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Clinical Trial Protocol

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Future ONCOLOGY

ASTEFANIA: adjuvant ado-trastuzumab emtansine and atezolizumab for high-risk, HER2-positive breast cancer

Sara A Hurvitz*[,1], Thomas Bachelot[2], Giampaolo Bianchini[3], Nadia Harbeck[4], Sherene Loi[5,6], Yeon Hee Park[7], Aleix Prat[8], Leslie Gilham[9], Thomas Boulet[10], Nino Gochitashvili[11], Estefania Monturus[10], Chiara Lambertini[10], Beatrice Nyawira[10], Adam Knott[11], Eleonora Restuccia[10] & Peter Schmid[12]

[1]University of California Los Angeles/Jonsson Comprehensive Cancer Centre (UCLA/JCCC), 10833 Le Conte Ave., Los Angeles, CA 900024, USA

[2]Centre Léon Bérard, 28 Prom. Léa et Napoléon Bullukian, 69008, Lyon, France

[3]IRCCS Ospedale San Raffaele, Via Olgettina, 60, Milano, MI, 20132, Italy

[4]Department of Gynecology & Obstetrics & CCC Munich, Breast Center, LMU University Hospital, Marchioninistraße 15, 81377, Munich, Germany

[5]Division of Cancer Research, Peter MacCallum Cancer Centre, 305 Grattan Street, Melbourne, Victoria, 3000, Australia

[6]The Sir Peter MacCallum Department of Medical Oncology, University of Melbourne, Grattan Street, Parkville, Victoria, 3010, Australia

[7]Samsung Medical Center, 81 Irwon-ro, Gangnam-gu, Seoul, South Korea

[8]Hospital Clínic Barcelona, C. de Villarroel, 170, 08036, Barcelona, Spain

[9]Patient advocate, Melbourne, VIC, Australia

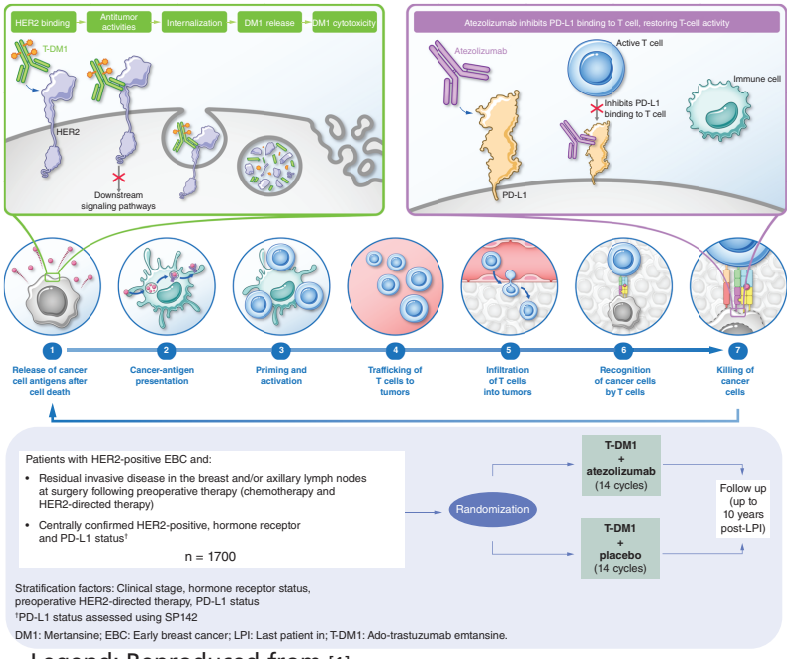
[10]F.Hoffmann-La Roche Ltd, Gartenstrasse 9, CH-4052, Basel, Switzerland

[11]Roche Products Limited, Hexagon Place, Shire Park, Falcon Way, Welwyn Garden City, AL7 1TW, UK

[12]Barts Cancer Institute, Queen Mary University of London, Charterhouse Square, London, EC1M 5PZ, UK

*Author for correspondence: Tel.: +310 998 4747; shurvitz@mednet.ucla.edu

Graphical abstract:



Legend: Reproduced from [1].

There is a strong rationale for combining HER2-targeted therapies with cancer immunotherapy to increase efficacy in breast cancer, particularly in the early-stage setting, where the immune system has not been weakened by heavy pretreatment. ASTEFANIA aims to evaluate the efficacy of adjuvant atezolizumab in combination with ado-trastuzumab emtansine in patients with high-risk, HER2-positive early breast cancer and residual disease following HER2-based neoadjuvant therapy. Eligible patients will be randomized to receive ado-trastuzumab emtansine in combination with either atezolizumab or placebo for 14 cycles within 12 weeks of primary surgery. The primary outcome is invasive disease-free survival and secondary outcomes include additional efficacy end points, safety and pharmacokinetics. The study plans to enroll 1700 patients across 32 counties.

Clinical Trial Registration: NCT04873362 (ClinicalTrials.gov)

First draft submitted: 11 May 2022; Accepted for publication: 12 August 2022; Published online: 16 November 2022

Keywords: ado-trastuzumab emtansine • atezolizumab • cancer immunotherapy • early breast cancer • HER2

Introduction to the trial

ASsessment of Trastuzumab Emtansine For AdjuvaNt therapy In HER2-positive breast cancer (BC) with Atezolizumab (ASTEFANIA; NCT04873362) is a phase III study evaluating the efficacy and safety of adjuvant ado-trastuzumab emtansine (T-DM1) in combination with either atezolizumab or placebo for patients with HER2-positive BC at high risk of recurrence following preoperative therapy, sponsored by F Hoffmann-La Roche Ltd (Basel, Switzerland).

Background & rationale

In 2020, an estimated 2.3 million new diagnoses of BC were recorded, making it the most common invasive malignancy in the world [2]. It has been estimated that 15–20% of BC have gene amplification or protein overexpression of HER2, leading to aggressive disease biology [3]. While current HER2-targeted therapies have significantly improved patient outcomes, up to one in four women with HER2-positive early BC experience recurrence or death within 10–11 years of diagnosis, despite HER2-targeted treatment with trastuzumab-based regimens [4–6].

T-DM1 is an antibody–drug conjugate comprised of trastuzumab linked to a cytotoxic agent, mertansine (DM1), using the stable linker 4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate. As such, T-DM1 binds to HER2 with an affinity similar to that of trastuzumab. T-DM1 undergoes receptor-mediated internalization following binding, thereby releasing DM1 into the cell and inducing cytotoxicity by inhibiting microtubule assembly. This results in cell cycle arrest and tumor-cell apoptosis [7]. HER2-targeted antibodies have demonstrated an ability to engage the innate and adaptive immune systems, contributing to their clinical activity [8]. In this context, the trastuzumab component of T-DM1 may also induce an immune response via antibody-dependent cell-mediated cytotoxicity. Moreover, the killing of cancer cells causes release of cancer cell antigens, further stimulating an immune response with the invasion of T cells (Figure 1) [9].

The KATHERINE study (NCT01772472) compared T-DM1 with trastuzumab in the adjuvant setting, in patients with residual invasive disease after trastuzumab plus taxane-based treatment administered in the neoadjuvant setting. Interim analyses showed that T-DM1 significantly reduced the risk of recurrence of invasive BC or death by 50% when compared with trastuzumab [10]. T-DM1 improved the 3-year invasive disease-free survival (IDFS) rate compared with trastuzumab (88.3 vs 77.0%). The study established T-DM1 as the standard of care in this setting. In KATHERINE, T-DM1 improved outcomes for patients regardless of the subgroup analyzed; however, despite the treatment benefit, certain patients were identified as having a particularly high risk of recurrence despite T-DM1 treatment, including those who enrolled with inoperable disease and those with operable, hormone receptor-negative and node-positive disease (3-year IDFS was 76.0% with T-DM1 in both groups). This highlighted the unmet need for therapies to improve outcomes for certain high-risk populations [11]. Incorporating a cancer immunotherapy to the existing T-DM1 treatment schedule is currently being explored as a potentially effective measure to address this need.

Cancer cells can overexpress PD-L1, an immune checkpoint, on the extracellular surface. PD-L1 binds to PD-1 and B7-1 proteins on the extracellular surface of T cells, causing their deactivation and thereby allowing cancer cells to evade the immune system. Atezolizumab is a monoclonal antibody that targets and selectively inhibits

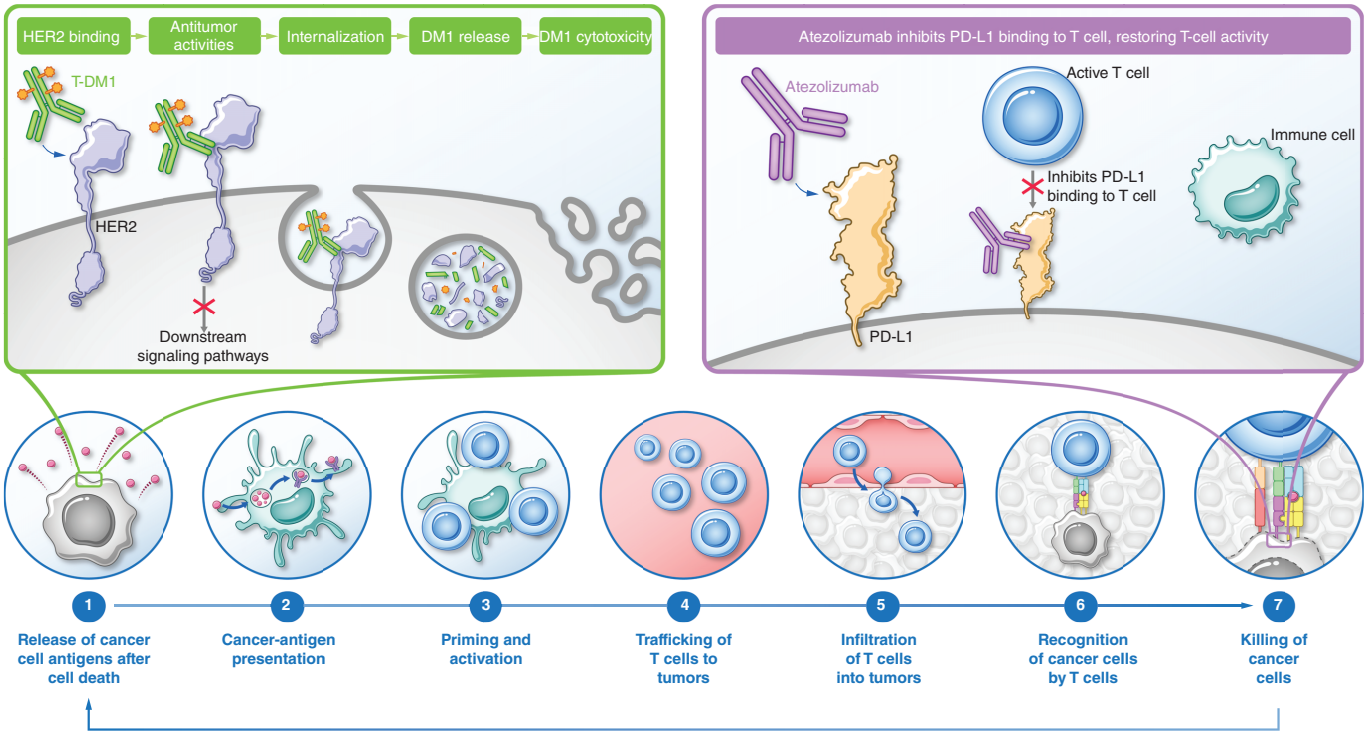


Figure 1. Proposed impact of the combination of T-DM1 and atezolizumab on the cancer-immunity cycle. T-DM1 is an antibody–drug conjugate that binds to HER2. Following binding T-DM1 undergoes receptor-mediated internalization, thereby releasing DM1 into the cell and inducing cytotoxicity by inhibiting microtubule assembly, resulting in tumor cell apoptosis and the release of cancer cell antigens. Atezolizumab is a monoclonal antibody that targets and selectively inhibits PD-L1 on cancer cells, preventing PD-L1 from binding to PD-1 and allowing T-cell activation.
DM1: Mertansine; T-DM1: Ado-trastuzumab emtansine.

PD-L1 on cancer cells, preventing PD-L1 from binding to PD-1 and allowing T-cell activation (Figure 1) [12]. A proof-of-concept trial (PANACEA; NCT02129556) demonstrated that combining trastuzumab with an anti-PD-1 immune checkpoint inhibitor, pembrolizumab, had limited results in patients with trastuzumab-resistant, heavily pretreated, HER2-positive advanced BC. Notably, there were no objective responses observed in patients with PD-L1 negative tumors and a 15% overall response rate in those with PD-L1-positive tumors [13]. Primary and metastatic tumors are biologically different entities, with most metastatic tumors demonstrating an immune-depleted state; therefore, immune-based therapy in the early setting may be more effective when the host immune system has not been exhausted by multiple rounds of cytotoxic therapy and when the tumor has undergone less editing [14–16]. For this reason, the optimal time for cancer immunotherapy administration may be the early setting.

Keyriched-1 (NCT03988036) was the first study to investigate a chemotherapy-free neoadjuvant treatment regimen for a cancer immunotherapy. It examined pembrolizumab plus the HER2-targeted therapies, trastuzumab and pertuzumab, in the neoadjuvant setting for HER2-enriched early breast cancer (EBC). At the primary analysis, the triplet regimen showed a centrally confirmed pathologic complete response (pCR) rate of 46% (95% CI: 0.31–0.62) [17]. Neo-PATH (NCT03991878) investigated the combination of cancer immunotherapy with atezolizumab plus HER2-targeted therapies and chemotherapy in the neoadjuvant setting. In this trial, atezolizumab, docetaxel, trastuzumab and pertuzumab led to a pCR rate of 61% in the intention-to-treat population [18].

Despite these promising results, there is a rationale for selecting long-term efficacy end points (such as IDFS) to be used as the primary end points in trials of cancer immunotherapy, rather than response-based end points such as pCR and response rates. The failure of IMpassion050 (NCT03726879) to demonstrate an improvement in its primary pCR end point (a decrease in pCR rate of 0.33% compared with placebo) [19], adds to the existing evidence against use of response-based end points to assess the efficacy of cancer immunotherapy. Indeed, in triple negative BC (TNBC) both KEYNOTE-522 (NCT03036488) and GeparNuevo (NCT02685059) demonstrated only a modest increase in pCR rate (by 7.5 and 9.2%, respectively) but a clinical meaningful benefit in terms

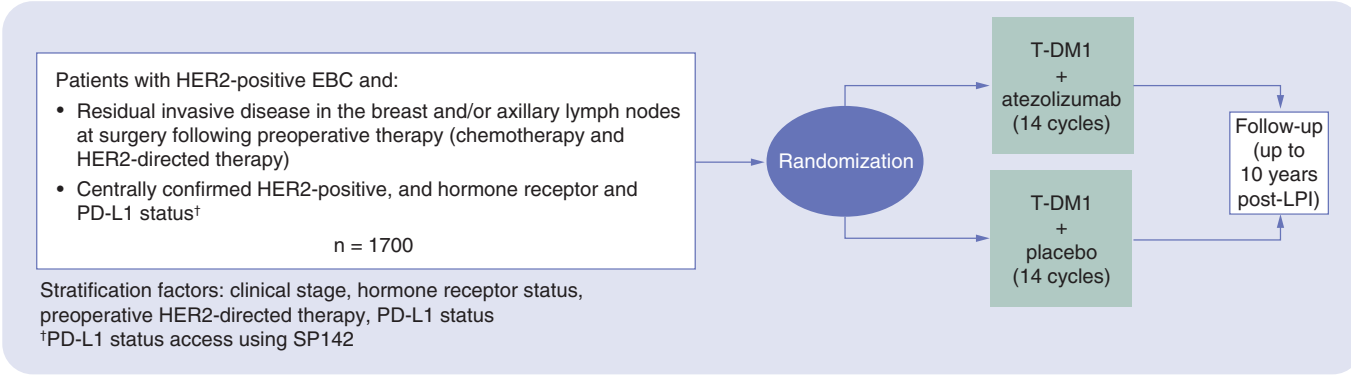


Figure 2. Study design. ASTEFANIA is a phase III study evaluating the efficacy and safety of adjuvant T-DM1 in combination with either atezolizumab or placebo for patients with HER2-positive BC at high risk of recurrence following preoperative therapy.
EBC: Early breast cancer; LPI: Last patient in; T-DM1: Ado-trastuzumab emtansine [1].

of long-term outcomes (KEYNOTE-522 event-free survival hazard ratio (HR) was 0.63 and GeparNuevo 3-year IDFS HR was 0.54) [16,20–25]. This modest increase in pCR alongside meaningfully improved long-term outcomes might be explained, at least in part, by the different risk of recurrence in patients with residual disease depending on whether they had received cancer immunotherapy or placebo; distant recurrence in each arm was 3.4 and 5.9%, respectively [21]. Unlike chemotherapy, cancer immunotherapies exert their effects on the immune system and may take time to mount an antitumor immune response, resulting in moderate changes in short-term, response-based outcomes despite possible long-term remissions and survival benefit [19,26,27]. All of these considerations suggest that pCR might not be the most appropriate and reliable end point for assessing the benefit of cancer immunotherapy.

In preclinical models, T-DM1 has demonstrated enhanced T-cell responses, including increased T-cell infiltration and complete tumor rejection when combined with CTLA-4 and PD-1-blocking antibodies [7]. Figure 1 summarizes the potential combined effect of T-DM1 and atezolizumab in the cancer-immunity cycle. The phase II KATE2 trial (NCT02924883) evaluated T-DM1 plus atezolizumab in patients with HER2-positive locally advanced or metastatic BC. While the overall study did not meet its primary end point in the intention-to-treat population, exploratory analyses in subgroups of patients with PD-L1-positive disease showed that the addition of atezolizumab to T-DM1 (as compared with the addition of placebo to T-DM1) resulted in longer median progression-free survival (8.5 vs 4.1 months; HR: 0.60; 95% CI: 0.32–1.11) and higher 1-year overall survival rates (94 vs 88%; HR: 0.57, 95% CI: 0.23–1.42) [28]. These promising results in a PD-L1 positive population supported the design of the ongoing KATE3 trial (NCT04740918) in locally advanced BC, and provide an additional rationale for testing this combination in the early setting. The advantages shown in preclinical studies together with supporting clinical data from KATE2 suggest that the combination of cancer immunotherapy and T-DM1 may have a greater benefit in HER2-positive EBC.

In TNBC, cancer immunotherapies have been broadly investigated and have shown benefit in the early-stage setting. In that context, the benefit of immune checkpoint inhibitors was seen regardless of PD-L1 status [22,23,29,30]. This contrasts with the treatment of TNBC and HER2-positive BC in the metastatic setting, where immune checkpoint inhibitors seem to benefit only those patients with PD-L1-positive tumors [28,31,32]. These findings support the evaluation of immune checkpoint inhibitors in patients with EBC regardless of PD-L1 status. They also support the use of longer-term outcomes, such as IDFS, over pCR in this setting.

Design

Study design

ASTEFANIA is a phase III, randomized, double-blind, multicenter study in patients with centrally confirmed HER2-positive EBC with residual invasive disease at surgery after neoadjuvant chemotherapy and HER2-targeted treatment. The study design is shown in Figure 2.

Eligibility criteria

Patients with high-risk, centrally confirmed HER2-positive EBC with residual invasive disease are eligible for this study; further inclusion and exclusion criteria can be found in Box 1. Tumor tissue samples are submitted for central

Box 1. Key inclusion and exclusion criteria.

Key inclusion criteria
- Histologically confirmed invasive breast carcinoma
- Centrally confirmed HER2-positive disease
- Centrally confirmed PD-L1 and hormone receptor status
- Clinical stage at presentation: cT4/any N/M0, any cT/N2–3/M0 or cT1–3/N0–1/M0 (patients with cT1mi/T1a/T1b/N0 are not eligible)†
- Patients with cT1-3/N0-1 disease at presentation must have pathologic evidence of residual invasive carcinoma in axillary lymph node(s) at surgery‡
- Patients with cT4/any N/M0 or cT1–3/N2–3/M0 disease at presentation must have pathologic evidence of residual invasive carcinoma in the breast and/or axillary lymph node(s) at surgery‡
- Completion of preoperative systemic chemotherapy including ≥9 weeks of taxane and 9 weeks of trastuzumab (anthracycline and/or additional HER2-targeted agents are permitted)
- ≤12 weeks between primary surgery and randomization
- ECOG PS 0 or 1
- Screening LVEF ≥50% with no decrease of >15% from prechemotherapy; if no prechemotherapy LVEF, screening LVEF ≥55%
- Life expectancy of ≥6 months
- Adequate hematologic and end-organ function

Key exclusion criteria
- Stage IV BC
- An overall response of disease progression according to the investigator at the conclusion of preoperative systemic therapy
- Prior treatment with T-DM1, atezolizumab or other immune checkpoint inhibitor(s)
- History of exposure to various cumulative doses of anthracyclines
- History of other malignancy <5 years prior to screening, except for appropriately treated carcinoma *in situ* of the cervix, non melanoma skin carcinoma, stage I uterine cancer or ductal carcinoma *in situ*
- Current grade ≥2 peripheral neuropathy
- History of idiopathic pulmonary fibrosis, organizing pneumonia or pneumonitis
- History of or active autoimmune disease or immune deficiency
- Treatment with immunostimulatory or immunosuppressive agents
- Cardiopulmonary dysfunction
- Any known active liver disease

†Clinical presentation according to American Joint Committee on Cancer Cancer Staging Manual, 8th Edition [33].
BC: Breast cancer; ECOG PS: Eastern Cooperative Oncology Group Performance Status; LVEF: Left ventricular ejection fraction.

confirmation of HER2-positive, and PD-L1 and hormone receptor status. HER2-positive status is defined by an immunohistochemistry score of 3+ and/or an *in situ* hybridization-positive status (ratio of ≥2.0 for the number of *HER2* gene copies to the number of chromosome 17 copies). PD-L1 is centrally confirmed using the VENTANA PD-L1 SP142 immunohistochemical assay (Ventana Medical Systems, Inc., AZ, USA), where PD-L1-positivity is defined as *PD-L1* expression on ≥1% of the tumor area of tumor-infiltrating immune cells (IC 1/2/3) and PD-L1-negativity as IC <1% (IC 0). Hormone receptor status will be centrally confirmed as estrogen receptor (ER)- or progesterone receptor (PgR)-positive, or ER- and PgR-negative.

Planned sample size

The target study sample size is 1700 patients across 304 sites in 32 countries, as shown in Figure 3.

Planned study period

The follow-up is planned for approximately 10 years from last patient in. The sample size of the study is primarily driven by the analysis of IDFS.

Study procedures

Within 12 weeks of primary surgery, patients will be randomized 1:1 to T-DM1 plus atezolizumab or T-DM1 plus placebo, using the following stratification factors: clinical stage at presentation (stage cT4/any N/M0 or any cT/N2–3/M0 vs stage cT1–3/N0–1/M0), hormone receptor status (ER- or PgR-positive vs ER-, PgR-negative,

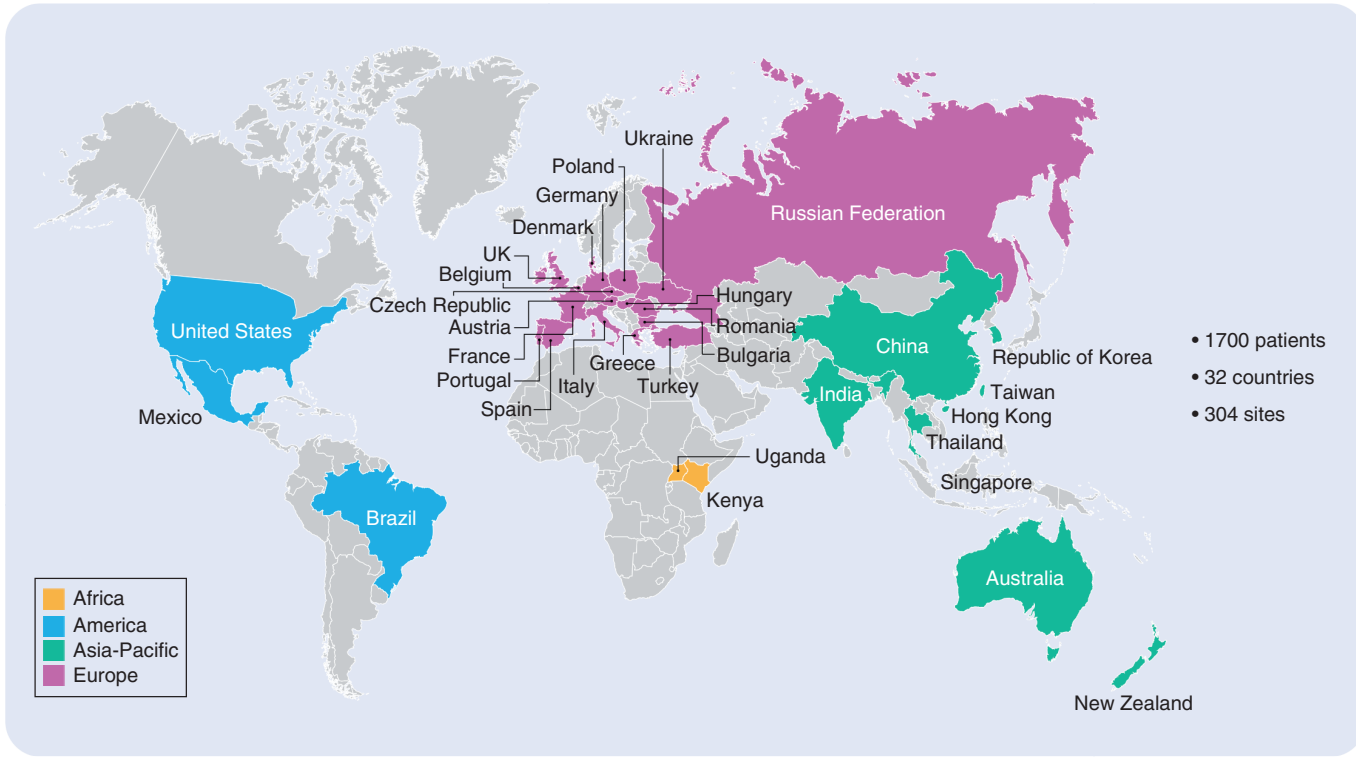


Figure 3. Study sites. ASTEFANIA's target sample size is 1700 patients across 304 sites in 32 countries [1].

Table 1. Study efficacy end points.

End points	Time frame
Primary	
IDFS, defined as the time from randomization to the date of the first occurrence of: ipsilateral invasive BC recurrence, ipsilateral locoregional invasive BC recurrence, contralateral invasive BC, distant recurrence, or death from any cause	From randomization up to 10 years post-LPI
Secondary	
IDFS including second primary non breast invasive cancer	From randomization up to 10 years post-LPI
DFS, including all IDFS events and contralateral or ipsilateral ductal carcinoma or second primary non breast invasive cancer	From randomization up to 10 years post-LPI
DRFI	From randomization up to 10 years post-LPI
OS	From randomization up to 10 years post-LPI
Proportion of patients with clinically meaningful deterioration in GHS/QoL, physical, role, and cognitive function scores, as assessed using the EORTC QLQ-C30	From baseline until 2 years after study treatment completion/discontinuation visit (~3 years)
Mean absolute scores in GHS/QoL, physical, role and cognitive function, as assessed using the EORTC QLQ-C30 and mean change from baseline scores in GHS/QoL, physical, role and cognitive function, as assessed using the EORTC QLQ-C30	From baseline until 2 years after study treatment completion/discontinuation visit (~3 years)

BC: Breast cancer; DFS: Disease-free survival; DRFI: Distant recurrence-free interval; EORTC QLQ-C30: European Organization for Research and Treatment of Cancer Quality of Life of Cancer Patients Questionnaire; GHS/QoL: Global Health Status/Quality of Life; IDFS: Invasive disease-free survival; LPI: Last patient in; OS: Overall survival.

and unknown), preoperative HER2-targeted therapy (trastuzumab vs trastuzumab plus additional HER2-directed agents), and PD-L1 status (IC 0 vs IC 1/2/3).

Patients in the respective arms will receive T-DM1 3.6 mg/kg via intravenous (iv.) infusion every 3 weeks plus atezolizumab or placebo 1200 mg iv. every 3 weeks for 14 cycles. Patients will receive an iv. infusion of atezolizumab/placebo prior to the iv. infusion of T-DM1 on day 1 of each 21-day cycle. Trastuzumab will be used to complete 14 cycles of study treatment if T-DM1 is discontinued for toxicity not considered to be related to the trastuzumab component of T-DM1.

Table 2. Study immunogenicity end points.

End points	Time frame
C_{max} and C_{min} for atezolizumab	Day 1 of cycles 1 and 4 pre-infusion, day 1 of cycles 1 and 4 after 30 min postinfusion, day 1 of cycles 2, 3 and 8 pre-infusion (cycle = 21 days) and at study treatment completion/ discontinuation visit (~11 months after cycle 1 day 1)
C_{max} and C_{min} for T-DM1	Day 1 of cycles 1 and 4 pre-infusion, day 1 of cycles 1 and 4 after 30 min postinfusion, (cycle = 21 days) and at study treatment completion/ discontinuation visit (~11 months after cycle 1 day 1)
C_{max} for DM1	Day 1 of cycles 1 and 4 pre-infusion, day 1 of cycles 1 and 4 after 30 min postinfusion, (cycle = 21 days)
C_{max} and C_{min} for total trastuzumab	Day 1 of cycles 1 and 4 pre-infusion, day 1 of cycles 1 and 4 after 30 min postinfusion, (cycle = 21 days) and at study treatment completion/ discontinuation visit (~11 months after cycle 1 day 1)
Percentage of patients with ADAs to atezolizumab	Day 1 of cycles 1 and 4 pre-infusion, day 1 of cycles 2, 3 and 8 (cycle = 21 days) and at study treatment completion/ discontinuation visit (~11 months after cycle 1 day 1)
Percentage of patients with ADAs to T-DM1	Day 1 of cycles 1 and 4 pre-infusion, and at study treatment completion/discontinuation visit (~11 months after cycle 1 day 1)

ADA: Antidrug antibody; C_{max}: Maximum serum concentration; C_{min}: Minimum serum concentration; DM1: Mertansine; T-DM1: Ado-trastuzumab emtansine.

Outcome measures/end points

The efficacy and immunogenicity end points, with assessment schedule, are listed in Tables 1 & 2, respectively. The safety end points include incidence and severity of adverse events, with severity determined according to the National Cancer Institutes Common Terminology Criteria for Adverse Events Version 5.0 (NCI-CTCAE v5.0), from baseline up to 10 years from last patient in.

Statistics

The log-rank test will be used to compare IDFS between the treatment arms, according to the protocol defined stratification factors. The Cox proportional hazards model, stratified by the protocol-defined stratification factors, will be used to estimate the HR between the two treatment arms and its 95% CI.

Conclusion

The combination of HER2-targeted therapy and cancer immunotherapy, such as T-DM1 plus atezolizumab, may offer increased potency to the immune response in HER2-positive EBC. ASTEFANIA aims to evaluate the efficacy of T-DM1 plus atezolizumab in patients with high-risk, HER2-positive EBC with residual disease after neoadjuvant HER2-targeted therapy plus chemotherapy.

Executive summary

HER2-positive breast cancer

- HER2-positive breast cancer (BC) accounts for 15–20% of BC.
- Ado-trastuzumab emtansine (T-DM1) is an antibody–drug conjugate that consists of trastuzumab linked to a cytotoxic agent, mertansine (DM1). T-DM1 binds to HER2 receptors and induces antitumor activity, which kills cancer cells, causing the release of cancer cell antigens, in turn causing an immune system response that results in enhanced T-cell activation.
- KATHERINE (NCT01772472) studied adjuvant T-DM1 in patients with HER2-positive primary BC who had residual tumors; subgroup analyses established an unmet need in patients with inoperable disease, or with operable, hormone-receptor negative and node-positive disease.

Cancer immunotherapy plus HER2-targeted therapies

- Compared with early breast cancer (EBC), metastatic BC typically involves a weakened immune system; therefore, cancer immunotherapy potentially offers greater potency in EBC.
- The immune checkpoint programmed death-ligand 1 (PD-L1) is expressed on cancer cells binding to T cells and in turn prevents T-cell activity.
- Atezolizumab is a cancer immunotherapy that inhibits PD-L1 and therefore prevents PD-L1 from binding to T cells and allows T-cell activation.
- Preclinical models of the combination of T-DM1 and cancer immunotherapies have shown a strong antitumor effect.

ASTEFANIA study design

- ASTEFANIA is a phase III study evaluating the efficacy and safety of adjuvant atezolizumab or placebo and T-DM1 for patients with HER2-positive EBC at high risk of recurrence following preoperative therapy, sponsored by F Hoffmann-La Roche Ltd (Basel, Switzerland).
- Eligible patients will receive T-DM1 3.6 mg/kg via intravenous infusion every 3-weeks, plus atezolizumab or placebo 1200 mg intravenously every 3 weeks for 14 cycles.

- A total of 1700 patients across 304 sites in 32 countries across Africa, America, Asia-Pacific and Europe will be enrolled.

ASTEFANIA study end points

- The primary end point is invasive disease-free survival (DFS), defined as time from randomization to the date of the first occurrence of ipsilateral invasive breast tumor recurrence, ipsilateral locoregional invasive BC recurrence, contralateral invasive breast cancer, distant recurrence or death from any cause.
- The secondary end points include invasive DFS (including second primary non breast invasive cancer), DFS, distant recurrence-free interval, overall survival, safety, patient-reported outcomes, pharmacokinetics and immunogenicity.

Author contributions

SA Hurvitz, T Bachelot, N Harbeck, YH Park, A Prat, T Boulet, N Gochitashvili, E Monturus, C Lambertini, B Nyawira, A Knott, G Bianchini, E Restuccia and P Schmid contributed to conceptualization. S Loi, YH Park, T Boulet, N Gochitashvili, E Monturus, C Lambertini, B Nyawira, A Knott, G Bianchini, E Restuccia and L Gilham contributed to methodology. SA Hurvitz, N Harbeck, S Loi and P Schmid contributed to investigation and supervision. SA Hurvitz, A Knott, E Restuccia and P Schmid contributed to resources. All authors contributed to manuscript review and revisions and all provided final approval of the manuscript. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Ethical conduct of research

This study is being conducted in full conformance with the ICH E6 guideline for GCP and the principles of the Declaration of Helsinki, or the applicable laws and regulations of the country in which the research is conducted; whichever affords the greater

protection to the individual. The study complies with the requirements of the ICH E2A guideline (Clinical Safety Data Management: Definitions and Standards for Expedited Reporting). Studies conducted in the United States or under a US Investigational New Drug Application comply with US FDA regulations and applicable local, state and federal laws. Studies conducted in the EU or European Economic Area comply with the EU Clinical Trial Directive (2001/20/EC) and applicable local, regional and national laws.

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