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Rational and trial design of FASCINATE-N: a prospective, randomized, precision-based umbrella trial

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Abstract

Background: With our growing insight into the molecular heterogeneity and biological characteristics of breast cancer, individualized treatment is the future of cancer treatment. In this prospective Fudan University Shanghai Cancer Center Breast Cancer Precision Platform Series study – neoadjuvant therapy (FASCINATE-N) trial, we classify breast cancer patients using multiomic characteristics into different subtypes to evaluate the efficacy of precision-based targeted therapies compared to standard neoadjuvant chemotherapy.

Methods and design: The FASCINATE-N trial is a prospective, randomized, precision-based umbrella trial that plans to enroll 716 women with early breast cancer. After enrollment, patients will first be divided into three groups: hormone receptor (HR)+/human epidermal growth factor receptor 2 (HER2)–, HER2+, and HR–/HER2–. The HR+/HER2– patients are further stratified using fusion and clustering of similarity network fusion (SNF) algorithm into four subtypes; HER2+ patients are divided into HR+/HER2+ and HR–/HER2+ subtypes; and HR–/HER2– patients are stratified using the Fudan University Shanghai Cancer Center classification. For the assignment of drugs to patients, Bayesian methods of adaptive randomization will be used. The primary endpoint is pathological complete response rate; secondary endpoints include 3-year invasive disease-free survival, overall response rate, and toxicities according to common terminology criteria for adverse events (CTCAE) scale version 4.0 and the ratio of patients with complete cell cycle arrest (Ki67 $<$ 2.7%) in HR+/HER2+ breast cancer.

Discussion: The goal of our trial is to test the efficacy of our subtyping-based treatment in a neoadjuvant setting and to conduct a pilot study into the efficacy of targeted therapies within each precision-based subtype. The precision-based treatment arm can be updated with the refinement of our subtyping method, the discovery of new targets, and the development of novel targeted drugs. Our trial offers a unique opportunity to provide patients with individualized neoadjuvant therapy and test promising novel treatments that may further benefit patients.

Trial registration: ClinicalTrials.gov identifier: NCT05582499 (https://classic.clinicaltrials.gov/ct2/show/NCT05582499).

Plain language summary

Rational and trial design of FASCINATE-N (Fudan University Shanghai Cancer Center Breast Cancer Precision Platform Series study- neoadjuvant therapy): a prospective, randomized, precision-based umbrella trial

Our FASCINATE-N trial is a prospective, randomized, precision-based umbrella trial that plans to enroll 716 women with early breast cancer. We will first divide patients

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into three groups: hormone receptor (HR)+/human epidermal growth factor receptor 2 (HER2)–, HER2+, and HR–/HER2–. Then, we will further classify patients using multiomic characteristics into different subtypes to evaluate the efficacy of precision-based targeted therapies compared to standard neoadjuvant chemotherapy. The goal of our trial is to test the efficacy of our subtyping-based treatment in a neoadjuvant setting and to conduct a pilot study into the efficacy of targeted therapies within each precision-based subtype. The precision-based treatment arm can be updated with the refinement of our subtyping method, the discovery of new targets and the development of novel targeted drugs. Our trial offers a unique opportunity to provide patients with individualized neoadjuvant therapy and test promising novel treatments that may further benefit patients.

Keywords: breast cancer, neoadjuvant treatment, precision-based subtype, precision-based treatment, umbrella trial

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Background

Breast cancer has the highest incidence among female patients worldwide and greatly endangers women's health. According to the World Health Organization and GLOBOCAN, in 2020, there were more than 2.26 million new cases of breast cancer cases, ranking first among female malignant tumors, with over 684,000 cancer deaths.[1,2] Breast cancer is a highly heterogeneous disease, where even tumors with the same histological morphology have different molecular biological behaviors.[3,4] Based on gene expression profiles and molecular biological characteristics, breast cancer is divided into four subtypes: luminal A, luminal B, HER2− enriched, and triple-negative type,[3,5] and current clinical guidelines recommend the treatment of breast cancer according to molecular subtypes.[6]

Neoadjuvant therapy refers to the systemic treatment of breast cancer prior to definitive surgical therapy to reduce tumor burden and improve overall survival.[6,7] It was originally used for its impact on surgery, downstaging tumors, and allowing breast-conserving surgery rather than mastectomy. Neoadjuvant therapy also reduces the activity of tumor cells and the possibility of intraoperative metastasis and is equivalent to the *in vivo* experiment of systemic treatment to observe the effectiveness of therapeutic agents for individual patients, allowing for treatment adjustments so that a more effective regimen can be adopted after surgical treatment. In recent years, great progress has been made in neoadjuvant therapy research, with novel drugs, expanding indications, and continuous improvement in individualized treatment. Neoadjuvant therapy has also been utilized as an *in vivo* model to test novel antitumor drugs, provide more accurate efficacy data, and speed drug approval. However, there are still many unmet needs in the field of neoadjuvant treatment. Current clinical trials mainly attempt to increase the pathological complete response (pCR) rate by adding additional treatment based on standard chemotherapy. While this logic is widely accepted, the question remains as to whether it is beneficial for the overall population. For example, in the TRAIN-2 trial, nine cycles of paclitaxel, carboplatin, trastuzumab, and pertuzumab were used for the neoadjuvant treatment of HER2−-positive patients to achieve a pCR rate of 68%.[8,9] In subtypes that already have a relatively high pCR rate, the number of patients that benefit from additional treatment is limited and would result in the 'overtreatment' of the remaining patients. Although we do not approve of excessive treatment in our pursuit of a higher pCR rate, we lack reliable methods to screen for patients who may benefit from additional therapy. Clinicopathological characteristics do not offer enough accuracy for screening and fail to offer personalized response prediction. Meanwhile, in trials such as PHERGAIN and ADAPT HER2+HR−[10–12] that attempt to de-escalate treatment, there was not a clear means of handpicking patients aside from lower tumor burden and early clinical stage and cannot predict the biological characteristics of the tumor. Better

prediction of tumor response to neoadjuvant chemotherapy is needed to direct the escalation or de-escalation of neoadjuvant strategies. For example, in WSG-ADAPT,[13,14] attempts were made to establish early predictive surrogate markers (e.g. Oncotype-DX and Ki-67) for therapy response under a short induction treatment to maximally individualize therapy and avoid unnecessary toxicity by ineffective treatment. Similar attempts have been made in other trials such as POETIC and GeparTrio with limited degrees of success.[15–17]

Currently, basing patient selection criteria on the traditional four molecular subtypes is becoming increasingly inefficient. With our growing insight into the molecular heterogeneity and biological characteristics of breast cancer, individualized treatment is the future of cancer treatment, and a neoadjuvant setting may be ideal in our exploration to uncover patients who may most benefit from precision treatment. While numerous novel oncology drugs are being proposed, it would be difficult to make any breakthrough if we are to attempt treatment without screening with predictive biomarkers, and yet if we were to narrow our sights to individual biomarkers, even ones with a relatively high rate of gene mutations such as *PI3KCA* or *BRCA*, it would be difficult to gather a sufficient patient sample. Our proposed solution for this problem is to classify patients into treatment-relevant subgroups rather than relying on any one biomarker. Using this method, we can screen for likely patients and maintain an adequate patient pool at the same time. Our previous work in the molecular subtyping of triple-negative breast cancer (TNBC) and HR+/HER2− breast cancer has set the groundwork for the exploration of precision medicine in the neoadjuvant setting.

Objectives

In this FASCINATE-N trial, we integrate findings from our laboratory and previous clinical trials to classify patients by their multiomic characteristics into different subtypes according to genetic aberrations and expression signatures. We refer to the I-SPY2 trial and its unique approach toward the rapid, focused clinical development of paired oncologic therapies and biomarkers.[18–20] Through this method, we hope to uncover predictive and prognostic biomarkers that may direct us in choosing the most suitable treatment regimen for each patient, and explore the future paradigm for using molecular classification to guide the development of precision treatment and clinical practice.

Design and methods

FASCINATE-N (protocol version 1.0, August 12th, 2022) is a prospective, platform research addressing the individualization of neoadjuvant decision-making in early breast cancer patients based on clinical subtype. We expect refinement of subtyping methods and treatment arms may evolve following the update of basic translational research, the discovery of new targets, and the development of novel targeted drugs. Each subtrial will utilize the subtype-specific treatment to establish individualized therapy approaches and assess early therapy response.

The trial plans to enroll 716 women with early primary breast cancer who will receive neoadjuvant treatment according to their tumor subtype and randomization results. Patients treated at Fudan University Shanghai Cancer Center (FUSCC) whose diagnostic core biopsy shows a histologically confirmed unilateral primary invasive carcinoma of the breast and are recommended by their physician to undergo neoadjuvant therapy will be informed about the FASCINATE-N trial and asked to participate. Patients will be asked to sign informed consent forms for inclusion in the trial and blood and tissue sample donation. Only if both informed consent forms are obtained, and inclusion and exclusion criteria are not violated, will the patient be ready for trial registration. Patients who are not registered prior to any trial-related procedure cannot be accepted for the trial at a later time. The reporting of this study conforms to the CONSORT statement[21] (Supplemental Table 1).

Eligibility

Female patients with histologically confirmed unilateral primary invasive carcinoma of the breast aged 18–70 years are eligible if they are candidates for neoadjuvant chemotherapy with clinical stage T2-4, N1-3, and have no clinical evidence for distant metastasis. Patients are required to have at least one measurable lesion according to Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST v1.1). Estrogen receptor, progesterone receptor, and HER2 status must be known and measured by immunohistochemistry (IHC) and HER2 fluorescence *in situ* hybridization (FISH) when applicable. Patients must not be pregnant nor lactating,

fertile female subjects are required to use a medically approved contraceptive method for the duration of the study treatment and at least 3 months after the last use of the study drug. Patients are required to have the ability to understand and sign a written informed consent. Written informed consent must be obtained prior to any protocol-specific procedures and must be documented together with the expected cooperation and accessibility of the patients for the treatment and follow-up according to local regulatory requirements. Patients must also be able to tolerate the treatment, as indicated by normal laboratory values and proper organ function.

Patients must not have had previous cytotoxic chemotherapy, endocrine therapy, biological therapy, or radiotherapy for any reason. Patients with New York Heart Association grade II or above heart disease, severe systemic infections, or other serious diseases are excluded. The patient must not have a known allergy or hypersensitivity reaction to the study drug or incorporated substances used for treatment. Prior malignancy with disease-free survival of less than 5 years (except curatively treated cured cervical carcinoma *in situ* and non-melanoma skin) as well as patients of childbearing age who refuse to take appropriate contraceptive measures during the course of the study are not allowed. Concurrent treatment with other experimental drugs or participation in another interventional clinical trial within 30 days prior to trial entry is prohibited. Patients who are judged by the investigator to be of poor compliance or not able to consent will be excluded. The CONSORT flow diagram for enrollment, eligibility verification, treatment allocation, randomization, and analysis is shown in Figure 1.

Patient stratification

Standard biomarker signatures, HR status (+ or −) and HER2 status (+ or −), are used to first divide patients into three groups: HR+/HER2−, HER2+, and HR−/HER2−. HR+/HER2− patients are further stratified using fusion and clustering of similarity network fusion (SNF) algorithm into four subtypes: SNF1 (classic luminal type), SNF2 (immune-mediated type), SNF3 (proliferative type), and SNF4 (receptor tyrosine kinase-driven type). Our previous study has shown that each subtype has unique multiomics and clinicopathological features, which can be summarized as follows: SNF1 has a high rate of *PIK3CA* mutation, a low rate of *TP53* mutation, and is mainly PAM50 luminal A/luminal B subtypes; SNF2 has a high rate of *TP53* mutation, immune cell enrichment, high expression of immune checkpoint molecules, and contains more PAM50 HER2/Basal subtypes; SNF3 is dominated by PAM50 luminal B subtype, with more *CCND1*/*FGFR1*/*MDM2* copy number gains and cell cycle pathway activations; and SNF4 is shown to have the worst prognosis and high expression of signatures associated with receptor protein kinase and structural extracellular matrix.[22] HER2+ patients are divided into HR+/HER2+ and HR−/HER2+ according to HR status. HR−/HER2− patients are stratified using FUSCC classification based on immunohistochemical markers androgen receptor (AR), cluster of differentiation 8 (CD8), and Forkhead Box C1 (FOXC1) as IHC-based immunomodulatory (IHC-IM; AR− and CD8+) and IHC-based basal-like immune-suppressed (IHC-BLIS; AR−, CD8−, and FOXC1+).[23] HER2− low was defined as HER2 IHC (1+) and IHC (2+) with FISH (−); HER2− negative was defined as HER2 IHC (0).

Overall clinical trial design

The primary goal of our trial is to test the efficacy of our subtyping-based treatment in a neoadjuvant setting and to evaluate the efficacy of targeted therapies within subtypes. The overall trial design for FASCINATE-N is an umbrella that allows for randomization into either experimental or active comparator arm. The trial consists of three main cohorts: HR+/Her2−, HER2+, and HR−/HER2−. Each cohort is further stratified into different subtypes as described above, and patients of each subgroup are randomized at a 1:1 ratio into either the precision-based treatment group or the active comparator group. Randomization is done by the investigator through an interactive web response system. Patients and investigators are aware of the treatment group assignment. The minimum sample size for each precision-based treatment group is 20 patients. To obtain information about treatment effects as early as possible, relationships between pathologic complete response and baseline markers will be modeled, and outcomes assessed continually during the trial. Treatment

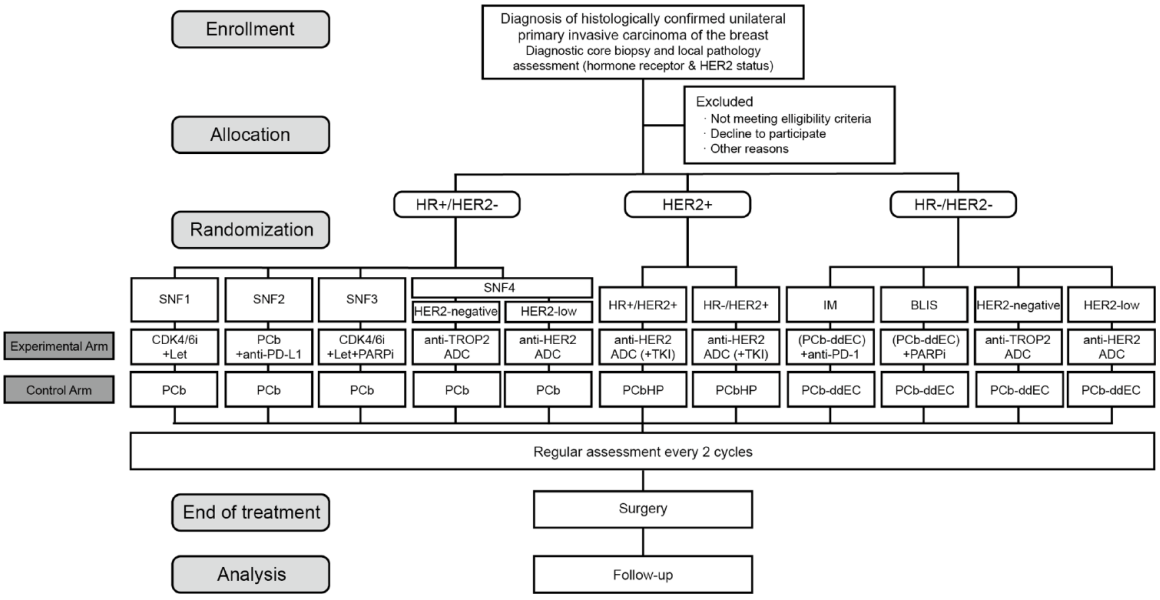

Figure 1. FASCINATE-N CONSORT flow diagram. Patients are allocated to one of three subgroups depending on HR and HER2 status. Randomization is always subject to the respective subgroups after allocation. The patient will be treated according to their disease. Follow-up is scheduled for 3 years following registration.
ADC, antibody–drug conjugate; anti-PD-1, anti-programmed death 1 antibody; anti-PD-L1, anti-programmed death ligand 1 antibody; CDK4/6i, cyclin-dependent kinases 4/6 inhibitor; HER2, human epidermal growth factor receptor 2; HR, hormone receptor; Let, letrozole; PARPi, poly adenosine diphosphate-ribose polymerase inhibitor; PCb, six cycles of nab-paclitaxel combined with carboplatin; PCb-ddEC, four cycles of nab-paclitaxel combined with carboplatin, followed by four cycles of dose-dense epirubicin combined with cyclophosphamide; PCbHP, six cycles of nab-paclitaxel combined with carboplatin, trastuzumab, and pertuzumab; SNF, similarity network fusion; TKI, tyrosine kinase inhibitor; TROP2, trophoblast cell-surface antigen 2.

dosage may be adjusted or even discontinued if intolerable toxicity or any other medically important events occur according to the investigator's clinical judgment. Patients are not allowed to undergo radiotherapy for breast cancer prior to curative surgery. Throughout the course of this study, the curative effect will be evaluated regularly according to the Bayesian monitoring method whenever a precision-based treatment group enrolls 20 patients.[24] Each drug's Bayesian predictive probability of being successful in a phase III confirmatory trial will be calculated for each treatment arm. Specifically, if the probability of a precision-based treatment arm achieving a higher pCR rate than its corresponding active comparator is greater than 95%, primary and secondary efficacy endpoints and safety endpoints will be analyzed and the treatment arm will graduate from the trial, allowing it to be tested in smaller phase III trials. The graduation of precision-based treatment will not affect the enrollment of the corresponding cohort. Meanwhile, if the probability of a precision-based treatment achieving a higher pCR rate than its corresponding active comparator is less than 5%, primary and secondary efficacy endpoints and safety endpoints will be analyzed and the treatment arm will be dropped from the trial for futility. New drugs will be added to the precision treatment groups after feasibility evaluation, as previous drugs are graduated or dropped, and the trial will be modified accordingly.

Based on the statistical models, each drug will be tested on a minimum of 20 patients and a maximum of 120 patients. Following the initial core biopsy, blood sample draw, and relevant imaging exams to determine biomarker signature and eligibility, patients will be stratified according to breast cancer subtype and randomized. After two cycles of the assigned treatment, patients will undergo a repeat core biopsy and tumor response assessment. The patient will be assessed according to RECIST v1.1 criteria every two cycles until the completion of neoadjuvant treatment, and a blood sample draw will be performed prior

to surgery. Tumor tissue will be collected at the surgery to assess whether the patient has a pathologic complete response. This is the primary trial endpoint but patients will also be followed for disease-free survival and overall survival.

Endpoints and sample size estimation

The primary endpoint of the study is the pCR rate. Sample sizes will be calculated based on each of the three cohorts, taking two-sided $\alpha = 0.10$. In HR+/HER2−, the pCR rate of the active comparator group is set at 10%, the improvement of the pCR rate in the precision-based treatment group is supposed to be 10%, the power is set as 70%, and the dropout rate of each group 5%. The estimated sample size in each group is 118 patients, and a total of 236 patients are planned to be enrolled in the HR+/HER2− cohort. In HER2+ and HR−/HER2− breast cancer, the pCR rate of the active comparator group is set at 50%, the improvement of the pCR rate in the precision-based treatment group is supposed to be 15%, the power is set as 75%, and the dropout rate of each group 5%. The estimated sample size of each group is 120 patients, and a total of 240 patients are planned to be enrolled for both the HER2+ and HR−/HER2− cohorts.

Treatment

HR+/HER2− cohort

HR+/HER2− patients are divided into subgroups using SNF subtyping. Precision-based treatment is as follows: SNF1 receives an oral cyclin-dependent kinase (CDK) 4/6 inhibitor, dalpicilib 150 mg orally once a day from day 1 to day 21 in a 28-day per cycle, and letrozole 2.5 mg orally once a day daily with goserelin (for premenopausal patients) 3.6 mg i.m. every 4 weeks for six cycles. SNF2 receives an anti-programmed death ligand 1 (PD-L1) antibody, SHR-1316 (10 mg/m^2 i.v. once every 2 weeks), nab-paclitaxel (100 mg/m^2 i.v. day 1, day 8, and day 21 in a 28-day per cycle) combined with carboplatin (AUC 1.5 i.v. day 1, day 8, and day 21 in a 28-day per cycle) for six cycles. SNF3 receives a poly adenosine diphosphate-ribose polymerase (PARP) inhibitor, fluzoparib 50 mg orally twice a day daily, dalpicilib 150 mg orally once a day from day 1 to day 21 in a 28-day per cycle, and letrozole 2.5 mg orally once a day daily with goserelin (for premenopausal patients) 3.6 mg i.m. every 4 weeks for six cycles. HR+/HER2− negative SNF4 patients receive SHR-A1921, a trophoblast cell-surface antigen 2 (TROP2) antibody–drug conjugate (ADC) 3 mg/kg i.v. day 1 every 3 weeks for eight cycles; while HR+/HER2− low SNF4 patients receive an anti-HER2 ADC, SHR-A1811 4.8 mg/kg i.v. day 1 every 3 weeks for eight cycles. Active comparator for all subgroups is nab-paclitaxel (100 mg/m^2 i.v. day 1, day 8, and day 21 in a 28-day per cycle) combined with carboplatin (AUC 1.5 i.v. day 1, day 8, and day 21 in a 28-day per cycle) for six cycles.

HER2+ cohort

Patients with HER2+ disease are divided according to HR status. Precision-based treatment is SHR-A1811 4.8 mg/kg i.v. day 1 every 3 weeks for eight cycles with or without an irreversible dual pan-Erbb receptor tyrosine kinase inhibitor (TKI), pyrotinib 240 mg orally once a day daily. The active comparator is nab-paclitaxel (100 mg/m^2 i.v. day 1, day 8, and day 21 in a 28-day per cycle) combined with carboplatin (AUC 1.5 i.v. day 1, day 8, and day 21 in a 28-day per cycle), trastuzumab (initial dose 8 mg/kg, subsequent dose 6 mg/kg i.v. every 3 weeks), and pertuzumab (initial dose 840 mg, subsequent dose 420 mg i.v. every 3 weeks) for six cycles.

HR−/HER2− cohort

HR−/HER2− patients are divided into subgroups using the FUSCC classification. Precision-based treatment for the immunomodulatory (IM) subtype is the addition of an anti-programmed death-1 (PD-1) antibody, camrelizumab 200 mg i.v. once every 2 weeks to standard chemotherapy. Precision-based treatment for the basal-like immune suppressed (BLIS) subtype is the addition of fluzoparib 50 mg orally twice a day daily to standard chemotherapy. Precision-based treatment for HR−/HER2− low is SHR-A1811 4.8 mg/kg i.v. day 1 every 3 weeks for eight cycles, and SHR-A1921 3.0 mg/kg i.v. day 1 every 3 weeks for eight cycles for HR−/HER2− negative patients. Active comparator or standard chemotherapy for all subgroups is four cycles of weekly nab-paclitaxel (100 mg/m^2 i.v. day 1, day 8, and day 21 in a 28-day per cycle) combined with carboplatin (AUC 1.5 i.v. day 1, day 8, and day 21 in a 28-day per cycle) followed by four cycles of

dose-dense epirubicin (80–90 mg/m^2 i.v. every 2 weeks) combined with cyclophosphamide (500 mg/m^2 i.v. every 2 weeks).

Investigational drugs

Investigational drugs are chosen based on therapeutic targets uncovered by basic and translational research from our center. Candidate drugs are required to have been tested and found safe in at least one phase I clinical trial, and there should be evidence of its potential efficacy against breast cancer from preclinical or clinical studies. Investigational drugs are contributed by pharmaceutical companies.

Assessment

The primary endpoint of the pCR rate is defined as the disappearance of all invasive cancer in the breast after the completion of neoadjuvant chemotherapy. Secondary endpoints include 3-year invasive disease-free survival, overall response rate (ORR), toxicities according to common terminology criteria for adverse events scale version 4.0, and the ratio of patients with complete cell cycle arrest (Ki67 $<$ 2.7%) in the HR+/HER2+ breast cancer. Secondary endpoints regarding translational research will include tissue sample collection and peripheral blood collection.

Core biopsies, ultrasounds, and breast magnetic resonance imaging (MRI) are performed at baseline and after two cycles of therapy. Chest computed tomography (CT), brain MRI or CT, and emission computed tomography or positron emission tomography (PET) are conducted before treatment to rule out metastatic breast cancer. Subsequent imaging is performed at two-cycle intervals. In patients who consented to additional examinations, breast and axillary PET is conducted at baseline and after one cycle of treatment, and additional examinations are conducted if needed. Assessment of response is performed according to RECIST v1.1. Surgical specimens are analyzed for response by trained local pathologists. Next-generation sequencing panel of targeted genes is conducted on baseline tumor biopsy samples for biomarker analysis.

Follow-up

The timing of follow-up visits is based on the date of neoadjuvant treatment initiation and curative surgery. Pre-surgical follow-up visits are scheduled at the start of neoadjuvant treatment and after every two cycles of treatment for tumor response assessment. Post-surgical follow-up visits are scheduled at months 3, 9, 12, 15, 18, 24, 30, and 36 or until relapse to document event-free survival, overall survival, further therapies, toxicities, local relapse, second primary malignancy, and first treatment for metastatic or second primary breast cancer. Patients continue post-surgical follow-up and are followed for survival once a year thereafter. Patients who relapse or suffer from second primary malignancy will only be followed for survival.

Discussion

Currently, neoadjuvant therapy indications in early and locally advanced breast cancer are mainly based on molecular subtype and clinical-pathological factors. However, based on discoveries made in recent years, we know that merely dividing breast cancers into four molecular subtypes for treatment is no longer enough. Further classification and subtyping are required to increase treatment efficacy while minimizing the proportion of patients that receive 'unneeded' treatment or are 'undertreated'. The original aspiration of the FASCINATE-N trial was to prove that our novel subtyping model and precision-based treatment will allow us to increase the overall pCR rate of breast cancer, to do so without the overtreatment of patients who would not benefit from escalated therapy, and to find a method to effectively screen for patients who may avoid the toxicity of chemotherapy.

While there have been numerous methods of breast cancer classification in the past, there is no solid evidence to pronounce any one method as the golden standard.[25–27] Our subtyping model combines the classic molecular subtyping with multiomic data gathered from basic and translational studies to better classify patients into treatable subgroups. Further subtyping of HR+/HER2− breast cancer is based on research conducted by Jin *et al.*,[22] where a large-scale multiomics cohort of 579 HR+/HER2− breast cancer patients was established. Through integrative analysis of somatic copy number aberrations, somatic mutations, transcriptome, proteomics, metabolomics, and single-cell RNA sequencing data, four novel molecular subtypes were identified within HR+/HER2− breast cancer showing distinct biological and clinical features, which indicated subtype-specific therapeutic strategies.

For potential clinical translation, convolutional neural network models were developed through deep learning algorithms to discriminate these subtypes based on digital pathology. Their integrative molecular classification provided novel insights into the molecular heterogeneity of HR+/HER2− breast cancer and their findings are now being utilized and tested in our study. Further subtyping of TNBC is based on previous research by Jiang *et al.*,[28] which presented the multiomic profiling of 465 Chinese patients with TNBCs and provided the largest genomically characterized TNBC dataset to date. TNBCs were classified into four mRNA subtypes with distinct molecular features, genomic aberrations that drive each TNBC mRNA subtype were identified, and provided additional insights into TNBC heterogeneity and potential therapeutic options. Subsequently, a surrogate IHC-based classification method was devised as a simplified approach to classify TNBCs into molecular subtypes for clinical use, and this method was utilized in the previous FUTURE trial in metastatic breast cancer[29,30] and our current FASCINATE-N study. The phase Ib/II FUTURE trial confirmed the feasibility of a biopsy-mandated, subtyping-based, and genomic biomarker-guided therapy in heavily pretreated refractory metastatic TNBCs. The trial indicated for the first time the potential role of TNBC subtyping and genomic testing in targeted therapy of refractory metastatic TNBCs. Furthermore, the FUTURE trial demonstrated favorable outcomes, ORR and disease control rates of the 69 enrolled patients were 29.0% and 42.0%, respectively. The fact that the FUTURE trial was able to achieve a favorable efficacy despite the enrollment of heavily pretreated patients, suggests that the combination of TNBC subtyping and genomic sequencing during screening may greatly benefit the precision treatment of refractory metastatic TNBCs, and this is also what we hope to see in our FASCINATE-N trial. Currently, we classify HER2+ breast cancer subtypes by HR status to assign treatment arms but additional biomarkers may be included upon further discovery.

In clinical practice, more and more high-risk patients receive neoadjuvant therapy, and patients who achieve pCR after neoadjuvant therapy have a better survival prognosis. Although there have been many promising novel treatment targets and oncology drugs, the process of drug development and regulatory review remains time-consuming and expensive. Prior to our study, other studies such as the ADAPT[11,12] and I-SPY2[18,19,31,32] trials have pioneered the targeted treatment of breast cancer patients guided by biomarkers, greatly innovating the neoadjuvant treatment of patients. The ADAPT umbrella trial consists of dynamic testing of early therapy response and will recruit 4936 patients according to their respective breast cancer subtype in four distinct sub-trials focusing on the identification of early surrogate markers for therapy success in the neoadjuvant setting. The I-SPY2 trial was first presented as a unique approach toward the rapid, focused clinical development of paired oncologic therapies and biomarkers. It aimed to quickly determine the efficacy of new treatment methods and treatment combinations and to establish new imaging and molecular diagnosis and typing methods that can evaluate efficacy. The special feature of I-SPY2 was that it evaluates the effectiveness of the new therapy through an adaptive random method based on Bayesian theory and could promptly discover the efficacy of different subtypes of tumors on this new agent. The advantage of this type of research is that it reduces the scale of the trial and speeds up drug development, which is a model of step-up treatment. However, the trial also had its limitations, for example, there is controversy regarding whether paclitaxel was suitable for combination with the study's experimental agents. There is also concern that the intrinsic subtype of the control group and experimental group may not be balanced.

Our FASCINATE-N trial, performed in the neoadjuvant setting, focuses on women with early breast cancer identified at a stage when a cure is possible but neoadjuvant is recommended. The adaptive design approach not only provides a model for rapid assessment of novel phase II drugs and identification of effective drugs and drug combinations but also allows us to test the efficacy of our subtyping and determine which patients will benefit most from novel treatment regimes. While our statistical design is similar to the I-SPY2 trial, the basis of chemotherapy for our control group is nab-paclitaxel and carboplatin, so our experimental group is compared against a sufficiently powerful chemotherapy regimen. In addition, we conduct prospective multiomic analysis to ensure the balance of patient and tumor characteristics and guide us in an individualized approach to patient treatment. For example, the TNBC IHC-IM subtype exhibits an immune-inflamed phenotype characterized by the infiltration of CD8+ T cells into tumor

parenchyma, suggesting its susceptibleness to immune therapy. For HR+/HER2− SNF1 who are sensitive to endocrine therapy and may potentially omit chemotherapy, we test the effectiveness of neoadjuvant endocrine therapy against chemotherapy. The unique characteristics of each subtype offer potential targets for individualized treatments.

In this study, we sought to include promising treatment options for all three subgroups. In TNBC patients, previous results from IMpassion130 showed that the addition of atezolizumab to nab-paclitaxel prolonged progression-free survival among patients with metastatic TNBC in both the intention-to-treat population and the anti-PD-L1-positive subgroup.[33] In the neoadjuvant setting, the GeparNuevo trial showed that the addition of PD-L1 inhibitor durvalumab to neoadjuvant chemotherapy led to an increase in the pCR rate by 9% ($p=0.287$).[34] Whereas the KEYNOTE-522 trial showed that the addition of anti-PD-1 antibody pembrolizumab in neoadjuvant chemotherapy can significantly improve the pCR rate of TNBC.[35] In our study, we sought to further screen TNBC patients using our FUSCC classification so that we may select with greater accuracy the subpopulation of patients most likely to benefit from immunotherapy and achieve higher pCR rates, and to exempt patients who are unlikely to gain additional benefit from immunotherapy. The treatment efficacy of anti-HER2 treatment in HER2-low metastatic breast cancer was reported in the DESTINY-Breast04 trial,[36] trastuzumab deruxtecan resulted in significantly longer progression-free and overall survival than the physician's choice of chemotherapy, suggesting its potential to improve treatment outcome for patients historically categorized as 'HER2− negative' and providing us with a new direction in treatment research. The above results led us to incorporate immune checkpoint inhibition and anti-HER2 ADC in our precision-based treatment arms to better understand cancer cell-intrinsic and microenvironmental factors that may help in the selection of patients with the highest likelihood of benefit from these treatments. In HR+/HER2− breast cancer, multiple studies have shown that targeted therapies such as PARP inhibitors, CDK4/6, and immune checkpoint inhibitors can improve survival in HR+/HER2− breast cancer.[19,32] However, the promise of drug targets and the biological correlations between different biomarkers remains uncertain. The I-SPY2 trial has reported data on neoadjuvant immunotherapy for HR+/HER2− patients at high risk according to MammaPrint, the addition of pembrolizumab to chemotherapy achieved pCR rates of 30% *versus* 13% with standard chemotherapy.[19] Additional data from I-SPY2 showed that the addition of durvalumab and olaparib to paclitaxel improved pCR rate in HER2− negative patients as compared with standard chemotherapy, especially in a subset of high-risk HR+/HER2− (estimated pCR 22% with control *versus* 64% with durvalumab/olaparib).[32] Indicating that immunotherapy for high-risk luminal-like disease is worth exploring, and thus its incorporation into our treatment arms. At the same time, we have also included treatment arms aiming to explore the efficacy of anti-HER2 ADC in HR+/HER2− low subgroups. In HER2+ patients, our main research target is to assess anti-HER2 ADC's efficacy, especially when combined with TKI. A phase II clinical trial, NSABP Foundation Trial FB-10, studied the safety and tolerability of T-DM1 plus neratinib in patients with metastatic HER2-positive breast cancer and reported that responses occurred at all neratinib doses.[37] A similar strategy is also being tested in the DESTINY-Breast07 study, which will investigate the safety, tolerability, and antitumor activity of trastuzumab deruxtecan in combination with tucatinib in patients with HER2-positive metastatic breast cancer (NCT04538742).

As we have previously mentioned, this study evaluates the effectiveness of new therapy through an adaptive random method based on Bayesian theory. The advantage of this method is that patients can be assigned to the subgroup with the best efficacy according to the treatment outcome of previously enrolled patients, allowing beneficial subgroups to be found at an earlier timepoint, and for ineffective subgroups to be discarded at the earliest timepoint. Our trial offers a unique opportunity to provide patients with individualized therapy and test promising novel treatments that may further benefit patients. The primary goal of our trial is to confirm the accuracy of our subtyping method and to conduct a pilot study into the efficacy of each precision-based treatment arm. Future directions of our platform include the incorporation of artificial intelligence-based imaging systems as well as novel concepts and compounds so that we may continuously improve our precision treatment under this system. The precision-based treatment arm can be updated with the advance of basic translational

research in our center, especially the refinement of our subtyping method, the discovery of new targets, and the development of novel targeted drugs. The knowledge and information that we gain as the trial proceeds will guide us in our further research and allow us to fine-tune our subtyping schema and treatment arms to better benefit subsequent patients.

Declarations

Ethics approval and consent to participate

The FASCINATE-N trial is conducted in accordance with the Declaration of Helsinki, the Guideline for Good Clinical Practice of the International Conference on Harmonization, and all applicable Chinese laws and requirements. The trial was approved by the Ethics Committee of FUSCC on September 28th, 2022. This trial will be conducted under ethical, scientific, and medical standards that protect the rights and welfare of participants. Eligible individuals are provided with informed consent forms, all of which were approved by the Ethics Committee of FUSCC in the currently applicable version at the time of recruitment. Written informed consent was obtained from all patients.

Consent for publication

Informed consent has been obtained from the participants involved.

Author contributions

Wen-Jia Zuo: Data curation; Formal analysis; Investigation; Resources; Writing – original draft; Writing – review & editing.

Li Chen: Data curation; Formal analysis; Investigation; Resources; Writing – review & editing.

Yu Shen: Formal analysis; Methodology; Writing – review & editing.

Zhong-Hua Wang: Conceptualization; Investigation; Resources; Writing – review & editing.

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Competing interests

The authors declare that there is no conflict of interest.

Availability of data and materials

Data for the FASCINATE-N trial are not yet available. Upon completion of the trial, the data that support the findings of this study will be available from the corresponding author upon reasonable request. All data provided will be anonymized to respect the privacy of patients who have participated in the trial in line with applicable laws and regulations.

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Supplemental material

Supplemental material for this article is available online.

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