21-003046

Comprehensive Single-Cell Transcriptional Analysis of Aromatase Inhibitor-Resistant Breast Cancer

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Comprehensive Single-Cell Transcriptional Analysis of Aromatase Inhibitor-Resistant Breast Cancer

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Study Product: Aromatase inhibitor, Letrozole

Protocol Number: (IRBe) IRB 21-003046

√ Study contributor(s) not responsible for patient care.
* Grant holder

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List of Abbreviations

LIST OF ABBREVIATIONS

AE	Adverse Event/Adverse Experience
CFR	Code of Federal Regulations
CRF	Case Report Form
DSMB	Data and Safety Monitoring Board
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
IND	Investigational New Drug Application
IRB	Institutional Review Board
PHI	Protected Health Information
PI	Principal Investigator
SAE	Serious Adverse Event/Serious Adverse Experience
SOP	Standard Operating Procedure

Study Summary

Title	Comprehensive Single-Cell Transcriptional Analysis of Aromatase Inhibitor-Resistant Breast Cancer
Running Title	Single cell sequencing for aromatase inhibitor-resistant breast cancer.
Phase	Pilot
Subject Participation Duration	2-8 weeks
Single or Multi-Site	Single site
Objectives	To comprehensively evaluate differences in tumor microenvironment subpopulations in AI-sensitive vs. AI-resistant HR+ breast cancer.
Number of Subjects	50 patients
Diagnosis and Main Inclusion Criteria	1. Female ≥ 18 years. 2. Postmenopausal and suitable to receive aromatase inhibitor as per physician's discretion. 3. Histologically confirmed un-resected operable invasive adenocarcinoma of the breast ≥ 0.5 cm with estrogen receptor (ER) and/or progesterone receptor (PR) positive ≥ 10%, and no human epidermal growth factor receptor 2 (HER2) amplification or overexpression. 4. Patients must not have received any prior chemotherapy, radiation therapy, or endocrine therapy for their current breast cancer. Patients who received tamoxifen or raloxifene or another agent for prevention of breast cancer may be included.
Study Product, Dose, Route, Regimen	Letrozole 2.5 mg oral daily (standard of care)
Duration of Administration	2-8 weeks
Reference therapy	N/A
Statistical Methodology	This is a pilot study to evaluate the difference in tumor microenvironment subpopulation in AI-sensitive vs. AI-resistant HR+ breast cancer.

1 Introduction

This document is a protocol for a human research study. This study will be carried out in accordance with the applicable United States government regulations and Mayo Clinic research policies and procedures.

Aromatase inhibitor is currently the cornerstone standard of care endocrine therapy for patients with hormone receptor positive breast cancer. This is a pilot study to evaluate in-depth transcriptomic analysis using single cell sequencing technology to evaluate the effects of aromatase inhibitor not only in cancer breast cell but also noncancerous cells in the tumor microenvironment.

1.1 Background

In-depth transcriptomic analysis for each subpopulation in the tumor microenvironment of pre-treatment and post-treatment tumor samples obtained from AI-sensitive vs. AI-resistant patients will be performed using single-nucleus RNA-sequencing (snRNA-seq) (Figure 1). Re-clustering of stromal cells into sub-clusters will be undertaken to uncover hidden transcriptomic heterogeneity and to identify transcription factor regulation signatures that define these sub-clusters. Validation of transcriptome data will be performed using qPCR and immunofluorescence staining of cultured flow-sorted cells from human tissue sections and immunohistochemistry of these sections. We also seek to study the interactions between spatially distinct stromal sub-clusters and other cells (tumor, immune and endothelial) that will be studied by annotating our dataset with curated human ligand-receptor pairs. In turn, this will shed light on the role of stromal sub-clusters on modulating immune activation vs. tolerance/evasion via recruitment/exclusion and/or (dys)regulation (exhaustion/activation) of specific immune subpopulations including T-regs, cytotoxic T cells, and tumor-associated macrophages. Furthermore, peripheral blood will also be collected at baseline and after AI treatment to evaluate systemic changes of immune response after AI in responders vs. nonresponders.

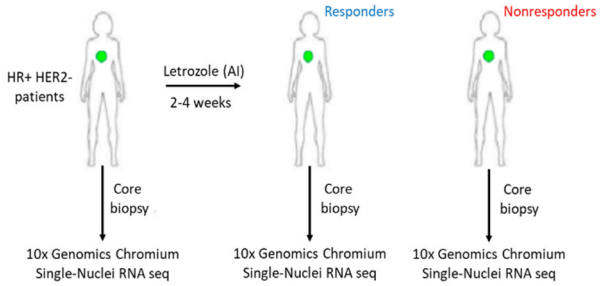


Figure 1. The schematic representations of the study: The collection of pre-treated biopsies from breast cancer patients with estrogen receptor positive (ER+) and human epidermal growth receptor 2 negative (HER2-) is shown. The obtained surgical specimens from good responders and bad responders (progressors) will be analyzed and compared with pre-treated samples. All analyses will be carried out through single nucleus RNA-sequencing.

We had experience working with patient samples with triple-negative breast cancer (TNBC). The purpose of the study is to show the impact of different combinations of chemotherapeutics on tumor microenvironment remodeling in bad responders (chemotherapy-resistant). Trichrome staining (a staining method to detect total collagen) was performed on both biopsies (before treatment) and surgical specimens (after treatment), which were obtained from the same patients, to compare the collagen expression level. Our findings showed that chemotherapeutics cause fibrosis, represented by higher collagen expression in the surgical specimens in comparison to collagen expression in biopsies (Figure 2) (1,2).

In another study, we aimed to analyze the fibroblast

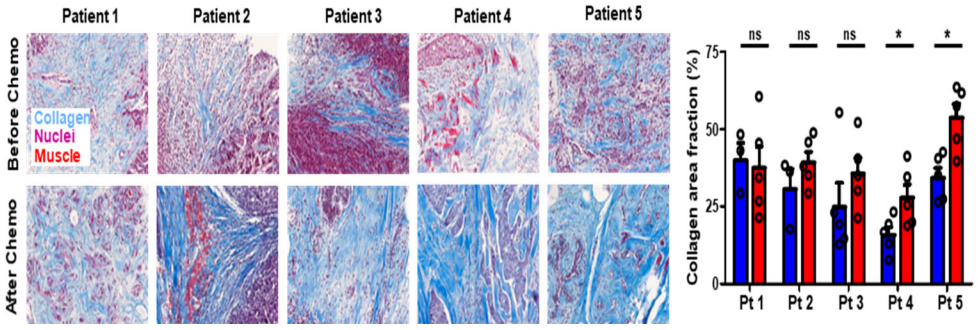


Figure 2. Trichrome staining of samples obtained from disease progressor patients with TNBC: Representative images show that different combination chemotherapy causes an increase in collagen content in the tumor microenvironment (*p<0.01).

subpopulations in doxorubicin (dox) sensitive vs. resistant murine TNBC models. For this study, doxorubicin-resistant murine triple-negative 4T1 cells were developed and inoculated into a mouse mammary fat pad. Tumors were taken, fibroblasts were isolated and analyzed with 10x genomics single-cell RNA-sequencing. Our findings showed that the fibroblasts which were isolated from dox-resistant tumors activated overexpression of collagen-related genes (Figure 3).

There have been several studies addressing the issues on the impact of chemotherapeutics on different cell populations in breast tumors (3, 4). The amount of infiltrated immune cell populations into breast tumors, for example, has been strongly related to the response to chemotherapy (5). However, not much is known about the impact of AIs on the cell populations of breast tumors. A very appreciated effort from Miller and colleagues reported their findings on patients who developed letrozole resistance (6). This study, however, was unfortunately limited by bulk RNA-based gene expression profiling.

In our project, by the help of single nuclei RNA-sequencing, we will be able to analyze the expression profile of the different type of tumor cell populations before and after letrozole treatment. This will provide an intensive analysis identifying novel markers predicting for response and understanding molecular mechanisms of resistance.

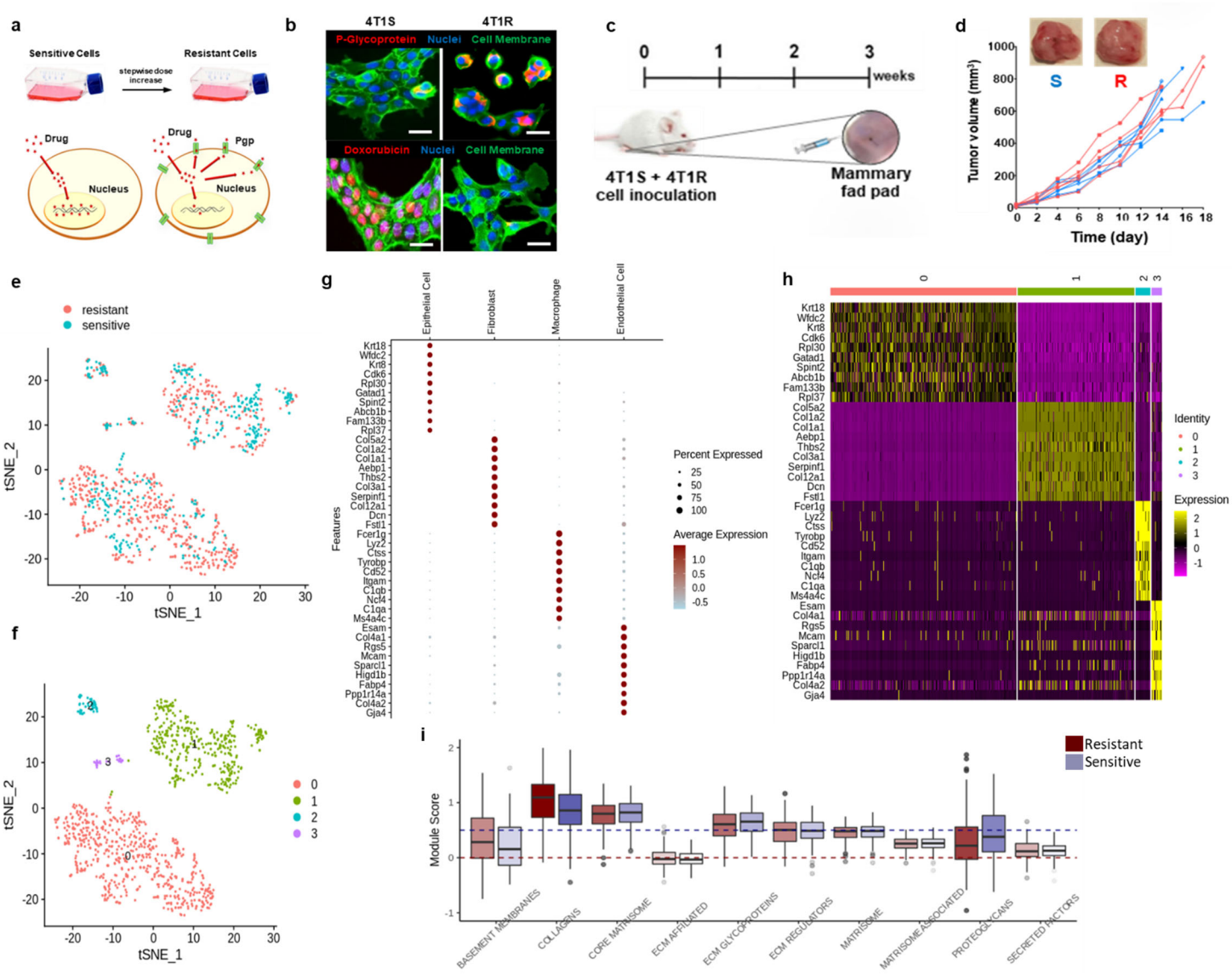


Figure 3. Development of doxorubicin resistant 4T1 cells and the gene expression profile of fibroblasts isolated from resistant 4T1 tumor: The development of doxorubicin resistant 4T1 cells from sensitive cells through stepwise dose increase and schematic explanation of resistant cancer cells are shown (a). Representative microscopy images demonstrate p-glycoprotein (red) expression and intracellular uptake of doxorubicin (red) in sensitive and resistant 4T1 cells (b). The schematic representation shows inoculation of sensitive and resistant 4T1 cells into mammary fat pad (c). Similar growth rate of sensitive and resistant tumors is shown (d). The analysis of different cell populations in sensitive and resistant tumors is shown (e). Four clusters, 0; epithelial cells, 1; fibroblasts, 2; macrophages and 3; endothelial cells are shown (f). Selected top 10 genes in cell clusters are shown (f). The heat-map analysis of top 10 genes for each cluster is shown (h)These results show that the fibroblasts overexpress extracellular matrix related protein, such as collagens, which can cause fibrosis (i).

1.2 Investigational Agent

Letrozole is an aromatase inhibitor, which is a commonly used endocrine therapy for breast cancer as per standard of care. Letrozole is an oral agent commonly supplied as a tablet.

2 Study Objectives

Our approach is to study the impact of AIs on not only cancerous cells but also on noncancerous cells in the tumor microenvironment. We seek to characterize factors discriminating non-responders from responders to AI early during treatment by analyzing different cells. We also aim to work on the impact of AIs on the immune landscape, as a noncancerous cell subpopulation, of AI-sensitive and AI-resistant tumors. This, we believe, will contribute to the current knowledge to improve the therapeutic efficacy of current treatment modalities, including chemotherapy and immunotherapy. Accordingly, we hypothesize that snRNA-seq can uncover hidden transcriptomic heterogeneity and underlying preexisting mechanisms driving AI resistance when analyzed at the single-cell level. Moreover, our research will also shed more light on the impact of estrogen deprivation on the immune landscape with AI treatment.

Primary Objective

1. To comprehensively evaluate differences in tumor microenvironment subpopulations in AI-sensitive vs. AI-resistant HR+ breast cancer.

Secondary Objective

1. To assess the effects of estrogen deprivation on the systemic immune response.
2. To assess the effects of estrogen deprivation on tumor immune microenvironment.

3 Study Design

This is a pilot study to evaluate the effects of aromatase inhibitor using single cell sequencing technology to evaluate both cancerous cells and tumor microenvironment.

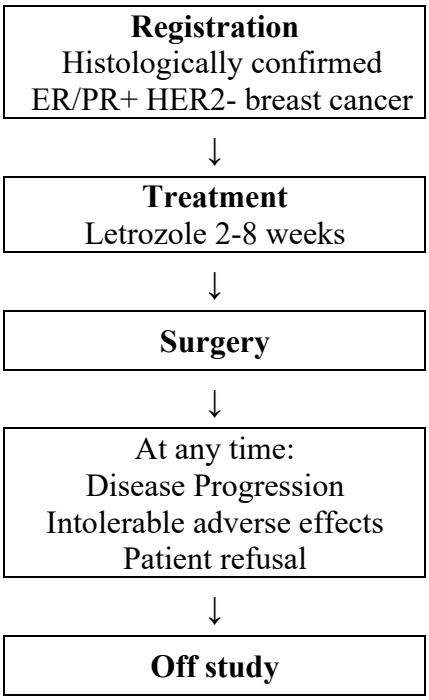
3.1 General Description

This is a prospective study to collect tissue samples from patients with early-stage hormone receptor-positive HER2-negative breast cancer. Patients with newly diagnosed hormone receptor-positive HER2-negative invasive breast cancer will be approached by the study team prior to surgery. If there is no indication for neoadjuvant therapy and the patient can proceed with surgery, the patient will be registered and start on aromatase inhibitor with letrozole 2.5 mg oral daily for 2-8 weeks until surgery as part of their standard of care. Both archival biopsy and surgical specimens will also be collected for single-cell sequencing. For patients who screen fail and do not consent to future research, tissue and blood will be discarded.

Peripheral blood prior to and after letrozole treatment will also be collected.

Estrogen receptor, progesterone receptor, HER2, and Ki-67 will be evaluated in both biopsy and surgical specimens as per standard of care. Tumors with persistent elevation of Ki-67 ≥ 10% after

2-8 weeks of letrozole will be classified as non-responders, and tumors with Ki-67 < 10% after treatment will be classified as responders.



3.2 Number of Subjects

Up to 50 patients

3.3 Duration of Participation

2-8 weeks

3.4 Primary Study Endpoints

To comprehensively evaluate differences in tumor microenvironment subpopulations in AI-sensitive vs. AI-resistant HR+ breast cancer.

3.5 Secondary Study Endpoints

1. To assess the effects of estrogen deprivation on the systemic immune response.
2. To assess the effects of estrogen deprivation on tumor immune microenvironment.

4 Subject Selection Enrollment and Withdrawal

4.1 Inclusion Criteria

Screening inclusion criteria

1. Female ≥ 18 years.
2. Postmenopausal and suitable to receive aromatase inhibitor as per physician's discretion.
3. Histologically confirmed un-resected operable invasive adenocarcinoma of the breast ≥ 0.5 cm with estrogen receptor (ER) and/or progesterone receptor (PR) positive ≥ 10%, and no human epidermal growth factor receptor 2 (HER2) amplification or overexpression.
4. Patients must not have received any prior chemotherapy, radiation therapy, or endocrine therapy for their current breast cancer. Patients who received tamoxifen or raloxifene or another agent for prevention of breast cancer may be included.
5. Willing and able to provide research tissue samples.
6. Willing and able to provide research blood samples.

4.2 Exclusion Criteria

1. Immunocompromised patients including patients known to be HIV positive or those on chronic steroids.

NOTE: Must be off systemic steroids at least 14 days prior to pre-registration. However, topical steroids, inhalants or steroid eye drops are permitted.

2. Known history of active autoimmune disease that has required systemic treatment within ≤ 30 days (i.e., with use of disease modifying agents, corticosteroids, or immunosuppressive drugs) prior to pre-registration.

NOTE: Replacement therapy (e.g., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency) is not considered a form of systemic treatment. Patients with vitiligo, Graves disease, or psoriasis not requiring systemic treatment within the past 30 days are not excluded. Patients with Celiac disease controlled with diet modification are not excluded.

4.3 Subject Recruitment, Enrollment and Screening

This is a prospective study to collect tissue samples from patients with early-stage hormone receptor-positive HER2-negative breast cancer. Patients with newly diagnosed hormone receptor-positive HER2-negative invasive breast cancer will be approached by the study team prior to surgery. Patients will be screened by a study team member through provider list or schedule list. Patients will be contacted preferably via a phone call prior to their visit or in person at the time of their visit. Patients will be consented either electronically remotely via email or in person at the time of their visit by a study team member.

4.4 Early Withdrawal of Subjects

4.4.1 When and How to Withdraw Subjects

Patients will be withdrawn from the study prior to completing all study related procedures if:

- Disease progression
- Intolerable adverse effects

- Patient decision to withdraw from the study
- Failure to adhere to protocol requirements, including refusal of surgery and noncompliance to letrozole ≥ 5 consecutive days.

4.4.2 Data Collection and Follow-up for Withdrawn Subjects

A patient is deemed a withdrawal if he/she is removed from the study for any reason before any study treatment is given. On-study material and the End of Active Treatment/Cancel Notification Form must be submitted. No further data submission is necessary.

5 Study Drug

5.1 Description

Letrozole is a standard of care aromatase inhibitor used to treat hormone receptor positive breast cancer.

5.2 Treatment Regimen

Letrozole 2.5 mg oral daily dose will be used for 2-8 weeks prior to surgery.

5.3 Drug Accountability

Drug accountability will be performed via patient pill diaries and patient appointments.

6 Study Procedures

This is a prospective study to collect tissue samples from patients with early-stage hormone receptor-positive HER2-negative breast cancer. Patients with newly diagnosed hormone receptor-positive HER2-negative invasive breast cancer will be approached by the study team prior to surgery. Once pathology confirms the diagnosis for hormone receptor-positive HER2 negative invasive breast cancer, the patient will be evaluated by breast surgeons. If there is no indication for neoadjuvant therapy and the patient can proceed with surgery within 2-8 weeks, the patient will be considered registered and start on aromatase inhibitor with letrozole 2.5 mg oral daily for 2-8 weeks until surgery as part of their standard of care. Patients will take letrozole until within 48 hours prior to surgery. Both archival biopsy and surgical specimens will also be collected for single-cell sequencing.

Peripheral blood will be drawn at two timepoints: 1.) prior to letrozole treatment and 2.) at least two weeks after the start of letrozole treatment, but prior to surgery.

Estrogen receptor, progesterone receptor, HER2, and Ki-67 will be evaluated in both biopsy and surgical specimen as per standard of care. Tumors with persistent elevation of Ki-67 ≥ 10% after

2-8 weeks of letrozole will be classified as non-responders, and tumors with Ki-67 < 10% after treatment will be classified as responders.

This study is not utilizing the Cancer Center Registration Application. Patients will be considered enrolled upon completion of the ICF in PTrax. However, patients will not be considered registered within the Cancer Center until they have met all eligibility criteria and have a status of accrued in PTrax.

6.1 Test Schedule

Tests and Procedures	Screening	Baseline (Day 1 of Letrozole)	After 2-8 weeks of Letrozole	Surgery[2]
Visit Window	**-30 days from baseline**	**-7 days**		
	Standard of Care Procedures			
Consent	X			
History and Physical		X	X[5]	
Adverse event assessment		X	X[5]	
Tissue collection	X[1]			X[1]
	Research Procedures			
Blood samples - Sodium heparin (green top) 10 mL x 6 tubes - Serum (red top) 10 mL x 1 tube		X[3]	X[3]	

[1] Archival sample collection from the standard of care biopsy and surgical resection at least 5 x 5 uM slides will be collected.
[2] Surgery should occur less than 48 hours after the last dose of letrozole.
[3] Completed at two timepoints: 1.) prior to letrozole treatment and 2.) at least two weeks after the start of letrozole treatment, but prior to surgery.

6.2 Sample Processing

All biopsy and surgical specimens will be snap frozen by BAP/PRC or CRC team. Samples will be kept at -80ºC and held for pick up.

Blood samples will be processed by BAP/PRC. PBMC, serum, and plasma will be aliquoted, kept at -80^{0}C, and held for pick up.

Statistical Plan

6.3 Sample Size Determination

This is a pilot study to evaluate the difference in tumor microenvironment subpopulation in AI-sensitive vs. AI-resistant HR+ breast cancer. Therefore, power calculation will not be required.
Statistical Methods

Secondary analysis: Sequenced data will go through secondary analysis in department of bioinformatics in Mayo Clinic Rochester campus. The estimated duration is 3 weeks for this analysis.

Tertiary analysis: The last step of data analysis will be performed by myself and our collaboration partners of Dr. Daniel Wickland and Dr. Xue Wang.

Endpoints

Specific Aim 1: To comprehensively evaluate differences in tumor microenvironment subpopulations in AI-sensitive vs. AI-resistant HR+ breast cancer.

Specific Aim 2: To assess the effects of estrogen deprivation on the immune microenvironment.

- Sub Aim 2A: To assess the effects of estrogen deprivation on the systemic immune response.
- Sub Aim 2B: To assess the effects of estrogen deprivation on tumor immune microenvironment.

7 Safety and Adverse Events

7.1 Definitions

Unanticipated Problems Involving Risk to Subjects or Others (UPIRTSO)
Any unanticipated problem or adverse event that meets the following three criteria:

- Serious: Serious problems or events that results in significant harm, (which may be physical, psychological, financial, social, economic, or legal) or increased risk for the subject or others (including individuals who are not research subjects). These include: (1) death; (2) life threatening adverse experience; (3) hospitalization - inpatient, new, or prolonged; (4) disability/incapacity - persistent or significant; (5) birth defect/anomaly; (6) breach of confidentiality and (7) other problems, events, or new information (i.e. publications, DSMB reports, interim findings, product labeling change) that in the opinion of the local investigator may adversely affect the rights, safety, or welfare of the subjects or others, or substantially compromise the research data, **AND**
- Unanticipated: (i.e. unexpected) problems or events are those that are not already described as potential risks in the protocol, consent document, not listed in the Investigator's Brochure, or not part of an underlying disease. A problem or event is "unanticipated" when

it was unforeseeable at the time of its occurrence. A problem or event is "unanticipated" when it occurs at an increased frequency or at an increased severity than expected, AND

- Related: A problem or event is "related" if it is possibly related to the research procedures.

Adverse Event
An untoward or undesirable experience associated with the use of a medical product (i.e. drug, device, biologic) in a patient or research subject.

Serious Adverse Event
Adverse events are classified as serious or non-serious. Serious problems/events can be well defined and include;

- death
- life threatening adverse experience
- inpatient hospitalization or prolongation of existing hospitalization
- persistent or significant disability or incapacity
- substantial disruption of the ability to conduct normal life functions
- birth defect/congenital anomaly

and/or per protocol may be problems/events that in the opinion of the sponsor-investigator may have adversely affected the rights, safety, or welfare of the subjects or others, or substantially compromised the research data.

All adverse events that do not meet any of the criteria for serious, should be regarded as **non-serious adverse events**.

Adverse Event Reporting Period
For this study, the study treatment follow-up period is defined as 2-8 weeks while on letrozole up until surgery, or until early discontinuation of letrozole. The study period during which adverse events must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up.

Hospitalization, Prolonged Hospitalization or Surgery
Any adverse event that results in hospitalization or prolonged hospitalization at least possibly related to the study should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for an adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event and are not considered to be SAEs in the following circumstances:

- Planned hospitalizations required by the protocol, including breast biopsy and surgery.
- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should **not** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful
- Hospitalization for elective procedures unrelated to the current disease and/or treatment on this trial

- Hospitalization for insertion of access for administration of a drug
- Hospitalization for routine maintenance of a device (*e.g.*, battery replacement) that was in place before study entry
- Hospitalization, or other serious outcomes for signs and symptoms of progression of cancer.

7.2 Recording of Adverse Events

Adverse events will be assessed at baseline and after at least 2-8 weeks of Letrozole. The patient will be contacted by the coordinator via phone call, if not seen in clinic, to assess adverse events, particularly: hot flashes and arthralgia (joint pain). If patient reports ≥ grade 3 adverse events, the study coordinator will notify the provider to follow-up with the patient within 2 business days to provide further guidance. In case of an emergency, patient will be instructed to seek immediate medical attention and go to the emergency room, and the study coordinator will notify the provider immediately.

All adverse events at least possibly related occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been ultimately determined that the study treatment or participation is not the probable cause. Serious adverse events at least possibly related that are still ongoing at the end of the study period must be followed up by the coordinator via phone call, if not seen in clinic, to determine the final outcome or 30 days after last dose of letrozole. Any serious adverse event that occurs during the Adverse Event Reporting Period and is considered to be at least possibly related to the study treatment or study participation should be recorded and reported according to the Mayo IRB Policy and Procedures.

CTCAE Version 5.0 (v5.0: November 27, 2017)
https://ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm will be used.

7.3 Reporting of Serious Adverse Events and Unanticipated Problems

When an adverse event has been identified, the study team will take appropriate action necessary to protect the study participant and then complete the Study Adverse Event Worksheet and log. The sponsor-investigator will evaluate the event and determine the necessary follow-up and reporting required.

7.3.1 Sponsor-Investigator reporting: notifying the Mayo IRB

The sponsor-investigator will report to the Mayo IRB any UPIRTSOs and Non-UPIRTSOs at least possibly related to the study according to the Mayo IRB Policy and Procedures.

Information below will be collected:

- Subject's name:
- Medical record number:
- Disease/histology (if applicable):
- The date the adverse event occurred:
- Description of the adverse event:
- Relationship of the adverse event to the research (drug, procedure, or intervention*):

- If the adverse event was expected:
- The severity of the adverse event: (use a table to define severity scale 1-5**)
- If any intervention was necessary:
- Resolution: (was the incident resolved spontaneously, or after discontinuing treatment)
- Date of Resolution:

The sponsor-investigator will review all adverse event reports to determine if specific reports need to be made to the IRB . The sponsor-investigator will sign and date the adverse event report when it is reviewed. For this protocol, only SAEs/UPIRTSOs at least possibly related to the study will be reported to the IRB.

The relationship of an AE to the Investigational Drug is a clinical decision by the sponsor-investigator (S-I) based on all available information at the time of the completion of the form and is graded as follows:

1. Not related: a reaction for which sufficient information exists to indicate the etiology is unrelated to the study drug; the subject did not receive the study medication or the temporal sequence of the AE onset relative to administration of the study medication is not reasonable or the event is c1early related to other factors such as the subject' s clinical state, therapeutic intervention or concomitant therapy.

2. Unlikely: a clinical event, including laboratory test abnormality, with a temporal relationship to drug administration which makes a causal relationship improbable and in which other drugs, chemicals, or underlying disease provide plausible explanations.

3. Possible: a clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the drug but which could also be explained by concurrent disease or other drugs or chemicals; information on drug withdrawals may be lacking are unclear.

4. Probable: a clinical event including laboratory test abnormality, with a reasonable time sequence to administration of the drug, unlikely to be attributed to concurrent disease or other drugs or chemicals and which follows a clinically reasonable response on withdrawal (de-challenge): re-challenge information is not required to fulfill this definition.

5. Definite: a reaction that follows a reasonable temporal sequence from administration of the drug, or in which the drug level has been established in body fluids or tissues, that follows a known or expected response pattern to the suspected drug, and that is confirmed by improvement on stopping or reducing the dosage of the drug, and reappearance of the reaction on repeated exposure (re-challenge).

8 Data Handling and Record Keeping

8.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (long term survival status that the subject is alive) at the end of their scheduled study period.

8.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

8.3 Data Collection

This study will use REDCap for data collection.

9 Study Monitoring

The principal investigator(s) and the study statistician will review the study monthly to identify accrual, adverse event, and any endpoint problems that might be developing.

10 Ethical Considerations

This study is to be conducted according to United States government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted local Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study. The decision of the IRB concerning the conduct of the study will be made in writing to the sponsor-investigator before the commencement of this study.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the approved IRB consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or the subject's legally authorized representative, and the individual obtaining the informed consent.

11 Budget

This study is funded through a grant from Mayo Clinic.

11.1 Costs charged to patient

- Routine clinical care
- Biopsy
- Surgery
- Letrozole 2.5 mg

11.2 Test to be research funded

- Research blood draw
- Tumor tissue sequencing

12 References

1. Echeverria, G.V., Ge, Z., Seth, S., Zhang, X., Jeter-Jones, S., Zhou, X., Cai, S., Tu, Y., McCoy, A., Peoples, M. and Sun, Y., 2019. Resistance to neoadjuvant chemotherapy in triple-negative breast cancer mediated by a reversible drug-tolerant state. Science translational medicine, 11(488).
2. Saatci, O., Kaymak, A., Raza, U., Ersan, P.G., Akbulut, O., Banister, C.E., Sikirzhytski, V., Tokat, U.M., Aykut, G., Ansari, S.A. and Dogan, H.T., 2020. Targeting lysyl oxidase (LOX) overcomes chemotherapy resistance in triple negative breast cancer. Nature communications, 11(1), pp.1-17.
3. Nguyen, Q.H., Pervolarakis, N., Blake, K., Ma, D., Davis, R.T., James, N., Phung, A.T., Willey, E., Kumar, R., Jabart, E. and Driver, I., 2018. Profiling human breast epithelial cells using single cell RNA sequencing identifies cell diversity. Nature communications, 9(1), pp.1-12.
4. Kim, C., Gao, R., Sei, E., Brandt, R., Hartman, J., Hatschek, T., Crosetto, N., Foukakis, T. and Navin, N.E., 2018. Chemoresistance evolution in triple-negative breast cancer delineated by single-cell sequencing. Cell, 173(4), pp.879-893.
5. Denkert, C., von Minckwitz, G., Darb-Esfahani, S., Lederer, B., Heppner, B.I., Weber, K.E., Budczies, J., Huober, J., Klauschen, F., Furlanetto, J. and Schmitt, W.D., 2018. Tumour-infiltrating lymphocytes and prognosis in different subtypes of breast cancer: a

pooled analysis of 3771 patients treated with neoadjuvant therapy. The lancet oncology, 19(1), pp.40-50.

6. Miller, W.R., Larionov, A., Renshaw, L., Anderson, T.J., Walker, J.R., Krause, A., Sing, T., Evans, D.B. and Dixon, J.M., 2009. Gene expression profiles differentiating between breast cancers clinically responsive or resistant to letrozole. *J Clin Oncol*, *27*(9), pp.1382-1387.
7. Zheng GXY, Terry JM, Belgrader P, Ryvkin P, Bent ZW, Wilson R, et al. Massively parallel digital transcriptional profiling of single cells. Nature Communications. 2017;8(1):14049
8. Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, et al. STAR: ultrafast universal RNA-seq aligner. Bioinformatics (Oxford, England). 2013;29(1):15-21. Epub 2012/10/25.
9. Lun ATL, Riesenfeld S, Andrews T, Dao TP, Gomes T, Marioni JC, et al. EmptyDrops: distinguishing cells from empty droplets in droplet-based single-cell RNA sequencing data. Genome Biol. 2019;20(1):63.
10. Stuart T, Butler A, Hoffman P, Hafemeister C, Papalexi E, Mauck WM, 3rd, et al. Comprehensive Integration of Single-Cell Data. Cell. 2019;177(7):1888-902.e21. Epub 2019/06/11.
11. Finak G, McDavid A, Yajima M, Deng J, Gersuk V, Shalek AK, et al. MAST: a flexible statistical framework for assessing transcriptional changes and characterizing heterogeneity in single-cell RNA sequencing data. Genome Biol. 2015;16:278.
12. Liberzon A, Subramanian A, Pinchback R, Thorvaldsdóttir H, Tamayo P, Mesirov JP. Molecular signatures database (MSigDB) 3.0. Bioinformatics (Oxford, England). 2011;27(12):1739-40. Epub 2011/05/05.