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# FINAL STUDY REPORT

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Full title of the trial:	A Phase II Prospective Imaging Study Evaluating the Utility of Pre-treatment Zirconium-89 Labelled Trastuzumab PET/CT and an Early FDG-PET/CT Response to Identify Patients with Advanced HER-2 Positive Breast Cancer Unlikely to Benefit from a Novel Anti-HER2 Therapy: T-DM1
Short title of the trial:	ZEPHIR
EudraCT number:	2011-005437-39
Sponsor protocol number:	MO27955/IJBMNTDM1
ClinicalTrials.gov Number:	NCT01565200
Sponsor	Institut Jules Bordet Rue Meylemeersch 90, 1070 Anderlecht Belgique/België
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Report date	12 March 2025



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## APPROVAL

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	<p>Netherlands) on a metastatic lesion was acceptable (in case it is not available for the primary tumour).</p> <p>According to publications, abstracts and a recent meta-analysis, it was highly recommended to biopsy a carefully selected and accessible metastatic site before any new treatment initiation, especially before first metastatic treatment line. This was the reason why in this trial, a biopsy of a metastatic site was required before the T-DM1 start. If the biopsy turned out to be not contributive (absence of malignant cells) a new biopsy was optional.</p> <p>It had to be anticipated that, in a few patients, small, asymptomatic brain metastases was be picked up by <sup>89</sup>Zr-trastuzumab PET imaging. These patients underwent a brain MRI prior to their first T-DM1 course as well as a detailed neurologic examination. These evaluations had to be repeated every 3 cycles until the patient was taken off protocol because of progressive disease outside the brain. In case T-DM1 was stopped for toxicity, the treating oncologist, after consultation with the team, was free to decide whether or not to propose radiotherapeutic treatment of the brain metastases at this point.</p> <p>Progressive disease in the brain on imaging or appearance of new symptoms or worsening of pre-existing minor symptoms was prompt consideration for radiotherapy treatment (either by gammaknife or whole brain radiotherapy) with or without steroid therapy</p>
<p><b>SCIENTIFIC BACKGROUND</b></p>	<p><b>1. Disease Information</b></p> <p>Amplification of the HER2 gene occurs in approximately 20% to 25% of primary human breast cancers and typically results in overexpression of the HER2 protein at &gt;1 million copies per cell. Such tumours are considered “HER2-positive” and are associated with aggressive growth and poor clinical outcome. Trastuzumab (Herceptin®; Genentech Inc, South San Francisco, CA), a humanized monoclonal antibody directed against the extracellular domain of HER2, is associated with substantial activity as a single agent and with improved survival when combined to chemotherapy (mostly taxane) in the metastatic setting as well as in the adjuvant setting. While trastuzumab’s mechanism of action is not fully understood, treatment benefits are attributed to inhibition of constitutive HER2 signaling and induction of antibody-dependent cellular cytotoxicity. The tyrosine kinase inhibitor lapatinib, in combination with capecitabine, is indicated for patients with advanced or metastatic HER2-positive breast cancer after prior trastuzumab, anthracycline, and taxane treatment. Unfortunately, all patients with metastatic disease ultimately progress on HER2-directed therapies, although nonclinical studies suggest that high levels of HER2 expression persist after progression. Therefore, a significant need for effective, alternative therapies remains for these patients.</p>



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## 2. Therapeutic Information

Trastuzumab-DM1 (T-DM1) is a novel anti-HER2 antibody-drug conjugate (ADC) in development for treatment of patients with HER2-positive breast cancer. T-DM1 combines the HER2-targeting properties of trastuzumab with intracellular delivery of DM1, a highly potent derivative of the antimicrotubule agent maytansine. DM1 binds to tubulin and inhibits microtubule assembly with greater potency than vincristine or vinblastine. In T-DM1, trastuzumab and DM1 are covalently linked via the thioether linker (N-maleimidomethyl) cyclohexane-1-carboxylate (MCC). The stability of MCC, compared with disulfide linkers, was shown to strongly contribute to the favourable activity and toxicity profiles of T-DM1 in preclinical testing (data on file, Genentech, South San Francisco, CA); exposure of HER2-positive tumours to DM1 is maximized, whereas exposure of normal tissue is minimized. Additionally, T-DM1 seems to retain the antitumor properties of trastuzumab, including flagging HER2-positive tumour cells for destruction by antibody-dependent cellular cytotoxicity and inhibiting HER2 signaling (data on file, Genentech, South San Francisco, CA).

A phase I dose-escalation study evaluated dosing schedules of T-DM1 in patients with HER2-positive Metastatic Breast Cancer (MBC) who had experienced progression on trastuzumab-based therapy. Most adverse events (AEs) attributed to T-DM1 were  $\leq$  Grade 2. The maximum-tolerated dose of T-DM1 was 3.6 mg/kg every 3 weeks, based on the dose limiting toxicity of Grade 4 thrombocytopenia at 4.8 mg/kg. For the 15 patients treated at the maximum-tolerated dose, median progression free survival (PFS) was 10.4 months, and four of nine (44%) patients with measurable disease had an objective response. The pharmacokinetics of T-DM1 were characterized by relatively slow clearance, a small volume of distribution (limited to plasma volume), and a half-life of approximately 4 days. Systemic DM1 exposure was low (average of approximately 5 ng/mL maximum plasma levels). On the basis of these results, a phase II study (TDM4258g) was initiated to evaluate T-DM1 treatment in patients with HER2-positive MBC who experienced progression on HER2-directed therapy. This study with a follow-up of  $\geq 12$  months among 112 treated patients showed an objective response rate by independent assessment of 25.9% (95% CI, 18.4% to 34.4%). Median duration of response was not reached as a result of insufficient events (lower limit of 95% CI, 6.2 months), and median progression-free survival time was 4.6 months (95% CI, 3.9 to 8.6 months). The response rates were higher among patients with confirmed HER2-positive tumours (immunohistochemistry 3+ or fluorescent in situ hybridization positive) by retrospective central testing (n=74). Higher response rates were also observed in patients whose tumours expressed  $\geq$  median HER2 levels by quantitative reverse transcriptase polymerase chain reaction for HER2 expression, compared with patients who had less than median HER2 levels. T-DM1 was well tolerated with no dose-limiting cardiotoxicity. Most adverse events were Grade 1 or 2; the most frequent Grade  $\geq 3$  AEs were hypokalemia (8.9%), thrombocytopenia (8.0%), and fatigue (4.5%).



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	<p>In the randomised phase II trial of T-DM1 versus docetaxel+trastuzumab (TDM4450g), results show that T-DM1 has a superior antitumor activity (5 months gain in median PFS, <math>p=0.035</math>) compared to the more traditional combination of a taxane with trastuzumab as well as a much improved safety profile with a lower global incidence of adverse events, minimal alopecia and no neutropenic fever. Occasionally patients develop Grade 3 thrombocytopenia and/or hepatotoxicity but these side effects are reversible and manageable with dose reductions. So far there is no indication that T-DM1 is associated with an increased risk of declined left ventricular ejection fractions or clinical congestive heart failure as compared to taxane-trastuzumab regimens (Data presented at ECCO-ESMO 2011 congress).</p> <p>T-DM1 has recently been granted FDA and EMA approval for use in taxane and trastuzumab pre-treated patients with advanced disease, following the demonstration of a superior therapeutic index over the combination of capecitabine and lapatinib. The EMILIA randomized study, indeed, showed in 980 women a 35% reduction in the risk of disease progression favouring T-DM1, translating into an absolute 3 months gain in median time to progression (HR for PFS 0.650, <math>p</math> value <math>&lt;0.0001</math>; median PFS of 9.6 months versus 6.4 months). The study also showed improved survival and safety clearly favoured T-DM1.</p>
<b>RATIONALE</b>	<p><b>1. Rationale for HER2 imaging</b></p> <p>The ability to safely and accurately predict the presence or absence of the target is essential in the era of molecular targeted therapies, especially when some data indicate that HER2 status of a tumour can vary during the course of the disease and even in the same patient between distinct tumour lesions. This explains why a number of experts recommend to biopsy newly occurring lesions during the course of the disease. The advantages of imaging these receptors using a labelled antibody over the conventional methods (biopsy) would be:</p> <ul style="list-style-type: none"> <li>▪ The non-invasiveness</li> <li>▪ The possibility to evaluate lesions unsuitable for a biopsy</li> <li>▪ The ability to measure receptor expression for the entire disease burden, thereby avoiding the sampling error that can occur with heterogeneous receptor expression</li> </ul> <p>By confronting the imaging results from the FDG-PET/CT and the <math>^{89}\text{Zr}</math>-trastuzumab PET/CT (also called HER2 PET/CT), one expects to identify metastatic sites with “low” or “absent” HER2 expression, unlikely to benefit from HER2 targeted therapies and particularly from a conjugate which will not get internalized in the absence of an intact HER2 receptor to which trastuzumab can bind. It is thought that up to 30% of HER2 positive tumours overexpress a truncated HER2 receptor to which trastuzumab cannot bind.</p> <p>A feasibility study in Groningen enrolled 14 patients with HER2 positive metastatic breast cancer and explored different scan protocols of <math>^{89}\text{Zr}</math>-trastuzumab. The highest tumour to background ratio and thus the best</p>



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	<p>detection rate was obtained on images taken 4-5 days after the injection of <math>^{89}\text{Zr}</math>-trastuzumab. Moreover “optimal” PET-scan results in trastuzumab-naïve patients (defined as patients with a trastuzumab-free interval of <math>\geq 6</math> months) required a 50 mg dose of <math>^{89}\text{Zr}</math>-trastuzumab while patients already on trastuzumab could be imaged in an adequate way with a 10 mg dose.</p> <p><b>2. Rationale for FDG PET/CT</b></p> <p>Objective response assessment, a key element in evaluating benefit of treatment in solid tumours, relies exclusively on size criteria using morphological imaging (CT, MRI). While largely standardized and improved by WHO, RECIST 1.0 and 1.1 criteria, radiological assessment requires to wait 6 to 9 weeks after treatment initiation. In case of treatment failure, the patient will have been treated unnecessarily and will have run the risk of experiencing a full range of treatment-induced toxicities.</p> <p>With tumour shrinkage being the final step in a complex cascade of cellular and subcellular alterations induced by therapy, changes in the metabolism of cellular energy should predict treatment response/ non-response earlier than the changes in tumour size. Metabolism-based imaging, especially using positron emission tomography (PET) coupled with a computerized tomography (CT) module (PET-CT) and 18F-fluorodeoxyglucose (FDG) as a surrogate marker of tumour glycolytic activity, has been proposed as a suitable tool to assess tumour response/non-response to treatment as early as after one or two treatment cycles.</p> <p>In patients with HER2 positive breast cancer, however, the experience on FDG PET response during anti-HER2 therapy is limited. The most consistent results come from the NEOALTTO trial, which compared in the preoperative setting, 3 antiHER2 strategies, namely trastuzumab, lapatinib or their combination given alone for 6 weeks and then for 3 months with weekly paclitaxel prior to surgery. Impressive clinical responses were already noted with the anti-HER2 agents alone and the pathological complete response (pCR) at surgery went from 25-29% in the single anti-HER2 arms to 51% for the arm exploring dual HER2 targeting. 86 of the 450 NEOALTTO patients were included in a FDG-PET/CT substudy with functional imaging performed on day 0, 15 and 45 of the biological window. Preliminary results indicate that early metabolic response can be seen after 2 weeks of treatment, which are strongly predictive for the response after 6 weeks of treatment. Moreover, pCR rates are higher in responders at week 2 (<math>p=0.12</math>) and week 6 (<math>p=0.05</math>) (ESMO 2011 abstract 5.013).</p> <p>The working hypothesis of the current study is that early FDG-PET/CT is particularly accurate in detecting lesions unlikely to show a significant response to HER2 targeted drugs, even if high HER2 expression: indeed molecular aberrations downstream of HER2 can also explain “resistance”</p>
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	<p>to trastuzumab and HER2 positive cancer cells may show de novo resistance to T-DM1 cytotoxic agent after its internalization.</p> <p><b>3. Rationale for combining HER2 imaging with FDG-PET/CT</b></p> <p>Metabolism-based imaging, using <math>^{18}\text{F}</math>-fluorodeoxyglucose (FDG) as a surrogate marker of tumour glycolytic activity, has been proposed as a suitable tool to assess tumour response or non-response to treatment as early as after one treatment cycle. Given the limited prior knowledge on the diagnostic accuracy of <math>^{89}\text{Zr}</math>-trastuzumab PET/CT, we propose to prospectively evaluate if FDG uptake changes seen in patients with metastatic breast cancer after a single course of T-DM1 can be used to predict inability of the treatment to induce an objective response (RECIST 1.1 and 1.0 based).</p> <p>Another reason for combining both FDG-PET/CT and <math>^{89}\text{Zr}</math>-trastuzumab is to identify the most interesting lesions to biopsy for the translational component, namely FDG positive lesions but <math>^{89}\text{Zr}</math>-trastuzumab negative.</p> <p><b>4. Laboratory translational research</b></p> <p>According to publications, abstracts and a recent meta-analysis, it is highly recommended to biopsy a carefully selected and accessible metastatic site before any new treatment initiation, especially before first metastatic treatment line. This is the reason why in this trial, a biopsy of a metastatic site will be required.</p>
<b>OBJECTIVES</b>	<p><b>Primary objective:</b></p> <p>The primary objective is to show that pre-treatment <math>^{89}\text{Zr}</math>-trastuzumab PET/CT is able to select lesions not responding morphologically from treatment with T-DM1 (applying RECIST 1.0 criteria)</p> <p><b>Secondary objectives on a lesion-based analysis:</b></p> <ol style="list-style-type: none"> <li>1. Early FDG-PET/CT</li> </ol> <p>The objective is to show that early FDG PET/CT (performed after one cycle of T-DM1 just before the second cycle) is able to select lesions not responding from treatment with T-DM1 according to metabolic and morphological response criteria post 3 cycles of T-DM1.</p> <ol style="list-style-type: none"> <li>2. <math>^{89}\text{Zr}</math>-trastuzumab PET/CT</li> </ol> <p>The objective is to show that <math>^{89}\text{Zr}</math>-trastuzumab PET/CT is able to select lesions not responding from treatment with T-DM1 according to metabolic response criteria post 3 cycles of T-DM1</p> <ol style="list-style-type: none"> <li>3. Combination of <math>^{89}\text{Zr}</math>-trastuzumab PET/CT and Early FDG-PET/CT</li> </ol> <p>The objective is to show that a lesion with no/faint uptake on <math>^{89}\text{Zr}</math>-trastuzumab PET/CT and not responding metabolically on the early FDG-PET/CT will not respond according to metabolic and morphological criteria after 3 cycles of T-DM1.</p> <p>Exploratory objective on a patient based analysis :</p>



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	To assess the distribution of time to treatment failure.
<b>INCLUSION CRITERIA</b>	<ol style="list-style-type: none"> <li>The patient must have histologically confirmed HER2 positive invasive carcinoma of the breast in the reference laboratory of the participating center. HER2 positive criteria to be applied are those used in the participating countries: <ul style="list-style-type: none"> <li>Belgium: FISH amplification ratio <math>\geq 2</math> in the reference laboratory of the participating center</li> <li>The Netherlands: IHC 3+ or FISH ratio <math>\geq 2</math> in the reference laboratory of the participating center</li> </ul> </li> <li>The patient must have documented progressive disease and present with at least 2 non-bone “target” metastatic lesions, unequivocally of neoplastic origin with <ul style="list-style-type: none"> <li>a transaxial diameter greater than 2 cm on the screening diagnostic CT/MRI for all non-bone lesions except lymphnodes</li> <li>a short axis greater than 1,5 cm for lymphnodes on the screening diagnostic CT/MRI</li> </ul> <p>These two lesions should not be confluent with adjacent lesions and not have been irradiated previously.</p> </li> <li>A concurrent biopsy of a metastatic site is mandatory (with two formalin fixed paraffin embedded (FFPE) core sample and two snap frozen tumour sample) after progression has been documented and before inclusion and the patient agrees with the procedure.</li> <li>Primary tumour blocks (or 11 unstained slides) available for confirmatory central laboratory HER2 testing in Institut Jules Bordet. If available, a snap frozen sample of the primary tumour will also be centralized in Institut Jules Bordet.</li> <li>Age <math>\geq 18</math> years</li> <li>Eastern Cooperative Oncology Group (ECOG) performance status (PS) 0 to 1</li> <li>No significant cardiac history and current LVEF <math>\geq 50\%</math></li> <li>Adequate organ function, evidenced by the following laboratory results: <ul style="list-style-type: none"> <li>Absolute neutrophil count <math>&gt; 1,500</math> cells/mm<sup>3</sup></li> <li>Platelet count <math>&gt; 100,000</math> cells/mm<sup>3</sup></li> <li>Hemoglobin <math>&gt; 9</math> g/dL</li> <li>AST(SGOT) and ALT (SGPT) <math>&lt; 2.5 \times</math> ULN</li> <li>Total Bilirubin <math>\leq 1.5 \times</math> ULN unless the patient has documented Gilbert’s syndrome. Patients with known Gilbert’s Syndrome should have direct bilirubin within normal limits.</li> <li>Serum alkaline phosphatase <math>\leq 2.5 \times</math> ULN. Patients with bone metastases: alkaline phosphatase <math>\leq 5 \times</math> ULN</li> <li>Serum creatinine <math>&lt; 2.0</math> mg/dL or <math>177 \mu\text{mol/L}</math></li> <li>International normalized ratio (INR) and activated partial thromboplastin time or partial thromboplastin time (aPTT or</li> </ul> </li> </ol>



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	<p>PTT) &lt; 1.5 ULN (unless on therapeutic anti-coagulation except vitamin K antagonists which are prohibited in this study)</p> <ol style="list-style-type: none"> <li>Absence of any psychological, familial, sociological or geographical condition potentially hampering compliance with the study protocol and follow-up schedule; those conditions should be discussed with the patient before registration in the trial.</li> <li>For women of childbearing potential, a serum pregnancy test will be done (and it must be negative) and an agreement to use a highly-effective form of contraception during all the study and at least the following 7 months will be obtained.</li> <li>Signed written informed consent obtained prior to any study specific procedure.</li> <li>Completion of all necessary baseline surgical, laboratory and imaging investigations prior to patient inclusion.</li> </ol>
<b>EXCLUSION CRITERIA</b>	<ol style="list-style-type: none"> <li>Patients with bone only metastases are not eligible.</li> <li>Diffuse liver (<math>\geq 50\%</math>) involvement on imaging.</li> <li>Patients with brain metastasis as the sole site of metastatic disease and/or are symptomatic or require therapy to control symptoms NB: Brain metastasis are allowed provided they are asymptomatic and/or controlled by previous radiotherapy. In case of recent prior brain radiotherapy, there must be evidence on MRI imaging of brain metastatic control for at least 6 weeks since the end of radiotherapy. Moreover, the patient should be at the end of corticosteroid therapy and be clinically asymptomatic.</li> <li>Current uncontrolled hypertension despite medication intake (systolic &gt; 150 mmHg and/or diastolic &gt; 100 mmHg)</li> <li>Current unstable angina</li> <li>History of symptomatic CHF of any New York Heart Association (NYHA) criteria or ventricular arrhythmia that requires treatment</li> <li>History of myocardial infarction within the last 6 months</li> <li>History of a decrease in LVEF to &lt; 40% or symptomatic CHF with previous trastuzumab treatment</li> <li>Current dyspnea at rest due to complications of advanced malignancy, or other diseases that require continuous oxygen therapy</li> <li>Current severe, uncontrolled systemic disease (e.g., clinically significant cardiovascular, pulmonary, or metabolic disease; wound healing disorders; ulcers; or bone fractures)</li> <li>History of other malignancy within the last 5 years, except for appropriately treated carcinoma in situ of the cervix, non-melanoma</li> </ol>



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	<p>skin carcinoma, Stage I uterine cancer, or other cancers with a similar outcome as those previously mentioned</p> <p>12. Pregnant or lactating women</p> <p>13. Concurrent, serious, uncontrolled infections or current known infection with HIV, active hepatitis B and/or hepatitis C.</p> <p>14. Known prior severe hypersensitivity to trastuzumab</p> <p>15. Patient who received lapatinib within the 15 days prior to 89Zr-Trastuzumab injection</p> <p>16. Patient under a prohibited concomitant therapy, including vitamin K antagonist (see Section 7.1.7 concomitant therapy)</p> <p>17. Patients with a peripheral neuropathy Grade 3 or higher</p>
<b>INVESTIGATIONAL MEDICINAL PRODUCTS</b>	<ul style="list-style-type: none"> <li>Co-injection of 50 mg trastuzumab and 37 MBq±10% <sup>89</sup>Zr-trastuzumab</li> <li>T-DM1: 3.6 mg/kg, IV, every 3 weeks until disease progression</li> </ul>
<b>INDICATION OF USE</b>	HER2 positive metastatic breast cancer
<b>TARGETED POPULATION</b>	Patients with locally recurrent (not amenable to resection with curative intent) or metastatic HER2 positive breast cancer presenting ≥ 2 metastatic lesions fulfilling imaging criteria (definition in protocol) and eligible to receive T-DM1
<b>PARTICIPATING COUNTRY/-IES</b>	Belgium and The Netherlands
<b>START DATE OF THE TRIAL</b>	05/03/2012
<b>PARTICIPATING SITES NUMBER</b>	5
<b>LENGTH OF THE STUDY</b>	<ul style="list-style-type: none"> <li>Actual start date of recruitment to the protocol: 07/05/2012</li> <li>Actual date stop date of recruitment to the protocol: 24/02/2017</li> <li>Long term follow-up planned? Yes, for efficacy– Duration: until disease progression only for patients who stopped T-DM1 for toxicity reason(s)</li> </ul>
<b>INDEPENDENT DATA MONITORING COMMITTEE</b>	No
<b>PROTECTION OF TRIAL SUBJECTS</b>	To ensure patient safety during treatment, in the event of treatment-related toxicity, a dose delay or dose reduction to 3 or 2.4 mg/kg was allowed. Additionally, concomitant therapy, such as: bisphosphonates and other bone supportive agents, palliative radiotherapy for painful



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	bone metastases and gamma knife or radiotherapy for symptomatic brain metastases, was allowed as supportive measure.
<b>STATISTICAL METHODS USED</b>	<p>The study's underlying hypothesis was that a lesion with negative uptake on baseline HER2 PET/CT would not respond anatomically to T-DM1.</p> <p>Using a single-stage Fleming-A'Hern design with a one-sided test (5% type I error) and power of 80% to test the null hypothesis of an NPV of &lt;85% versus the alternative NPV of <math>\geq 95\%</math>, we calculated that 60 HER2-negative and RECIST 1.0 measurable lesions would need to be examined. The secondary objectives aimed to show that HER2 PET/CT, early FDG PET/CT, and the combination of both HER2 PET/CT and early FDG PET/CT would be able to select lesions not responding to treatment according to metabolic and anatomic response criteria post three cycles of T-DM1. To get a power of 90% with a one-sided test (5% type I error) for testing the null hypothesis of NPV &lt; 85% versus the alternative hypothesis of NPV <math>\geq 95\%</math>, we calculated that 76 mNR FDG-positive lesions on the early FDG PET/CT would need to be examined. The NPVs for both primary and secondary objectives were calculated and reported together with an associated exact 95% confidence interval (CI). Finally, for the exploratory objective of our study, we defined TTF as the time from the start of T-DM1 to discontinuation for any reason, including disease progression (clinical or image-based), treatment toxicity, and death. For the correlation between TTF and imaging results, patients who discontinued T-DM1 for any other reason than progression were censored. The distribution of TTF was estimated by the Kaplan-Meier method. For comparison, the data were fitted with Cox regression models. Hazard ratios (HRs) are reported with 95% CI. The statistical significance level was set at 5% (two-sided).</p>
<b>RESULT ANALYSIS STAGE</b>	<p>Final</p> <p>Date final analysis: 08/12/2022</p>
<b>PRIMARY COMPLETION DATA</b>	<ul style="list-style-type: none"> <li>• Is this the analysis of the primary completion data? Yes</li> <li>• Primary completion date: 03/07/2017</li> </ul>
<b>GLOBAL END OF TRIAL DATE</b>	<ul style="list-style-type: none"> <li>• Global end of trial reached? Yes</li> <li>• Global end of trial date: 09/04/2024</li> </ul>
<b>PREMATURE END OF TRIAL</b>	No



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## 2 POPULATION OF TRIAL SUBJECTS

The actual number of subjects enrolled in the ZEPHIR trial per country is

Country	Number of subjects
Belgium	49
The Netherlands	41

The number of subjects enrolled per age group is displayed in the below table.

Age of subjects	Number of subjects
In utero	-
Preterm newborn - gestational age <37 wk	-
Newborns (0-27 days)	-
Infants and toddlers (28 days - 23 months)	-
Children (2-11 years)	-
Adolescents (12-17 years)	-
Adults (between 18 and 64 years)	-
From 65 years	72
From 65 to 84 years	18
85 years and over	0

## 3 SUBJECT DISPOSITION

### 3.1 Recruitment

<b>Recruitment details</b>	Between 07/05/2012 and 24/02/2017, subjects were recruited in 5 participating sites in 2 countries (Belgium and the Netherlands).
<b>Screening details</b>	Once informed consent was signed, inclusion and exclusion criteria were double checked to identify any screening failure. They were reported in the trial registration tool.



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### 3.2 Post assignment period(s)

<b>Period title:</b>	Overall trial
<b>Baseline period:</b>	Yes
<b>Allocation method:</b>	Not applicable
<b>Blinding used:</b>	<p>Patients and investigators were not blinded regarding administered treatment.</p> <p>The HER2–PET/CT and ‘early’ FDG–PET/CT results were blinded to the treating oncologist.</p>
<b>Roles blinded:</b>	Investigators for imaging results.
<b>Blinding implementation details:</b>	The nuclearist physicians kept their assessments confidential until the analysis of the trial results.
<b>Arms</b>	Single arm study.
Arm title: Not applicable as there is only one cohort in this study	
<b>Arm description:</b>	<p>The treatment was approved in the indication (T-DM1).</p> <p>The search about the predictive value of HER2 PET and early FDG-PET on treatment efficacy was investigated in a single arm study</p>
<b>Arm type:</b>	Experimental
<b>IMP arm information :</b>	<ul style="list-style-type: none"> <li>Co-injection of 50 mg trastuzumab and 37 MBq±10% <sup>89</sup>Zr-trastuzumab</li> <li>T-DM1: 3.6 mg/kg, IV, every 3 weeks until disease progression</li> </ul>
<b>Numbers of subjects in the arm</b>	
<b>Started</b>	90 subjects were entered in the trial and were scheduled to receive T-DM1, 7 subjects did never receive the treatment as planned in the protocol.
<b>Completed</b>	<p>83</p> <p>T-DM1 had to be given until failure (disease progression or toxicity).</p> <p>At the time of analysis, T-DM1 had been discontinued in all subjects, with four still in follow-up receiving trastuzumab only (n = 2) or</p>



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	trastuzumab and hormonal therapy with letrozole (n = 2).
Not completed	Treatment was completed in all subjects. Thirteen subjects had to stop treatment due to toxicity.
Adverse event, not serious	10
Adverse event, serious fatal	0
Adverse event, serious non-fatal	1
Consent withdrawn by subject	1 (at subject request)
Physician decision	1
Protocol violation	0

#### 4 BASELINE CHARACTERISTICS

Baseline characteristics reporting groups	
Reporting group title	Whole study cohort
Reporting group description	All 90 subjects that were included in the study
<b>Number of subjects</b>	90
<b>Age categories</b>	
<i>Units: Subjects</i>	
In utero	-
Preterm newborn - gestational age <37 wk	-
Newborns (0-27 days)	-
Infants and toddlers (28 days - 23 months)	-
Children (2-11 years)	-
Adolescents (12-17 years)	-
Adults (between 18 and 64 years)	72
From 65 years	18



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From 65 to 84 years	18
85 years and over	0
<b>Age continuous</b>	
<i>Units: years</i>	
Median	54
Full range (min-max)	30-78
<b>Gender</b>	
<i>Units: Subjects</i>	
Female	90
Male	0

Other baseline characteristics of the subjects are provided in the table below:

Baseline subjects ' characteristics	
<b>ECOG - n subjects</b>	
0	46
1	44
<b>Disease type at screening</b>	
Visceral	84
Non-visceral	6
<b>History of brain metastases - n subjects</b>	20
<b>HER2-positivity based on primary tumor - n subjects</b>	84
confirmed by reference lab	82
not confirmed by reference lab	2
FISH+ only	4
IHC 2+ with FISH+	11
IHC 3+ with FISH+	42



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ULB



IHC 3+ (no FISH+)	27
<b>HER2-positivity based on metastatic biopsy - n subjects</b>	6
confirmed by reference lab	6
not confirmed by reference lab	0
FISH+ only	0
IHC 2+ with FISH+	3
IHC 3+ with FISH+	2
IHC 3+ (no FISH+)	1
<b>Hormone receptor status - n subjects</b>	
ER+ or PR+ or both	63
ER- and PR-	27
<b>Prior systemic therapies for advanced disease</b>	
Yes - n subjects	83
No - n subjects	7
Median number of lines (range)	3 (0-11)
Median number of lines including trastuzumab (range)	2 (0-7)
Tyrosine kinase inhibitor - number of subjects	24
Pertuzumab - n subjects	6
HSP90 inhibitor - n subjects	4
n: number. ICH: immunohistochemistry. FISH: fluorescence in-situ hybridization. ER: estrogen receptor. PR: progesterone receptor. HSP90: Heat shock protein.	

## 5 END POINTS

Anatomic response as assessed by RECIST 1.1 after 3 T-DM1 cycles.

Time to treatment failure as defined as time interval between T-DM1 start and treatment stop whatever the cause.



## 5.1.1 Statistical analysis of end points

### 5.1.1.1 Primary endpoint

The primary endpoint was analysed using as level of analysis a tumoral lesion.

The explanatory variables were HER2 PET, early and late FDG PETs.

Statistical analysis title	Analyse of response using a lesion-based analysis	Analyse type	Other
		Comment	
Statistical analysis description	<p>Our main objective was to evaluate the ability of HER2 PET/CT to predict, before initiation of treatment, tumor lesions unlikely to respond anatomically to T-DM1. The ability of HER2 PET/CT to predict metabolic response after three cycles of T-DM1 was also explored. In addition, we analyzed how an early FDG PET/CT, alone or combined with the pre-treatment HER2 PET/CT, can identify tumor lesions that will not respond (anatomically and metabolically) after three T-DM1 cycles.</p> <p>Sample size:</p> <p>The primary goal was to establish the negative predictive value, lesion-based, of <sup>89</sup>Zr-trastuzumab PET/CT defined as the number of lesions classified as HER2 negative not responding (stable or progressive) after 3 cycles of T-DM1 using RECIST classification</p> <p>A negative HER2 lesion is defined as a lesion that shows no/faint <sup>89</sup>Zr-trastuzumab uptake on PET (the lesion cannot/almost not be differentiated from the background activity). A NPV of ≥95% (alternative hypothesis) would be of considerable practical value whereas one less than 85% (null hypothesis) has no practical application in this context.</p> <p>Using a single-stage Fleming-A'Hern design with a one-sided test (5% type I error) and power of 80% to test the null hypothesis of an NPV of &lt; 85% versus the alternative NPV of ≥ 95%, we calculated that 60 HER2-negative and RECIST 1.0 measurable lesions would need to be examined.</p> <p>The secondary objectives aimed to show that HER2 PET/CT, early FDG PET/CT, and the combination of both HER2 PET/CT and early FDG PET/CT would be able to select lesions not responding to treatment according to metabolic and anatomic response criteria post three cycles of T-DM1. To get a power of 90% with a one-sided test (5% type I error) for testing the null hypothesis</p>		



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	of NPV < 85% versus the alternative hypothesis of NPV ≥ 95%, we calculated that 76 mNR FDG-positive lesions on the early FDG PET/CT would need to be examined. The NPVs for both primary and secondary objectives were calculated and reported together with an associated exact 95% confidence interval (CI).				
Comparison group	The comparisons were done on responding and non-responding lesions.				
Number of subjects	Three hundred eighty-eight tumor lesions were selected on baseline FDG PET/CT. HER2 lesion-based classification was performed for 383, as 5 were excluded due to HER2 PET/CT image artifacts. The distribution among HER2 classes is the following: over one-third of all lesions (39%) were categorized as HER2-negative: 84/383 (22%) in class 1, and 64/383 (17%) in class 2, reflecting considerable heterogeneity of HER2 overexpression as detected on HER2 targeted imaging.				
Analysis specification	Pre-specified				
Parameter estimate					
Point estimate	Negative predictive value, positive predictive value				
Confidence interval	Level	95%	Sides	2	
Parameter type	Proportion				
Variability estimate	Standard error of the parameter estimates.				

Three hundred eighty-eight tumor lesions were selected on baseline FDG PET/CT. HER2 lesion-based classification was performed for 383, as 5 were excluded due to HER2 PET/CT image artifacts. Over one-third of all lesions (39%) were categorized as HER2-negative: 84/383 (22%) in class 1, and 64/383 (17%) in class 2, reflecting considerable heterogeneity of HER2 overexpression as detected on HER2 targeted imaging.



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The anatomic response was evaluable for 265/383 target lesions (measurable per RECIST1.0), of which 93 (35%) were HER2-negative. HER2 PET/CT correctly identified 75/93 HER2-negative lesions as anatomically NR after three T-DM1 cycles with an NPV of 81% (Table 1).

Late metabolic response was evaluable in 377 out of 383 lesions (6 lesions excluded due to disease progression before late FDG PET/CT was performed). The PPV and NPV of HER2 PET/CT for the lesion-based late metabolic response were 86% and 63%, respectively (Table 2).

The predictive values of early FDG PET/CT response alone and in combination with the pre-treatment HER2 PET/CT in selecting non-responding lesions, according to anatomic and metabolic response criteria, are detailed in Tables 3.A and 3.B, respectively. Briefly, out of 109 lesions classified as mNR on the early FDG PET/CT, only 21 showed anatomic response on the diagnostic CT after three T-DM1 cycles, giving NPV of 81% for the early FDG PET/CT lesion-based evaluation. When combining both molecular imaging results, the NPV for the absence of anatomic response after three T-DM1 cycles was 91%. The combination of HER2 and early FDG PET/CT resulted in an NPV of 84% and PPV of 97% for the late metabolic response.

**Table 1.** Relation between HER2 classification of tumor lesions and anatomic response measurements

	Classification	n lesions	Anatomic lesion response after three T-DM1 cycles		PPV	NPV
			R	NR		
HER2 PET/CT	+	172	123	49	72% <sup>1</sup>	
	-	93	18	75		81% <sup>2</sup>

Exact 95% confidence intervals as follows: <sup>1</sup>:64%-78%, <sup>2</sup>:71%-88%.

n: number. HER2+: HER2-positive lesions (class 3 and 4). HER2-: HER2-negative lesions (class 1 and 2). R: anatomically responding lesions. NR: anatomically non-responding lesions.



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Table 2 - Relation between HER2 classification of tumor lesions and late metabolic response assessment

	Classification	n lesions	Metabolic lesion response after three T-DM1 cycles		PPV	NPV
			mR	mNR		
HER2 PET/CT	+	235	202	33	86% <sup>1</sup>	
	-	142	53	89		63% <sup>2</sup>

Exact 95% confidence intervals as follows: <sup>1</sup>: 81%-90%, <sup>2</sup>: 54%-71%.

Table 3A - Relation between early metabolic response of tumor lesions and anatomic response measurements and late metabolic response assessment

	Classification	n lesions	Anatomic lesion response after three T-DM1 cycles		PPV	NPV
			R	NR		
Early FDG PET/CT	mR	158	120	38	76% <sup>1</sup>	
	mNR	109	21	88		81% <sup>2</sup>



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	Classification	n lesions	Metabolic lesion response after three T-DM1 cycles		PPV	NPV
			mR	mNR		
Early FDG PET/CT	mR	225	210	15	93% <sup>3</sup>	
	mNR	157	49	108		69% <sup>4</sup>
Exact 95% confidence intervals as follows: <sup>1</sup> : 68%-82%, <sup>2</sup> : 72%-88%, <sup>3</sup> : 89%-96%, <sup>4</sup> : 61%-76%.						
Table 3B. Relation between the combination of HER2 classification and early metabolic response of tumor lesions, and anatomic response measurements and late metabolic response assessment						
	Classification	n lesions	Anatomic lesion response after three T-DM1 cycles		PPV	NPV
			R	NR		
	+ / mR	130	108	22	83% <sup>1</sup>	
HER2 PET/CT / Early FDG PET/CT	+ / mNR	42	15	27		
	- / mR	28	12	16		
	- / mNR	65	6	59		91% <sup>2</sup>
	Classification	n lesions	Metabolic lesion response after three T-DM1 cycles		PPV	NPV
			mR	mNR		



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	+ / mR	176	170	6	97% <sup>3</sup>	
HER2 PET/CT / Early FDG PET/CT	+ / mNR	59	32	27		
	- / mR	47	38	9		
	- / mNR	95	15	80		84% <sup>4</sup>
Exact 95% confidence intervals as follows: <sup>1</sup> : 74%-88%, <sup>2</sup> : 81%-97%, <sup>3</sup> : 93%-99%, <sup>4</sup> : 75%-91%.						
n: number. mR: metabolically responding lesions. mNR: metabolically non-responding lesions. R: anatomically responding lesions. NR: anatomically non-responding lesions.						

### 5.1.1.2 Exploratory endpoint

The exploratory endpoint, time to treatment failure was analysed using as level of analysis a subject.

The explanatory variables were HER2 PET, early and late FDG PETs.

Statistical analysis title	Analyse of time to treatment failure using a subject-based analysis	Analyse type	Other
		Comment	
Statistical analysis description	Time to treatment failure as a time-to-event variable.		
Comparison group	The analysis was done according to the subjects' classification based on baseline HER2 PET and on early FDG PET.		
Number of subjects	83 subjects having received treatment		
Analysis specification	Pre-specified		



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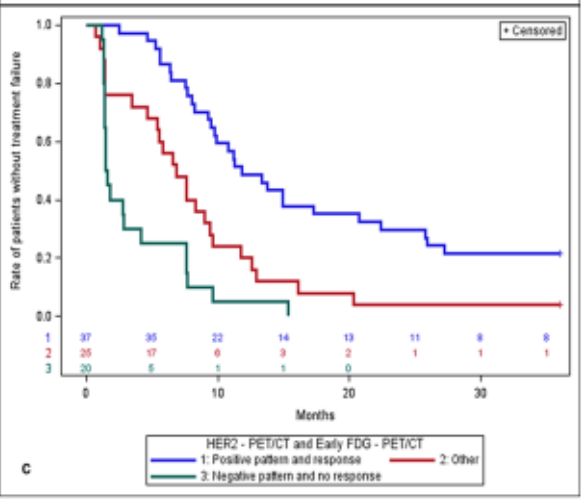
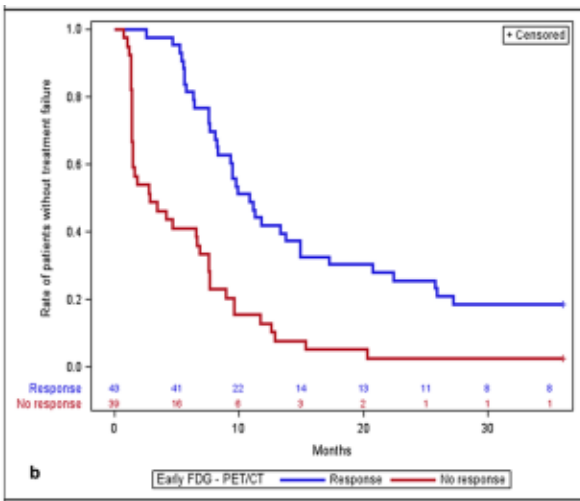
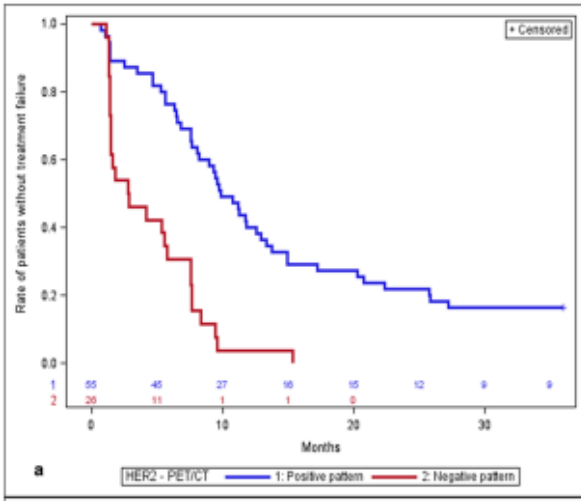


Statistical hypothesis test			
p-value	<0.05 (two-sided)		Comment
Method	Kaplan-Meier estimates, Cox regression analyses		
Parameter estimate			
Point estimate	Hazard ratio		
Confidence interval	Level	95%	
Parameter type	Cox proportional hazard; Hazard ratio (HR)		
Variability estimate	Standard error of the regression coefficient.		

The molecular imaging results of all subjects receiving T-DM1 (n = 83) were correlated with treatment discontinuation. Median TTF was 9.9 months (95% CI: 7.7 – 12.9 months) for the HER2-positive patients versus 2.8 months (95% CI: 1.4 – 5.8 months) in the HER2-negative group of subjects, with an HR of 3.7 (95% CI: 2.19 – 6.35,  $p < 0.0001$ ) using the HER2-positive group as reference. According to the early metabolic response assessment, the median TTF was 10.8 months in early metabolic responders (95% CI: 8.2 months – 14.9) and 2.8 months (95% CI: 1.4 – 6.8 months) for the early metabolic non-responders (HR of 3.0, 95% CI: 1.9 – 4.8,  $p < 0.0001$ ). When combining both imaging modalities, HER2-positive and early metabolic responding subjects had a median TTF of 11.8 months (95% CI: 9.5 – 20.8 months), compared to the HER2-negative and early metabolic non-responding patients who had a median TTF of 1.5 months (95% CI: 1.4 – 4.2 months). Subjects with discordance between HER2-positivity and the early metabolic response (as defined above) demonstrated a median TTF of 6.8 months (95% CI: 4.7 – 9.4 months). Using the double-positive group as a reference, the HRs for the double-negative and discordant groups were 5.8 (95% CI: 3.1 – 10.8) and 2.6 (95% CI: 1.5 – 4.5), respectively (overall  $p < 0.0001$ ).



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## 6 ADVERSE EVENTS

### 6.1 Adverse events information

<b>Timeframe for reporting adverse events</b>	<p>After informed consent, but prior to initiation of study medications (D0: <sup>89</sup>Zr-Trastuzumab injection), only SAEs caused by a protocol-mandated intervention were collected (e.g. SAEs related to invasive procedures such as biopsies, medication washout or no treatment run-in).</p> <p>From initiation of study medications (D0: <sup>89</sup>Zr-trastuzumab injection) until 30 days after the last T-DM1 dose, all AEs and SAEs were collected.</p> <p>After this period, only SAEs that were reasonably related to participation in the study had to be reported.</p>
<b>Adverse event reporting additional description</b>	<p>Progression of underlying malignancy will not be reported as an (serious) adverse event if it is clearly consistent with the suspected progression of the underlying cancer as defined by RECIST 1.1 or FDG PET/CT (after 3 cycles of T-DM1).</p> <p>Hospitalization due solely to the progression of underlying malignancy should NOT be reported as a serious adverse event. Clinical symptoms of progression may be reported as (serious) adverse events if the symptom cannot be determined as exclusively due to the progression of the underlying malignancy, or does not fit the expected pattern of progression for the disease under study.</p>
<b>Dictionary used for adverse event reporting</b>	MedDRA 27.1
<b>Assessment type</b>	Systematic
<b>Reporting group title(s)</b>	Safety analysis for all treated subjects
<b>Reporting group description:</b>	All subjects included in the trial



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## 6.2 Serious adverse events (SAEs)

<b>Number of subjects exposed</b>	90
<b>Number of subjects affected by SAEs</b>	20
<b>Number of death (all causes)</b>	1 (for disease progression)
<b>Number of deaths resulting from adverse events</b>	0

Thirty-three serious AEs were reported for 20 subjects, with 11 possibly related to T-DM1, including thrombocytopenia (n = 2), pyrexia and chills (n = 4), anal abscess, pulmonary tumor hemorrhage, cognitive disorder, seizure and supraventricular tachycardia (all n = 1).

MedDRA Primary SOC <i>MedDRA PT</i>	Number of subjects affected	All SAE occurrences	SAEs occurrences related to <sup>89</sup> Zr- trastuzumab or trastuzumab	SAEs occurrences related to T-DM1	Number of fatalities
<b>Blood and lymphatic system disorders</b>					
<i>Thrombocytopenia</i>	2	2		2	0
<b>Cardiac disorders</b>					
<i>Cardiac failure chronic</i>	1	1			0
<i>Pericardial effusion</i>	1	1			0
<i>Supraventricular tachycardia</i>	1	1		1	0
<b>Gastrointestinal disorders</b>					
<i>Abdominal hernia</i>	1	1			0
<b>General disorders and administration site conditions</b>					
<i>Chills</i>	1	1		1	0
<i>Pyrexia</i>	1	3		3	0
<b>Immune system disorders</b>					
<i>Drug hypersensitivity</i>	1	1	1		0
<i>Hypersensitivity</i>	1	1			0
<b>Infections and infestations</b>					
<i>Anal abscess</i>	1	1		1	0
<i>Bacteraemia</i>	1	1			0
<i>Cellulitis</i>	1	1			0
<i>Erysipelas</i>	1	1			0
<i>Pneumonia</i>	1	1			0
<i>Soft tissue infection</i>	1	1			0
<i>Upper respiratory tract infection</i>	1	1			0
<i>Urinary tract infection</i>	1	1			0
<i>Viral infection</i>	1	1			0
<b>Metabolism and nutrition disorders</b>					
<i>Hypercalcaemia</i>	1	1			0
<b>Neoplasms benign, malignant and unspecified (incl cysts and polyps)</b>					
<i>Tumour haemorrhage</i>	1	1		1	0



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MedDRA Primary SOC <i>MedDRA PT</i>	Number of subjects affected	All SAE occurrences	SAEs occurrences related to <sup>89</sup> Zr- trastuzumab or trastuzumab	SAEs occurrences related to T-DM1	Number of fatalities
<b>Nervous system disorders</b>					
<i>Cerebrovascular accident</i>	1	1			0
<i>Cognitive disorder</i>	1	1		1	0
<i>Seizure</i>	3	4		1	0
<b>Psychiatric disorders</b>					
<i>Mental disorder</i>	1	2			0
<b>Respiratory, thoracic and mediastinal disorders</b>					
<i>Dyspnoea</i>	1	1			0
<i>Pleural effusion</i>	1	1			0

### 6.3 Non-serious adverse events

Number of subjects exposed	90
Number of subjects affected by non-SAEs	86
Frequency threshold for reporting non-SAEs	0%

MedDRA Primary SOC <i>MedDRA PT</i>	Number of subjects affected	All AE occurrences	AEs occurrences related to <sup>89</sup> Zr-trastuzumab or trastuzumab	AEs occurrences related to T-DM1
<b>Blood and lymphatic system disorders</b>				
<i>Anaemia</i>	9	9	1	7
<i>Leukocytosis</i>	1	1		
<i>Leukopenia</i>	3	4		4
<i>Microcytosis</i>	1	1		
<i>Neutropenia</i>	1	1		1
<i>Thrombocytopenia</i>	12	21		20
<b>Cardiac disorders</b>				
<i>Extrasystoles</i>	2	2		2
<i>Palpitations</i>	4	12		9
<i>Ventricular extrasystoles</i>	2	2		2
<b>Ear and labyrinth disorders</b>				
<i>Tinnitus</i>	1	1		
<i>Vertigo</i>	5	9		4
<b>Endocrine disorders</b>				
<i>Goitre</i>	1	1		
<b>Eye disorders</b>				
<i>Binocular eye movement disorder</i>	1	1		
<i>Blepharitis</i>	1	1		
<i>Dry eye</i>	10	13		11
<i>Erythema of eyelid</i>	1	1	1	



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MedDRA Primary SOC <i>MedDRA PT</i>	Number of subjects affected	All AE occurrences	AEs occurrences related to <sup>89</sup> Zr-trastuzumab or trastuzumab	AEs occurrences related to T-DM1
<i>Eye disorder</i>	3	3		1
<i>Eye swelling</i>	1	1		
<i>Glaucoma</i>	1	1		1
<i>Keratitis</i>	2	2		2
<i>Lacrimation increased</i>	6	7		6
<i>Macular degeneration</i>	1	1		
<i>Scleral haemorrhage</i>	1	1		1
<i>Vision blurred</i>	7	7		4
<i>Visual acuity reduced</i>	1	1		1
<i>Visual field defect</i>	1	1		
<b>Gastrointestinal disorders</b>				
<i>Abdominal distension</i>	6	7		3
<i>Abdominal pain</i>	5	7	1	2
<i>Abdominal pain lower</i>	2	2		2
<i>Abdominal pain upper</i>	7	10		5
<i>Constipation</i>	32	70	1	47
<i>Diarrhoea</i>	20	38	1	29
<i>Dry mouth</i>	15	22		17
<i>Dyspepsia</i>	4	10		6
<i>Faeces discoloured</i>	1	1	1	
<i>Gastritis</i>	2	2		
<i>Gastrooesophageal reflux disease</i>	1	1		
<i>Gingival bleeding</i>	5	9		9
<i>Gingival pain</i>	2	4		4
<i>Glossitis</i>	1	1		1
<i>Haematochezia</i>	1	1		1
<i>Haemorrhoidal haemorrhage</i>	1	1		
<i>Haemorrhoids</i>	1	1		
<i>Lip swelling</i>	1	1		
<i>Mouth haemorrhage</i>	1	1		1
<i>Mouth ulceration</i>	1	1		1
<i>Nausea</i>	43	126	1	110
<i>Odynophagia</i>	3	3		2
<i>Periodontal disease</i>	1	1		
<i>Stomatitis</i>	10	12		10
<i>Toothache</i>	4	4		1
<i>Vomiting</i>	20	33		22
<b>General disorders and administration site conditions</b>				
<i>Asthenia</i>	1	1		1
<i>Catheter site inflammation</i>	1	1		1
<i>Chest pain</i>	4	4		
<i>Chills</i>	26	44	8	31



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MedDRA Primary SOC <i>MedDRA PT</i>	Number of subjects affected	All AE occurrences	AEs occurrences related to <sup>89</sup> Zr-trastuzumab or trastuzumab	AEs occurrences related to T-DM1
<i>Fatigue</i>	53	292	1	282
<i>Gait disturbance</i>	2	2		1
<i>Hypothermia</i>	1	1		
<i>Influenza like illness</i>	19	24		9
<i>Injection site nodule</i>	1	1		
<i>Localised oedema</i>	1	1		
<i>Malaise</i>	9	14		12
<i>Mucosal dryness</i>	3	5		5
<i>Mucosal inflammation</i>	7	7		6
<i>Oedema</i>	6	7		4
<i>Oedema peripheral</i>	13	23		15
<i>Pain</i>	14	16		10
<i>Pyrexia</i>	21	37		31
<b>Hepatobiliary disorders</b>				
<i>Cholelithiasis</i>	1	1		
<i>Cholestasis</i>	1	1		
<i>Hepatic function abnormal</i>	1	1	1	1
<i>Hepatic pain</i>	4	4	1	
<i>Hepatitis</i>	1	1		
<i>Hyperbilirubinaemia</i>	1	1		
<b>Immune system disorders</b>				
<i>Drug hypersensitivity</i>	1	1		
<i>Mite allergy</i>	1	1		
<b>Infections and infestations</b>				
<i>Bronchitis</i>	4	5		2
<i>Candida infection</i>	1	1		1
<i>Cellulitis</i>	1	2		1
<i>Conjunctivitis</i>	5	7		6
<i>Cystitis</i>	2	4		4
<i>Device related infection</i>	1	1		
<i>Escherichia urinary tract infection</i>	2	2		
<i>Folliculitis</i>	1	1		
<i>Fungal infection</i>	1	1		1
<i>Furuncle</i>	1	1		1
<i>Gastroenteritis</i>	3	3		1
<i>Gastroenteritis viral</i>	1	1		
<i>Gingivitis</i>	1	1	1	1
<i>Herpes zoster</i>	1	1		
<i>Infection</i>	2	2		1
<i>Influenza</i>	10	12		2
<i>Localised infection</i>	1	1		
<i>Nasopharyngitis</i>	6	7		2
<i>Oral candidiasis</i>	1	1		1



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MedDRA Primary SOC <i>MedDRA PT</i>	Number of subjects affected	All AE occurrences	AEs occurrences related to <sup>89</sup> Zr-trastuzumab or trastuzumab	AEs occurrences related to T-DM1
<i>Oral herpes</i>	4	4		2
<i>Otitis media</i>	1	1		
<i>Pharyngitis</i>	4	5		2
<i>Pneumonia</i>	2	2	1	1
<i>Postoperative wound infection</i>	1	1		
<i>Respiratory tract infection</i>	6	8		3
<i>Rhinitis</i>	2	7		4
<i>Rhinotracheitis</i>	1	1		1
<i>Sinusitis</i>	9	14		7
<i>Skin infection</i>	1	1		
<i>Soft tissue infection</i>	1	1		
<i>Tonsillitis</i>	1	1		1
<i>Tooth infection</i>	1	1		1
<i>Tracheitis</i>	1	1		1
<i>Upper respiratory tract infection</i>	12	14		4
<i>Urinary tract infection</i>	13	48		16
<i>Vaginal infection</i>	1	1		1
<i>Viral infection</i>	3	3		1
<i>Viral upper respiratory tract infection</i>	1	1		
<b>Injury, poisoning and procedural complications</b>				
<i>Allergic transfusion reaction</i>	1	1		
<i>Contusion</i>	9	23		21
<i>Ear canal injury</i>	1	1		
<i>Fall</i>	1	2		
<i>Foot fracture</i>	1	1		
<i>Foreign body</i>	1	1		
<i>Fracture</i>	1	1		
<i>Infusion related reaction</i>	2	2	2	
<i>Limb injury</i>	1	1		
<i>Procedural pain</i>	1	1		
<i>Radiation neuropathy</i>	1	1		
<i>Radiation skin injury</i>	1	1		
<i>Scar</i>	1	1		
<i>Scratch</i>	1	1		
<i>Wrist fracture</i>	2	2		
<b>Investigations</b>				
<i>Alanine aminotransferase increased</i>	23	32	1	27



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<i>Aspartate aminotransferase increased</i>	28	36		31
<i>Blood alkaline phosphatase increased</i>	10	14		8
<i>Blood bilirubin increased</i>	4	5		3
<i>Blood cholesterol increased</i>	1	1		
<i>Blood creatinine increased</i>	1	1		
<i>Blood lactate dehydrogenase increased</i>	1	1		
<i>Ejection fraction decreased</i>	8	10		10
<i>Gamma-glutamyltransferase increased</i>	21	27	2	18
<i>Heart rate decreased</i>	1	1		
<i>Menstruation normal</i>	1	1		
<i>Neutrophil count decreased</i>	2	2	1	2
<i>Platelet count decreased</i>	15	32		32
<i>Serum ferritin decreased</i>	1	1		1
<i>Weight decreased</i>	2	2		
<b>Metabolism and nutrition disorders</b>				
<i>Decreased appetite</i>	22	33		25
<i>Diabetes mellitus</i>	1	1		
<i>Fluid retention</i>	1	1		1
<i>Folate deficiency</i>	1	1		
<i>Hypercalcaemia</i>	2	2		
<i>Hyperglycaemia</i>	2	2		
<i>Hypocalcaemia</i>	2	3		1
<i>Hypokalaemia</i>	2	3		1
<i>Hyponatraemia</i>	3	5		1
<i>Iron deficiency</i>	2	2		
<i>Vitamin D deficiency</i>	1	1		
<b>Musculoskeletal and connective tissue disorders</b>				
<i>Arthralgia</i>	20	43		24
<i>Back pain</i>	9	18		4
<i>Bone pain</i>	4	4		1
<i>Bursitis</i>	2	2		1
<i>Muscle spasms</i>	8	14		10
<i>Muscular weakness</i>	2	2		



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MedDRA Primary SOC <i>MedDRA PT</i>	Number of subjects affected	All AE occurrences	AEs occurrences related to <sup>89</sup> Zr-trastuzumab or trastuzumab	AEs occurrences related to T-DM1
<i>Musculoskeletal chest pain</i>	1	1		
<i>Musculoskeletal pain</i>	2	5		4
<i>Musculoskeletal stiffness</i>	1	1		
<i>Myalgia</i>	19	33		27
<i>Neck pain</i>	3	4		1
<i>Osteoarthritis</i>	1	1		
<i>Osteonecrosis</i>	1	1		
<i>Pain in extremity</i>	15	27		15
<i>Periarthritis</i>	1	2		
<i>Sacral pain</i>	1	1		
<i>Temporomandibular joint syndrome</i>	1	1		
<i>Tendonitis</i>	1	1		
<b>Neoplasms benign, malignant and unspecified (incl cysts and polyps)</b>				
<i>Haemangioma</i>	2	2		2
<i>Tumour pain</i>	1	1		
<b>Nervous system disorders</b>				
<i>Amnesia</i>	3	3		
<i>Aphasia</i>	1	1		
<i>Ataxia</i>	1	1		
<i>Balance disorder</i>	1	1		
<i>Burning sensation</i>	1	1		1
<i>Carpal tunnel syndrome</i>	2	2		
<i>Coordination abnormal</i>	1	1		1
<i>Disturbance in attention</i>	1	1		
<i>Dizziness</i>	11	27		16
<i>Dysaesthesia</i>	1	1		
<i>Dysgeusia</i>	9	9		9
<i>Headache</i>	34	202	2	155
<i>Hypoaesthesia</i>	3	3		1
<i>Nervous system disorder</i>	3	3		1
<i>Neuropathy peripheral</i>	16	19		13
<i>Neurotoxicity</i>	1	1		
<i>Paraesthesia</i>	6	6		6
<i>Paraesthesia oral</i>	1	1		
<i>Parkinson's disease</i>	1	1		
<i>Peripheral motor neuropathy</i>	1	1		
<i>Peripheral sensory neuropathy</i>	6	6		4



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MedDRA Primary SOC <i>MedDRA PT</i>	Number of subjects affected	All AE occurrences	AEs occurrences related to <sup>89</sup> Zr-trastuzumab or trastuzumab	AEs occurrences related to T-DM1
<i>Restless legs syndrome</i>	1	1		1
<i>Syncope</i>	4	4		3
<i>Taste disorder</i>	2	2		2
<i>Tremor</i>	1	1		
<b>Psychiatric disorders</b>				
<i>Anxiety</i>	4	4		
<i>Confusional state</i>	1	1		1
<i>Depression</i>	6	7		1
<i>Hallucination, visual</i>	1	1		
<i>Insomnia</i>	1	1		1
<i>Sleep disorder</i>	1	1		
<b>Renal and urinary disorders</b>				
<i>Dysuria</i>	2	2		1
<i>Haematuria</i>	1	1		
<i>Micturition urgency</i>	1	1		1
<i>Urinary incontinence</i>	3	4		
<i>Urinary retention</i>	1	1		
<b>Reproductive system and breast disorders</b>				
<i>Adnexa uteri pain</i>	1	1		
<i>Breast pain</i>	2	1		
<i>Genital atrophy</i>	1	1		
<i>Genital burning sensation</i>	1	1		
<i>Genital haemorrhage</i>	1	1		1
<i>Vaginal haemorrhage</i>	2	2		2
<i>Vulvovaginal dryness</i>	1	1		1
<b>Respiratory, thoracic and mediastinal disorders</b>				
<i>Bronchial obstruction</i>	1	1		
<i>Cough</i>	13	21	1	12
<i>Dysphonia</i>	4	5		2
<i>Dyspnoea</i>	14	21		12
<i>Dyspnoea exertional</i>	1	1		
<i>Epistaxis</i>	28	92	1	89
<i>Haemoptysis</i>	3	4		4
<i>Hypersensitivity pneumonitis</i>	1	1	1	1
<i>Interstitial lung disease</i>	1	1		1
<i>Nasal congestion</i>	1	1		
<i>Nasal obstruction</i>	1	1		
<i>Nasal ulcer</i>	1	3		3
<i>Oropharyngeal pain</i>	1	1		
<i>Rhinitis allergic</i>	1	1		
<i>Rhinorrhoea</i>	3	5		2
<i>Sinus disorder</i>	1	1		1
<i>Sinus pain</i>	1	1		



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MedDRA Primary SOC <i>MedDRA PT</i>	Number of subjects affected	All AE occurrences	AEs occurrences related to <sup>89</sup> Zr-trastuzumab or trastuzumab	AEs occurrences related to T-DM1
<i>Sneezing</i>	1	1		
<b>Skin and subcutaneous tissue disorders</b>				
<i>Alopecia</i>	14	14		11
<i>Dermatitis allergic</i>	1	1		
<i>Dermatitis contact</i>	1	1		
<i>Dry skin</i>	2	4		3
<i>Eczema</i>	1	1		1
<i>Erythema</i>	4	5		2
<i>Hyperhidrosis</i>	1	1	1	
<i>Nail disorder</i>	2	2		2
<i>Nail ridging</i>	2	2		2
<i>Night sweats</i>	1	1		1
<i>Onychoclasia</i>	4	5		5
<i>Pain of skin</i>	1	1		1
<i>Petechiae</i>	1	1		1
<i>Photosensitivity reaction</i>	1	1		
<i>Polymorphic light eruption</i>	1	1		
<i>Pruritus</i>	5	5		1
<i>Purpura</i>	1	1		
<i>Rash</i>	8	10	1	7
<i>Rash maculo-papular</i>	4	4	1	1
<i>Skin discolouration</i>	1	1		
<i>Skin disorder</i>	1	1		
<i>Skin fissures</i>	1	1	1	1
<i>Skin irritation</i>	2	2		1
<i>Skin lesion</i>	2	3		3
<i>Spider naevus</i>	1	1		1
<i>Umbilical erythema</i>	1	1		
<b>Vascular disorders</b>				
<i>Arteriosclerosis</i>	1	1		
<i>Circulatory collapse</i>	1	1		
<i>Haematoma</i>	5	9		8
<i>Haemorrhage</i>	2	3		3
<i>Hot flush</i>	6	12		7
<i>Hypertension</i>	6	8		
<i>Hypotension</i>	1	1		
<i>Lymphoedema</i>	1	1		
<i>Phlebitis</i>	2	2		1
<i>White coat hypertension</i>	1	1		

35 adverse reactions (ARs) related either to <sup>89</sup>Zr-trastuzumab or trastuzumab were reported. The sponsor considered that these ARs are related to the cold trastuzumab co-administered with the tracer.



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Grade  $\geq 3$  AEs due to T-DM1 were reported in 48 subjects (53.3%) (Frequency threshold for reporting non-SAEs=5%), among which the most common were fatigue (11.1%), hypertension (7.8%), and increased gamma-glutamyl transferase (16.7%), leading to dose reductions in 20 subjects.

MedDRA SOC	MedDRA PT	N subjects	N AEs (Grade 3,4)	N AEs related
Investigations	GGT increase	15	15	11
General disorders and administration site conditions	Fatigue	6	10	9
Vascular disorders	Hypertension	5	7	0

## 7 ADDITIONAL INFORMATION

### 7.1 Global substantial modifications

The global substantial modifications are summarised in the below tables:

#### Belgium

Reference	Date	Description
1	EC: 21 <sup>st</sup> June 2012, CA: 22 <sup>nd</sup> June 2012	New/updated protocol and ICF
2	EC: 20 <sup>th</sup> Nov 2012, CA: 4 <sup>th</sup> Dec 2012	New/updated protocol and ICF, addition of sites
3	EC: 3 <sup>rd</sup> April 2014, CA: 10 <sup>th</sup> April 2014, FANC: 18 <sup>th</sup> April 2014	New/updated protocol and ICF
AMD-0004	CA: 05/08/2015, EC: 09/07/2015	New/updated protocol and ICF
AMD-0007	EC: 01/10/2015	New/updated ICF (addendum)
AMD-0009	CA: 11/05/2016, EC: 02/06/2016	RSI change
AMD-0014	EC: 23/03/2017, CA: 28/02/2017	Temp halt recr
AMD-0016	CA: 20/04/2017, EC: 20/04/2017	Change RSI
AMD-0021	CA: 10/07/2017, EC: 13/07/2017	New/updated protocol and ICF (addendum)
AMD-0047	EC: 06/12/2018	New/updated ICF (addendum)
AMD-0057	CA: 11/04/2019, EC: 02/05/2019	RSI change
AMD-0096	CA: 15/01/2021, EC: 21/01/2021	RSI
AMD-0126	CA: 21/10/2021	Move of Bordet
AMD-0138	EC: 09/03/2022	New insurance certificate

#### The Netherlands

Reference	Date	Description
M12.122031	EC: 2 <sup>nd</sup> August 2012	New/updated protocol and ICF
M12.126685	EC: 12 <sup>th</sup> Nov 2012	New/updated protocol and ICF
M12.126819	EC: 15 <sup>th</sup> Nov 2012	Addition of sites



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M13.130108	EC: 18 <sup>th</sup> Jan 2014	New/updated ICF
M14.156128	EC: 27 <sup>th</sup> May 2014	New/updated protocol and ICF
M14.162603	EC: 2 <sup>nd</sup> Oct 2014	New/updated protocol and ICF
M15.185759	EC: 23 <sup>rd</sup> Dec 2015	New/updated protocol and ICF
M16.190932	EC: 6 <sup>th</sup> April 2016	Updated IB
M16.193153	EC: 24 <sup>th</sup> May 2016	RSI change
M17.209939	EC: 3 <sup>rd</sup> April 2017	Temp halt of recr
M17.211070	EC: 25 <sup>th</sup> April 2017	Change of RSI
M17.219114	EC: 13 <sup>th</sup> Oct 2017	New/updated protocol and ICF (addendum)
M18.233113	EC: 6 <sup>th</sup> July 2018	Updated IB
M19.224372	21 <sup>st</sup> Jan 2019	New/updated ICF (addendum)
M19.231963	23 <sup>rd</sup> May 2019	RSI change
M21.273702	12 <sup>th</sup> April 2021	New/updated protocol and ICF

## 7.2 Global interruptions and re-starts

There were no global interruptions to the trial.

## 7.3 Limitations, addressing sources of potential bias and imprecisions and caveats

There were no limitations and caveats.