STUDY PROTOCOL

A Phase III, randomized, double blind (patient and assessor blinded), two armed, parallel, active controlled, non-inferiority clinical trial to compare therapeutic efficacy and safety of AryoTrust™ (produced by AryoGen Pharmed) and Herceptin® (the reference drug, produced by Roche Company) as control drug in treating HER2-positive Breast Cancer.

Name of Test Drug:	AryoTrust
Phase:	III
Methodology:	Randomized, two-armed, double-blind (patient and assessor blinded), parallel, active controlled, non-inferiority clinical trial
Sponsor:	AryoGen Pharmed No.140. Corner of Tajbakhsh Street 24th Kilometer of Tehran-Karaj Makhsous Road, Alborz, Iran.
Sponsor Representatives:	Dr. Araz Sabzevari Orchid Pharmed company CEO
NCT Number:	NCT03425656
Protocol Date	23 Dec 2017

Table Contents

Confidential Information

The confidential information in the following document is provided to you as an investigator, auditor, and sponsor for review by you, your staff and an ethics committee and food and drug administration. By accepting this document, you agree that the information contained herein will not be disclosed to others without written authorization from AryoGen Pharmed Co., except to the extent necessary to obtain informed consent from those persons to whom the products may be administered. By signing below, the investigator agrees to adhere to the protocol as outlined and agrees that any changes to the protocol must be approved by AryoGen Pharmed Co., prior to seeking approval from the Independent Ethics Committee (IEC).

This study will be conducted in accordance with Good Clinical Practices (GCPs), the Declaration of Helsinki, local, ethical, and legal requirements.

Chief Investigator: Dr. Seyed Reza Safaeinodehi, Tehran University of Medical Sciences

Signature**: …….**

Date**: ……/……. /……**

Sponsor Representative: Dr. Araz Sabzevari, Orchid Pharmed company CEO

Signature**: …….**

Date**: ……/……. /… ……**

CRO Trial Representative: Dr. Hamed Hosseini, Tehran University of Medical Sciences

Signature**: …….**

Date**: ……/……. /… ……**

□ **Confirmed**	□**Conditionally confirmed**	□ **Not Confirmed**
Comments:		

Date:

Abbreviations

Abbreviation	Stand for
IEC	Independent Ethics Committee
GCP	Good Clinical Practices
IRCT	Iranian Registry of Clinical Trial
CRF	Case Report Form
ICH	International Conference on Harmonization
TSC	Trial Steering Committee
HER2	Human epidermal growth factor receptor 2
FISH	Fluorescence In Situ Hybridization
IHC	Immunohistochemistry
AC	Adriamycin, cyclophosphamide
pCR	pathologic Complete Response
cCR	clinical Complete Response
cPR	clinical Partial Response
cNC	clinically No Change
cPD	clinical Progressive Disease
cOR	clinical Objective Response
SOPs	Standard Operating Procedures
ER	Estrogen Receptor
ECG	Electrocardiography
LVEF	Left Ventricular Ejection Fraction
AST	Aspartate Aminotransferase
ULN	Upper Limit of Normal
ICF	Informed Consent Form

SIL	Subject Identification Log
AE	Adverse Effect
CBC	Complete Blood Count
Plt	Platelet
CRO	Contract Research Organization
PI	Principal Investigator

Administrative information

Title

A Phase III, randomized, two-armed, patient-outcome assessor-data analyzer blinded, parallel active controlled non-Inferiority clinical trial study of AryoTrust (AryoGen Pharmed Trastuzumab) efficacy and safety in Human Epidermal Growth Factor Receptor 2–Positive breast cancer in comparison to Herceptin® (Genentech/Roche) control.

Trial registration

It is planned to register this protocol to Iranian Registry of Clinical Trial (IRCT) http://IRCT.ir

Registration code in IRCT: IRCT201606226135N7

Ethics committee code has been received in 1395/04/01 from Tehran University of Medical Sciences with the number of IR.TUMS.REC.1395.2730.

Ethics committee code has been received in 1395/04/27 from Isfahan University of Medical Sciences with the number of IR.MUI.REC.1395.4.041.

Clinical Trial Authorization (CTA) from Iran food and drug administration of Iran with the number of 665/65516 in 1395/04/19.

Protocol version

Version 4.3, Date: 2017/12/23

Funding

AryoTrust (AryoGen Pharmed) will be manufactured by AryoGen Pharmed Company and Herceptin® will be provided by AryoGen Pharmed Company. All trial costs will be provided by AryoGen Pharmed Company.

Roles and Responsibilities

Sponsor:

AryoGen Pharmed Company

Contact address: AryoGen Pharmed, No. 140, Corner of Tajbakhsh St., 24th Km Tehran-Karaj Makhsous Road, Alborz, Iran

Tel: 0098 26 36106480-4

Fax: 0098 26 36104644

Zip Code: 3164819711

Sponsor representative: Dr. Araz Sabzvari

Contact address: Orchid Pharmed company, No 2, Khorasani dead-end, Derakhti St., Dadman Blvd., Shahrak Gharb, Tehran, Iran.

Telephone: 00982143473000 Extension: 400

Role:

- Preparing final draft of study protocol
- Obtaining necessary approvals from external organizations for the conduct of study
- Providing standard operational procedures (SOP) for principle investigators in participating centers
- Providing quality controlled drugs to be used in the trial and delivery to participating centers
- Funding provision for all activities foreseen in study protocol via signing contracts with principal investigator
- Providing compensation for patients who may be adversely affected by participating in the trial
- Providing insurance coverage for all study participants
- Providing necessary training for staff
- Recruiting necessary workforce to conduct monitoring of the trial
- All expenses will be the responsibility of the sponsor.

- Audit of study sites to review the process of conducting the study and report the cases of violations from the protocol.

Representatives of the sponsor to conduct an audit to monitor the quality of the study:

Name: Dr. Somayeh Amini, Pharmacist, Medical Department Manager

Contact address: Orchid Pharmed company, No 2, Khorasani dead-end, Derakhti St., Dadman Blvd., Shahrak Gharb, Tehran, Iran.

Telephone: 00982143473000 Extension: 211

Signature:

Name: Dr. Nasim Anjidani, Pharmacist, Clinical Trial department Manager

Contact address: Orchid Pharmed company, 5th floor, No 74, Hafezi St., Farahzadi Blvd., Shahrak Gharb, Tehran, Iran.

Telephone: 00982143473000 Extension: 510

Signature:

Name: Dr. Sahar Pedram, Pharmacist, Clinical Research Associate

Contact address: Orchid Pharmed company, 5th floor, No 74, Hafezi St., Farahzadi Blvd., Shahrak Gharb, Tehran, Iran.

Telephone: 00982143473000 Extension: 530

Signature:

Name: Mrs. Elham Farhang, Clinical Research Coordinator

Contact address: Orchid Pharmed company, 5th floor, No 74, Hafezi St., Farahzadi Blvd., Shahrak Gharb, Tehran, Iran.

Telephone: 00982143473000

Roles:

- Visiting study sites to review the process of the protocol and ensure that the study is conducted in accordance with the protocol and GCP principles (at least one or two of these persons will attend each visit).
- Check the site's facilities for the performance and continuation of the clinical trials and take the necessary measures to provide them (to ensure that enough drugs are available to continue the study, to ensure that enough CRF and consent forms are met, and other requirements for the performance of the study).
- Persistent training of site personnel for protocol performance.
- Checking CRFs and matching them with source data and filling out the query for noncompliance and reminding them to correct them.
- Provide reports of non-compliances and facilities required by the site and submit it to the company's managers.

Representative of the sponsor for external monitoring on quality control of the study:

Name: CRO Trial

CRO representative: Dr. Hamed Hosseini, Tehran university of medical sciences

Contact address: Tehran university of medical sciences, Institute of Research and Research Centers, Keshavarz Blvd., North Kargar St., Tehran, Iran.

Telephone: 00982188963546

Signature:

CRO monitor: Dr. Ayat Ahmadi, Tehran university of medical sciences

Contact address: Tehran university of medical sciences, Institute of Research and Research Centers, Keshavarz Blvd., North Kargar St., Tehran, Iran.

Telephone: 00982188963546

Signature:

Roles:

- Preparing a randomization file

- Conducting a 70% monitoring in all of the sites participating in the study according to GCP Guidelines, 108 patients were diagnosed in patient recruitment centers.
- Supervision over the study and Data Management
- Performing Statistical analysis and Preparing or approving the project either in middle or the end of the study
- Cooperation with company to perform Monitoring visits
- Cooperation for answering the methodology queries
- Regular visiting of all patient sites of Tehran

Chief investigator:

Name: Dr. Seyed Reza Safaeinodehi

Contact Address: Imam Khomeini Hospital Complex, Tehran, IRAN.

Telephone: 00982166581593

Role:

- ✓ Conducting the study according to the agreed protocol
- ✓ Setting up a team for this purpose
- ✓ All clinical examinations
- ✓ Filling out the CRF
- ✓ Responsible for conducting trial according to ICH-GCP in sites.
- ✓ Organizing training events for their existing and newly recruited team members wherever necessary
- ✓ Provision of suitable storage and delivery for drugs used in the trial
- ✓ Cooperation with monitors during the conduct of the study
- ✓ No part of this trial could be published without the prior agreement with sponsor. (This agreement must be signed by PI and sponsor before the study initiation.)
- ✓ Responsible to provide training for staff at the study site
- ✓ Patients care
- ✓ Notification of AEs and ADRs to sponsor and regulatory according to the protocol schedule and regulatory requirements.

Signature:

Principal Investigators:

Roles:

- Conducting the study according to the agreed protocol
- Setting up a team for this purpose
- Organizing training events for their existing and newly recruited team members whenever necessary
- Provision of suitable venues for patients admission
- Provision of suitable storage for drugs used in the trial
- Cooperation with monitors during the conduct of the study
- All medical documents related to the trial should be sent to AryoGen Pharmed Company at the end of the trial. A copy may be kept by principle investigator
- No part of this trial could be published without the prior agreement of AryoGen Pharmed Company.

In all of these centers, patient recruitment is performed.

- **Emam Khomeini Hospital**

Principal Investigator:

- Dr. Farhad Shahi, Tehran University of Medical Sciences
- Dr. Mehrzad Mirzania, Tehran University of Medical Sciences
- Dr. Farahnaz Vafaeizadeh, Tehran University of Medical Sciences

Contact Address: Hematology and oncology center, Valiasr hospital, Imam Khomeini Therapeutic Complex, Keshavarz blvd., Tehran, Iran.

Telephone: 00982166581593

- **Mehrad Hospital**

Principal Investigator: Dr. Jahangir Rafaat, Tehran University of Medical Sciences.

Contact Address: Miremad St. Mottahari St., Tehran, Iran.

Telephone: 00982188747401

- **Masoud Clinic**

Principal Investigator: Dr. Masoud Iravani, Tehran University of Medical Science

Contact Address: No. 144., 19th St., after Jalal Al Ahmad Highway, North Kargar St., Tehran, Iran.

Telephone: 00982188336300

- **Jahad Daneshgahi Clinic**

Principal Investigator: Dr. Safa Najar Najafi, Tehran University of Medical Sciences.

Contact Address: No. 45., West Shahid Nazari St. Aboureyhan St., Enghelab St., Tehran, Iran.

Telephone: 00982166404050

- **Hazrate Rasoul Hospital**

Principal Investigator: Dr. Nafiseh Ansarinejad, Iran University of Medical Sciences

Contact Address: Niayesh St., Satarkhan St., Tehran, Iran.

Telephone: 00982166515001

- **Emam Reza Hospital**

Principal Investigator: Dr. Abolghasem Allahyari, Mashhad University of Medical Sciences.

Contact Address: Emam Reza square, Ebne Sina St., Mashhad, Khorasan Razavi.

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- **Dr. Mehrdad Payandeh Clinic**

Principal Investigator: Dr. Mehrdad Payandeh, Kermanshah University of Medical Sciences.

Contact Address: No 101, Fakhr Razi Alley, Shahrdari square, Modares St., Kermanshah, Iran.

Telephone: 00983837282400

- **Dr. Mohammadreza Mortazavi Zadeh Clinic**

Principal Investigator: Dr. Mohammadreza Mortazavi Zadeh, Yazd University of Medical Sciences.

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- **Shahid Faghihi Hospital**

Principal Investigator: Dr. Shirin Haghighatju, Shiraz University of Medical Sciences.

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Telephone: 00987132351087

- **Shafa Hospital**

Principal Investigator:

- Dr. Mehran Hosseinzadeh, Ahwaz University of Medical Sciences.

- Dr. Mohammad Segholeslami, Ahwaz University of Medical Sciences.

- Dr. Ahmad Ahmad Zadeh Deilami, Ahwaz University of Medical Sciences.

Contact Address: Golestan St., Ahwaz, Khoozestan, Iran.

Telephone: 00986133743281

- **Javad-al-aeme Hospital**

Principal Investigator: Dr. Vahid Moazed, Kerman University of Medical Sciences.

Contact Address: aboozar 25, Kerman, Kerman Province

Telephone: 00983432510025

- **Park Clinic**

Principal Investigator: Dr. Behjat Kalantari, Kerman University of Medical Sciences.

Contact Address: Sadi St, Kerman, Kerman Province

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- **Toos Hospital**

Principal Investigator:

- Dr. Saeed Salimi, Iran University of Medical Sciences, Iran University of Medical Sciences.

- Dr. Mohsen Razavi, Iran University of Medical Sciences, Iran University of Medical Sciences.

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- **Aliebne Abitaleb Hospital**

Principal Investigator: Dr. Seyed Mehdi Hashemi, Zahedan University of Medical Sciences.

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Telephone: 00985433295570

- **Tohid Hospital**

Principal Investigator: Dr. Bayazid Ghaderi, Sanandaj University of Medical Sciences.

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- **Baqiyatallah Hospital**

Principal Investigator: Dr.Mojtaba Qadyani, Shahid Beheshti University of Medical Sciences.

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- **Booali Hospital**

Principal Investigator:

- Dr. Arash Jenabian, Azad University of Medical Sciences.

- Dr. Somaye khamsi, Azad University of Medical Sciences.

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- **Sheikh Mofid Clinic**

Principal Investigator: Dr. Ali Hajigholami, Isfahan University of Medical Sciences.

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statistics advisers

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Name: Dr. Ali Akhlaghi

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Role: Monitor data entry into the database, analyzes the results.

Scientific steering committee

Name: Dr. Reza Safaeinodehi, Oncologist, Tehran University of Medical Sciences.

Contact address: Oncology and hematology center, Emam Khomeini hospital, Keshavarz BLVD., Tehran University of Medical Sciences, Tehran, Iran.

Telephone: 00982166581593

Name: Dr. Farhad Shahi, Oncologist, Tehran University of Medical Sciences.

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Telephone: 00982166581593

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Name: Dr. Hamed Hosseini, Tehran University of Medical Sciences.

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Telephone: 00982188963546

Representatives of the sponsor to attend the meeting (at least one of the following will attend each meeting):

Name: Dr. Somayeh Amini, Pharmacist, Medical Department Manager

Address: Orchid Pharmed company, No 2, Khorasani deadend, Derakhti St., Dadman blvd., Shahrak Gharb, Tehran, Iran.

Telephone: 00982143473000 Extension: 211

Signature:

Name: Dr. Nasim Anjidani, Pharmacist, Clinical Trial Department Manager

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Telephone: 00982143473000 Extension: 510

Signature:

Name: Dr. Sahar Pedram, Pharmacist, Clinical Research Associate

Address: Orchid Pharmed company, 5th floor, No 74, Hafezi St., Farahzadi blvd., Shahrak Gharb, Tehran, Iran.

Telephone: 00982143473000 Extension: 510

Signature:

Roles:

1- Establishing coordination among researchers in study centers.
2- Monitor and review the reports provided by the sponsor and CRO.
3- Signing of the protocol and protocol amendments and reports submitted to the Food and Drug Administration of Iran and ethics committees on behalf of other professors.
4- Adopting a proper decision to address the conditions in which the profit-risk balance for participants in the study, either individually or in general, conflicts. With regard to safety information, these decisions may include the identification of the relationship between the drug and the unwanted event observed, the adoption of appropriate strategies for the continuation of study and the departure of the person who is no longer eligible for the study.
5- Make the right decision to decode the drug.
6- Establishment of meetings every two to three months (Due to the low rate of patient recruitment, the frequency of the sessions will be every two to three months, and in case of any serious complications, especially suspected complications, these sessions will be held as soon as possible.).

Introduction

Background

Breast cancer is a disease in which certain cells in the breast become abnormal and multiply uncontrollably to form a tumor. Breast cancer is the second most commonly diagnosed cancer in women. About one in eight women in the United States will develop invasive breast cancer in her lifetime. Researchers estimate that more than 230,000 new cases of invasive breast cancer will be diagnosed in U.S. women in 2015[1].

Some genes and the proteins they make can influence how a breast cancer behaves and how it might respond to a specific treatment. Human epidermal growth factor receptor 2 (HER2) is one of such genes that can play a role in the development of breast cancer [2]. The HER2 gene expresses HER2 protein receptors which help to control how a healthy breast cell grows, divides, and repairs itself. This gene is over expressed in 25% to 30% of breast cancers, suggesting a role for overexpression in tumorigenesis. This overexpression is most commonly the result of gene amplification that happens when the HER2 gene doesn't work correctly and makes too many copies of itself. These extra HER2 genes tell breast cells to make too many HER2 receptors (HER2 protein overexpression). All these process make breast cells grow and divide in an uncontrolled way [3].

Breast cancers with HER2 gene amplification or HER2 protein overexpression are called HER2-positive in the pathology report. HER2-positive breast cancers tend to grow faster and are more likely to spread and relapse compared to HER2-negative breast cancers. Several lines of evidence support the role of HER2 overexpression in the pathogenesis and poor clinical outcome of human tumors. A number of these studies have shown that breast cancers that overexpress HER2 have a more aggressive course and higher relapse and mortality rates. HER2 overexpression in retrospective analyses of adjuvant studies was also associated with resistance to cyclophosphamide, methotrexate, and fluorouracil chemotherapy [4]. HER2 can be determined by any of several methods. The most commonly used methods are the Fluorescence in situ hybridization (FISH), which detects gene amplification by measuring the number of copies of the HER2 gene in the nuclei of tumor cells and Immunohistochemistry (IHC), which measures the number of HER2 receptors on the cell surface and therefore detects receptor overexpression [5]

Treatment

Several murine monoclonal antibodies against the extracellular domain of the HER2 protein were found to inhibit the proliferation of human cancer cells that overexpressed HER2, both in vitro and in vivo [6]. In the 1980s, a monoclonal antibody against HER-2 was developed. This antibody that

inhibited tumor growth, was called trastuzumab. In 1998, trastuzumab (Herceptin; Genentech Inc. South San Francisco, CA) as a humanized monoclonal antibody directed against the extracellular domain of HER2 was approved for the treatment of metastatic breast cancer [7]. In 2005, the results of five adjuvant trials evaluating trastuzumab, involving >10,000 women, were presented. Despite differences in study design and short follow-ups of only 1 to 2 years, these studies showed the same remarkable results, adjuvant trastuzumab therapy halves the recurrence rate and reduces mortality by 30% [8]. This monoclonal antibody as a single agent has modest antitumor activity [9] but it had synergistic effects when used in combination with cisplatin, carboplatin, docetaxel and ionizing radiation and had additive effects when used with doxorubicin, cyclophosphamide, and paclitaxel [7]. In phase III randomized trials, trastuzumab in combination with standard chemotherapy (paclitaxel, docetaxel or doxorubicin, and cyclophosphamide combinations) demonstrated improvement in time to progression, overall response, duration of response and a favorable impact on survival compared with the same chemotherapy alone as therapy for metastatic breast cancer overexpressing HER2 [10]. Women with breast cancers that overexpress HER2 are at greater risk for disease progression and death than women whose tumors do not overexpress HER2. Herceptin® (Genentech/Roche), is a proprietary name of trastuzumab which blocks downstream signaling of HER2 and substantially improves the efficacy of chemotherapy in women with metastatic and early-stage HER2-positive breast cancers. Although Cardiac dysfunction was observed in 27% of patients treated with trastuzumab and anthracycline-based combination therapy benefits seem to outweigh risks and the ensuing congestive heart failure is generally reversible [11]. For women with newly diagnosed breast cancer, a practical benefit of preoperative therapy is that it will downstage the primary tumor in most patients, allowing a higher rate of breast conservation. It also provides an in vivo assessment of tumor response to the particular drug regimen and, hence, an opportunity to optimize therapy. Furthermore, by using pathologic Complete Response (pCR) assessment, after preoperative therapy, an appropriate surrogate of long-term disease-free survival is available. It is hypothesized that a regimen that produces higher rates of pCR in the neoadjuvant treatment setting will also result in higher rates of long-term outcomes [4].

Trastuzumab common adverse events

- Flu- like symptoms like mild fever, chills, headache, limb pain, weakness and lethargy
- Skin rush
- Nausea, vomiting, diarrhea
- Decreased left ventricular ejection fraction (LVEF)
- Dyspnea, cough

- Infusion reactions

Rationale for the study

The enhanced antitumor activity of trastuzumab has been shown in several studies. Given the high prices of this antibody as a medicine in Iran, AryoGen Pharmed (Pharmaceutical Company in Tehran, Iran) decided to produce this drug in the country. We designed this study in which patients with HER2-positive locally advanced or inflammatory breast cancer will be randomly assigned to either Herceptin® (Genentech/Roche trastuzumab) or AryoTrust (AryoGen Pharmed trastuzumab). Both groups will receive 4 doses of Adriamycin, cyclophosphamide (AC) every 14 days, proceeded by 4 cycles of Docetaxel + trastuzumab every 21 days. The main goal of this study is to demonstrate that the AryoTrust (AryoGen Pharmed trastuzumab) is not inferior to Herceptin® (Genentech/Roche trastuzumab) in term of pCR rate. In addition, the efficacy characteristics, other than pCR, and safety outcomes of AryoTrust vs. Herceptin® after 4 cycles of infusion in breast cancer volunteers will be assessed.

Objectives

Main objective

- To verify the non-inferiority of AryoTrust (AryoGen Pharmed trastuzumab) vs. Herceptin® (Genentech/Roche trastuzumab), both given concomitantly with docetaxel after Adriamycin plus cyclophosphamide in the neoadjuvant setting according to pathological clinical response and immunogenicity assay in patients with Human Epidermal Growth Factor Receptor 2–Positive breast cancer.

Primary objective

- The primary objective of this study is to verify the non-inferiority of AryoTrust vs. Herceptin®, given concomitantly with docetaxel after Adriamycin plus cyclophosphamide in the neoadjuvant setting according to pathological complete response (pCR) rate in patients with Human Epidermal Growth Factor Receptor 2–Positive breast cancer.

Secondary objectives

- To evaluate non-significance between AryoTrust and Herceptin®, given concomitantly with docetaxel after Adriamycin plus cyclophosphamide in the neoadjuvant setting according to clinical objective response (cOR), clinical complete response (cCR), clinical partial response (cPR), clinically no change (cNC), clinical progressive disease (cPD), breast conservation rate.
- To evaluate the safety of AryoTrust vs. Herceptin®, given concomitantly with docetaxel after Adriamycin plus cyclophosphamide in the neoadjuvant setting in patients with Human Epidermal Growth Factor Receptor 2–Positive breast cancer.
- To assess the immunogenicity of AryoTrust vs. Herceptin®, given concomitantly with docetaxel after Adriamycin plus cyclophosphamide in the neoadjuvant setting in patients with Human Epidermal Growth Factor Receptor 2–Positive breast cancer.

Methods

Trial Design

This is a Phase III, randomized, two-armed, patient-outcome assessor-data analyzer blinded, parallel active controlled non-Inferiority clinical trial study with a 1:1 allocation.

Study setting

This trial will be initiated from July 2016 and 108 patients with breast cancer recruited from the following centers. All centers implement same protocol and procedures by using same SOPs which will be accompanied by training of personnel at the beginning, throughout and at the end of the trial. Regular and strict monitoring visits are provided to ensure all processes will be carried out in accordance with GCP. Probable variation of eligibility criteria and evaluation criteria will be resolved through investigator meetings.

Eligibility criteria

Inclusion criteria:

- 18-70 years old female patients
- Patients with newly diagnosed stage III (locally advanced) or inoperable stage II (due to sizes larger than 5 cm or high tumor to breast ratio) tumors are candidates for participation. (Appendix 2)
- Willing and able to sign an informed consent
- Pathological diagnosis of adenocarcinoma of the breast
- ECOG status of 0-1
- With any ER/PR status
- HER2 positive (Immunohistochemical (IHC) 3+ intensity, amplification of the HER2 gene on fluorescence in situ hybridization (FISH+) or HER2 positive results of Chromogenic in situ hybridization (CISH)).

NOTE: ductal carcinoma in situ (DCIS) components should not be considered in the determination of degree of IHC staining or FISH amplification.

Exclusion Criteria

- Clinical or radiologic evidence of metastatic disease
- History of any other malignancy including previous breast cancer, second non-breast malignant disease
- History of previous chemotherapy
- Left ventricular ejection fraction [LVEF] <55% confirmed by echocardiogram within 3 months before registration, Any prior myocardial infarction, History of documented congestive heart failure (CHF),Any prior history of arrhythmia or cardiac valvular

disease requiring medications or clinically significant, Current use of medications for treatment of angina pectoris, Current uncontrolled hypertension (diastolic>100 mmHg or systolic>200 mmHg), A severe conduction abnormality (having pacemaker or diagnosed by the ECG) and any other significant cardiovascular disease.

- Hematologic abnormalities including baseline Absolute Neutrophil Count (ANC) of ≤1,500/µL or platelet count ≤ 100,000/µL
- Liver dysfunction including: (baseline)
  - Alanine amino transferase (ALT) and/or aspartate amino transferase (AST) ≥ 3 Upper Limit Normal (ULN)
  - Alkaline phosphatase (ALP) ≥3 ULN
  - serum total bilirubin > 1.5 ULN
- Renal dysfunction, defined as serum creatinine ≥2.5 mg/dL
- Pregnant, lactating women or women of childbearing potential who are not willing to use adequate contraception

Intervention

Intervention group:

All patients are scheduled to receive AC-Docetaxel+Trastuzumab regimen as detailed bellow:

Doxorubicin + Cyclophosphamide phase:

Cycle length: 14 days. Total cycles: 4 cycles			
Drug	***Dose and route***	***Administration***	***Given on days***
Doxorubicin	60 mg/m^2 IV	Dilute with normal saline (NS) to a final concentration of 2 mg/mL and administered as an IV bolus over three to five minutes.	Day 1
Cyclophosphamide	600 mg/m^2 IV	Dilute with 250 to 500 mL NS or D5W and administer over 30 to 60 minutes.	Day 1

- If there is a change in body weight of at least 10 percent, dose should be recalculated for all drugs.

Pre-treatment considerations

- Hydration:

  Patients receiving cyclophosphamide should maintain adequate oral hydration (2 to 3 L/day during administration and for one to two days thereafter) and void frequently to reduce the risk of haemorrhagic cystitis.

- Emesis risk:

  HIGH (>90 percent frequency of emesis).

  Aprepitant 125 mg PO pre-chemotherapy on Day 1 and 80 mg PO post-chemotherapy once daily on Days 2 and 3

  Granisetron 1 mg or 0.01 mg/kg IV

  Dexamethasone 12 mg IV 8 mg oral or IV daily; days 2-3

- Vesicant/irritant properties:

  Doxorubicin is a vesicant and can cause significant tissue damage if an extravasation occurs. For peripheral infusions, the IV line should be recently placed into a large, intact vein, with good blood return established immediately prior to starting the infusion.

  The IV or catheter site should be continuously monitored throughout drug administration infusion. If extravasation occurs, apply ice to the site.

- Cardiac issues:

  A baseline assessment of LVEF is recommended, with periodic reassessment of during therapy. The risk of doxorubicin-associated cardiac dysfunction is related to cumulative dose.

Monitoring parameters

- CBC with differential and platelet count every two weeks prior to each treatment cycle.
- Serum electrolytes and liver and renal function tests every two weeks prior to each treatment cycle.

- During treatment with AC and Docetaxel, assess line site periodically during infusion of chemotherapy for signs and symptoms of extravasation.
- Myelotoxicity:

  Subsequent cycles should be delayed until the absolute neutrophil count is greater than 1000/microL and platelet count greater than 100,000/microL.

ANC ($\times 10^9$/L)		Platelets ($\times 10^9$/L)	Dose (both drugs)
≥ 1.5	And	≥ 90	100%
1.0-1.49	or	70-90	75%
< 1	or	< 70	delay

If there is more than a three-week delay in treatment, a dose reduction of 25 percent is recommended.

Dose adjustment for hepatic or renal dysfunction

Doxorubicin

Renal Impairment

CrCl <50 mL/minute: No dosage adjustment necessary.

Hemodialysis: Supplemental dose is not necessary.

Hepatic Impairment

The manufacturers' labeling recommends the following adjustments:

Serum bilirubin 1.2 to 3 mg/dL: Administer 50% of dose.

Serum bilirubin 3.1 to 5 mg/dL: Administer 25% of dose.

Severe hepatic impairment (Child-Pugh class C or bilirubin >5 mg/dL): Use is contraindicated.

ALT/AST		Bilirubin (micromole/L)	Dose
2-3 × ULN	Or	-	75%
>3 × ULN		20-50	50%
-		51-85	25%
-		> 85	Do not administer

Cyclophosphamide

Renal Impairment

Cl_{cr} ≥10 mL/minute: No dosage adjustment required.

Cl_{cr} <10 mL/minute: Administer 75% of normal dose.

Creatinine clearance (ml/min)	Cyclophosphamide dose
≥ 10	100%
< 10	75%

Hepatic Impairment

Serum bilirubin 3.1 to 5 mg/dL or transaminases >3 times ULN: Administer 75% of dose.

Serum bilirubin >5 mg/dL: Avoid use.

Docetaxel + AryoTrust phase:

Cycle length: 21 days. Total cycles: 4 cycles		
Drug	***Dose and route***	***Administration***
Docetaxel	100 mg/m^2 IV	Dilute in 250-500* mL NS or D5W Administer I.V. infusion over 1-hour through nonsorbing polyethylene lined (non-DEHP) tubing
AryoTrust	8 mg/kg IV loading dose (at cycle 1), followed by 6 mg/kg at subsequent cycles	IV infusion in 250ml NS over 90 minutes for first dose (observe for 1 hour after infusion) IV infusion in 250 mL NS over 1 hour on the second dose. Observe for 30 minutes post infusion • IV infusion in 250 mL NS over 30 min on all subsequent doses if no adverse reactions. Observe for 30 min post infusion **DO NOT** mix with D5W, and **DO NOT** infuse as an IV push or bolus.

* use 250 mL for doses 74-185 mg, use 500 mL for doses greater than 185 mg

- If there is a change in body weight of at least 10 percent, dose should be recalculated for all drugs.

Pretreatment considerations

- **Prophylaxis for infusion reactions:**

  Dexamethasone 8 mg PO bid for 3 days, starting one day prior to each Docetaxel administration. Patient must receive minimum of 3 doses pre-treatment. Additional antiemetic not usually required.

  Trastuzumab may also cause infusion reactions. These can be severe, though most are mild; they typically occur during or within 24 hours of the first infusion. The most common symptoms are fever, chills, nausea, headache, abdominal pain, and dyspnea; less often are vomiting, dizziness, rash, rhinitis, or hypotension. Most clinicians do not routinely premedicate prior to the first trastuzumab dose.

  NOTE: Trastuzumab infusion should be discontinued for anaphylaxis (bronchospasm, hypotension, angioedema).

- **Infection prophylaxis:**

  filgrastim (G-CSF) 5 mcg/kg/day Days 3 to 10 reduce filgrastim treatment duration if ANC greater than $10*10^{10}$ or intolerable bone pain.

- **Dose adjustment for baseline liver or renal dysfunction:**

  The available data suggest that disposition of trastuzumab is not altered based on serum creatinine (≤2 mg/dL).

- **Cardiac issues:**

  Trastuzumab is associated with cardiotoxicity, and reassessment of LVEF prior to therapy (after completion of AC) is indicated.

- **Pulmonary issues:**

  Trastuzumab has been associated with serious pulmonary toxicity (including acute respiratory distress syndrome [ARDS] and interstitial pneumonitis) and should be used with caution in patients with preexisting pulmonary disease.

Monitoring parameters

- CBC with differential and platelet count weekly during treatment with Docetaxel.
- Serum electrolytes and liver and renal function tests prior to each treatment cycle of Docetaxel.
- Assess changes in neurologic function prior to each treatment cycle of Docetaxel.
- Assess cardiac function prior to AC and prior to initiation of trastuzumab.
- Assess cardiac function prior to trastuzumab and after 4 cycles of trastuzumab.
- During treatment with docetaxel, assess line site periodically during infusion of chemotherapy for signs and symptoms of extravasation.

Suggested dose alterations for toxicity

- **Myelotoxicity:**

  Subsequent cycles should be delayed until the absolute neutrophil count is greater than 1000/microL and platelet count greater than 100,000/microL.

  If there is more than a three-week delay in treatment, a dose reduction of 25 percent is recommended.

ANC ($\times 10^9$/L)		Platelet ($\times 10^9$/L)	Dose (Docetaxel)	Dose after neutropenic sepsis on Docetaxel
≥ 1.5	and	>90	100%	75%
1.0-1.49	Or	70-90	75%	Delay
< 1	Or	<70	delay	Delay

• **Dose adjustment for hepatic or renal dysfunction:**

Docetaxel

Renal impairment

No dose modification is indicated

Hepatic impairment

Alkaline phosphatase		AST +/or ALT	dose
< 2.5 ULN	And	≤ 1.5 ULN	100%
2.5-5 ULN	And	1.6-6 ULN	75%
> 5 ULN	or	>5 ULN	delay

Trastuzumab

Renal Impairment

There are no dosage adjustments provided in the manufacturer's labeling, although data suggest that the disposition of trastuzumab is not altered based on serum creatinine (up to 2 mg/dL)

Hepatic Impairment

There are no dosage adjustments provided in the manufacturer's labeling.

• **Cardiotoxicity:**

Asymptomatic patients-Trastuzumab continuation based on serial LVEFs

Relationship of LVEF to LLN	Absolute Decrease of less than 10 points from baseline	Absolute Decrease of 10-15 points from baseline	Absolute Decrease of greater than or equal to 16 points from baseline
Within normal limits	Continue	Continue	Hold*
1-5 points below LLN	Continue	Hold*	Hold*
Greater than or equal to 6 points below LLN	Continue*	Hold*	Hold*

- * repeat LVEF assessment after 3-4 weeks, consider cardiac assessment
- If criteria for continuation are met-resume trastuzumab
- If 2 consecutive holds or a total of 3 holds occur, discontinue trastuzumab

Symptomatic patients:

- Symptomatic patients with evidence of cardiac dysfunction should have trastuzumab discontinued.

- **Pulmonary toxicity:**

  Discontinue trastuzumab if patients develop ARDS, interstitial pneumonitis, or pulmonary fibrosis.

Control Group:

All patients are scheduled to receive AC-Docetaxel+Trastuzumab regimen as detailed bellow:

Doxorubicin + Cyclophosphamide phase:

Cycle length: 14 days. Total cycles: 4 cycles			
Drug	***Dose and route***	***Administration***	***Given on days***
Doxorubicin	60 mg/m^2 IV	Dilute with normal saline (NS) to a final concentration of 2 mg/mL and administered as an IV bolus over three to five minutes .	Day 1
Cyclophosphamide	600 mg/m^2 IV	Dilute with 250 to 500 mL NS or D5W and administer over 30 to 60 minutes.	Day 1

- If there is a change in body weight of at least 10 percent, dose should be recalculated for all drugs.

Pre-treatment considerations

- Hydration:

  Patients receiving cyclophosphamide should maintain adequate oral hydration (2 to 3 L/day during administration and for one to two days thereafter) and void frequently to reduce the risk of haemorrhagic cystitis.

- Emesis risk:

  HIGH (>90 percent frequency of emesis).

  aprepitant 125 mg PO pre-chemotherapy on Day 1 and 80 mg PO post-chemotherapy once daily on Days 2 and 3

  Granisetron 1 mg or 0.01 mg/kg IV

  Dexamethasone 12 mg IV 8 mg oral or IV daily; days 2-3

- Vesicant/irritant properties:

  Doxorubicin is a vesicant and can cause significant tissue damage if an extravasation occurs. For peripheral infusions, the IV line should be recently placed into a large, intact vein, with good blood return established immediately prior to starting the infusion.

  The IV or catheter site should be continuously monitored throughout drug administration infusion. If extravasation occurs, apply ice to the site.

- Cardiac issues:

  A baseline assessment of LVEF is recommended, with periodic reassessment of during therapy. The risk of doxorubicin-associated cardiac dysfunction is related to cumulative dose.

Monitoring parameters

- CBC with differential and platelet count every two weeks prior to each treatment cycle.
- Serum electrolytes and liver and renal function tests every two weeks prior to each treatment cycle.
- During treatment with AC and Docetaxel, assess line site periodically during infusion of chemotherapy for signs and symptoms of extravasation.
- Myelotoxicity:

  Subsequent cycles should be delayed until the absolute neutrophil count is greater than 1000/microL and platelet count greater than 100,000/microL.

ANC ($\times 10^9$/L)		Platelets ($\times 10^9$/L)	Dose (both drugs)
≥ 1.5	And	≥ 90	100%
1.0-1.49	or	70-90	75%
< 1	or	< 70	delay

  If there is more than a three-week delay in treatment, a dose reduction of 25 percent is recommended.

Dose adjustment for hepatic or renal dysfunction

Doxorubicin

Renal Impairment

CrCl <50 mL/minute: No dosage adjustment necessary.

Hemodialysis: Supplemental dose is not necessary.

Hepatic Impairment

The manufacturers' labeling recommends the following adjustments:

Serum bilirubin 1.2 to 3 mg/dL: Administer 50% of dose.

Serum bilirubin 3.1 to 5 mg/dL: Administer 25% of dose.

Severe hepatic impairment (Child-Pugh class C or bilirubin >5 mg/dL): Use is contraindicated.

ALT/AST		Bilirubin (micromole/L)	Dose
2-3 × ULN	Or	-	75%
>3 × ULN		20-50	50%
-		51-85	25%
-		> 85	Do not administer

Cyclophosphamide

Renal Impairment

Cl_{cr} ≥10 mL/minute: No dosage adjustment required.

Cl_{cr} <10 mL/minute: Administer 75% of normal dose.

Creatinine clearance (ml/min)	Cyclophosphamide dose
≥ 10	100%
< 10	75%

Hepatic Impairment

Serum bilirubin 3.1 to 5 mg/dL or transaminases >3 times ULN: Administer 75% of dose.

Serum bilirubin >5 mg/mL: Avoid use.

Docetaxel + Herceptin® phase:

Cycle length: 21 days. Total cycles: 4 cycles		
Drug	***Dose and route***	***Administration***
Docetaxel	100 mg/m^2 IV	Dilute in 250-500* mL NS or D5W Administer I.V. infusion over 1-hour through nonsorbing polyethylene lined (non-DEHP) tubing
Herceptin®	8 mg/kg IV loading dose (at cycle 1), followed by 6 mg/kg at subsequent cycles	IV infusion in 250ml NS over 90 minutes for first dose (observe for 1 hour after infusion) IV infusion in 250 mL NS over 1 hour on the second dose. Observe for 30 minutes post infusion • IV infusion in 250 mL NS over 30 min on all subsequent doses if no adverse reactions. Observe for 30 min post infusion **DO NOT** mix with D5W, and **DO NOT** infuse as an IV push or bolus.

* use 250 mL for doses 74-185 mg, use 500 mL for doses greater than 185 mg

- If there is a change in body weight of at least 10 percent, dose should be recalculated for all drugs.

Pretreatment considerations

- **Prophylaxis for infusion reactions:**

  Dexamethasone 8 mg PO bid for 3 days, starting one day prior to each Docetaxel administration. Patient must receive minimum of 3 doses pre-treatment. Additional antiemetic not usually required.

  Trastuzumab may also cause infusion reactions. These can be severe, though most are mild; they typically occur during or within 24 hours of the first infusion. The most common symptoms are fever, chills, nausea, headache, abdominal pain, and dyspnea; less often are vomiting, dizziness, rash, rhinitis, or hypotension. Most clinicians do not routinely premedicate prior to the first trastuzumab dose.

  NOTE: Trastuzumab infusion should be discontinued for anaphylaxis (bronchospasm, hypotension, angioedema).

- **Infection prophylaxis:**

  filgrastim (G-CSF) 5 mcg/kg/day Days 3 to 10 reduce filgrastim treatment duration if ANC greater than 10 or intolerable bone pain

- **Dose adjustment for baseline liver or renal dysfunction:**

  The available data suggest that disposition of trastuzumab is not altered based on serum creatinine (≤2 mg/dL).

- **Cardiac issues:**

  Trastuzumab is associated with cardiotoxicity, and reassessment of LVEF prior to therapy (after completion of AC) is indicated.

- **Pulmonary issues:**

  Trastuzumab has been associated with serious pulmonary toxicity (including acute respiratory distress syndrome [ARDS] and interstitial pneumonitis) and should be used with caution in patients with preexisting pulmonary disease.

Monitoring parameters

- CBC with differential and platelet count weekly during treatment with Docetaxel.
- Serum electrolytes and liver and renal function tests prior to each treatment cycle of Docetaxel.
- Assess changes in neurologic function prior to each treatment cycle of Docetaxel.
- Assess cardiac function prior to AC and prior to initiation of trastuzumab.
- Assess cardiac function prior to trastuzumab and after 4 cycles of trastuzumab.
- During treatment with docetaxel, assess line site periodically during infusion of chemotherapy for signs and symptoms of extravasation.

Suggested dose alterations for toxicity

- **Myelotoxicity:**

  Subsequent cycles should be delayed until the absolute neutrophil count is greater than 1000/microL and platelet count greater than 100,000/microL.

  If there is more than a three-week delay in treatment, a dose reduction of 25 percent is recommended.

ANC (× 10^9/L)		Platelet (× 10^9/L)	Dose (Docetaxel)	Dose after neutropenic sepsis on Docetaxel
≥ 1.5	and	>90	100%	75%
1.0-1.49	Or	70-90	75%	Delay
< 1	Or	<70	delay	delay

Dose adjustment for hepatic or renal dysfunction

Docetaxel

Renal impairment

No dose modification is indicated

Hepatic impairment

Alkaline phosphatase		AST +/or ALT	dose
< 2.5 ULN	And	≤ 1.5 ULN	100%
2.5-5 ULN	And	1.6-6 ULN	75%

> 5 ULN	or	>5 ULN	delay

Trastuzumab

Renal Impairment

There are no dosage adjustments provided in the manufacturer's labeling, although data suggest that the disposition of trastuzumab is not altered based on serum creatinine (up to 2 mg/dL)

Hepatic Impairment

There are no dosage adjustments provided in the manufacturer's labeling.

• **Cardiotoxicity:**

Asymptomatic patients-Trastuzumab continuation based on serial LVEFs

Relationship of LVEF to LLN	Absolute Decrease of less than 10 points from baseline	Absolute Decrease of 10-15 points from baseline	Absolute Decrease of greater than or equal to 16 points from baseline
Within normal limits	Continue	Continue	Hold*
1-5 points below LLN	Continue	Hold*	Hold*
Greater than or equal to 6 points below LLN	Continue*	Hold*	Hold*

- * repeat LVEF assessment after 3-4 weeks, consider cardiac assessment
- If criteria for continuation are met-resume trastuzumab
- If 2 consecutive holds or a total of 3 holds occur, discontinue trastuzumab

Symptomatic patients:

- Symptomatic patients with evidence of cardiac dysfunction should have trastuzumab discontinued.

• **Pulmonary toxicity:**

Discontinue trastuzumab if patients develop ARDS, interstitial pneumonitis, or pulmonary fibrosis.

Outcomes

Primary outcomes

- Pathologic Complete Response (pCR) defined as "the absence of residual invasive cancer on hematoxylin and eosin evaluation of the complete resected breast specimen and all sampled regional lymph nodes following completion of neoadjuvant systemic therapy"[1].

Secondary outcomes

- clinical Complete Response (cCR)[2]: Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm
- clinical Partial Response (cPR)[3]: At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters
- clinically Stable Disease (cSD)[4]: Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest diameters while on study.
- clinical Progressive Disease (cPD)[5]: At least 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progression)
- clinical Objective Response(cOR): (cOR= cCR+ cPR)
- breast conservation rate

Safety Outcomes

All safety measures including Left Ventricular Ejection Fraction (LVEF), Anemia, Thrombocytopenia, Leucopenia, Neutropenia, Febrile neutropenia, Fever without neutropenia, Nausea, Vomiting, Diarrhea, Stomatitis, Mucositis, Dysphagia/esophagitis, Conjunctivitis, Allergic reactions, Edema, Asthenia, Hot flushes, Alopecia, Hand-foot syndrome, Nail changes, Sensory neuropathy and any unexpected serious adverse events should be assessed, recorded and reported.

[1]FDA: Guidance for Industry, Pathological Complete Response in Neoadjuvant Treatment of High-Risk Early-Stage Breast Cancer: Use as an Endpoint to Support Accelerated Approval.
[2] New response evaluation criteria in solid tumors: Revised RECIST guideline (version 1.1) [3]
New response evaluation criteria in solid tumors: Revised RECIST guideline (version 1.1) [4]
New response evaluation criteria in solid tumors: Revised RECIST guideline (version 1.1) [5]
New response evaluation criteria in solid tumors: Revised RECIST guideline (version 1.1)

Immunogenicity

Samples for immunogenicity assessment (antidrug antibody [ADA] and neutralizing antibody [nAb]) will be collected at the beginning of the first trastuzumab administration (visit 6), (visit 7), (visit 9) and 3 weeks after the surgery visit (visit 12) All patients in each treatment arm were considered evaluable for ADA response to trastuzumab. The evaluation of both arms immunogenicity used tiered strategies to detect, confirm, and characterize ADA responses. Each tiered strategy included the following elements:

- A screening assay to detect anti-trastuzumab antibodies;
- A confirmatory assay to assess the specificity of screen positive samples by competition with excess trastuzumab;
- A titration assay to determine anti trastuzumab antibody titers for confirmed positive samples. Serial dilutions of all confirmed positives were performed in order to estimate the magnitude of a positive response. The highest dilution that produced a signal above the screening assay cut point multiplied by the assay minimum required dilution (1:10) was deemed the end-point titer and was expressed as a dilution factor (reciprocal of the dilution). Any potential effects of ADAs on the efficacy (pharmacokinetics and pathologic complete response [pCR]) and safety (administration-related reactions [ARRs]) of trastuzumab were explored using descriptive statistics.

Termination policy

This decision is the responsibility of the scientific steering committee and should be approved by the Food and Drug Administration of Iran, the Ethics Committee of Tehran University of Medical Sciences and the Ethics Committee of Isfahan University of Medical Sciences.

Participant timelines

Activity No.	Activity list	Visit No	0	1	2	3	4	5	6	7	8	9	10	11	12
		Timeline (weeks)	- 3 to 0	1	3	5	7	9	10	13	16	19	21	23	26
1	Eligibility criteria Medical History Physical Examination Laboratory tests Imaging for cancer staging		X												
2	Informed consent		X												
3	Demographic and baseline characteristics		X												
4	Sonography, MRI or mammography (within last week is acceptable)		X					X					X		
5	Echocardiography for determination of LVEF		X					X					X		
6	Randomization							X							
7	Adverse Events evaluation/recording/reporting			X	X	X	X	X	X	X	X	X	X	X	X
8	CBC, BUN, Cr, ALT, AST, ALK, Bilirubin		X		X	X	X	X	X	X	X	X	X		
9	Doxorubicin + Cyclophosphamide			X	X	X	X								
10	Immunogenicity sampling								X	X		X			X
11	Intervention (Docetaxel + Trastuzumab)								X	X	X	X			
12	Surgery, specimen collection, pathological assessment													X	

Sample size

Using a non-inferiority margin of 0.25 for the primary end point, a sample size of 48 in each group achieves 82% power to detect a non-inferiority margin difference between the group proportions for the case the reference group proportion was considered as 0.60. The treatment group proportion was assumed to be 0.35 under the null hypothesis of inferiority. The test statistic used for sample size calculation is the one-sided Z test (unpooled) and the significance level of the test is targeted at 0.05. Considering 0.10 losses to follow up, final sample size is 54 in each group.

Recruitment

This study has two arms and 108 subjects will participate:

1. Group I: subjects will receive AryoTrust (produced by AryoGen Pharmed) IV infusion from 6th to 9th visit.
2. Group II: subjects will receive Herceptin® (the reference drug, produced by Genentech/Roche) IV infusion from 6th to 9th visit.

Randomization

The randomization is based on blocked randomization. Patient's allocation will be carried on 1:1 allocation ratio by 27 blocks (length of each block is 4). Randomization sequence will be generated using the random generation command in Microsoft Excel (RANDBETWEEN). Once the randomization has been made, a unique code will be assigned to each eligible patient, what she will be identified throughout the study by. The assigned code will be made up of four letters (corresponding to the first two letters of the patient's first name and the first two letters of the patient's family name), three digits (site code), two first letters of the drug's generic name which is followed by the study phase (e.g. TR3 for Trastuzumab phase 3 study) and four digits (corresponding to the randomization number).

A code will be allocated to each package of Trastuzumab. **This code consists of two English letters and a digit and is a unique code for each patient.** As a result, when the randomization number is unique, each patient will receive a unique package of the drug which will be determined through the randomization process. **Sequence generation is one of the "Trial" CRO's responsibilities**

Allocation (concealment process)

Randomization process will not be exposed to those who are conducting the study and will be provided via telephone call for each consecutive eligible patient after the identification characteristics of each eligible patient have been recorded by the randomization center. Since the randomization code is unique, the next sequence is not predictable for site personnel. The allocation of randomization code will be performed in fifth visit and after all inclusion criteria and none of exclusion criteria were met and signing the informed consent was done.

Blinding

Both trastuzumab products are indistinguishable for patients and health care providers. So it will be possible to make patients blind about the treatment group which they have been allocated to. In addition to this, the outcome evaluators and data managers (data analyzer) will not be aware of patients' allocations.

Treatment compliance

Patient education will be done to reduce the lack of adherence to treatment in each visit. Each patient has a reminder call before the visit. In addition to this, The nurse will administere all investigational product only to subjects included in the study following the procedures set out in the study protocol. Subjects do not receive home-take doses. The date of dosing will be recorded. In every monitoring session of the trial, drug accountability data and also information relating to patients' injections at appropriate times will be checked by the monitor. If an injection is delayed, the reason will be checked.

The sponsor's monitor tracked vials received and used and retained all unused vials

Treatment discontinuation

The reasons for discontinuation of the drug should be clearly stated in the CRF. Study participants may be excluded from the study for the following reasons:

- Patient dissatisfaction
- Failure to adhere to treatment includes refusing to study drug requirements, refusing to perform the procedures listed in the study protocol or using drugs that are prohibited for the patient.

- Patients who do not complete all four courses of AC and all four periods of the Trastuzumab + Docetaxel regimen according to the schedule (Standard dose modification less than 20% or a delay of less than 1 week is acceptable).
- Pregnancy or suspected pregnancy
- The occurrence of any blood, hepatic or renal complications requiring treatment discontinuation.
- The onset of any side effect that the investigator considers necessary for the patient to leave the study
- After 4 AC courses, if the LVEF level does not exceed 15% of its baseline level or below 55%, patients will be able to continue treatment with Trastuzumab + Docetaxel regimen.
- Failure to follow the patient

Data management

Data collection methods

Data collection will be electronic-based and the data will be recorded in eCRF. Adequate heed will be given to collect accurate and valid data. Investigators are responsible for completing the eCRF in study centers. The sites will be equipped with personal computers or tablets. Only the investigator or the person assigned by the investigator will have access to the database for data entry. ”Trial CRO” and sponsor is responsible to set up a regular monitoring scheme by qualified staff.

Data management

The chief investigator, principal investigators, and other personnel assigned by the investigators will be responsible for eCRF data entry. Each investigator will be given a specific username and password. The chief investigator and principal investigators must not disclose the data obtained from the study. All of the Investigators are responsible for keeping the study data safe. Sending and receiving the patients' information must be done considering safety and security procedures. “Trial” CRO and sponsor is responsible for planning a monitoring which will be conducted by qualified personnel in order to check the eCRF for discrepancies with the patient source documents. The monitors or auditors are not able to change eCRF data. However, they can make queries for blatant mistakes in data entry and the investigator is responsible for rectifying such mistakes or answering to the queries. The history of modification in eCRF data will be recorded meticulously and can be observed by the monitors or auditors.

Data monitoring

The objectives of the data quality control are:

- To ensure the existence of the patients and the respect of ethics (including signed patient informed consent)
- To detect the issues (including systematic errors) as early as possible for appropriate measures to be taken
- To ensure the validity of the data

To meet these objectives, quality control should be applied via the following activities:

The schedule of site quality control evaluation, performed by the monitoring team must be explained to the investigators at the time of site initiation and agreed upon. QC will be performed in study sites by the monitoring team who are in charge of planning for action plans to improve study site quality.

QC of the study site will be carried out during two main monitoring visits (30% and 70% of study completion) and several periodic monitoring visits throughout the study.

In every monitoring visit the following items will be evaluated according to a pre-prepared check list and the quality control report will be completed.

- eCRF forms will be assessed in terms of completeness, the quality of data entry and accordance with source data.
- Informed consent forms signed by the patient and the physician for all the patients who have gone through the screening visit
- Evaluation of key variables regarding the wrong and missing data

Statistical methods & Statistical Analyses

Analysis Populations

The primary population for evaluating efficacy will be based on the per-protocol analysis. On the other hand, all safety analysis will be based on the safety population. Safety population is an approach in which all patients will be analyzed according to the treatment which they actually received (as-treated analysis).

Missing data

QC and QA criteria will be adopted to hold up missing data at minimum. Source documents will be retained in study sites and their data will be retrieved at request. The listwise dropping will be used if the rate of main outcome (pCR) was missed out of the final data.

Covariate Adjustment

The primary statistical analyses for outcome variables will be unadjusted.

Trial profile

All patients who provide informed consent will be accounted for in the final statistical report. A CONSORT style flow diagram will illustrate patient progression through the trial from initial screening for eligibility to completion of the final primary outcome assessment. Number (percentage) by treatment group will be given for patients in the PPS population, reasons for study withdrawal, and major protocol deviations and violations.

Patient characteristics and baseline comparisons

Data will be described informatively, according to study's objectives. Categorical variables will be summarized by frequencies and percentages. Continuous variables will be summarized by mean and standard deviation or quartiles.

Analysis of the primary outcome

Treatment differences in proportions will be calculated for these outcomes. A 95% two-sided confidence interval will be constructed and the upper bound to determine non-inferiority with a 2.5% significance level will be used.

Analysis of secondary outcomes

Frequency and proportions will be calculated for all secondary efficacy endpoints include clinical complete response (cCR), clinical partial response (cPR), clinically stable disease (cSD), clinical progressive disease (cPD), clinical objective response (cOR), breast conservation rate.

Safety analysis

All safety data will be analyzed descriptively by each treatment group. Adverse events will be reported as incidence. Absolute and relative frequencies of AEs will be reported. Laboratory data for hematology and clinical chemistry will be plotted by treatment groups during the study. The

frequency of changes with respect to normal ranges between baseline and endpoint will be tabulated. Frequencies of clinically noteworthy values (defined in the statistical analysis plan) occurring during the study will also be given. Shifts from normal to abnormal between baseline and endpoint will be evaluated.

Moreover, the mean and SD of baseline vital signs will be reported. Shifts from normal to abnormal between baseline and endpoint will be evaluated for the physical examination.

Monitoring

Site initiation visit

SIV will be performed to ensure that the facility and medications required for the trial are available in the study site and the investigators and staff involved in the study are aware of study objectives and GCP principles. In the initiation visit the role of the monitor, CRA, project manager, and the auditor will be defined clearly. After the introduction of the roles and responsibilities, staff will be taught the protocol details including timelines, sample size, eligibility criteria, protocol conformity, deviation, and reporting. The training should be documented. The eCRF details, ICD process, source documentation, randomization and serious adverse event reporting will be discussed and documented as well.

Site monitoring visit

site monitoring visit will be performed to ensure the conduct of the study according to approved protocol and GCP principles. Before the trial starts, monitoring visits will be planned for each study site and will be confirmed by the respective PI through email or letter. The monitoring visits during the study will be scheduled in order to visit when the planned recruitment of patients will be in its 30% and 70% progress. Sponsor is responsible for the 30% monitoring visit and “Trial” CRO is in charge of the 70% visit. In each visit, study elements will be monitored including source data, consent forms, trial medicinal products accountability, adverse events, protocol compliance, team qualification, and training. The monitoring report will be prepared and reported to both PI and sponsor preferably in an arranged meeting.

Study closeout

study closeout visit will be performed to ensure the proper documentation of the data and return of the medicinal product and the equipments related to the trial. After the last visit of the last participant, site close-out will be scheduled in a meeting and in the presence of the PI. The

site close-out visit will start with a brief meeting with the PI and a decision will be made regarding the disposal of the remaining investigational medicinal product. The study documents will be available at the trial site. A copy all safety reports will remain with the PI and one copy will be rendered to the sponsor. There will be a brief closure meeting with the investigator at the site and the site close-out report will be prepared after the visit.

Adverse events

Adverse Event will be considered any medical event presented by the subjects involved in the study, which do not necessarily have a causal relation with the treatment in study. It will be reported as an adverse effect any symptom, sign (including any abnormal laboratory determination) or temporary disease associated to the use if the drug in study, whether they are or not etiologically related to it. The medical conditions present before the beginning of the study will only be considered as adverse events if they worsen during the study and cannot be attributable to the natural evolution of the disease. It will be considered a Serious Adverse Event (SAE) if the event:

- ✓ results in death;
- ✓ implies a death risk;
- ✓ requires hospitalization;
- ✓ extends prior hospitalization;
- ✓ results in persistent or significant incapacity;
- ✓ produces a congenital anomaly or malformation, or
- ✓ requires medical or surgical intervention to avoid a permanent damage.

AE classification based on its severity:

AE will be classified according to its severity in relation to the guidelines established in the Common Terminology Criteria for Adverse Events (CTCAE, version 5.0, published on November 27, 2017). Grade refers to the severity of the AE. The CTCAE displays Grades 1 through 5 with unique clinical descriptions of severity for each AE based on this general guideline:

Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

Grade 2: Moderate; minimal, local or noninvasive intervention indicated; limiting ageappropriate instrumental ADL*.

Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**.

Grade 4: Life-threatening consequences; urgent intervention indicated.

Grade 5: Death related to AE.

* A Semi-colon indicates 'or' within the description of the grade.

* Activities of Daily Living (ADL)

*Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

**Self-care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

AE classification based on its relation with the treatment in study:

In order to establish the relation between the AE or the DAR and the treatment in study, the following definitions will be considered:

- ✓ **Certain**: a clinical event including alterations in the laboratory tests manifesting with a reasonable temporal sequence related to the administration of the drug and cannot be explained by the current disease, or by other drugs or substances. The response to the drug suppression (released; dechallenge) should be clinically plausible. The event should be final from a pharmacological point of view, using, if necessary, a conclusive re-exposure procedure.
- ✓ **Probable/Likely**: a clinical event, including alterations in the laboratory tests manifesting with a reasonable temporal sequence related to the administration of the drug, which is unlikely to be attributed to the current disease, or other drugs and substances, and which when releasing the drug (dechallenge) a clinically reasonable response appears. No information on re-exposure (rechallenge) is required to assign this definition.
- ✓ **Possible**: a clinical event, including alteration in the laboratory tests manifesting with a reasonable temporal sequence related to the administration of the drug, but can also be explained by the concurrent disease, or by other drugs and substances. The information regarding the release of the drug may be missing or unclear.
- ✓ **Unlikely**: a clinical event, including alterations in the laboratory tests manifesting with an improbable temporal sequence related to the administration of the drug, and can be explained in a more plausible way by the concurrent disease, or by other drugs or substances.
- ✓ **Conditional/Unclassified**: a clinical event, including alterations in the laboratory tests, notified as an adverse reaction, of which it is essential to obtain more data in order to make a proper evaluation, or the additional data are under examination.

- ✓ **Non evaluable/Unclassifiable**: a notification that suggests an adverse reaction, but cannot be judged because the information is insufficient or contradictory and cannot be verified or completed in its data.

WHO-UMC Causality Categories

Causality term	Assessment criteria*
Certain	• Event or laboratory test abnormality, with plausible time relationship to drug intake • Cannot be explained by disease or other drugs • Response to withdrawal plausible (pharmacologically, pathologically) • Event definitive pharmacologically or phenomenological (i.e. an objective and specific medical disorder or a recognized pharmacological phenomenon) • Rechallenge satisfactory, if necessary
Probable/ Likely	• Event or laboratory test abnormality, with reasonable time relationship to drug intake • Unlikely to be attributed to disease or other drugs • Response to withdrawal clinically reasonable • Rechallenge not required
Possible	• Event or laboratory test abnormality, with reasonable time relationship to drug intake • Could also be explained by disease or other drugs • Information on drug withdrawal may be lacking or unclear
Unlikely	• Event or laboratory test abnormality, with a time to drug intake that makes a relationship improbable (but not impossible) • Disease or other drugs provide plausible explanations
Conditional/ Unclassified	• Event or laboratory test abnormality • More data for proper assessment needed, or • Additional data under examination
Unassessable/ Unclassifiable	• Report suggesting an adverse reaction • Cannot be judged because information is insufficient or contradictory • Data cannot be supplemented or verified

*All points should be reasonably complied with

AEs Recording

All the undesirable and unexpected AE that follow the administration of the drug will be accurately recorded in the medical record of the patient and in the corresponding section of the CRF. The event description should be recorded, as well as the temporal sequence regarding to the administration of the drug, its duration, the procedures performed for the diagnosis if appropriate, the results of the repeated exposure and the qualification made by the Researcher as regards its severity and its relation to the administered drug.

AE Reporting Responsibilities

Principle investigator responsibilities:

- The investigator must report any SAE/R, which results in death or is life-threatening, to the sponsor and IEC within the maximum of 24 hours by fax, email or etc.

- The investigator must immediately and not later than seven calendar days report those SAE/Rs which are not life-threatening or do not result in death but may put subjects in danger or require intervention (medical or surgical) to prevent life-threatening outcomes. These SAE/R need to be reported to the sponsor and IEC as soon as possible but not later than seven calendar days of having taken notice of the SAE/R.

- The investigator should report to the sponsor and IEC all predictable adverse events of investigational medicinal product including for example injection site reactions, in case of patient withdrawal from study or adverse event with a greater frequency than expected.

Sponsor responsibilities:

- SUSARs which result in death, or are life-threatening; need to be reported by the sponsor as soon as possible but not later than seven calendar days, to IEC and IFDA, using CIOMS form (Suspect Adverse Reaction Report Form). A follow-up report is to be submitted within 15 calendar days.

- The sponsor must report SUSARs which are not life-threatening or do not result in death, but may put subjects in danger or require intervention (medical or surgical) to prevent life-threatening outcomes, as soon as possible but not later than 15 calendar days, to IEC and IFDA, using CIOMS form (Suspect Adverse Reaction Report Form). The follow-up report considering the relation between the investigational medicinal product and an adverse event is to be submitted as soon as possible.

- All the reports and follow-up results of SAE/R need to be reported to IFDA within maximum 15 calendar days after sponsor awareness of SAE/R.

- If the severity or frequency of predictable adverse events, for example, injection site reactions, results in patient withdrawal from the study, or in case of higher incidence than expected, the sponsor must report it to IFDA within maximum 15 calendar days.

- The sponsor must report all the information regarding the SAE and serious ADR that are reported during the course of the study and recommendations of investigators related to increasing of study risk for subjects to Food and Drug Administration of Iran within 15 calendar days.

- The sponsor must report to IFDA all recommendations from investigators about possible increased risk of adverse events or participants, within maximum 15 calendar days.

Follow-up

- The Investigator should take all appropriate measures to ensure the safety of the patients, notably he/she should follow up the outcome of any Adverse Events (clinical signs, laboratory values or other, etc.) until the return to normal or consolidation of the patient's condition;
- In case of any Serious Adverse Event, the patient must be followed up until clinical recovery is complete and laboratory results have returned to normal, or until progression has been stabilized. This may imply that follow-up will continue after the patient has left the study and that additional investigations may be requested by the Monitoring Team
- In case of any Serious Adverse Event brought to the attention of the Investigator at any time after cessation of Investigational Product and considered by him/her to be caused by the Investigational Product with a reasonable possibility, this should be reported to the Monitoring Team.

Withdrawal of patients:

Participants may be withdrawn from the study for any of the following reasons:

- Withdrawal of consent by the patient

- Noncompliance, including refusal of study medical requirements, refusal of procedures as stated in the study protocol, or use of prohibited medications
- In the case of suspected pregnancy, a pregnancy test for Beta hCG will be requested, and if the test is positive, the patient will be excluded from the study
- The occurrence of an undesirable event that causes the investigator to consider the patient's exclusion from the study
- Not possible to follow the patient's condition (Loss to follow-up)

The reason(s) for withdrawal should be stated clearly in the eCRF.

Patient Admission Criteria

Since all injections during the study are performed under the supervision of the nurse/nurses who have been trained, we will ensure that patients who are present at all injection days and whose injections are approved in the CRF, has an admission to the treatment.

Auditing

Regular auditing will be carried out to ensure strict adherence to the study protocol. the auditor will be determined and introduced to regulatory authorities by the sponsor.
The Audit reports will be prepared for the sponsor and the results will be reported to chief investigator as well.
Auditors will be responsible for:

- Regular visits to the study centers to inspect the sufficiency of existing structures and processes
- Identifying training needs of study site staff
- Making necessary arrangements to set up training courses
- Monitoring adherence to all procedures foreseen in the study protocol
- Preparing written report from each visit
- Certifying the quality and reliability of the monitoring visits during the study
- Certifying the quality and reliability of the study conduction by investigators and staff
- Certifying the quality and veracity of the data to be reported to legal bodies

Ethics and dissemination

Research ethics approval

- Ethics committee approval is mandatory for start of this study
- No patient will be recruited to this study without a signed informed consent.
- Patients will be informed that they can leave the study anytime they desire with no need for any explanation.

- To ensure the confidentiality in case a form is lost, the name and surname of the patients will not appear on any forms.
- Adverse effect report forms will be evaluated after every visit. The research team is responsible for dealing with the immediate aftermath of any adverse event regardless of the event being directly related to the medication that is being studied.
- Before initiation of the trial, it will be reviewed with IFDA. The protocol, CRF, information for patients and informed consent form will be submitted to the ethics committee of Tehran university of medical sciences and Isfahan University of medical sciences, for review and approval according to international regulatory guidelines.

Consent

The investigator will thoroughly explain the purpose of the study to the patient. The patient will be provided with an information sheet and will be given sufficient time and opportunity to inquire about the details of the study and to decide whether or not to participate in the study, e.g. to give permission to use their data for investigative purposes, knowing their information will remain confidential. The informed consent form should be signed and dated by the patient and the person with whom they discuss the information regarding the consent form. The investigator will explain that the patient is completely free to refuse to give permission for his/her data to be used or to withdraw from the trial at any time and for any reason. Similarly, the investigator and/or sponsor will be free to withdraw the patient at any time for administrative reasons. Any other requirements necessary for the protection of the human rights of the patient will also be explained, according to GCP guidelines, declaration of Helsinki and local regulation for clinical trials.

Confidentiality

All study-related information will be stored securely at the study site. To ensure confidentiality, randomization codes will be used on all the reports, gathered data, information regarding the study progress and administrative forms. All records that contain names or other personal identifiers, such as subject identification form and informed consent forms, will be stored separately from study records. All local databases will be secured with password-protected access systems. Forms, lists, logbooks, appointment books, and any other lists which link participant's randomization code to other identifying information will be stored in a separate, locked file in an area with limited access. All laboratory and other test results will be kept strictly confidential. All counseling and blood sampling will be conducted in private rooms, and study staff will be required to preserve the confidentiality of all participants.

Amendment

Any modifications to the protocol which may impact on the conduct of the study, the potential benefit of the patient or may affect patient safety, including changes to study objectives, study design, patient population, sample size, study procedures, or significant administrative aspects will require a formal amendment to the protocol. Such amendment will be agreed upon by chief Investigator and AryoGen Pharmed company and should be approved by IFDA and the Ethics Committee prior to implementation. Administrative changes to the protocol are minor corrections and/or clarifications that have no effect on the way the study is to be conducted. These administrative changes will be agreed upon by chief Investigator and AryoGen Pharmed company and will be documented in a memorandum.

Declaration of interests

The presence or absence of any kind of financial and or nonfinancial relationship between the sponsor and chief investigator and principal investigators (with the exemption of this study contract) should be officially declared to IFDA as a written conflict of interest letter.

Funding organization

All expenses of this study including patients' treatment and medicines, study conduct and performing and research related injuries compensation will be provided by AryoGen Pharmed co.

Access to data

Chief investigator, sponsor and CRO Trial will have access to full dataset. In addition, ethic committee and regulatory organizations can access to data, if needed.

Ancillary and post-trial care

Ancillary care (related to trial) will be provided by principal investigator under sponsor support for participants.

Dissemination policy

No other publication is allowed before the primary publication. Any subsequent presentation or publication ((including the sub-studies) by a study team member must be approved by the steering committee and chief Investigator and the primary publication should be cited. The final decision to publish any manuscript/ abstract/ presentation will be made by chief Investigator and the sponsor after prior notice to the "steering committee for their review and comments.

Appendix 1: Informed consent materials

Informed Consent Form

Study title: A PHASE III, RANDOMIZED, TWO ARMED PATIENT-OUTCOME ASSESSOR-DATA ANALYZER BLINDED, PARALLEL ACTIVE CONTROLLED NON-INFERIORITY CLINICAL TRIAL STUDY OF ARYOTRUST (ARYOGEN PHARMED TRASTUZUMAB) EFFICACY AND SAFETY IN HUMAN EPIDERMAL GROWTH FACTOR RECEPTOR 2–POSITIVE BREAST CANCER IN COMPARISON TO HERCEPTIN® (GENENTECH/ROCHE) CONTROL.

Mr./Mrs. ……

We invite you to participate in the above-mentioned clinical study. Information about this research is provided in this sheet, and you are free to join this research or not.

You do not have to make an immediate decision and you are given a deadline to announce your opinion after consulting the research team and anyone you desire, about participation and your involvement in this research is entirely voluntarily. Before signing this consent, make sure that you understand all the information in this form and all of your questions are answered.
Researcher: Dr. Seyed Reza Safaie Nodehi

1- I know that purposes of this research are: the evaluation of the efficacy and safety of trastuzumab of AryoGen Pharmed company in comparison with trastuzumab of Genentech/Roche company.
2- I know that my participation in this study is completely voluntary and I am not obligated to participate in this research. I was assured that if I were not willing to participate in this research, I would not be deprived of routine diagnostic and therapeutic care and my therapeutic relationship with the health center and my physician will not get affected.
3- I know that even after agreeing to participate in the research, I can resign freely at any time after informing the researcher, without giving any reason and my withdrawal from the research will not deprive me of the usual treatment services.
4- This is how my participation is in this study:
   After the diagnosis of doctors about the need for using trastuzumab to treat my illness, and after agreeing to participate in the study, I will randomly be assigned to one of the two groups receiving trastuzumab of an Iranian company AryoGen Pharmed or trastuzumab of Genentech/Roche company.

I know that the other treatments and prescribed medications are similar between the two groups, and the randomization between the two groups is completely accidental and no one predetermines it. During the study, I must receive the prescribed medication and refer to the doctor's instructions for the tests and related visits on a regular basis. I know that the procedures in each visit are as follows: At the first session (visit 0), my health status is evaluated by the physician, and previous laboratory data and imaging results are monitored and recorded. Next, a blood sample of me is taken to assess the physical condition and health indicators. Then an echocardiographic test and a breast sonography will be done. If I have done a sonography, MRI or mammography within the last week, there is no need to do it again. If the results of the tests, imaging and evaluation of the physician were not inconsistent with my presence in the study, I will be included in the study and will receive the first dose of cyclophosphamide and adriamycin. My participation in the study includes two stages of treatment, in the first stage of 4 cycles (including visits one, two, three, and four), I go to the health center every two weeks and receive cyclophosphamide and adriamycin as treatment, except for the first session (visit 1), which is in the same time as I present the results of my previous laboratory test. In the next three sessions of this stage, a blood sample will be taken from me every time to evaluate my health and my ability to continue the study. At the end of these four sessions, an echocardiography and sonography will be done for me again, these results and the result of blood tests will be presented to my doctor for review and record in the next visit (visit 5). In visit 6, the second stage of treatment, which consists of 4 cycles, every 3 weeks, starts for me. In each session, I will receive docetaxel (100 mg per square meter of body surface area) and one of the two AryoTrust or Herceptin® (the first course is 8 mg per kilogram, and in the next 3 courses, 6 mg per kilogram). I know that AryoTrust is the trastuzumab drug which is produced by AryoGen Pharmed company in Iran, and the Herceptin® drug is the brand trastuzumab drug which is produced by Genentech/Roche Company. I will receive AryoTrust, If I am in the first group, and I will receive Herceptin® and if I am in the second group. I know that it's totally coincidental that I am selected for which group and I will not be aware of my group until the end of the study. My doctor explained to me that the process of choosing a therapeutic group unknowingly is completely scientific and is because of evaluating the drugs properly. My doctor also assured me that regardless of in which group of treatments I am, all the necessary procedures for appropriate treatment will be done for me, and wherever participation in this study is considered to be dangerous for my health, I will be excluded from the study and I will receive the appropriate treatment according to my health condition. I know that all medications in the two treatment groups are similar and the difference between the two groups is due to the difference in the company that makes the drug trastuzumab. At each of the treatment sessions, my health status is evaluated by the medical staff of the study, and in order to fully control my health conditions, in addition to the blood tests performed in each session (8 times total), in the first visit, the 5th visit and before the surgery (9th visit), echocardiography of the heart (3 times total)

and sonography (3 times total) will be done for me. The total number of visits is 10 sessions and the duration of the treatment is 21 or 23 weeks. In every visit, questions about my health conditions from the previous visit until now, are asked. At the end of the course of drug therapy, regardless of the treatment group, I will be referred for surgery. I know that after the end of the study (if I need to continue treatment), given the fact that the trastuzumab of both types (Iranian and brand) is available in the pharmaceutical market of the country, and given the insurance coverage of this drug, I can provide this drug from reputable pharmacies. I know that according to the principles of conducting clinical trials of AryoGen Pharmed company, the cost of trastuzumab will be paid up to the end of the study, and after the completion of the study, it will be my responsibility, like other patients.

5- The possible benefits of my participation in this research are as follows:

I receive free treatment for 23 weeks. Also, in each of the study groups, during this study, I will be examined and evaluated by the physicians with greater precision and sensitivity regarding my condition and the side effects of drugs. Also, the effects of my treatment will be carefully evaluated. After surgery, I will be informed of the outcome of my treatment, and the treatment outcomes will fully be explained to me, and my questions about my health status and the continuation of treatment will completely be answered. The contact number of the researcher or his authorized representative is available to me so that if I had a question during the study or if a health problem occurred to me, I can have immediate access to him.

6- The possible harms and adverse events of my participation in this research are as follows:

I have been informed that previous studies on AryoTrust have shown similar side effects as Herceptin®, and there is no evidence that AryoTrust has more adverse effects than Herceptin®. I know that receiving trastuzumab (AryoTrust or Herceptin®) in the first cycle may cause fever and chills and flu-like symptoms and there is only a small chance that these symptoms are severe and associated with nausea and skin rashes.

These complications will usually get milder in subsequent visits. Less likely, trastuzumab may cause cardiomyopathy and reduce heart's ability to transfer blood to organs, although in most cases it is not seen, or it is so insignificant that it does not cause a symptom, or only it causes symptoms such as transient shortness of breath, but in some cases it may also lead to congestive heart failure or cerebral stroke. During the study and after each session I receive medical treatment, health examinations and diagnostic laboratory tests will be done for me, as well as three times echocardiography in the first, fourth (the end of the first stage treatment) and the ninth visit (The end of the second stage of treatment and before surgery) will be done for me, and if the continuation of the study is dangerous for my health, I will be excluded from this treatment. In the case of mild flu-like syndromes, I will receive treatments for reducing the symptoms. If these complications cause disability for me, with the doctor's diagnosis, the interval between treatment cycles may get longer, or even the treatment can be discontinued for a

while. In the case of cardiac complications, temporary or permanent cessation of treatment, my heart status will be consulted with the help of a cardiologist. In the case of any complication that occurs for me in this study, the investigator will report them to the responsible authorities in accordance with the relevant laws. Also, me or my family should inform my doctor of any adverse event occurrence during the study (including events leading to hospitalization and death) as soon as possible. This undesirable event may not be due to the drug being studied, but in any case, I should inform my doctor as soon as possible. I know that if any complication occurs for me in this study, the investigator will report them to the responsible authorities in accordance with the relevant laws, and I will regularly be monitored by the treatment team for the incidence of these complications.

7- In the case of unwillingness to participate in the research, the usual services (therapeutic, diagnostic, etc.) for me will be provided which the benefits and harms are as follows:
In case of unwillingness to participate in the research, the usual services (therapeutic, diagnostic, etc.), for me will be provided which is the same treatment described in the second group of study (the Herceptin® group, trastuzumab of Roche company). A routine visit will be performed by the doctor and the usual care will be provided. My drugs will be prescribed to me by my physician diagnosis. The cost of laboratory testing and drugs will be paid by myself according to my insurance coverage, and I will be referred for surgery at the end of the treatment cycles. Also, because the treatment is routinely similar to that used in the study, the complications and drug adverse events are similar to the research.

8- I know that the researchers of this study will keep all of my information confidential and are only allowed to publish the overall and collective results of this research without mentioning my name and my profile.

9- I know that the Ethics Committee in my study is allowed to have access to my information to monitor my rights.

10- I know that I should not pay any of the costs below:
1-Trastuzumab drug cost (Iranian or brand), 2-Costs related to diminishing the complications related to the study drug, 3-Costs of diagnostic procedures related to the company in the study and related to the side effects of the drug studied, including laboratory tests, imaging, echocardiography and pathology, 4-The cost of the patient's share of other medications (after deducting the share of insurance) during the study in 26 weeks (about 6 months). In the case of no need for more diagnostic procedures, the number of diagnostic procedures is as follows: Total blood tests 8 times, Echocardiography (total 3 times) and Sonography (total 3 times).

11- I know that if during and after the research any physical and mental problems arose because of my participation in this research, it will be the responsibility of the researcher to treat the complications and the related damages.

I know that if I become hospitalized due to participation in this study, or a disability or any other unpleasant consequence occurs to me in this study, that if I did not attend this study, it would not have happened for me, the relevant compensation is AryoGen Pharmed company responsibility and I am insured by the AryoGen Pharmed company concerning the adverse events occurring for me because of my participation in the study.

12- Mrs. Elham Farhang has been introduced to me for answering my questions, and I was told that during the study any time a health problem occurred to me or if I had a question regarding participation in this research, I can share with her and ask for guidance. Her following address and phone number were given to me:
Address: Fifth floor, No 74, Near faculty of Nutrition, Hafezi Street, Shahrak Gharb, Tehran, Iran.
Telephone: 00982188078848
Cell phone: 00989129592162

13- I know that if I have a problem or objection to executors of the research or the research process, I can contact the Ethics Committee of the Tehran University of Medical Sciences at the address of: Room 605, 6th Floor, Central headquarters of Tehran University of Medical Sciences, Qods Street, Keshavarz Blvd., Tehran, Iran. Telephone: 009821-81633626 or Vice-Chancellor for Research in Isfahan Province with the telephone number of 031-36682407, and present my problem either verbally or in writing.

This form of information and informed consent is provided in two copies and will be signed by the researcher and me. A signed copy will be given to me and a signed copy will be given to the researcher.

I have read and understood the explanations mentioned above, and based on that, I declare my informed consent to participate in this research.

Participant signature:

I consider myself bound to comply with the obligations of the executor in the above provisions, and I undertake to work on the rights and safety of people participating in this research.

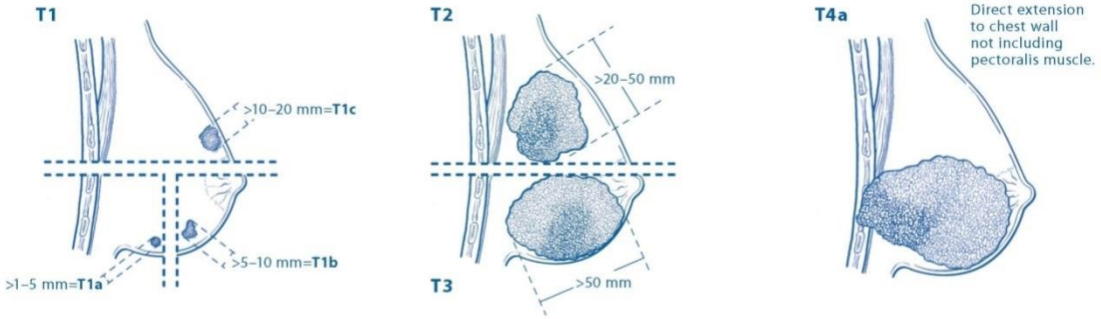
Researcher signature:

Appendix 2: breast cancer staging

American Joint Committee on Cancer

Breast Cancer Staging

7th EDITION



Primary Tumor (T)

- TX Primary tumor cannot be assessed
- T0 No evidence of primary tumor
- Tis Carcinoma in situ
- Tis (DCIS) Ductal carcinoma in situ
- Tis (LCIS) Lobular carcinoma in situ
- Tis (Paget's) Paget's disease of the nipple NOT associated with invasive carcinoma and/or carcinoma in situ (DCIS and/or LCIS) in the underlying breast parenchyma. Carcinomas in the breast parenchyma associated with Paget's disease are categorized based on the size and characteristics of the parenchymal disease, although the presence of Paget's disease should still be noted

- T1 Tumor ≤ 20 mm in greatest dimension
- T1mi Tumor ≤ 1 mm in greatest dimension
- T1a Tumor > 1 mm but ≤ 5 mm in greatest dimension
- T1b Tumor > 5 mm but ≤ 10 mm in greatest dimension
- T1c Tumor > 10 mm but ≤ 20 mm in greatest dimension
- T2 Tumor > 20 mm but ≤ 50 mm in greatest dimension
- T3 Tumor > 50 mm in greatest dimension

- T4 Tumor of any size with direct extension to the chest wall and/or to the skin (ulceration or skin nodules)
  Note: Invasion of the dermis alone does not qualify as T4
- T4a Extension to the chest wall, not including only pectoralis muscle adherence/invasion
- T4b Ulceration and/or ipsilateral satellite nodules and/or edema (including peau d'orange) of the skin, which do not meet the criteria for inflammatory carcinoma
- T4c Both T4a and T4b
- T4d Inflammatory carcinoma (see "Rules for Classification")

Distant Metastases (M)

- M0 No clinical or radiographic evidence of distant metastases
- cM0(i+) No clinical or radiographic evidence of distant metastases, but deposits of molecularly or microscopically detected tumor cells in circulating blood, bone marrow, or other nonregional nodal tissue that are no larger than 0.2 mm in a patient without symptoms or signs of metastases
- M1 Distant detectable metastases as determined by classic clinical and radiographic means and/or histologically proven larger than 0.2 mm

ANATOMIC STAGE/PROGNOSTIC GROUPS			
Stage 0	Tis	N0	M0
Stage IA	T1*	N0	M0
Stage IB	T0	N1mi	M0
	T1*	N1mi	M0
Stage IIA	T0	N1**	M0
	T1*	N1**	M0
	T2	N0	M0
Stage IIB	T2	N1	M0
	T3	N0	M0
Stage IIIA	T0	N2	M0
	T1*	N2	M0
	T2	N2	M0
	T3	N1	M0
	T3	N2	M0
Stage IIIB	T4	N0	M0
	T4	N1	M0
	T4	N2	M0
Stage IIIC	Any T	N3	M0
Stage IV	Any T	Any N	M1

Notes

* T1 includes T1mi.

** T0 and T1 tumors with nodal micrometastases only are excluded from Stage IIA and are classified Stage IB.

- M0 includes M0(i+).
- The designation pM0 is not valid; any M0 should be clinical.
- If a patient presents with M1 prior to neoadjuvant systemic therapy, the stage is considered Stage IV and remains Stage IV regardless of response to neoadjuvant therapy.
- Stage designation may be changed if postsurgical imaging studies reveal the presence of distant metastases, provided that the studies are carried out within 4 months of diagnosis in the absence of disease progression and provided that the patient has not received neoadjuvant therapy.
- Postneoadjuvant therapy is designated with "yc" or "yp" prefix. Of note, no stage group is assigned if there is a complete pathologic response (CR) to neoadjuvant therapy, for example, ypT0ypN0cM0.

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