primary study endpoint, as well as in trials where assessment of stable disease, tumour progression or time to progression analyses are undertaken, since all of these outcome measures are based on an assessment of anatomical tumour burden and its change on study. There are no assumptions in this paper about the proportion of patients meeting the criteria for any of these endpoints which will signal that an agent or treatment regimen is active: those definitions are dependent on type of cancer in which a trial is being undertaken and the specific agent(s) under study. Protocols must include appropriate statistical sections which define the efficacy parameters upon which the trial sample size and decision criteria are based. In addition to providing definitions and criteria for assessment of tumour response, this guideline also makes recommendations regarding standard reporting of the results of trials that utilise tumour response as an endpoint.

While these guidelines may be applied in malignant brain tumour studies, there are also separate criteria published for response assessment in that setting.[13] This guideline is *not* intended for use for studies of malignant lymphoma since international guidelines for response assessment in lymphoma are published separately.[14]

Finally, many oncologists in their daily clinical practice follow their patients' malignant disease by means of repeated imaging studies and make decisions about continued therapy on the basis of both objective and symptomatic criteria. It is not intended that these RECIST guidelines play a role in that decision making, except if determined appropriate by the treating oncologist.

3. Measurability of tumour at baseline

3.1. *Definitions*

At baseline, tumour lesions/lymph nodes will be categorised measurable or non-measurable as follows:

3.1.1. *Measurable*

Tumour 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 5 mm; see Appendix II on imaging guidance).
- 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.

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 (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the *short* axis will be measured and followed (see Schwartz et al. in this Special Issue[15]). See also notes below on 'Baseline documentation of target and non-target lesions' for information on lymph node measurement.

3.1.2. *Non-measurable*

All other lesions, including small lesions (longest diameter <10 mm or pathological lymph nodes with ⩾10 to <15 mm short axis) as well as truly non-measurable lesions. Lesions considered truly non-measurable include: leptomeningeal disease, ascites, pleural or pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, abdominal masses/abdominal organomegaly identified by physical exam that is not measurable by reproducible imaging techniques.

3.1.3. *Special considerations regarding lesion measurability*

Bone lesions, cystic lesions, and lesions previously treated with local therapy require particular comment:

Bone lesions:.

- Bone scan, PET scan or plain films are not considered adequate imaging techniques to measure bone lesions. However, these techniques can be used to confirm the presence or disappearance of bone lesions.
- Lytic bone lesions or mixed lytic-blastic lesions, with *identifiable soft tissue components*, that can be evaluated by cross sectional imaging techniques such as CT or MRI can be considered as measurable lesions if the *soft tissue component* meets the definition of measurability described above.
- Blastic bone lesions are non-measurable.

Cystic lesions:.

- Lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.
- 'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

Lesions with prior local treatment:

- Tumour lesions situated in a previously irradiated area, or in an area subjected to other loco-regional therapy, are usually not considered measurable unless there has been demonstrated progression in the lesion. Study protocols should detail the conditions under which such lesions would be considered measurable.

3.2. *Specifications by methods of measurements*

3.2.1. *Measurement of lesions*

All measurements should be recorded in metric notation, using calipers if clinically assessed. All baseline evaluations should be performed as close as possible to the treatment start and never more than 4 weeks before the beginning of the treatment.

3.2.2. *Method of assessment*

The same method of assessment and the same technique should be used to characterise each identified and reported lesion at baseline and during follow-up. Imaging based evaluation should always be done rather than clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions: Clinical lesions will only be considered measurable when they are superficial and ⩾10 mm diameter as assessed using calipers (e.g. skin nodules). For the case of skin lesions, documentation by colour photography including a ruler to estimate the size of the lesion is suggested. As noted above, when lesions can be evaluated by both clinical exam and imaging, imaging evaluation should be undertaken since it is more objective and may also be reviewed at the end of the study.

Chest X-ray: Chest CT is preferred over chest X-ray, particularly when progression is an important endpoint, since CT is more sensitive than X-ray, particularly in identifying new lesions. However, lesions on chest X-ray may be considered measurable if they are clearly defined and surrounded by aerated lung. See Appendix II for more details.

CT, MRI: CT is the best currently available and reproducible method to measure lesions selected for response assessment. This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. As is described in Appendix II, when CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans). More details concerning the use of both CT and MRI for assessment of objective tumour response evaluation are provided in Appendix II.

Ultrasound: Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next (described in greater detail in Appendix II). If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

Endoscopy, laparoscopy: The utilisation of these techniques for objective tumour evaluation is not advised. However, they can be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response or surgical resection is an endpoint.

Tumour markers: Tumour markers *alone* cannot be used to assess *objective* tumour response. If markers are initially above

the upper normal limit, however, they must normalise for a patient to be considered in complete response. Because tumour markers are disease specific, instructions for their measurement should be incorporated into protocols on a disease specific basis. Specific guidelines for both CA-125 response (in recurrent ovarian cancer) and PSA response (in recurrent prostate cancer), have been published.[16–18] In addition, the Gynecologic Cancer Intergroup has developed CA125 progression criteria which are to be integrated with objective tumour assessment for use in first-line trials in ovarian cancer.[19]

Cytology, histology: These techniques can be used to differentiate between PR and CR in rare cases if required by protocol (for example, residual lesions in tumour types such as germ cell tumours, where known residual benign tumours can remain). When effusions are known to be a potential adverse effect of treatment (e.g. with certain taxane compounds or angiogenesis inhibitors), the cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment can be considered if the measurable tumour has met criteria for response or stable disease in order to differentiate between response (or stable disease) and progressive disease.

4. Tumour response evaluation

4.1. *Assessment of overall tumour burden and measurable disease*

To assess objective response or future progression, it is necessary to estimate the *overall tumour burden at baseline* and use this as a comparator for subsequent measurements. Only patients with measurable disease at baseline should be included in protocols where objective tumour response is the primary endpoint. Measurable disease is defined by the presence of at least one measurable lesion (as detailed above in Section 3). In studies where the primary endpoint is tumour progression (either time to progression or proportion with progression at a fixed date), the protocol must specify if entry is restricted to those with measurable disease or whether patients having non-measurable disease only are also eligible.

4.2. *Baseline documentation of 'target' and 'non-target' lesions*

When more than one measurable lesion is present at baseline all lesions up to a maximum of five lesions total (and a maximum of two lesions per organ) representative of all involved organs should be identified as *target lesions* and will be recorded and measured at baseline (this means in instances where patients have only one or two organ sites involved a *maximum* of two and four lesions respectively will be recorded). For evidence to support the selection of only five target lesions, see analyses on a large prospective database in the article by Bogaerts et al.[10].

Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to *reproducible repeated measurements*. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. To illustrate this point see the example in Fig. 3 of Appendix II.

Lymph nodes merit special mention since they are normal anatomical structures which may be visible by imaging even if not involved by tumour. As noted in Section 3, pathological nodes which are defined as measurable and may be identified as target lesions must meet the criterion of a short axis of $\geqslant$15 mm by CT scan. Only the *short* axis of these nodes will contribute to the baseline sum. The short axis of the node is the diameter normally used by radiologists to judge if a node is involved by solid tumour. Nodal size is normally reported as two dimensions in the plane in which the image is obtained (for CT scan this is almost always the axial plane; for MRI the plane of acquisition may be axial, saggital or coronal). The smaller of these measures is the short axis. For example, an abdominal node which is reported as being 20 mm × 30 mm has a short axis of 20 mm and qualifies as a malignant, measurable node. In this example, 20 mm should be recorded as the node measurement (See also the example in Fig. 4 in Appendix II). All other pathological nodes (those with short axis $\geqslant$10 mm but <15 mm) should be considered non-target lesions. Nodes that have a short axis <10 mm are considered non-pathological and should not be recorded or followed.

A *sum of the diameters* (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the *baseline sum diameters*. If lymph nodes are to be included in the sum, then as noted above, only the *short* axis is added into the sum. The baseline sum diameters will be used as reference to further characterise any objective tumour regression in the measurable dimension of the disease.

All other lesions (or sites of disease) including pathological lymph nodes should be identified as *non-target lesions* and should also be recorded at baseline. Measurements are not required and these lesions should be followed as 'present', 'absent', or in rare cases 'unequivocal progression' (more details to follow). In addition, it is possible to record multiple non-target lesions involving the same organ as a single item on the case record form (e.g. 'multiple enlarged pelvic lymph nodes' or 'multiple liver metastases').

4.3. *Response criteria*

This section provides the definitions of the criteria used to determine objective tumour response for target lesions.

4.3.1. *Evaluation of target lesions*

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

Partial Response (PR): At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters.

Progressive Disease (PD): At least a 20% increase in the sum of diameters of target lesions, taking as reference the *smallest sum on study* (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (*Note:* the appearance of one or more new lesions is also considered progression).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

4.3.2. *Special notes on the assessment of target lesions*

Lymph nodes. Lymph nodes identified as target lesions should always have the actual short axis measurement recorded (measured in the same anatomical plane as the baseline examination), even if the nodes regress to below 10 mm on study. This means that when lymph nodes are included as target lesions, the 'sum' of lesions may not be zero even if complete response criteria are met, since a normal lymph node is defined as having a short axis of <10 mm. Case report forms or other data collection methods may therefore be designed to have target nodal lesions recorded in a separate section where, in order to qualify for CR, each node must achieve a short axis <10 mm. For PR, SD and PD, the actual short axis measurement of the nodes is to be included in the sum of target lesions.

Target lesions that become 'too small to measure'. While on study, all lesions (nodal and non-nodal) recorded at baseline should have their actual measurements recorded at each subsequent evaluation, even when very small (e.g. 2 mm). However, sometimes lesions or lymph nodes which are recorded as target lesions at baseline become so faint on CT scan that the radiologist may not feel comfortable assigning an exact measure and may report them as being 'too small to measure'. When this occurs it is important that a value be recorded on the case report form. If it is the opinion of the radiologist that the lesion has likely disappeared, the measurement should be recorded as 0 mm. If the lesion is believed to be present and is faintly seen but too small to measure, a default value of 5 mm should be assigned (*Note:* It is less likely that this rule will be used for lymph nodes since they usually have a definable size when normal and are frequently surrounded by fat such as in the retroperitoneum; however, if a lymph node is believed to be present and is faintly seen but too small to measure, a default value of 5 mm should be assigned in this circumstance as well). This default value is derived from the 5 mm CT slice thickness (but should not be changed with varying CT slice thickness). The measurement of these lesions is potentially non-reproducible, therefore providing this default value will prevent false responses or progressions based upon measurement error. To reiterate, however, if the radiologist is able to provide an actual measure, that should be recorded, even if it is below 5 mm.

Lesions that split or coalesce on treatment. As noted in Appendix II, when non-nodal lesions 'fragment', the longest diameters of the fragmented portions should be added together to calculate the target lesion sum. Similarly, as lesions coalesce, a plane between them may be maintained that would aid in obtaining maximal diameter measurements of each individual lesion. If the lesions have truly coalesced such that they are no longer separable, the vector of the longest diameter in this instance should be the maximal longest diameter for the 'coalesced lesion'.

4.3.3. *Evaluation of non-target lesions*

This section provides the definitions of the criteria used to determine the tumour response for the group of non-target lesions. While some non-target lesions may actually be measurable, they need not be measured and instead should be assessed only *qualitatively* at the time points specified in the protocol.

Complete Response (CR): Disappearance of all non-target lesions and normalisation of tumour marker level. All lymph nodes must be non-pathological in size (<10 mm short axis).

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumour marker level above the normal limits.

Progressive Disease (PD): *Unequivocal progression* (see comments below) of existing non-target lesions. (*Note:* the appearance of one or more new lesions is also considered progression).

4.3.4. *Special notes on assessment of progression of non-target disease*

The concept of progression of non-target disease requires additional explanation as follows:

When the patient also has measurable disease. In this setting, to achieve 'unequivocal progression' on the basis of the non-target disease, there must be an overall level of substantial worsening in non-target disease such that, even in presence of SD or PR in target disease, the overall tumour burden has increased sufficiently to merit discontinuation of therapy (see examples in Appendix II and further details below). A modest 'increase' in the size of one or more non-target lesions is usually not sufficient to quality for unequivocal progression status. The designation of overall progression solely on the basis of change in non-target disease in the face of SD or PR of target disease will therefore be extremely rare.

When the patient has only non-measurable disease. This circumstance arises in some phase III trials when it is not a criterion of study entry to have measurable disease. The same general concepts apply here as noted above, however, in this instance there is no measurable disease assessment to factor into the interpretation of an increase in non-measurable disease burden. Because worsening in non-target disease cannot be easily quantified (by definition: if all lesions are truly non-measurable) a useful test that can be applied when assessing patients for unequivocal progression is to consider if the increase in overall disease burden based on the change in non-measurable disease is comparable in magnitude to the increase that would be required to declare PD for measurable disease: i.e. an increase in tumour burden representing an additional 73% increase in 'volume' (which is equivalent to a 20% increase diameter in a measurable lesion). Examples include an increase in a pleural effusion from 'trace' to 'large', an increase in lymphangitic

disease from localised to widespread, or may be described in protocols as 'sufficient to require a change in therapy'. Some illustrative examples are shown in Figs. 5 and 6 in Appendix II. If 'unequivocal progression' is seen, the patient should be considered to have had overall PD at that point. While it would be ideal to have objective criteria to apply to non-measurable disease, the very nature of that disease makes it impossible to do so, therefore the increase must be substantial.

4.3.5. *New lesions*

The appearance of new malignant lesions denotes disease progression; therefore, some comments on detection of new lesions are important. There are no specific criteria for the identification of new radiographic lesions; however, the finding of a new lesion should be unequivocal: i.e. not attributable to differences in scanning technique, change in imaging modality or findings thought to represent something other than tumour (for example, some 'new' bone lesions may be simply healing or flare of pre-existing lesions). This is particularly important when the patient's baseline lesions show partial or complete response. For example, necrosis of a liver lesion may be reported on a CT scan report as a 'new' cystic lesion, which it is not.

A lesion identified on a follow-up study in an anatomical location that was *not* scanned at baseline is considered a new lesion and will indicate disease progression. An example of this is the patient who has visceral disease at baseline and while on study has a CT or MRI brain ordered which reveals metastases. The patient's brain metastases are considered to be evidence of PD even if he/she did not have brain imaging at baseline.

If a new lesion is equivocal, for example because of its small size, continued therapy and follow-up evaluation will clarify if it represents truly new disease. If repeat scans confirm there is definitely a new lesion, then progression should be declared using the date of the initial scan.

While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

a. Negative FDG-PET at baseline, with a positive[1] FDG-PET at follow-up is a sign of PD based on a new lesion.
b. No FDG-PET at baseline and a positive FDG-PET at follow-up:
   If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD.
   If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan).
   If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.

[1] A 'positive' FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

4.4. *Evaluation of best overall response*

The best overall response is the best response recorded from the start of the study treatment until the end of treatment taking into account any requirement for confirmation. On occasion a response may not be documented until after the end of therapy so protocols should be clear if post-treatment assessments are to be considered in determination of best overall response. Protocols must specify how any new therapy introduced before progression will affect best response designation. The patient's best overall response assignment will depend on the findings of both target and non-target disease and will also take into consideration the appearance of new lesions. Furthermore, depending on the nature of the study and the protocol requirements, it may also require confirmatory measurement (see Section 4.6). Specifically, in non-randomised trials where response is the primary endpoint, confirmation of PR or CR is needed to deem either one the 'best overall response'. This is described further below.

4.4.1. *Time point response*

It is assumed that at each protocol specified time point, a response assessment occurs. Table 1 on the next page provides a summary of the overall response status calculation at each time point for patients who have measurable disease at baseline.

When patients have non-measurable (therefore non-target) disease only, Table 2 is to be used.

4.4.2. *Missing assessments and inevaluable designation*

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. For example, if a patient had a baseline sum of 50 mm with three measured lesions and at follow-up only two lesions were assessed, but those gave a sum of 80 mm, the patient will have achieved PD status, regardless of the contribution of the missing lesion.

4.4.3. *Best overall response: all time points*

The *best overall response* is determined once all the data for the patient is known.

Best response determination in trials where confirmation of complete or partial response IS NOT required: Best response in these trials is defined as the best response across all time points (for example, a patient who has SD at first assessment, PR at second assessment, and PD on last assessment has a best overall response of PR). When SD is believed to be best response, it must also meet the protocol specified minimum time from baseline. If the minimum time is not met when SD is otherwise the best time point response, the patient's best response depends on the subsequent assessments. For example, a patient who has SD at first assessment, PD at second and does not meet minimum duration for SD, will have a best response of PD. The same patient lost to follow-up after the first SD assessment would be considered inevaluable.

Table 1 – Time point response: patients with target (+/– non-target) disease.

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

CR = complete response, PR = partial response, SD = stable disease, PD = progressive disease, and NE = inevaluable.

Table 2 – Time point response: patients with non-target disease only.

Non-target lesions	New lesions	Overall response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD[a]
Not all evaluated	No	NE
Unequivocal PD	Yes or No	PD
Any	Yes	PD

CR = complete response, PD = progressive disease, and NE = inevaluable.

a 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised.

Best response determination in trials where confirmation of complete or partial response IS required: Complete or partial responses may be claimed only if the criteria for each are met at a subsequent time point as specified in the protocol (generally 4 weeks later). In this circumstance, the best overall response can be interpreted as in Table 3.

4.4.4. *Special notes on response assessment*

When nodal disease is included in the sum of target lesions and the nodes decrease to 'normal' size (<10 mm), they may still have a measurement reported on scans. This measurement should be recorded even though the nodes are normal in order not to overstate progression should it be based on increase in size of the nodes. As noted earlier, this means that patients with CR may not have a total sum of 'zero' on the case report form (CRF).

In trials where confirmation of response is required, repeated 'NE' time point assessments may complicate best response determination. The analysis plan for the trial must address how missing data/assessments will be addressed in determination of response and progression. For example, in most trials it is reasonable to consider a patient with time point responses of PR-NE-PR as a confirmed response.

Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as 'symptomatic deterioration'. Every effort should be made to document objective progression even after discontinuation of treatment. Symptomatic deterioration is *not* a descriptor of an objective response: it is a reason for stopping study therapy. The objective response status of such patients is to be determined by evaluation of target and non-target disease as shown in Tables 1–3.

Conditions that define 'early progression, early death and inevaluability' are study specific and should be clearly described in each protocol (depending on treatment duration, treatment periodicity).

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

Table 3 – Best overall response when confirmation of CR and PR required.

Overall response First time point	Overall response Subsequent time point	BEST overall response
CR	CR	CR
CR	PR	SD, PD or PR[a]
CR	SD	SD provided minimum criteria for SD duration met, otherwise, PD
CR	PD	SD provided minimum criteria for SD duration met, otherwise, PD
CR	NE	SD provided minimum criteria for SD duration met, otherwise NE
PR	CR	PR
PR	PR	PR
PR	SD	SD
PR	PD	SD provided minimum criteria for SD duration met, otherwise, PD
PR	NE	SD provided minimum criteria for SD duration met, otherwise NE
NE	NE	NE

CR = complete response, PR = partial response, SD = stable disease, PD = progressive disease, and NE = inevaluable.

a If a CR is truly met at first time point, then any disease seen at a subsequent time point, even disease meeting PR criteria relative to baseline, makes the disease PD at that point (since disease must have reappeared after CR). Best response would depend on whether minimum duration for SD was met. However, sometimes 'CR' may be claimed when subsequent scans suggest small lesions were likely still present and in fact the patient had PR, not CR at the first time point. Under these circumstances, the original CR should be changed to PR and the best response is PR.

needle aspirate/biopsy) before assigning a status of complete response. FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

For equivocal findings of progression (e.g. very small and uncertain new lesions; cystic changes or necrosis in existing lesions), treatment may continue until the next scheduled assessment. If at the next scheduled assessment, progression is confirmed, the date of progression should be the earlier date when progression was suspected.

4.5. *Frequency of tumour re-evaluation*

Frequency of tumour re-evaluation while on treatment should be protocol specific and adapted to the type and schedule of treatment. However, in the context of phase II studies where the beneficial effect of therapy is not known, follow-up every 6–8 weeks (timed to coincide with the end of a cycle) is reasonable. Smaller or greater time intervals than these could be justified in specific regimens or circumstances. The protocol should specify which organ sites are to be evaluated at baseline (usually those most likely to be involved with metastatic disease for the tumour type under study) and how often evaluations are repeated. Normally, all target and non-target sites are evaluated at each assessment. In selected circumstances certain non-target organs may be evaluated less frequently. For example, bone scans may need to be repeated only when complete response is identified in target disease or when progression in bone is suspected.

After the end of the treatment, the need for repetitive tumour evaluations depends on whether the trial has as a goal the response rate or the time to an event (progression/death). If 'time to an event' (e.g. time to progression, disease-free survival, progression-free survival) is the main endpoint of the study, then routine scheduled re-evaluation of protocol specified sites of disease is warranted. In randomised comparative trials in particular, the scheduled assessments should be performed as identified on a calendar schedule (for example: every 6–8 weeks on treatment or every 3–4 months after treatment) and should not be affected by delays in therapy, drug holidays or any other events that might lead to imbalance in a treatment arm in the timing of disease assessment.

4.6. *Confirmatory measurement/duration of response*

4.6.1. *Confirmation*

In non-randomised trials where response is the primary endpoint, confirmation of PR and CR is required to ensure responses identified are not the result of measurement error. This will also permit appropriate interpretation of results in the context of historical data where response has traditionally required confirmation in such trials (see the paper by Bogaerts et al. in this Special Issue[10]). However, in all other circumstances, i.e. in randomised trials (phase II or III) or studies where stable disease or progression are the primary endpoints, confirmation of response is not required since it will not add value to the interpretation of trial results. However, elimination of the requirement for response confirmation may increase the importance of central review to protect against bias, in particular in studies which are not blinded.

In the case of SD, measurements must have met the SD criteria at least once after study entry at a minimum interval (in general not less than 6–8 weeks) that is defined in the study protocol.

4.6.2. *Duration of overall response*

The duration of overall response is measured from the time measurement criteria are first met for CR/PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded on study).

The duration of overall complete response is measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectively documented.

4.6.3. *Duration of stable disease*

Stable disease is measured from the start of the treatment (in randomised trials, from date of randomisation) until the criteria for progression are met, taking as reference the *smallest sum on study* (if the baseline sum is the smallest, this is the reference for calculation of PD).

The clinical relevance of the duration of stable disease varies in different studies and diseases. If the proportion of patients achieving stable disease for a minimum period of time is an endpoint of importance in a particular trial, the protocol should specify the minimal time interval required between two measurements for determination of stable disease.

Note: The duration of response and stable disease as well as the progression-free survival are influenced by the frequency of follow-up after baseline evaluation. It is not in the scope of this guideline to define a standard follow-up frequency. The frequency should take into account many parameters including disease types and stages, treatment periodicity and standard practice. However, these limitations of the precision of the measured endpoint should be taken into account if comparisons between trials are to be made.

4.7. *Progression-free survival/proportion progression-free*

4.7.1. *Phase II trials*

This guideline is focused primarily on the use of objective response endpoints for phase II trials. In some circumstances, 'response rate' may not be the optimal method to assess the potential anticancer activity of new agents/regimens. In such cases 'progression-free survival' (PFS) or the 'proportion progression-free' at landmark time points, might be considered appropriate alternatives to provide an initial signal of biologic effect of new agents. It is clear, however, that in an uncontrolled trial, these measures are subject to criticism since an apparently promising observation may be related to biological factors such as patient selection and not the impact of the intervention. Thus, phase II screening trials utilising these endpoints are best designed with a randomised control. Exceptions may exist

where the behaviour patterns of certain cancers are so consistent (and usually consistently poor), that a non-randomised trial is justifiable (see for example van Glabbeke et al.[20]). However, in these cases it will be essential to document with care the basis for estimating the expected PFS or proportion progression-free in the absence of a treatment effect.

4.7.2. *Phase III trials*

Phase III trials in advanced cancers are increasingly designed to evaluate progression-free survival or time to progression as the primary outcome of interest. Assessment of progression is relatively straightforward if the protocol requires all patients to have measurable disease. However, restricting entry to this subset of patients is subject to criticism: it may result in a trial where the results are less likely to be generalisable if, in the disease under study, a substantial proportion of patients would be excluded. Moreover, the restriction to entry will slow recruitment to the study. Increasingly, therefore, trials allow entry of both patients with measurable disease as well as those with non-measurable disease only. In this circumstance, care must be taken to explicitly describe the findings which would qualify for progressive disease for those patients *without* measurable lesions. Furthermore, in this setting, protocols must indicate if the maximum number of recorded target lesions for those patients with measurable disease may be relaxed from five to three (based on the data found in Bogaerts et al.[10] and Moskowitz et al.[11]). As found in the 'special notes on assessment of progression', these guidelines offer recommendations for assessment of progression in this setting. Furthermore, if available, validated tumour marker measures of progression (as has been proposed for ovarian cancer) may be useful to integrate into the definition of progression. Centralised blinded review of imaging studies or of source imaging reports to verify 'unequivocal progression' may be needed if important drug development or drug approval decisions are to be based on the study outcome. Finally, as noted earlier, because the date of progression is subject to ascertainment bias, timing of investigations in study arms should be the same. The article by Dancey et al. in this special issue[21] provides a more detailed discussion of the assessment of progression in randomised trials.

4.8. *Independent review of response and progression*

For trials where *objective response* (CR + PR) is the primary endpoint, and in particular where key drug development decisions are based on the observation of a minimum number of responders, it is recommended that all claimed responses be reviewed by an expert(s) independent of the study. If the study is a randomised trial, ideally reviewers should be blinded to treatment assignment. Simultaneous review of the patients' files and radiological images is the best approach.

Independent review of progression presents some more complex issues: for example, there are statistical problems with the use of central-review-based progression time in place of investigator-based progression time due to the potential introduction of informative censoring when the former precedes the latter. An overview of these factors and other lessons learned from independent review is provided in an article by Ford et al. in this special issue.[22]

4.9. *Reporting best response results*

4.9.1. *Phase II trials*

When response is the primary endpoint, and thus all patients must have measurable disease to enter the trial, all patients included in the study must be accounted for in the report of the results, even if there are major protocol treatment deviations or if they are not evaluable. Each patient will be assigned one of the following categories:

1. Complete response
2. Partial response
3. Stable disease
4. Progression
5. Inevaluable for response: specify reasons (for example: early death, malignant disease; early death, toxicity; tumour assessments not repeated/incomplete; other (specify)).

Normally, all *eligible* patients should be included in the denominator for the calculation of the response rate for phase II trials (in some protocols it will be appropriate to include all treated patients). It is generally preferred that 95% two-sided confidence limits are given for the calculated response rate. Trial conclusions should be based on the response rate for all eligible (or all treated) patients and should *not* be based on a selected 'evaluable' subset.

4.9.2. *Phase III trials*

Response evaluation in phase III trials may be an indicator of the relative anti-tumour activity of the treatments evaluated and is almost always a secondary endpoint. Observed differences in response rate may not predict the clinically relevant therapeutic benefit for the population studied. If objective response is selected as a primary endpoint for a phase III study (only in circumstances where a direct relationship between objective tumour response and a clinically relevant therapeutic benefit can be unambiguously demonstrated for the population studied), the same criteria as those applying to phase II trials should be used and all patients entered should have at least one measurable lesion.

In those many cases where response is a secondary endpoint and not all trial patients have measurable disease, the method for reporting overall best response rates must be pre-specified in the protocol. In practice, response rate may be reported using either an 'intent to treat' analysis (all randomised patients in the denominator) or an analysis where only the subset of patients with measurable disease at baseline are included. The protocol should clearly specify how response results will be reported, including any subset analyses that are planned.

The original version of RECIST suggested that in phase III trials one could write protocols using a 'relaxed' interpretation of the RECIST guidelines (for example, reducing the number of lesions measured) but this should no longer be done since these revised guidelines have been amended in such a way that it is clear how these criteria should be applied for all trials in which anatomical assessment of tumour response or progression are endpoints.

Appendix I. Summary of major changes RECIST 1.0 to RECIST 1.1

	RECIST 1.0	RECIST 1.1	Rationale	Reference in special issue (if applicable)
Minimum size measurable lesions	CT: 10 mm spiral 20 mm non-spiral	CT 10 mm; delete reference to spiral scan	Most scans used have 5 mm or less slice thickness Clearer to give instruction based on slice interval if it is greater than 5 mm	
	Clinical: 20 mm	Clinical: 10 mm (must be measurable with calipers)	Caliper measurement will make this reliable	
	Lymph node: not mentioned	CT: ⩾15 mm short axis for target ⩾10–<15 mm for non-target <10 mm is non-pathological	Since nodes are normal structure need to define pathological enlargement. Short axis is most sensitive	Schwartz et al.[15]
Special considerations on lesion measurability	–	Notes included on bone lesions, cystic lesions	Clarify frequently asked questions	
Overall tumour burden	10 lesions (5 per organ)	5 lesions (2 per organ)	Data warehouse analysis shows no loss of information if lesion number reduced from 10 to 5. A maximum of 2 lesions per organ yields sufficient representation per disease site	Bogaerts et al.[10]
Response criteria target disease	CR lymph node not mentioned	CR lymph nodes must be <10 mm short axis	In keeping with normal size of nodes	Schwartz et al.[15]
	PD 20% increase over smallest sum on study or new lesions	PD 20% increase over smallest sum on study (including baseline if that is smallest) and at least 5 mm increase or new lesions	Clarification that if baseline measurement is smaller than any on study measurement, it is reference against which PD is assessed 5 mm absolute increase to guard against over calling PD when total sum is very small and 20% increase is within measurement error	
Response criteria non-target disease	'unequivocal progression' considered as PD	More detailed description of 'unequivocal progression' to indicate that it should not normally trump target disease status. It must be representative of overall disease status change, not a single lesion increase	Confusion with RECIST 1.0 where some were considering PD if 'increase' in any non-target lesion, even when target disease is stable or responding	
New lesions	–	New section on New lesions	To provide guidance on when a lesion is considered new (and thus PD)	
Overall response	Table integrated target and non-target lesions	Two tables: one integrating target and non-target and the other of non-target only	To account for the fact that RECIST criteria are now being used in trials where PFS is the endpoint and not all patients have measurable (target) disease at baseline	Dancey et al.[21]

		Special notes: How to assess and measure lymph nodes CR in face of residual tissue Discussion of 'equivocal' progression	Frequently asked questions on these topics	
Confirmatory measure	For CR and PR: criteria must be met again 4 weeks after initial documentation	Retain this requirement ONLY for non-randomised trials with primary endpoint of response	Data warehouse shows that response rates rise when confirmation is eliminated, but the only circumstance where this is important is in trials where there is no concurrent comparative control and where this measure is the primary endpoint	Bogaerts et al.[10]
Progression-free survival	General comments only	More specific comments on use of PFS (or proportion progression-free) as phase II endpoint Greater detail on PFS assessment in phase III trials	Increasing use of PFS in phase III trials requires guidance on assessment of PD in patients with non-measurable disease	Dancey et al.[21]
Reporting of response results	9 categories suggested for reporting phase II results	Divided into phase II and phase III 9 categories collapsed into 5 In phase III, guidance given about reporting response	Simplifies reporting and clarifies how to report phase II and III data consistently	
Response in phase III trials	More relaxed guidelines possible if protocol specified	This section removed and referenced in section above: no need to have different criteria for phase II and III	Simplification of response assessment by reducing number of lesions and eliminating need for confirmation in randomised studies where response is not the primary endpoint makes separate 'rules' unnecessary	
Imaging appendix	Appendix I	Appendix II: updated with detailed guidance on use of MRI, PET/CT Other practical guidance included	Evolving use of newer modalities addressed. Enhanced guidance in response to frequent questions and from radiology review experience	
New appendices		Appendix I: comparison of RECIST 1.0 and 1.1 Appendix III: frequently asked questions		

Conflict of interest statement

None declared.

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Appendix II. Specifications for standard anatomical radiological imaging

These protocols for image acquisition of computed tomography (CT) and magnetic resonance imaging (MRI) are recommendations intended for patients on clinical trials where RECIST assessment will be performed. Standardisation of imaging requirements and image acquisition parameters is ideal to allow for optimal comparability of subjects within a study and results between studies. These recommendations are designed to balance optimised image acquisition protocols with techniques that should be feasible to perform globally at imaging facilities in all types of radiology practices. These guidelines are not applicable to functional imaging techniques or volumetric assessment of tumour size.

Scanner quality control is highly recommended and should follow standard manufacturer and facility maintenance schedules using commercial phantoms. It is likely that for RECIST unidimensional measurements this will be adequate to produce reproducible measurements. Imaging quality control for CT includes an analysis of image noise and uniformity and CT number as well as spatial resolution. The frequency of quality control analysis is also variable and should focus on clinically relevant scanning parameters. Dose analysis is always important and the use of imaging should follow the ALARA principle, 'As Low As Reasonably Achievable', which refers to making every reasonable effort to maintain radiation exposures as far below the dose limits as possible.

Specific notes

Chest X-ray measurement of lesions surrounded by pulmonary parenchyma is feasible, but not preferable as the measurement represents a summation of densities. Furthermore, there is poor identification of new lesions within the chest on X-ray as compared with CT. Therefore, measurements of pulmonary parenchymal lesions as well as mediastinal disease are optimally performed with CT of the chest. MRI of the chest should only be performed in extenuating circumstances. Even if IV contrast cannot be administered (for example, in the situation of allergy to contrast), a non-contrast CT of the chest is still preferred over MRI or chest X-ray.

CT scans: CT scans of the chest, abdomen, and pelvis should be contiguous throughout all the anatomic region of interest. As a general rule, the minimum size of a measurable lesion at baseline should be no less than double the slice thickness and also have a minimum size of 10 mm (see below for minimum size when scanners have a slice thickness more than 5 mm). While the precise physics of lesion size and partial volume averaging is complex, lesions smaller than 10 mm may be difficult to accurately and reproducibly measure. While this rule is applicable to baseline scans, as lesions potentially decrease in size at follow-up CT studies, they should still be measured. Lesions which are reported as 'too small to measure' should be assigned a default measurement of 5 mm if they are still visible.

The most critical CT image acquisition parameters for optimal tumour evaluation using RECIST are *anatomic coverage, contrast administration, slice thickness, and reconstruction interval.*

a. *Anatomic coverage*: Optimal anatomic coverage for most solid tumours is the chest, abdomen and pelvis. Coverage should encompass all areas of known predilection for metastases in the disease under evaluation and

should additionally investigate areas that may be involved based on signs and symptoms of individual patients. Because a lesion later identified in a body part not scanned at baseline would be considered as a new lesion representing disease progression, careful consideration should be given to the extent of imaging coverage at baseline and at subsequent follow-up time points. This will enable better consistency not only of tumour measurements but also identification of new disease.

b. *IV contrast administration*: Optimal visualisation and measurement of metastases in solid tumours requires consistent administration (dose and rate) of IV contrast as well as timing of scanning. Typically, most abdominal imaging is performed during the portal venous phase and (optimally) about the same time frame after injection on each examination (see Fig. 1 for impact of different phase of IV contrast on lesion measurement). Most solid tumours may be scanned with a single phase after administration of contrast. While triphasic CT scans are sometimes performed on other types of vascular tumours to improve lesion conspicuity, for consistency and uniformity, we would recommend triphasic CT for hepatocellular and neuroendocrine tumours for which this scanning protocol is generally standard of care, and the improved temporal resolution of the triphasic scan will enhance the radiologists' ability to consistently and reproducibly measure these lesions. The precise dose and rate of IV contrast is dependent upon the CT scanning equipment, CT acquisition protocol, the type of contrast used, the available venous access and the medical condition of the patient. Therefore, the method of administration of intravenous contrast agents is variable. Rather than try to institute rigid rules regarding methods for administering contrast agents and the volume injected, it is appropriate to suggest that an adequate volume of a suitable contrast agent should be given so that the metastases are demonstrated to best effect and a *consistent method* is used on subsequent examinations for any given patient (ideally, this would be specified in the protocol or for an institution). It is very important that the same technique be used at baseline and on follow-up examinations for a given patient. This will greatly enhance the reproducibility of the tumour measurements. If prior to enrolment it is known a patient is not able to undergo CT scans with IV contrast due to allergy or renal insufficiency, the decision as to whether a non-contrast CT or MRI (with or without IV contrast) should be used to evaluate the subject at baseline and follow-up should be guided by the tumour type under investigation and the anatomic location of the disease. For patients who develop contraindications to contrast after baseline contrast CT is done, the decision as to whether non-contrast CT or MRI (enhanced or non-enhanced) should be performed should also be based on the tumour type, anatomic location of the disease and should be optimised to allow for comparison to the prior studies if possible. Each case should be discussed with the radiologist to determine if substitution of these other approaches is possible and, if not, the patient should be considered not evaluable from that point forward. Care must be taken in measurement of target lesions on a different modality and interpretation of non-target disease or new lesions, since the same lesion may appear to have a different size using a new modality (see Fig. 2 for a comparison of CT and MRI of the same lesion). Oral contrast is recommended to help visualise and differentiate structures in the abdomen.

c. *Slice thickness and reconstruction interval*: RECIST measurements may be performed at most clinically obtained slice thicknesses. It is recommended that CT scans be performed at 5 mm contiguous slice thickness or less and indeed this guideline presumes a minimum 5 mm thickness in recommendations for measurable lesion definition. Indeed, variations in slice thickness can have an impact on lesion measurement and on detection of new lesions. However, consideration should also be given for minimising radiation exposure. With these parameters, a minimum 10 mm lesion is considered measurable at baseline. Occasionally, institutions may perform medically acceptable scans at slice thicknesses *greater than* 5 mm. If this occurs, the minimum size of measurable lesions at baseline should be *twice the slice*

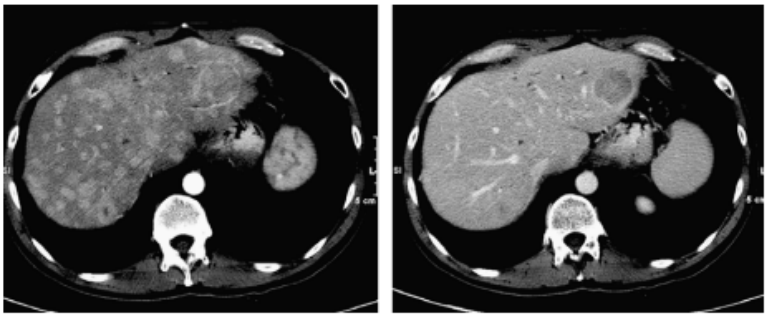

Fig. 1 – Difference in measurement/visualisation with different phases of IV contrast administration. Hypervascular metastases imaged in the arterial phase (left) and the portal venous phase (right). Note that the number of lesions visible differs greatly between the two phases of contrast administration as does any potential lesion measurement. Consistent CT scan acquisition, including phase of contrast administration, is important for optimal and reproducible tumour

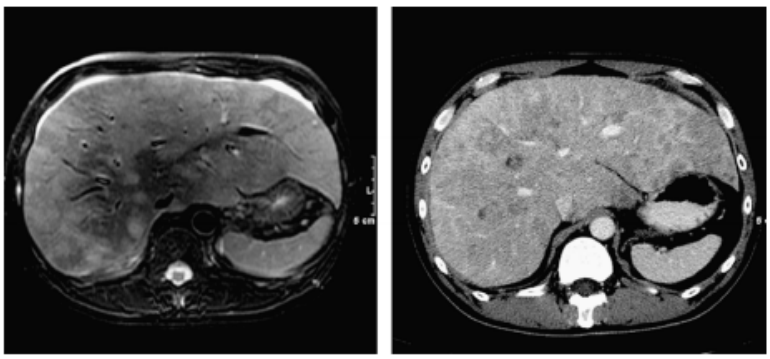

Fig. 2 – CT versus MRI of same lesions showing apparent 'progression' due only to differing method of measurement.

thickness of the baseline scans. Most contemporary CT scanners are multidetector which have many imaging options for these acquisition parameters.[23] The equipment vendor and scanning manual should be reviewed if there are any specific system questions.

d. *Alternative contrast agents*: There are a number of other, new contrast agents, some organ specific.[24] They may be used as part of patient care for instance, in liver lesion assessment, or lymph node characterisation[25], but *should not as yet be used in clinical trials.*

FDG-PET has gained acceptance as a valuable tool for detecting, staging and restaging several malignancies. Criteria for incorporating (or substituting) FDG-PET into anatomical assessment of tumour response in phase II trials are not yet available, though much research is ongoing. Nevertheless, FDG-PET is being used in many drug development trials both as a tool to assess therapeutic efficacy and also in assessment of progression. If FDG-PET scans are included in a protocol, by consensus, an FDG uptake period of 60 min prior to imaging has been decided as the most appropriate for imaging of patients with malignancy.[26] Whole-body acquisition is important since this allows for sampling of all areas of interest and can assess if new lesions have appeared thus determining the possibility of interval progression of disease. Images from the base of the skull to the level of the mid-thigh should be obtained 60 min post injection. PET camera specifications are variable and manufacturer specific, so every attempt should be made to use the same scanner, or the same model scanner, for serial scans on the same patient. Whole-body acquisitions can be performed in either 2- or 3-dimensional mode with attenuation correction, but the method chosen should be consistent across all patients and serial scans in the clinical trial.

PET/CT scans: Combined modality scanning such as with PET–CT is increasingly used in clinical care, and is a modality/technology that is in rapid evolution; therefore, the recommendations in this paper may change rather quickly with time. At present, low dose or attenuation correction CT portions of a combined PET–CT are of limited use in anatomically based efficacy assessments and it is therefore suggested that they should not be substituted for dedicated diagnostic contrast enhanced CT scans for anatomically based RECIST measurements. However, if a site can document that the CT performed as part of a PET–CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast) then the CT portion of the PET–CT can be used for RECIST measurements. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.

Ultrasound examinations should not be used in clinical trials to measure tumour regression or progression of lesions because the examination is necessarily subjective and operator dependent. The reasons for this are several: Entire examinations cannot be reproduced for independent review at a later date, and it must be assumed, whether or not it is the case, that the hard-copy films available represent a true and accurate reflection of events. Furthermore, if, for example, the only measurable lesion is in the para-aortic region of the abdomen and if gas in the bowel overlies the lesion, the lesion will not be detected because the ultrasound beam cannot penetrate the gas. Accordingly, the disease staging (or restaging for treatment evaluation) for this patient will not be accurate.

While evaluation of lesions by *physical examination* is also of limited reproducibility, it is permitted when lesions are superficial, at least 10 mm size, and can be assessed using calipers. In general, it is preferred if patients on clinical trials have at least one lesion that is measurable by CT. Other skin or palpable lesions may be measured on physical examination and be considered target lesions.

Use of *MRI* remains a complex issue. MRI has excellent contrast, spatial and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimised for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. Generally, axial imaging of the abdomen and pelvis with T1 and T2 weighted imaging along with gadolinium enhanced imaging should be performed. The field of view, matrix, number of excitations, phase encode steps, use of fat suppression and fast sequences should be optimised for the spe-

cific body part being imaged as well as the scanner utilised. It is beyond the scope of this document or appendix to prescribe specific MRI pulse sequence parameters for all scanners, body parts and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques if possible.

Selection of target lesions: In general, the *largest* lesions representative of involved organs (up to a maximum of two per organ and five total) are selected to follow as target lesions. However, in some cases, the largest lesions may not be easily measured and are not suitable for follow-up because of their configuration. In these cases, identification of the largest *most reproducible* lesions is advised. Fig. 3 provides an illustrative example where the largest lesion is not the most reproducible and another lesion is better to select and follow:

Measurement of lesions

The longest diameter of selected lesions should be measured in the plane in which the images were acquired. For body CT, this is the axial plane. In the event isotropic reconstructions are performed, measurements can be made on these reconstructed images; however, it should be cautioned that not all radiology sites are capable of producing isotropic reconstructions. This could lead to the undesirable situation of measurements in the axial plane at one assessment point and in a different plane at a subsequent assessment. There are some tumours, for instance paraspinal lesions, which are better measured in the coronal or sagittal plane. It would be acceptable to measure these lesions in these planes if the reconstructions in those planes were isotropic or the images were acquired with MRI in those planes. Using the same plane of evaluation, the maximal diameter of each target lesion should always be measured at subsequent follow-up time points even if this results in measuring the lesion at a different slice level or in a different orientation or vector compared with the baseline study. Software tools that calculate the maximal diameter for a perimeter of a tumour may be employed and may even reduce variability.

The only exception to the longest diameter rule is lymph node measurement. Because malignant nodes are identified by the length of their short axis, this is the guide used to determine not only whether they are pathological but is also the dimension measured for adding into the sum of target lesions. Fig. 4 illustrates this point: the large arrow identifies a malignant node: the shorter perpendicular axis is $\geqslant$15 mm and will be recorded. Close by (small arrow) there is a normal node: note here the long axis is greater than 10 mm but the short axis is well below 10 mm. This node should be considered non-pathological.

If a lesion disappears and reappears at a subsequent time point it should continue to be measured. However, the patient's response at the point in time when the lesion reappears will depend upon the status of his/her other lesions. For example, if the patient's tumour had reached a CR status and the lesion reappeared, then the patient would be considered PD at the time of reappearance. In contrast, if the tumour status was a PR or SD and one lesion which had disappeared then reappears, its maximal diameter should be added to the sum of the remaining lesions for a calculated response: in other words, the reappearance of an apparently 'disappeared' single lesion amongst many which remain is not in itself en-

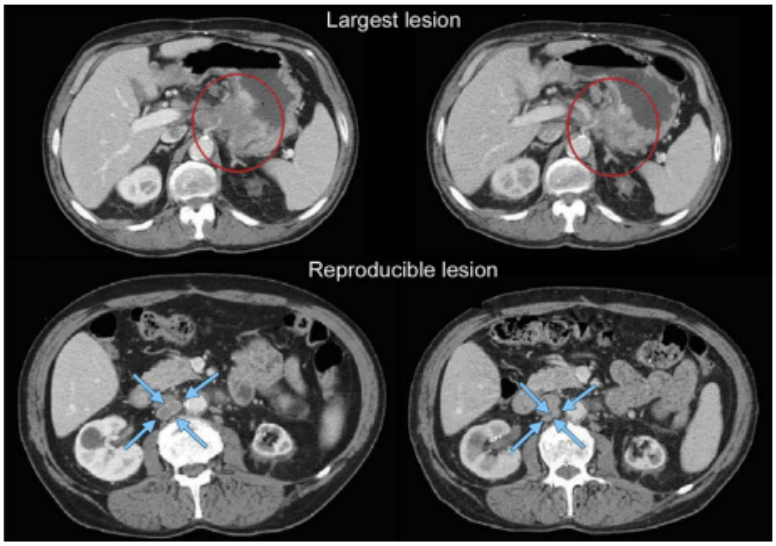

Fig. 3 – Largest lesion may not be most reproducible: most reproducible should be selected as target. In this example, the primary gastric lesion (circled at baseline and at follow-up in the top two images) may be able to be measured with thin section volumetric CT with the same degree of gastric distention at baseline and follow-up. However, this is potentially challenging to reproduce in a multicentre trial and if attempted should be done with careful imaging input and analysis. The most reproducible lesion is a lymph node (circled at baseline and at follow-up in the bottom two images).

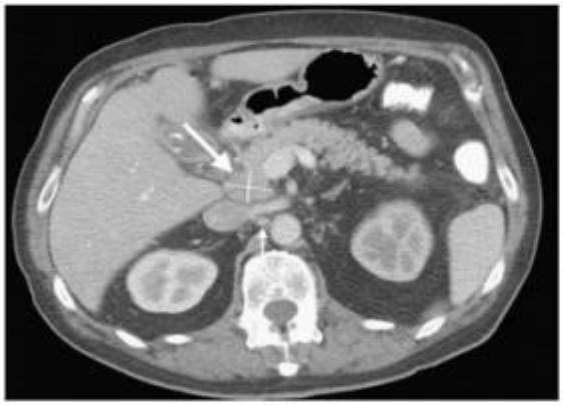

Fig. 4 – Lymph node assessment: large arrow illustrates a pathological node with the short axis shown as a solid line which should be measured and followed. Small arrow illustrates a non-pathological node which has a short axis <10 mm.

ough to qualify for PD: that requires the sum of all lesions to meet the PD criteria. The rationale for such a categorisation is based upon the realisation that most lesions do not actually 'disappear' but are not visualised because they are beyond the resolving power of the imaging modality employed.

The identification of the precise boundary definition of a lesion may be difficult especially when the lesion is embedded in an organ with a similar contrast such as the liver, pancreas, kidney, adrenal or spleen. Additionally, peritumoural oedema may surround a lesion and may be difficult to distinguish on certain modalities between this oedema and actual tumour. In fact, pathologically, the presence of tumour cells within the oedema region is variable. Therefore, it is most critical that the measurements be obtained in a reproducible manner from baseline and all subsequent follow-up timepoints. This is also a strong reason to consistently utilise the same imaging modality.

When lesions 'fragment', the individual lesion diameters should be added together to calculate the target lesion sum. Similarly, as lesions coalesce, a plane between them may be maintained that would aid in obtaining maximal diameter measurements of each individual lesion. If the lesions have truly coalesced such that they are no longer separable, the vector of the longest diameter in this instance should be the maximal longest diameter for the 'merged lesion'.

Progression of non-target lesions

To achieve 'unequivocal progression' there must be an *overall* level of substantial worsening in non-target disease that is of a magnitude that, even in the presence of SD or PR in target disease, the treating physician would feel it important to change therapy. Examples of unequivocal progression are shown in Figs. 5 and 6.

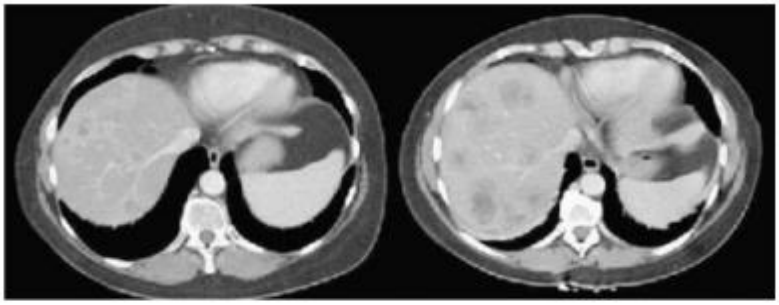

Fig. 5 – Example of unequivocal progression in non-target lesions in liver.

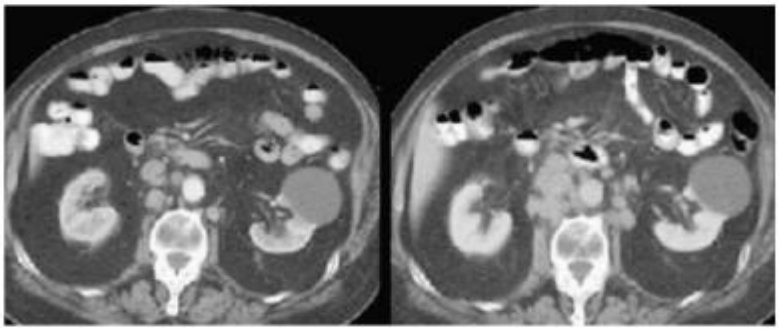

Fig. 6 – Example of unequivocal progression in non-target lesion (nodes).

Appendix III. Frequently asked questions

Question	Answer
What should be done if several unique lesions at baseline become confluent at a follow-up evaluation?	Measure the longest diameter of the confluent mass and record to add into the sum of the longest diameters
How large does a new lesion have to be to count as progression? Does any small subcentimetre lesion qualify, or should the lesion be at least measurable?	New lesions do not need to meet 'measurability criteria' to be considered valid. If it is clear on previous images (with the same technique) that a lesion was absent then its definitive appearance implies progression. If there is any doubt (because of the techniques or conditions) then it is suggested that treatment continue until next scheduled assessment when, generally, all should be clear. Either it gets bigger and the date of progression is the date of the first suspicion, or it disappears and one may then consider it an artefact with the support of the radiologists
How should one lesion be measured if on subsequent exams it is split into two?	Measure the longest diameter of each lesion and add this into the sum
Does the definition of progression depend on the status of all target lesions or only one?	As per the RECIST 1.1 guideline, progression requires a 20% increase in the sum of diameters of all target lesions AND a minimum absolute increase of 5 mm in the sum
Are RECIST criteria accepted by regulatory agencies?	Many cooperative groups and members of pharma were involved in preparing RECIST 1.0 and have adopted them. The FDA was consulted in their development and supports their use, though they don't require it. The European and Canadian regulatory authorities also participated and the RECIST criteria are now integrated in the European note for guidance for the development of anticancer agents. Many pharmaceutical companies are also using them. RECIST 1.1 was similarly widely distributed before publication
What is the criterion for a measurable lesion if the CT slice thickness is >5 mm?	RECIST 1.1 recommends that CT scans have a maximum slice thickness of 5 mm and the minimum size for a measurable lesion is twice that: 10 mm (even if slice thickness is <5 mm). If scanners with slice thickness >5 mm are used, the minimum lesion size must have a longest diameter twice the actual slice thickness
What should we record when target lesions become so small they are below the 10 mm 'measurable' size?	Target lesion measurability is defined at baseline. Thereafter, actual measurements, even if <10 mm, should be recorded. If lesions become very small, some radiologists indicate they are 'too small to measure'. This guideline advises that when this occurs, if the lesion is actually still present, a default measurement of 5 mm should be applied. If in fact the radiologist believes the lesion has gone, a default measurement of 0 mm should be recorded
If a patient has several lesions which have decreased in size to meet PR criteria and one has actually disappeared, does that patient have PD if the 'disappeared' lesion reappears?	Unless the sum meets the PD criteria, the reappearance of a lesion in the setting of PR (or SD) is not PD. The lesion should simply be added into the sum. If the patients had had a CR, clearly reappearance of an absent lesion would qualify for PD
When measuring the longest diameter of target lesions in response to treatment, is the same axis that was used initially used subsequently, even if there is a shape change to the lesion that may have produced a new longest diameter?	The longest diameter of the lesion should always be measured even if the actual axis is different from the one used to measure the lesion initially (or at different time point during follow-up) The only exception to this is lymph nodes: as per RECIST 1.1 the short axis should always be followed and as in the case of target lesions, the vector of the short axis may change on follow-up
Target lesions have been selected at baseline and followed but then one of these target lesions then becomes non-evaluable (i.e. different technique used) What is the effect this has on the other target lesions and the overall response?	What may be done in such cases is one of the following: (a) If the patient is still being treated, call the centre to be sure that future evaluations are done with the baseline technique so at least SOME courses are fully evaluable (b) If that is not possible, check if there IS a baseline exam by the same technique which was used to follow patients…in which case if you retrieve the baseline measures from that technique you retrieve the lesion evaluability (c) If neither (a) nor (b) is possible then it is a judgement call about whether you delete the lesion from all forms or consider the impact of the lesion overall is so important that its being non-evaluable makes the overall response interpretation inevaluable without it. Such a decision should be discussed in a review panel It is NOT recommended that the lesion be included in baseline sums and then excluded from follow-up sums since this biases in favour of a response

(continued on next page)

Appendix III – *continued*

Question	Answer
What if a single non-target lesion cannot be reviewed, for whatever reason; does this negate the overall assessment?	Sometimes the major contribution of a single non-target lesion may be in the setting of CR having otherwise been achieved: failure to examine one non-target in that setting will leave you unable to claim CR. It is also possible that the non-target lesion has undergone such substantial progression that it would override the target disease and render patient PD. However, this is very unlikely, especially if the rest of the measurable disease is stable or responding
A patient has a 32% decrease in sum cycle 2, a 28% decrease cycle 4 and a 33% decrease cycle 6. Does confirmation of PR have to take place in sequential scans or is a case like this confirmed PR?	It is not infrequent that tumour shrinkage hovers around the 30% mark. In this case, most would consider PR to have been confirmed looking at this overall case. Had there been two or three non-PR observations between the two time point PR responses, the most conservative approach would be to consider this case SD
In the setting of a breast cancer neoadjuvant study, would mammography not be used to assess lesions? Is CT preferred in this setting?	Neither CT nor mammography are optimal in this setting. MRI is the preferred modality to follow breast lesions in a neoadjuvant setting
A patient has a lesion measurable by clinical exam and by CT scan. Which should be followed?	CT scan. Always follow by imaging if that option exists since it can be reviewed and verified
A lesion which was solid at baseline has become necrotic in the centre. How should this be measured?	The longest diameter of the entire lesion should be followed. Eventually, necrotic lesions which are responding to treatment decrease in size. In reporting the results of trials, you may wish to report on this phenomenon if it is seen frequently since some agents (e.g. angiogenesis inhibitors) may produce this effect
If I am going to use MRI to follow disease, what is minimum size for measurability?	MRI may be substituted for contrast enhanced CT for some sites, but not lung. The minimum size for measurability is the same as for CT (10 mm) as long as the scans are performed with slice thickness of 5 mm and no gap. In the event the MRI is performed with thicker slices, the size of a measurable lesion at baseline should be two times the slice thickness. In the event there are inter-slice gaps, this also needs to be considered in determining the size of measurable lesions at baseline
Can PET–CT be used with RECIST?	At present, the low dose or attenuation correction CT portion of a combined PET–CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if your site has documented that the CT performed as part of a PET–CT is of the same diagnostic quality as a diagnostic CT (with IV and oral contrast) then the PET–CT can be used for RECIST measurements. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed

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19.5 Appendix 5: Summary of Product Characteristics (SPC)/Package Insert

For the most recent version of the locally applicable SPC see the following links:

EMA: http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/landing/epar_search.jsp&mid=WC0b01ac058001d124

USA: https://www.accessdata.fda.gov/scripts/cder/daf/

Belgium: http://bijsluiters.fagg-afmps.be

France: http://base-donnees-publique.medicaments.gouv.fr

Portugal: http://app7.infarmed.pt/infomed/pesquisa.php

Spain: https://www.aemps.gob.es/cima/inicial.do

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