SOLTI-1805 TOT-HER3 Study Concept: A Window-of-Opportunity Trial of Patritumab Deruxtecan, a HER3 Directed Antibody Drug Conjugate, in Patients With Early Breast Cancer

**Background:** Preclinical data support a key role for the human epidermal growth factor receptor 3 (HER3) pathway in hormone receptor (HR)–positive breast cancer. Recently, new HER3 directed antibody drug conjugates have shown activity in breast cancer. Given the need to better understand the molecular biology, tumor microenvironment, and mechanisms of drug resistance in breast cancer, we designed this window-of-opportunity study with the HER3 directed antibody drug conjugate patritumab deruxtecan (HER3-DXd; U3-1402).

**Trial Design:** Based on these data, a prospective, multicenter, single-arm, window-of-opportunity study was designed to evaluate the biological effect of patritumab deruxtecan in the treatment of naïve patients with HR-positive/HER2-negative early breast cancer whose primary tumors are ≥1 cm by ultrasound evaluation. Patients will be enrolled in four cohorts.
INTRODUCTION

HER3, encoded by the ERBB3 gene, is broadly expressed in various types of human cancer. HER3 has been associated with poor patient outcomes (1) and therapeutic agent resistance, including resistance to anti-EGFR, anti-HER2 inhibitors (2), and endocrine therapy (3, 4). HER3 belongs to the type I transmembrane tyrosine kinase family of receptors and activates intracellular signaling pathways, mainly the PI3K/AKT and MAPK/ERK pathways, upon dimerization with other HER family members (2, 5). These observations have resulted in the development of investigational HER3 directed agents in HER3-expressing breast cancer and other solid tumors.

Patritumab deruxtecan (HER3-DXd; U3-1402), a potential first-in-class HER3 directed antibody drug conjugate (ADC), is currently under development to act on these previously mentioned targets (6). In addition to its antitumor efficacy by binding HER3 ligand and the release of the cytotoxic payload in the tumor cells (7), patritumab deruxtecan enhanced the infiltration of innate and adaptive immune cells in preclinical models (8). These preclinical data have shown that patritumab deruxtecan can elicit potent antitumor immunity even in the setting of tumors insensitive to PD-1 and PD-L1 immune checkpoint inhibitors and that its efficacy is more pronounced in the presence of PD-1 inhibition, suggesting that patritumab deruxtecan sensitizes insensitive tumors to PD-1 blockade and has synergistic effects (8).

In the clinical setting, an early report of a clinical trial suggested that patritumab deruxtecan could be safely administered and it demonstrated promising antitumor efficacy (the overall response and the disease control rate were 42.9 and 90.5%, respectively) in heavily pretreated HER3-expressing metastatic breast cancer (9); these results are in accordance with more recent preliminary data from heavily pretreated EGFR-mutated non-small cell lung carcinoma patients, in whom the overall response rate was 25%, and the disease control rate was 70% (10).

Although no validated HER3 assay has been established to date, recent studies support the role of HER3 immunohistochemistry (IHC) as a potential biomarker (11–13). However, there are important limitations with IHC-based assays, such as different sensitivities of the antibodies used, their low dynamic range, their subjectivity in scoring, and their difficulty in establishing suitable cut-offs. Therefore, clinical implementation of a robust genomic assay would represent an important advancement. To overcome these limitations, we plan to test the prospective use of an mRNA-based ERBB3 expression assay using the nCounter platform (Nanostring Technologies, Seattle, USA) developed by our group (14).

The role of the host immune system in breast cancer is becoming an important topic to study for several reasons. First, the immune response has a fundamental role in the efficacy of drug therapy. In all breast cancer subtypes, baseline high TIL grade is associated with a significantly higher pCR rate after neoadjuvant chemotherapy (15). Second, the recent success of therapeutic agents capable of activating immune responses to cancer, such as anti-PD1/PDL1 or anti-CTLA4 inhibitors, allows innovative treatment strategies (16). Third, high tumor-infiltrating lymphocytes (TILs) counts and immune-related gene expression signatures in the primary tumor are consistently associated with better survival in triple-negative breast cancer and HER2-positive breast cancer (15, 17–19). On the other hand, the prognostic value of assessing TILs in HR-positive/HER2-negative breast cancer remains unclear according to a few studies (15, 20).

The TOT-HER3 (a window-of-opportunity study of patritumab deruxtecan, a HER3 directed ADC in operable breast cancer according to ERBB3 expression) trial is designed to assess whether a single dose of patritumab deruxtecan can increase immune infiltration and the lysis of tumor cells during short-term preoperative treatment in hormone receptor (HR)-positive/HER2-negative primary breast cancer. Short-term preoperative studies are a validated strategy for evaluating the impact of targeted therapies using the decrease in tumor cellularity and the increase in immune infiltration as a surrogate endpoint of treatment benefit (21, 22). The primary endpoint of TOT-HER3 is changes in the CelTIL score, a novel combined biomarker based on stromal TILs and tumor cellularity. Access to tumor tissue before and after the investigational treatment enables comprehensive analysis of biomarker changes, thus providing critical insights into the optimal patient population, biomarker predictive value, and potential mechanisms of primary resistance (23, 24).

METHODS

Study Design and Treatment

This is a prospective, multicenter, single-arm, window-of-opportunity study evaluating the biological effect of patritumab deruxtecan in treatment naïve patients with early breast cancer, whose primary tumors are ≥1 cm by ultrasound evaluation (Figure 1). The study will include up to 80 patients with HR-positive/HER2-negative tumors.
TABLE 1 | Main/key eligibility criteria.

| Inclusion Criteria                                                                 | Exclusion criteria                                                                                     |
|----------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------|
| 1. Written informed consent form.                                               | 1. Inoperable locally advanced or inflammatory (i.e., inoperable stage III) breast cancer.           |
| 2. Premenopausal or post-menopausal women and men, age ≥ 18 years.               | 2. Bilateral invasive breast cancer.                                                                   |
| 3. ECOG Performance Status 0–1.                                                  | 3. Patients in whom a primary tumor excisional biopsy was performed.                                  |
| 4. Histologically confirmed non-metastatic primary invasive adenocarcinoma of     | 4. Any prior treatment for primary actual invasive breast cancer.                                      |
| the breast untreated and recently diagnosed, with all the following              | 5. Prior treatment with a HER2 antibody, topoisomerase I inhibitor, with an ADC that consists of an    |
| characteristics:                                                                  | exatecan derivative that is a topoisomerase I inhibitor (e.g., DS-8201) and with a govitecan         |
| - At least one lesion that can be measured in at least 1 dimension with ≥ 1 cm   | derivative (e.g., IMMU-132).                                                                       |
| in the largest diameter measured by ultrasound.                                 | 6. Medical history of symptomatic congestive heart failure or serious cardiac arrhythmia requiring    |
| - Absence of distant metastasis (M0) as determined by institutional practice.  | treatment; myocardial infarction within 6 months prior to enrolment or unstable angina.             |
| - In the case of a multifocal or multicentric tumor, the largest lesion must be  | 7. QT interval corrected using Fridericia’s formula to > 450 ms in males and > 470 ms in females.     |
| ≥ 1 cm and designated the “target” lesion for all subsequent tumor evaluations  | 8. Any factor that increases the risk of corrected QT interval prolongation or risk of arrhythmic     |
| and biopsies.                                                                    | events, such as congenital long QT syndrome, family history of long QT syndrome, or unexplained       |
| 5. Patient must have biopsyable disease.                                         | sudden death under 40 years of age in first-degree relatives.                                         |
| 6. Estrogen (ER)-positive and/or Progesterone (PgR)-positive and HER2-negative    | 9. Medical history of clinically significant lung diseases or who are suspected to have these diseases  |
| tumor by the most recent American Society of Clinical Oncology—College of        | by imaging at the screening period.                                                                    |
| American Pathologists (ASCO-CAP) guidelines: ER and PgR defined as IHC nuclear   | 10. Clinically significant corneal disease.                                                          |
| staining >1% and HER2 negative locally assessed.                                 | 11. Known hypersensitivity to either the drug substance components or inactive ingredients in the      |
| 7. Ki67% ≥ 10% locally assessed.                                                 | drug product or history of severe hypersensitivity reactions to other monoclonal antibodies.        |
| 8. Available pretreatment FFPE core needle biopsy evaluable for PAM50 and ERBB3   | 12. Clinically severe pulmonary compromise resulting from intercurrent pulmonary illnesses including, |
| mRNA expression.                                                                 | but not limited to, any underlying pulmonary disorder and any autoimmune, connective tissue, or      |
| 9. Baseline LVEF ≥ 50%                                                          | inflammatory disorders with potential pulmonary involvement or prior pneumonectomy.                  |
| 10. Adequate organ functions                                                      |                                                                                                       |
| 11. 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.                                                |                                                                                                       |

Adult female patients (≥18 years old) with pre/post-menopausal status will be eligible if they have not been previously treated and have histologically confirmed stage I–IIIA invasive breast cancer, with primary tumors equal to or larger than 1 cm in diameter (as measured by ultrasound), clinical nodal status of 0–2, HR-positive and HER2-negative according to ASCO/CAP guidelines, and Ki67% ≥ 10% determined locally. Patients should also have an Eastern Cooperative Oncology Group (ECOG) performance status of 0–1 and adequate hematological counts, hepatic and renal function, and left ventricular ejection fraction ≥ 50%. Patients will be excluded if they have received prior anticancer therapy.

Detailed inclusion and exclusion criteria can be found in Table 1.

All patients will undergo pretreatment tumor tissue acquisition. Central determination of ERBB3 mRNA expression will be performed in FFPE core biopsies, and patients will be enrolled in four cohorts, according to the expression of ERBB3 based in quartiles and defined by the pre-specified cutoffs, to ensure a broad representation of HR-positive/HER2-negative tumors with different ERBB3 expression. The number of slots available per cohort will be limited to 20 patients each. After confirmation of all the eligibility criteria, patients will be enrolled, and a single dose of patritumab deruxtecan will be
administered by intravenous infusion at a dose of 6.4 mg/kg. A second optional biopsy will be performed in the same lesion 3–7 days after patritumab deruxtecan’s administration. A third biopsy post-treatment of the same lesion will be mandatory 21 (±3) days after the administration of patritumab deruxtecan, independently of the subsequent treatment. Thereafter, patients will be considered either for definitive surgery or primary medical treatment (e.g., neoadjuvant chemotherapy) at the discretion of the treating physician.

**Primary Endpoint—The CelTIL Score**

To answer the primary objective of the trial, we will evaluate CelTIL score differences between baseline and post-treatment samples in all patients regardless of their ERBB3 mRNA expression. The CelTIL score is based on the percentage (%) of tumor cellularity and the % of stromal TILs. Histopathologic analysis of the proportion of TILs will be done in whole sections of tumor tissue stained with hematoxylin and eosin (H&E). TILs will be quantified according to the 2014 guidelines developed by the International TILs Working Group (25). Percentages of TILs and tumor cellularity at baseline and D21 will be scored in slides of core biopsies from patients enrolled in the trial blinded from clinic–pathologic and outcome data.

The CelTIL score was developed based on day 15 tumor samples from the PAMELA trial (22). The neoadjuvant PAMELA trial treated 151 HER2+ breast cancer patients with trastuzumab-lapatinib (and endocrine therapy if HR-positive) (26). Tumor cellularity and the TILs score measured at day 15 following anti-Her2 therapy was associated with pathologic complete response (pCR). A combined score, CelTIL, considering both variables was derived: CelTIL score = −0.8 × tumor cellularity (in %) + 1.3 × TILs (in %). The CelTIL score was validated in the PAMELA (26) and LPT109096 (27) phase II neoadjuvant trials as an early readout of the probability of a pCR. High CelTIL scores identify tumors that have high immune infiltration and reduced tumor cellularity (22).

In a third study, the CelTIL score was performed in tumor samples of 196 patients with early-stage HER2-positive disease treated with standard trastuzumab-based chemotherapy from the NeoALTTO phase III trial (28). This study randomized 455 women with HER2-positive early breast cancer to lapatinib (Arm A), trastuzumab (Arm B), or trastuzumab and lapatinib (Arm C) for 6 weeks, followed by an assigned anti-HER2 treatment combined with paclitaxel weekly. The CelTIL score was independently associated with event free survival, overall survival, and pCR (29). Early and absolute changes in the CelTIL score following neoadjuvant therapy were associated with tumor shrinkage at surgery in other three neoadjuvant trials (30). Taken together, these results demonstrated that high TILs and low tumor cellularity following one cycle of treatment provided

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**TABLE 2 | Primary and secondary objectives and endpoints.**

| Primary objective | Primary endpoint |
|-------------------|------------------|
| To evaluate if one dose of U3-1402 increases the value of the CelTIL score between baseline and post-treatment samples in all included patients with early breast cancer. | Mean change in the CelTIL score per central assessment in paired samples after one dose of U3-1402 at C1D21 (±3). CelTIL score = −0.8 × tumor cellularity (in %) + 1.3 × TILs (in %). The minimum and maximum unscaled CelTIL scores will be −80 and 130. This unscaled CelTIL score will then be scaled to reflect a range from 0 to 100 points. |

| Secondary objectives | Secondary endpoints |
|----------------------|---------------------|
| To identify a significant increase in the CelTIL score after one dose of U3-1402 between baseline and post-treatment samples within each of the four ERBB3 cohorts. | Mean change in the CelTIL score at C1D21 of treatment in paired samples in ultralow, low, medium, and high ERBB3 cohorts. |
| To determine the association of the levels of baseline ERBB3 expression with changes in the CelTIL score after one dose of U3-1402 in all patients and within each ERBB3 cohort. | Correlation between ERBB3 mRNA baseline levels and changes in the CelTIL score at C1D21 in paired samples in all patients and in ultralow, low, medium, and high ERBB3 cohorts. |
| To determine the association of HER3 IHC expression with changes in the CelTIL score after a single dose of U3-1402 in all patients and within each ERBB3 cohort. | Correlation between HER3 IHC levels per central assessment and changes in the CelTIL score at C1D21 in paired samples in all patients and in ultralow, low, medium, and high ERBB3 cohorts. |
| To evaluate the changes in CelTIL across the four PAM50 intrinsic subtypes. | CelTIL score at the C1D21 score according to intrinsic subtype: Luminal A, Luminal B, HER2-enriched, and Basal-like subtypes. |
| To evaluate the antiproliferative activity of one dose of U3-1402 between baseline and post-treatment samples. | Complete Cell Cycle Arrest (CCCA) determined per central assessment by IHC Ki67<2.7% at C1D21. Differences in differential expression \[\text{mean suppression} = 100–[\text{geometric mean (post-treatment/pre-treatment 100)}]\] of proliferative genes \([\text{BIRC5, CCNB1, CDC20, CDC21, CEP55, KNTC2, MKI67, PTTG1, RRM2, TYMS, and UBE2C}]\). |
| To evaluate the association of ERBB3 mRNA expression with HER3 IHC expression. | Correlation coefficients between both biomarkers. |
| To evaluate the changes of HER3 expression. | HER3 IHC at baseline, at D3-D7 (optional), C1D21. |
| To describe the safety and tolerability of U3-1402. | Type, incidence, severity (as graded by the NCI CTCAE v. 5.0), seriousness, and attribution to the study medications of AEs and any laboratory abnormalities. |
independent and additional predictive information in patients with primary breast cancer following neoadjuvant treatment, also suggesting that CelTIL could be a surrogate for treatment efficacy in the neoadjuvant setting.

Secondary endpoints, summarized in Table 2, include mean change in the CelTIL score in ultralow, low, medium, and high ERBB3 cohorts, correlation between ERBB3 mRNA and HER3 IHC baseline levels and changes in the CelTIL score, the CelTIL score according to PAM50 intrinsic subtype, antiproliferative activity, and safety. Exploratory and translational research endpoints include the assessment of predictive and prognostic biomarkers.

**FIGURE 2** | Measurement of ERBB3 expression in breast cancer using the nCounter platform. (A) Box plots of ERBB3 gene expression in breast tumors as classified by hormone receptor and HER2 expression and intrinsic subtype. (B) Unsupervised hierarchical clustering using the 50 PAM50 genes and ERBB3 (rows) and 1,580 tumor samples (columns). Each colored square on the heatmap represents the relative median signature score for each sample with the highest expression being red, the lowest expression being green, and the average expression being black. (C) Pearson correlation between ERBB3, single genes, and PAM50 gene expression signatures evaluated in breast cancer samples.

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**Table 2**

| Endpoint Description | Endpoint Details |
|---------------------|------------------|
| Mean change in CelTIL score | Ultralow, low, medium, high ERBB3 cohorts |
| Correlation between ERBB3 mRNA and HER3 IHC baseline levels and changes in the CelTIL score | |
FIGURE 3 | Comparing ERBB3 expression across datasets | (A) Evaluation of ERBB3 cutoff in breast cancer samples from patients with early breast cancer included in IN-HOUSE, METABRIC, and TCGA. (B) Proportion of samples in each immunohistochemistry subtype based on the ERBB3 cohort. Each bar is colored according to the ERBB3 distribution in each cohort. (C) Correlation coefficients of proportions of tumor samples within each quartile between the three datasets. (D) Scatter plots of ERBB3 vs. ESR1 expression for samples from METABRIC, IN-HOUSE, and TCGA cohorts, colored by subtype. The three horizontal lines indicate the cutoffs of each cohort. Discontinued line in each figure represents the regression line. Pearson correlation coefficient (r) with significance (p-value) is presented in each figure.
Measuring ERBB3 mRNA

Each patient will be assigned to one of the four cohorts according to their ERBB3 mRNA expression in the baseline sample determined by the nCounter Platform. The cutoffs to be used in this trial were determined as follows.

To date, we have analyzed ERBB3 mRNA using the nCounter platform in 1,600 tumor samples using formalin-fixed paraffin-embedded tumor samples with IHC data. Among these samples with IHC data, 65% were HR-positive and 18% were HER2-positive. The IHC subtype distribution is as follows: (1) 51.9% HR-positive/HER2-negative, (2) 29.9% triple-negative breast cancer (TNBC), (3) 13.5% HR-positive/HER2-positive, and (4) 4.7% HR-negative/HER2-positive.

In this nCounter dataset, the range of ERBB3 mRNA expression has an 18.6-fold difference in gene expression (i.e., from the lowest to the highest ERBB3 value), and the interquartile range is 1.5 (in log base 2), which is equal to a difference in expression of 2.9-fold.

Large expression variability across and within each IHC-based and PAM50 subtype was observed. ERBB3 expression was statistically significantly higher in HR-positive tumors (P < 0.001; Figure 2A). ERBB3 expression varied statistically significantly according to the intrinsic subtype (P < 0.001; Figure 2A), with the Luminal A subtypes showing the highest median expression, followed by the Luminal B, HER2-enriched, and Basal-like.

Using quartiles, the proportion of ERBB3-high tumors within each IHC subtype ranged from 4% in TNBC to 36% in HR+HER2-negative when percentile 75th in the combined matrix was used as the cutoff to define ERBB3-high (Figure 2A).

Next, we explored the association of ERBB3 expression with PAM50 breast cancer-related genes in the combined matrix (Figure 2B). As expected, ERBB3 high correlated [correlation coefficients (r) > 0.50] with a group of five genes, including ESR1 and FOXA1, which are significantly enriched in luminal and hormone response biology. Concordant with this single-gene analysis, moderate correlation (r = 0.53) was found between ERBB3 and PAM50 Luminal A signature and negative correlation (r = −0.25) between ERBB3 and PAM50 Basal-like, proliferation, and risk of recurrence signatures (Figure 2C).

Evaluating ERBB3 Expression in Independent Datasets

In order to examine the consistency of the cutoff points, results from the in-house nCounter dataset were compared to two independent cohorts (i.e., METABRIC and TCGA datasets). METABRIC includes 1,992 breast cancer samples analyzed by the Illumina HT 12 IDATS platform, and TCGA includes 1,101 breast cancer samples analyzed by HiSeq Illumina sequencers (Figure 3A).

Using quartiles, Figure 3B shows the proportion of tumors within each quartile based on their IHC subtype between our in-house dataset, METABRIC, and TCGA. Figure 3C shows the correlation coefficients among the three datasets in the different IHC-group tumors. In HR-positive/HER2-negative, the correlation coefficients of the proportions between the three datasets were remarkably similar. In the other subtypes, the correlation coefficients among the datasets were between 0.49 and 0.99. A relationship between ERBB3 and ESR1 expression was seen to be moderately correlated across the three datasets (Figure 3D); the correlation coefficients among the datasets were between 0.51 and 0.59.

Statistical Analysis

The study would require a sample size of 72 (number of pairs) to achieve a power of 80% using a level of significance of 5% (two sided), for detecting a mean difference between pairs of 13 CellTIL score. It is assumed that the standard deviation of the differences is 38.6, which is the standard deviation observed in 403 patients with CellTIL data across the four SOLTI trials (30). Assuming a 10% drop-out or lack of available tissue, 80 patients will be recruited.

No formal statistical comparison will be carried out between cohorts. Statistical analyses will be performed to estimate the proportions or means (or medians) for all variables including confidence interval calculations.

CONCLUSION

We propose the TOT-HER3 study, the first window of opportunity trial to evaluate the biological effect of patritumab deruxtecan in patients with HR-positive/HER2-negative early breast cancer. High ERBB3 mRNA gene expression is observed across all subtypes of breast cancer, although it predominates in HR-positive/HER2-negative disease suggesting a role for HER3 directed therapies in this disease. We will analyze ERBB3 expression using a clinically applicable assay in FFPE primary tumors.

This information can provide insight for improving the design of future clinical trials in the HR-positive/HER2-negative breast cancer through the selection of patients who will mostly benefit from this drug. The use of a quantitative method such as ERBB3 mRNA expression, which offers the opportunity to identify different cutoffs, might potentially improve treatment personalization. In addition, the results of TOT-HER3 could help identify patients most likely to benefit from HER3 directed ADCs across cancer types.

DATA AVAILABILITY STATEMENT

Data from Breast tumor samples with available RNASEqV2 data at the TCGA portal was downloaded. Metabric expression data are available at the European Genome-Phenome Archive (https://ega-archive.org/), which is hosted by the European Bioinformatics Institute, under accession number EGAS00000000083. The rest of the data are available upon reasonable request.
AUTHOR CONTRIBUTIONS

All authors participated in the design and/or interpretation of the reported results and participated in the acquisition and/or analysis of data. In addition, all authors participated in drafting and/or revising the manuscript and provided administrative, technical, or supervisory support.

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