An Umbrella, Randomized, Controlled, Pre-Operative Trial Testing Integrative Subtype-Targeted Therapeutics in Estrogen Receptor-Positive, HER2-Negative Breast Cancer

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AMENDMENTS/SUMMARY OF CHANGES

Version	Description of Changes
1.1 (20-Oct-2020)	• Updated page numbers and section headers in the Table of Contents. • Made clerical and style changes throughout the protocol per Stanford SRC pre-review comments.
1.2 (12-Nov-2020)	• Combined the main umbrella protocol with treatment-specific appendices into one document. • Included Letrozole to Section 2.3 Rationale of the master section of the protocol.
1.3 (17-Nov-2020)	• Included an appendix with the IDS pharmacy address listed for each site. • Made clerical and grammatical changes throughout the protocol.
1.4 (18-Nov-2020)	• Made edits to the outcome measurement descriptions in Section 10, sample size description in Section 12.6 of the master protocol. • Made clerical and grammatical changes throughout the protocol.
1.5 (08-Dec-2020)	• Added stopping rules for toxicity and a futility rule for efficacy in Section 12.2 of the master protocol. • Added Section 3.2 (Dosing regimen) for treatment-specific appendices, and further clarified the dosing schedule for infigratinib in the footnote of the study calendars.
1.6 (26-Jan-2021)	• Amended Section 7.3 to require AEs of all grades to be required in AE reporting (not just Grades 3 or 4). • COST-FACIT survey has been removed and appropriate clerical changes made.
1.7 (02-Mar-2021)	• Removed part 2 of the treatment phase and its associated objectives. Appropriate clerical changes. • Combined integrative subtype 2 and 6 into one cohort. • Added appendix for integrative subtype 9 and typical-risk integrative subtype. • Typical-risk cohort will have three treatment arms. Appropriate clerical changes made. • Eligibility criteria changed to be ER-positive only (not PR-positive without ER positivity) and tumor size 1 cm or higher (rather than 1.5 cm or higher). • Revised statistical analytical approach from generalized estimating equations (GEE) to ANCOVA, based on FDA guidance document. • Added secondary objective to assess quantitative Ki67 reduction using digital pathology.

Version	Description of Changes
1.8 (29-Apr-2021)	• Removed the requirement of fasting labs for Cohort 2 screening. • Removed Ki67 < 2.7% as secondary endpoint. • Remove the secondary endpoint about digital path concordance. • Changed primary endpoint to be digital path (QuPath). • Clarified that pathologist assessing samples will be blinded to randomization. • Changed inclusion criteria for the pre-screening phase to include Ki67 ≥ 5%, if done locally. • Updated safety reporting guidelines in Sections 7.4 and 7.5 of the main "umbrella" protocol. • Removed futility stopping rule. • Included rationale and safety data for dose level chosen for infigratinib in Appendix F. • Updated treatment duration for IC2 and 6 (Appendix F) to be 18 days (-2, +3), and updated overall treatment duration throughout the main "umbrella" protocol. Added a D12 lab check accordingly to this cohort. • Simplified dose modification directions in all Cohort-specific appendices to reflect that there will only be 14 to 21 days of treatment (and thus holding drug more than 7 days typically should mean simply discontinuing drug). • Changed "pharmaceutical sponsor" to "pharmaceutical supplier" to reflect that the pharmaceutical companies are not the sponsor of this protocol. • Updated Appendix B (Sequencing Vendor's Order Requisition Form).
1.9 (03-Jun-2021) – (initial SRC approval)	• Updated schema of Appendix F in order to reflect the proper cohort labeling • Per SCI DSMC, clarified in Section 11.2 Data and Safety Monitoring Plan about the process of data review and communication.
1.10 (22-Mar-2022)	• IND and NCT numbers provided on cover page and throughout protocol. • Changed IC2/6 appendix to fulvestrant with or without zotatifin (manufacturer: eFFECTOR Therapeutics) throughout. • Changed from Tempus to Caris for sequencing to determine integrative subtype throughout. • Replaced Vandana Sundaram with Kate Miller as biostatistician. Added Lisa Kody as clinical research coordinator. • Adjustments to alpelisib dose modification plan so that therapy is discontinued rather than dose-reduced for certain Grade 3 adverse events, given altruistic clinical trial. • Integrated document navigation, as well as formatting and spelling corrections • Relabeled the overall Study Schema with therapeutic agents for each arm. • Updated treatment duration for Cohort 2 to be 14 days (-2, +7). • Modified study personnel roles for Protocol Director and Key Sub-investigator. • Removed redundant listing of eligibility criteria, added hyperlinks to checklists in corresponding appendices, and included information for protocol, participant and statement of eligibility for cohort-specific checklists.
1.11 (19-Apr-2022)	• Updated the investigational agent description and storage conditions for Cohort 2.

Version	Description of Changes
1.12 (28-Apr-2022)	• Updated the details in regard to the collection of correlatives studies in sections 2.5, 2.6.3, 8.1 and 8.2 • In section 11.3, clarified that the Stanford SRC and independent DSMB will judge the aggregate accrual and stopping rules respectively. • Removed the optional research plasma collection at the screening timepoint in each appendice's study calendar. This collection is mandatory at pre-screening.
2.0 (20-May-2022) (SRC approved)	• Added footnote to schema figure. • In the Synopsis and Schema, specified the number of participants to be enrolled in each cohort • Re-added figure D-1 that was removed in error in version 1.12. • Clarified that only one reviewer signature is needed for pre-screening eligibility review, and that it must be a site principal investigator or treating physician. • Clarified that the measurement for zotatifin should be based on the patient's weight in kilograms on treatment days. • Administrative, grammatical, and formatting changes
3.0 (9-Aug-2022)	• Provided a stopping rule for surgery delays for all cohorts • Exploratory analysis added for Cohort 1 per FDA recommendation • Added additional lab draws for glucose monitoring for Cohort 1 based on manufacturing packet and FDA recommendation • Added additional lab draws for creatine phosphokinase monitoring for Cohort 2 • Added lab tests at 30 Day Follow Up for Cohorts 1 and 2 • Added dose modification management for CPK elevation for Cohort 2 • Removed Dr Sledge as key sub-investigator • Administrative, grammatical, and formatting changes
4.0 (24-Aug-2022)	• On the basis of manufacturer Sanofi's decision to discontinue development of amcenestrant (17 August 2022), information and sections related to amcenestrant, ie, Cohorts 3 and 4, throughout the protocol are removed.
5.0 (16-Feb-2023) (SRC approved)	• Corrected the title of key investigator, Christina Curtis, PhD • Updated the dose, administration schedule and drug safety information of Zotatifin (Cohort 2 & 3) • Updated the main study schema and synopsis to include Cohort 3 Typical Risk. • Included an exploratory endpoint (PEPI score) in Sections 1.3, 10.3, 12.5 and list of abbreviations • Updated that the Stanford Data Safety Monitoring Committee (DSMC), in addition to TRIO-Global, will monitor Stanford sites • Referenced the Typical Risk arm in section 3.2 Treatment Eligibility, including updates to eligibility inclusion #1 in Appendix D-A • Updated Appendix D to add Typical Risk arm throughout • Removed mentions of UCLA and TRIO-US network • For zotatifin: added efficacy data, updated safety data, and recommendation for consideration of premedication with antiemetic • Formatting changes and typos fixed
	Adding the reason of change in version 5.0 per Stanford SRC's recommendation: • Re-introduction of the Typical Risk arm for comparison

Version	Description of Changes

6.0 (24-May-2023)	• Inserted eligibility criteria from the pre-screening phase into each treatment cohort's eligibility checklist, in order for all criteria to be triple-signed upon entry of the treatment phase
	• Prescreening:
	  o Clarified in Exclusion Criterion 2 that ovarian suppression for premenopausal women is allowed.
	• Cohort 1:
	  o Provided a window of administration that alpelisib can be taken from one day to another in section C 4.1.2.
	  o Added the window for triplicate electrocardiogram collection.
	  o In the Study Calendar, clarified that:
	  ▪ OncotypeDX will be recorded (if available).
	  ▪ Lab assessments do not have to be repeated if last performed within 72 hours.
	  ▪ The information to be collected for post-treatment tumor specimen.
	• Cohorts 2 & 3:
	  o In section D 1.3.4 and the Study Calendar, denoted that for subjects receiving zotatifin, it is preferred that fulvestrant be administered prior to zotatifin whenever possible.
	  o Updated pharmacokinetics data in section D 1.2.1.4, per the manufacturer.
	  o In section D 3.1.1, added a recommendation to consider holding statin and fenofibrate for those receiving zotatifin.
	  o In the Study Calendar, clarified that:
	  ▪ OncotypeDX will be recorded (if available).
	  ▪ Electrocardiogram is to be done as triplicate and the window for collection.
	  ▪ The information to be collected for post-treatment tumor specimen.
	  o Added nirmatrelvir to the list of strong CYP3A4 inhibitors in Appendix D-B, per the manufacturer.
	• Administrative, grammatical, and formatting changes

7.0 (20-Nov-2023)	• Provided additional information in sections 8.1 & 8.2 that specimen instructions can be found in specimen kits and lab manual. • Provided the definition of protocol deviation in section 11.4. • For Cohort 1, changed the dosage of Alpelisib from 300mg to 250mg. • Removed mentions of TRIO throughout the protocol. • Amended the total number of participating sites from "10 to 15" to "6 to 8". • Administrative, grammatical, and formatting changes

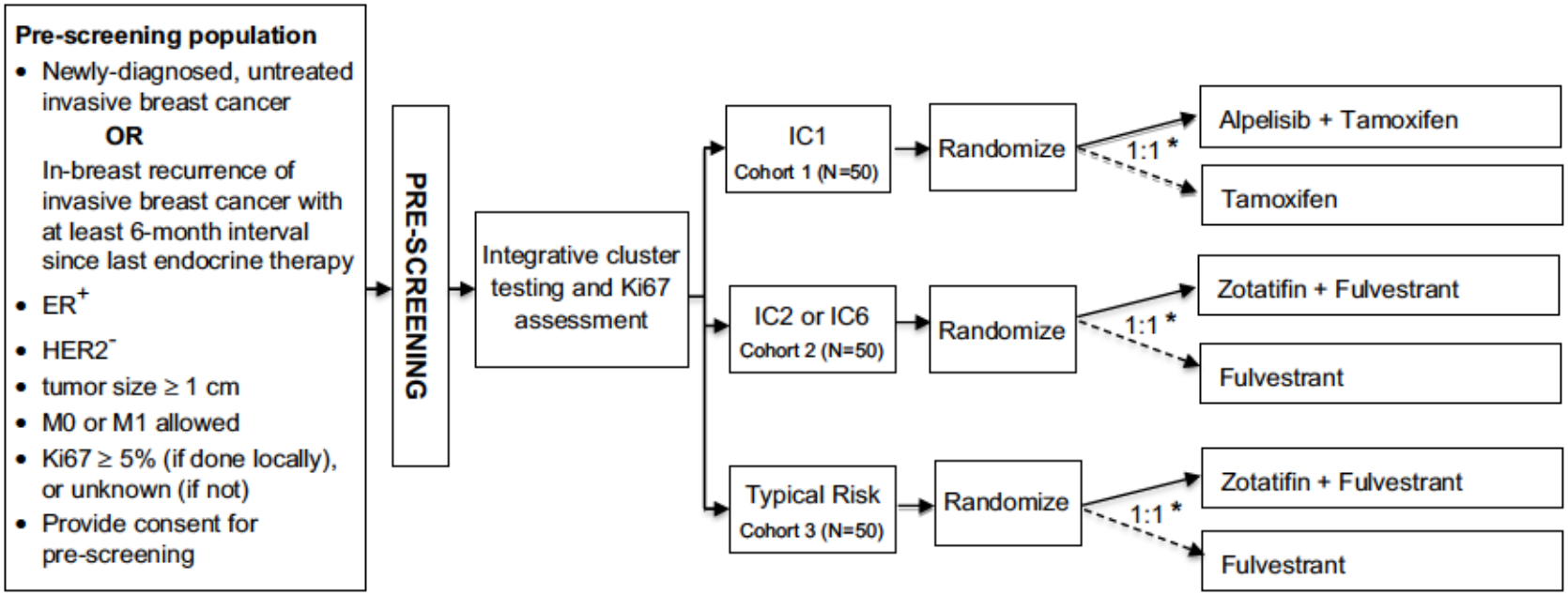
TABLE OF CONTENTS

PROTOCOL SYNOPSIS

TITLE	**An Umbrella, Randomized, Controlled, Pre-Operative Trial Testing Integrative Subtype-Targeted Therapeutics in Estrogen Receptor-Positive, HER2-Negative Breast Cancer**
STUDY PHASE	2
INDICATION	Estrogen receptor-positive (ER^+), HER2-negative ($HER2^-$) breast cancer
STUDY POPULATION	Patients with newly-diagnosed (stage I to IV) ER^+, $HER2^-$ breast cancer (BC), or in-breast recurrence of ER^+, $HER2^-$ BC (at least 6 months since last endocrine therapy), whose tumor size is ≥ 1 cm and has tumor marker of proliferation Ki67 ≥ 10%, and is of integrative subtype 1; 2; 6 or Typical Risk (3, 4, 7, 8). Ki67 is a intracellular marker of tumor proliferation that is assessed by immunohistochemistry.
INVESTIGATIONAL PRODUCTS and DOSAGES	Integrative subtype 1 (Cohort 1): Alpelisib tablets 250 mg/day by mouth Integrative subtypes 2 and 6 (Cohort 2) and Typical Risk (Cohort 3): Zotatifin 0.10 mg/kg x 1 administered by IV
ACTIVE CONTROL AGENTS AND DOSAGES	Cohort 1: Tamoxifen 20 mg/day by mouth (commercial supply) Cohort 2 & 3: Fulvestrant, two 250 mg intramuscular (IM) injections on Day 1. Fulvestrant is considered a standard mediation in this study, and is supplied as an IND-exempt agent.
PRIMARY OBJECTIVE	To evaluate the efficacy of investigational agent compared with standard endocrine therapy in reducing digital pathology software-assessed Ki67 values (using QuPath) from baseline to on-treatment biopsy after an appendix-specific treatment duration (ie, 14 days).
SECONDARY OBJECTIVE	To evaluate the efficacy of investigational agent compared with standard endocrine therapy on the proportion of subjects with Ki67 < 10% after an appendix-specific treatment duration (ie, 14 days).
TREATMENT SUMMARY	Subjects will be pre-screened to assess eligibility for participation in the treatment phase of the trial. In the treatment phase, enrolled subjects will be randomly assigned to either the investigational agent arm or the standard endocrine therapy arm. All subjects will be instructed to take their assigned therapy based on a duration specified in their respective appendix assignment, with the last day of treatment being the day prior to their surgery or scheduled biopsy. They will then undergo an on-treatment biopsy or definitive surgery.

SAMPLE SIZE	For each cohort, at least 50 subjects will be enrolled. Subjects will be randomized 1:1 to standard endocrine therapy alone or in combination with an IC-subtype specific investigational therapy. Covariate-adaptive randomization using a biased-coin minimization approach will be used. The co-variates of interest are prior endocrine therapy (yes/no) and planned treatment (core biopsy or definitive surgery). For the integrative subtype 2/6 cohort, an additional covariate of interest is integrative subtype.

SCHEMA



IC1 Cohort 1. See also Appendix C (REMOVED).
IC2 or 6 Cohort 2. See also Appendix D.
Typical Risk Cohort 3. See also Appendix D.
* Randomizations will be roughly 1:1, but may stratify

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

AE	Adverse event
ADA	American Diabetes Association
ALP	Alkaline Phosphatase
ALT	Alanine aminotransferase
ANCOVA	Analysis of covariance model
ASCO	American Society of Clinical Oncology
AST	Aspartate aminotransferase
BCRP	Breast Cancer Resistance Protein
CBC	Complete blood count
CRF	Case report/Record form
COPD	Chronic obstructive pulmonary disease
CPK	Creatine phosphokinase
CTCAE	Common Terminology Criteria for Adverse Events
DLT	Dose-limiting toxicity
DNA	Deoxyribonucleic acid
DSMB	Data Safety Monitoring Board (independent)
DSMC	Data Safety Monitoring Committee (Stanford)
DSP	Nanostring GEOMx Digital Spatial Profiling
DTI	Direct thrombin inhibitors
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
ER	Estrogen receptor
FDA	Food and Drug Administration
FFPE	Formalin-fixed paraffin-embedded
FPG	Fasting plasma glucose
GCP	Good clinical practice
GI	Gastrointestinal
HbA1c	Hemoglobin A1c
HER2	Human epidermal growth factor receptor 2
HIV	Human immunodeficiency virus
IC	Integrative cluster
IDS	Investigational Drug Service
IND	Investigational New Drug
IHC	Immunohistochemical

IRB	Institutional Review Board
LLN	Lower Limit of Normal
LS	Least-squared (eg, LS-means)
METABRIC	Molecular Taxonomy of Breast Cancer International Consortium
mITT	Modified intent-to-treat principle
NCI	National Cancer Institute
NTI	Narrow therapeutic index
PD	Protocol director
PEPI score	Preoperative endocrine prognostic index
PFS	Progression-free survival
PI	Principal investigator
PK	Pharmacokinetics
PLT	Platelets
RBG	Random blood glucose
SAE	Serious adverse event
SCI	Stanford Cancer Institute
SCI-CTO	Stanford Cancer Institute Clinical Trial Office
SERD	Selective estrogen receptor degrader
SJS	Stevens-Johnson Syndrome
SOP	Standard Operating Procedure
SRC	Stanford Review Committee
SUSAR	Serious, unexpected, suspected adverse reaction
TEAE	Treatment-emergent adverse event
TEN	Toxic Epidermal Necrolysis
ULN	Upper Limit of Normal
UP	Unanticipated problem

1. OBJECTIVES

This protocol outlines the structure and design of this phase 2 pre-operative umbrella, randomized, controlled, multi-center trial of biomarker-targeted therapy in subjects with estrogen receptor (ER)-positive, HER2-negative breast cancer.

1.1 Primary Objective

To evaluate the efficacy of an investigational agent compared with standard endocrine therapy, in reducing Ki67 values based on digital pathology (QuPath) from baseline to on-treatment biopsy after 14 days in ER-positive, HER2-negative tumors (tumor size ≥ 1 cm) with Ki67 ≥ 10%, for different integrative subtype categories identified at integrative subtype screening.

1.2. Secondary Objective

To evaluate the efficacy of an investigational agent compared with standard endocrine therapy, on the proportion of subjects with Ki67 < 10% after 14 days.

1.3 Exploratory Objectives

To compare the proportion of tumors with post-treatment modified PEPI score of 0 in the investigational versus standard endocrine therapy arms in those patients that undergo surgery after completion of protocol therapy.

Cohorts may have additional exploratory objectives that are defined in the cohort-specific appendix.

2. BACKGROUND

2.1. Study Disease

Breast cancer survival rates in early-stage, estrogen receptor-positive (ER^+), HER2-negative breast cancer have improved with modern oncology management, [1,2] but a sizable minority of patients eventually develop distant relapse and die from metastatic disease, and people with early-stage ER^+ disease face a persistent risk of distant recurrence and breast cancer death up to 20 years post-diagnosis. [3-8] These people are being failed by the current paradigm of endocrine therapy with or without chemotherapy as determined by risk ascertained at diagnosis. [9,10] New strategies are urgently needed to identify women who are at high risk of relapse from ER^+ breast cancer, and to reduce their risk.

An analysis was recently performed of 3,240 patients with 20 years of clinical follow-up, including 1,980 patients with accompanying molecular data from the METABRIC cohort. [11] The spatio-temporal patterns of relapse were delineated at unprecedented resolution across the immunohistochemical (IHC) subtypes, intrinsic subtypes (defined by PAM50) [12,13], and our previously validated 11 integrative clusters (IC), defined based on the integration of genomic copy number alterations and transcriptional profiles. [11,14] We observed important differences in recurrence rates amongst the integrative subtypes that were obscured in the IHC and PAM50 subtypes. Most notably, there was substantial variability in the risk of relapse amongst patients

with $ER^+/HER2^-$ breast cancer, where ICs 1, 2, 6, and 9 exhibited an exceedingly high (median 42 to 55%) risk of distant recurrence up to 20 years post-diagnosis (**Figure 1**).

The Molecular Taxonomy of Breast Cancer International Consortium (METABRIC) includes molecular tumor data from 1,980 early-stage breast cancer patients with 20 years of clinical follow-up. [15] Each of these tumors had genome-wide copy number as well as expression information, from which 11 breast cancer subtypes integrating these two categories of molecular information were derived. [11] These integrative cluster (IC) subtypes have been validated across multiple cohorts of early-stage breast tumors, and can be inferred using genome-wide copy number information, expression information, or both. [14] 8 subtypes correspond to predominantly estrogen receptor-positive (ER^+), HER2-negative tumors, of which 4 exhibit a highly characteristic pattern of genomic alteration, where a focal area of the genome is both gained and overexpressed. The 4 focal areas of amplification, and exemplar putative oncogenes located there, are: 17q23 (IC subtype 1); 11q13/14 (IC subtype subtype 2); 8p12 (IC subtype subtype 6); and 8q24 (IC subtype subtype 9). Intriguingly, this "firestorm" pattern mirrors that observed in another integrative subtype (IC subtype 5), which is comprised of classically HER2-positive breast cancers. Furthermore, it has been recently reported that these 4 "firestorm" ER^+ integrative subtypes exhibit an exceedingly high (median 42 to 55%) risk of distant recurrence up to 20 years post-diagnosis, [15] a risk comparable to IC subtype 5, the HER2-positive tumors, in the era prior to HER2-targeted therapy. Within METABRIC, these 4 high-risk subtypes collectively account for approximately 25% of all early-stage ER^+ tumors and the majority of distant recurrences. Integrative subtyping further identifies 4 categories of "typical-risk" ER^+ tumors: IC3, IC4, IC7, and IC8. These tumor subtypes account for approximately 75% of all $ER/HER2^-$ breast cancer, and while their risk is lower than the high-risk subtypes (and lower than that of triple-negative breast cancer, more consistent with the "typical" risks associated with $ER^+/HER2^-$ breast cancer), distant recurrences leading to death still occur. Better therapies are needed for all types of $ER^+/HER2^-$ breast cancer.

This study will evaluate specific integrative cluster (IC) subtypes via sub-protocols using different agents and regimens identified as possible effective against those subtypes. In the main "umbrella" protocol, the approach for identifying patients with specific integrative subtypes is presented, with the study design for investigating specific agents targeted to specific integrative subtype categories. Tumors will be classified into 1 of 5 possible integrative subtype categories: "typical-risk", representing integrative subtypes 3, 4, 7, and 8; IC1 (high-risk); IC2 (high-risk), IC6 (high-risk); or IC9 (high-risk). Subjects whose tumors classify as an integrative subtype corresponding to a currently-open treatment cohort will be eligible for randomization within that cohort. Based on IC subtype screening data, treatment assignment to a treatment regimen may or may not be available at any particular time.

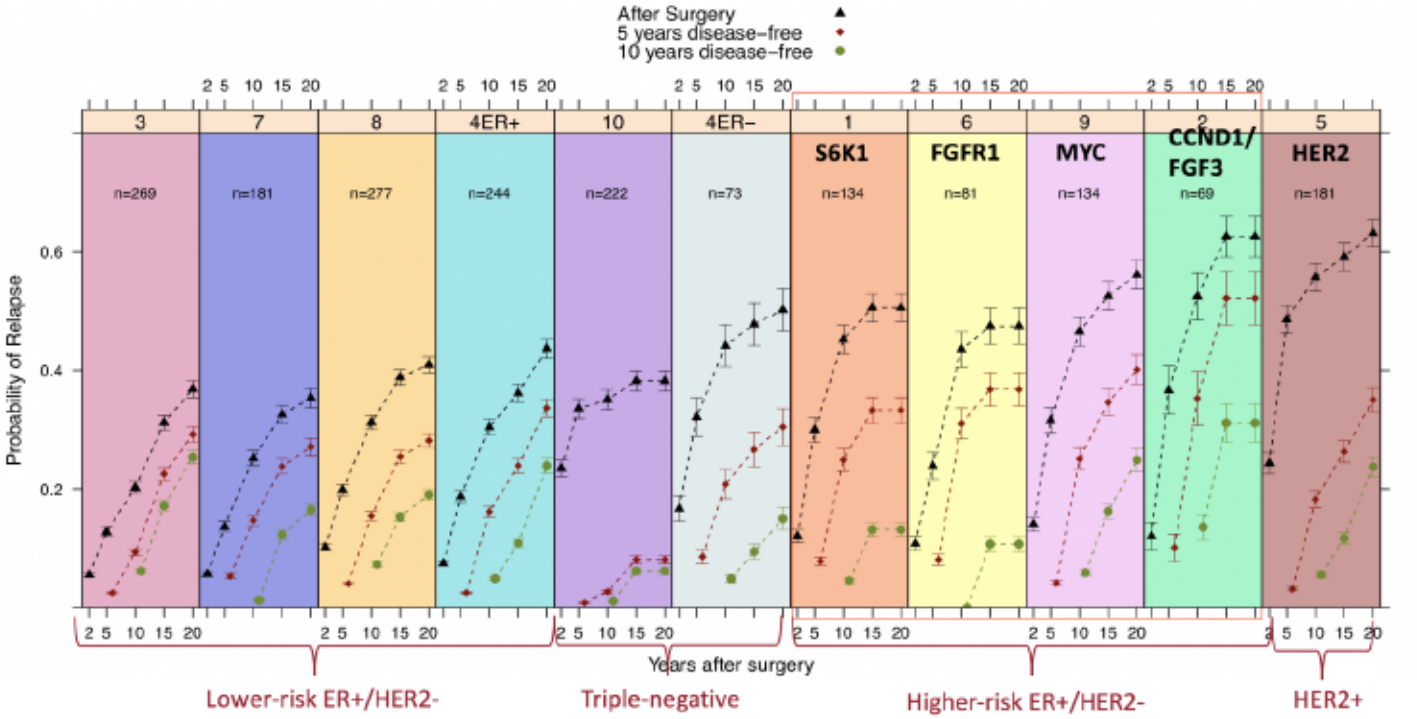


Figure 1. Probability of distant relapse over 20 years after diagnosis in the METABRIC cohort by integrative subtype. Black shows probability after definitive surgery, red after 5 years for patients who had not developed relapse at 5 years, and green after 10 years for patients who had not developed relapse at 10 years. 4 integrative subtypes predominantly comprised of estrogen receptor-positive, HER2-negative tumors exhibit markedly higher risk of relapse. Adapted from Rueda, *et al* (2019) *Nature*.

2.2. Study Agents

The investigational agents to be studied in this main "umbrella" protocol are described in Appendices C and D. Additional appendices will be added to this protocol if additional investigational agents are added to this trial.

2.2.1. Agent Selection

Agents in the investigational arm for each integrative subtype cohort were chosen based on the characteristic genomic/transcriptomic architecture for the integrative subtype tested in that cohort. For example, IC1 is characterized by a 17q23 copy number gain with concomitant overexpression, and thus a drug targeting putative driver genes encompassed in that amplicon will be tested for its efficacy in the IC1 subtype.

Specific details regarding regulatory status, mechanism of action, pharmacokinetics (PK), metabolism, potential for drug interactions, non-clinical experience, safety and efficacy are provided in each investigational agent-specific appendix.

2.2.2. Additional information

Further details are available in the Investigator's Brochures, which are included with the IRB submission. The study requires an Investigational New Drug application (IND). This study will be conducted under IND 161462.

2.3. Rationale

2.3.1. Rationale for use of investigational agents targeted for specific integrative subclusters

Details are provided in each agent-specific appendix.

2.3.2. Rationale for doses of the Study Agents

Tamoxifen

The doses and schedules selected for tamoxifen (20 mg/day by mouth) is FDA-approved for the treatment of ER^+ early-stage breast cancer.

Fulvestrant

The dose and schedule selected for fulvestrant (500 mg IM on Day 1) is consistent with its FDA-approved usage for the treatment of ER^+ advanced breast cancer.

2.3.3. Rationale for study design

Integrative subtyping not only identifies which patients are at high risk of relapse, but also provides information about what genetic alterations within the primary tumor may confer this risk. Each of the integrative subgroups is characterized by distinct copy number amplification events and concomitant overexpression of candidate drivers; namely IC1 [*RPS6KB1, PRR11* (17q23)]; IC2 [*FGF3/FGF4/FGF19*, *CCND1* (11q13)]; IC6 [*FGFR1* (8p12)]; IC9 [*MYC* (8q24)] with an enrichment for *NCOA3* gain/overexpression. [11] Furthermore, our robust statistical approach to combine copy number and expression information within tumors suggests that the pattern of overexpression and amplification seen in these four subtypes echoes the pattern of *ERBB2* (HER2) overexpression and amplification (**Figure 2**). Based on these results, we believe that targeted therapeutic strategies could prevent recurrences within these high-risk patient populations.

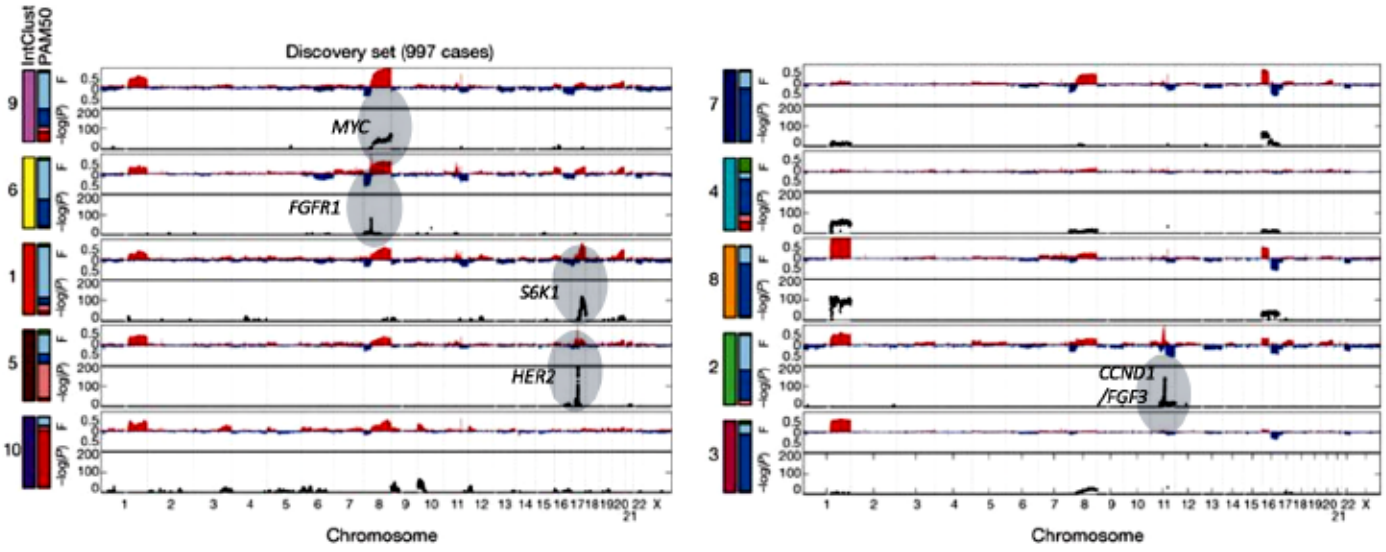


Figure 2. Each of the initially identified 10 integrative subtypes is shown, with adjacent stacked barplot highlighting PAM50 intrinsic subtype heterogeneity within each group. The upper plots show the frequencies of copy number changes (red is gained and blue is lost) in each subtype, and the lower plots show the subtype-specific association ($-\log_{10}$ *P*-value) of these changes. Adapted from Curtis, *et al* (2012) *Nature*.

Preoperative trials, where the proportion of tumor cells expressing Ki67 is measured before and on therapy, are increasingly recognized as powerful approaches to assess the efficacy of novel agents in early-stage ER-positive breast cancer, and to unravel the implicated biology and biomarkers. Of note, digital pathology software-assessed Ki67 (for example, using the open source software QuPath) is increasingly recognized as a reproducible endpoint. [16,17] Reduction in Ki67 on endocrine therapy, including after 14 to 18 days, consistently predicts outcome on an individual patient level. [18-20] In particular, reduction of Ki67 on endocrine therapy to less than 10% indicates good prognosis in $ER^+/HER2^-$ tumors [18,19] and has been used in a large randomized phase 3 study to determine whether subjects should continue on their assigned endocrine therapy or change therapeutic approach (ie, to chemotherapy). [21] A reduction in Ki67 with one agent compared to another also often indicates greater anti-tumor efficacy in clinically meaningful ways. For example, aromatase inhibitors are associated with a greater Ki67 reduction [22,23] and improved event-free survival [24,25] compared with tamoxifen, while the different aromatase inhibitors show equivalent Ki67 reduction [26] and event-free survival. [27] Notably, no difference in Ki67 reduction was seen between fulvestrant and aromatase inhibitors; [21] while fulvestrant does lead to modestly longer PFS in the metastatic setting than aromatase inhibitors, [28] the lack of a difference in Ki67 reduction suggests that an adjuvant trial might not be successful. In terms of targeted therapies, all three CDK4/6 inhibitors led to greater Ki67 reduction [29-31] and PFS in the metastatic setting [32-34] than did endocrine therapy alone, as did everolimus. [35,36]

In this umbrella trial, potential subjects' tumor integrative subtype will be ascertained from tumor sequencing data, along with centrally scored Ki67 immunohistochemistry. The results of these assays will determine for which integrative subtype cohort in the umbrella study, if any, the

subject is eligible. Each of the investigational agent protocols under the umbrella study will randomize subjects to either a standard endocrine therapy or a therapy targeted to the underlying integrative subtype for 14 days, and will compare Ki67 reduction between the arms to assess if the integrative subtype-targeted therapeutic approach leads to greater Ki67 reduction. The primary endpoint will be central, digital pathology software-assessed quantitative Ki67 reduction using QuPath. The rationales behind each of the integrative subtype-targeted therapeutic approaches are described in their respective appendices.

2.4. Integrative Subtyping

There are 11 validated integrative subtypes of breast cancer, defined initially from the METABRIC cohort based on the integration of genomic copy number alterations and transcriptional profiles. [11] It has subsequently been shown that these can be accurately inferred from expression profiling alone. [14] We can also accurately infer integrative subtype (IC1, IC2, IC6, IC9, or typical-risk) from whole-exome DNA sequencing. Whole-exome and whole-transcriptome sequencing will be performed by Caris Life Sciences ("Caris"), a CLIA-certified and CAP-accredited laboratory that generates a clinical report that can be shared with patients and their treating clinicians.

2.5. Study Design

Pre-Screening Phase

Potential subjects who preliminarily meet inclusion criteria and exclusion criteria as determined by study staff (for example, an investigator or clinical research coordinator) will meet with study staff to review the study and the informed consent for pre-screening. At this time, inclusion and exclusion criteria will be confirmed with the subjects and treating clinicians as necessary.

After the subject signs the informed consent for the pre-screening, they will provide a blood sample. In most cases, the breast tumor sample will be obtained from an existing pathologic specimen (for example, the diagnostic core biopsy). Some subjects may undergo a breast biopsy, either by palpation by a breast surgeon or under image-guidance by a breast radiologist, to obtain tumor tissue for testing. The study team will coordinate the shipping of the tumor and blood sample to Caris. Caris will complete tumor DNA and/or RNA sequencing and blood DNA and/or RNA sequencing per their standard protocol and will release the data to Stanford University. Study staff in Dr. Christina Curtis's laboratory will analyze these data to interpret integrative subtype. Caris will perform Ki67 immunohistochemistry and assess the percentage of tumor cells staining positive for Ki67. Stanford University will keep up to 10 additional sections of tissue for future studies, and will return the remainder of the tissue to its originating site.

If inadequate tissue is available for Caris analyses while preserving enough tissue for clinical assays, Caris staff will communicate with Stanford study staff that an additional biopsy would be needed to proceed with pre-screening, and Stanford study staff will coordinate with the site to determine if subject wishes to undergo this additional biopsy or withdraw from study.

Subjects with tumors whose integrative subtype screening results or Ki67 screening results do not confer eligibility for any currently enrolling cohort will be informed (either in-person or by telephone or video) and will continue with their planned clinical care.

Subjects who are eligible based on integrative subtype screening result and Ki67 screening result for a currently enrolling cohort will return for a second informed consent discussion (for study participation rather than for pre-screening).

Subjects who opt to receive their tumor sequencing results will receive the Caris clinical report.

As part of the informed consent, potential subjects will be given the option of consenting to the study team reviewing their medical records or contacting them or their local study team after their participation in the study (pre-screening and/or treatment phase) has ended. If they agree, investigators or study staff may access their medical records or contact them or the local study team to ascertain vital status, disease recurrence status, and other cancer-related history, to include pathology, imaging reports, laboratory reports, treatments, and other items present in the oncology notes. Potential subjects will also be given the option of consenting to the study team contacting them in the future for additional research studies.

Treatment Phase

The protocol is designed to identify specific integrative subtypes and evaluate integrative subtype-specific therapy for treating a disease, syndrome, or condition. Other key aspects:

- The interventional model is Parallel.
- The number of intervention arms for each integrative subtype is 2.
- The study will not be masked (not blinded).
- The study will have randomization with equal allocation for the two treatment arms (control and experimental) for each integrative subtype.

This phase 2 study will be conducted to evaluate the antiproliferative activity of investigational therapy as compared to standard endocrine therapy in a biomarker-selected population (integrative subtype-classifying tumors). Subjects will be randomized 1:1 to an IC-subtype-specific standard endocrine therapy alone or in combination with an investigational therapy. Covariate-adaptive randomization will be done using a biased-coin minimization approach to ensure balance across treatment arms. The co-variates of interest are prior endocrine therapy for breast cancer treatment or prevention (not hormone replacement therapy) vs no prior endocrine therapy; and planned on-treatment core biopsy vs definitive surgery. In the case of the IC2/IC6 cohort, an additional co-variate of interest is integrative subtype.

In the treatment phase of this study, subjects in each cohort will take their assigned therapies for 14 days (-2 days to +7 days) , after which an on-treatment breast biopsy or surgery will be performed. The primary and secondary objectives of the study will be assessed based on evaluation of the Ki67 score of the pre-treatment and on-treatment tumor samples.

2.6. Correlative Studies

2.6.1. Proteomic profiling

In exploratory work, we may examine proteomic and/or transcriptomic changes in tumor cells and the surrounding microenvironment *in situ* to delineate compensatory signaling pathways and mechanisms of response/resistance to the evaluated targeted agents. Paired pre- and post-treatment tissue samples from the trial may be profiled on the Nanostring GEOMx Digital Spatial Profiling (DSP) platform (Research Use Only) [37,38], with multiplexed ion beam imaging [39-41], or with other technologies. There is growing evidence that functional heterogeneity in the tumor and microenvironment contribute to treatment response, highlighting the importance of spatially resolved approaches. DSP enables geographic and phenotypic selection of tissue regions – for example, those that are enriched for pancytokeratin-positive tumor cells. Molecular changes observed with treatment may inform intelligent combinations of therapy in the future to overcome primary resistance or enhance treatment response.

2.6.2. Organoid cultures

Three-dimensional organoid cultures established from viable tumor cells have the potential to re-capitulate *in vitro* subject- and tumor-specific phenotypes, enabling the study of drug response and tumor evolution in the lab. [42-44] Tumor organoid cultures can be established as a renewable source of tissue for *in vitro* and *in vivo* experimentation; they are amenable to high-throughput drug screens and complementing xenograft-based approaches. Organoid culture can be used to evaluate the efficacy of novel therapeutic agents in specific genetic contexts. We may establish organoids from a subset of tumors in this study, allowing testing of novel drug combinations to improve response and overcome resistance in this high-risk subgroup. Furthermore, as these organoids can be treated *in vitro* with the same agents that the study subjects from whose tumors they were derived will be, we may be able to compare response between the *in vitro* and subjects. These experiments may inform our understanding of genomic determinants of sensitivity and resistance to targeted therapy, as well as the optimal combinations of therapies to produce response in biomarker-defined groups of breast tumors.

2.6.3. Cell-free DNA/RNA and tumor sequencing

Cell-free DNA and RNA is believed to be representative of the heterogeneity present even in micro-metastatic lesions. [45] Thus, it may better capture the spectrum of subclonal populations with the potential to grow in response to therapy than serial tumor biopsies. Furthermore, it has been shown that cell-free DNA sequencing can detect and characterize clonal dynamics in metastatic breast cancer. [46-48] Blood for cell-free DNA and/or RNA will be collected from subjects prior to initiation of study drug and if feasible, after approximately 2 weeks of therapy. Sequencing (eg, whole-exome or whole-transcriptomic) of the cell-free DNA extracted from these banked plasma samples may allow us to compare baseline changes between responders and non-responders. The banked buffy coat samples will allow us to identify somatic variants that act as driver mutations and compare them to a matched normal sample through tumor-normal sequencing, both prior to treatment and after approximately 2 weeks of therapy. These analyses may allow us to identify genetic mechanisms of resistance to the study drugs.

D. PARTICIPANT SELECTION AND ENROLLMENT PROCEDURES

Participants will be selected from multiple sites in the United States that are part of networks of academic and community sites including, but not limited to, Stanford University Medical Center.

No study-specific procedures will be conducted prior to initial consent (pre-screening consent), including for pre-screening. All subjects for all sites will be reported at the time of pre-screening consent to the coordinating site (Stanford) will be registered in the OnCore database.

3.1. Pre-Screening Phase Eligibility

The Participant Eligibility Checklist for the pre-screening phase is shown in **Appendix A**.

3.2. Treatment Phase Eligibility

The Participant Eligibility Checklist for each investigational agent for the treatment phase is included as part of the agent-specific appendix, as follows.

- Cohort 1 for IC1 Eligibility Checklist (See **Appendix C-A**) - REMOVED
- Cohort 2 for IC2 or IC6 (zotatifin and fulvestrant Eligibility Checklist (See **Appendix D-A**)
- Cohort 3 for Typical Risk (zotatifin and fulvestrant Eligibility Checklist (See **Appendix D-A**)

3.3. Informed Consent Process

Legally-authorized representatives (LARs) may not be used for consent, due to the requirements of the US Dept of Defense funding grant. The informed consent discussion may take place in-person or remotely (over video or the phone), and electronic signing by the subject may be used.

All subjects will be provided a consent form describing the pre-screening phase with sufficient information for subjects to make an informed decision regarding their participation. Those subjects that are preliminarily eligible for the treatment phase will then be provided a consent form specific to their cohort that describes the treatment phase of the study with sufficient information for subjects to make an informed decision regarding their participation. Subjects must sign the IRB-approved informed consent forms prior to participation in any study specific procedure. The subject must receive a copy of the signed and dated consent document. The original signed copy of the consent document must be retained in the medical record or research file.

3.4. Treatment Assignment and Randomization Procedures

Pre-Screening Phase

There will be no randomization for the pre-screening phase of this study

Treatment Phase

Subsequent to allocation to the treatment cohort based on integrative subtype (ie, IC1; IC2 or IC6; typical-risk), subjects will be randomized to standard treatment as a single agent, or

standard treatment in combination with the investigational therapy, with consideration for certain co-variates (see Section 12.1). The co-variates of interest are prior endocrine therapy for breast cancer treatment or prevention (not hormone replacement therapy) vs no prior endocrine therapy; and planned on-treatment core biopsy vs definitive surgery. For the IC2/IC6 cohort, an additional co-variate of interest is integrative subtype classification (IC2 or IC6).

3.5. Study Timeline

Primary Completion

Primary Completion for an individual subject is defined as the point in time when the sample is collected for the Ki67 determination, ie, 14 days after the start of treatment. Ki67 is a intracellular marker of tumor proliferation that is assessed by immunohistochemistry.

It is anticipated that pre-screening and enrollment of subjects will take 48 months.

Study Completion

It is anticipated that the study will complete follow-up of all subjects at 50 months. The study will end for each cohort when the last enrolled cohort subject has completed follow-up, specifically when the evaluation of the primary endpoint from the on-treatment tissue specimen has occurred and any required follow-up for drug-related toxicities has completed (including a 30-day safety follow-up).

4. TREATMENT PLAN

Treatment Phase

Subjects who undergo pre-screening and are found to be eligible for the treatment phase based on integrative subtype classification and breast tumor Ki67 score will return for an informed consent discussion at which point the study staff will review the informed consent for study participation in detail. No study-specific procedures, including for screening, will be conducted prior to initial consent. Inclusion and exclusion criteria will be confirmed with the subject and treating clinician as necessary at this time.

Subjects who preliminarily meet eligibility criteria and sign a Cohort-specific informed consent for the treatment phase will then undergo screening procedures, which may occur up to 14 days prior to Day 1 of treatment. Screening procedures will include cohort-specific procedures as described in the investigational agent-specific appendices. If not done during pre-screening, subjects may provide a blood sample for cell-free DNA/RNA analysis, with this blood sample to be sent to Caris. If the subject remains eligible based on the inclusion and exclusion criteria assessed by these measures, randomization will occur.

The treatment phase will then commence. Standard endocrine therapy (ie, tamoxifen or fulvestrant) should be prescribed and obtained according to treating institution's practices, and investigational agents dispensed by the investigational pharmacy. The biopsy or surgery will occur 15 days (between 13 and 22 days) after the first dose of study drug(s). For oral drugs, the final day of treatment will be 1 day prior to biopsy or surgery.

Subjects who are in the investigational agent study arm will undergo blood laboratory tests during the treatment period and on the day of surgery or core biopsy (or up to 2 days prior), and may provide a blood sample for cell-free DNA/RNA analysis on that day as well. For all participants, tumor tissue will be collected on day of surgery or core biopsy and sent to Caris for central Ki67 analysis and to Stanford to keep for other correlative analyses (see section 2.6).

There may be additional safety procedures that are cohort-specific and defined in the investigational agent-specific appendices.

4.1. Concomitant Medication Guidelines and Supportive Care Guidelines

Please refer to the investigational agent-specific appendix for details regarding concomitant medication and supportive care guidelines.

4.2. Criteria for Removal from Study

Subjects may withdraw from treatment at any time at their own request, or they may be withdrawn at the discretion of the investigator for safety, behavioral, or administrative reasons.

Reasons for subject discontinuation of study treatment may include:

- Completion of protocol procedures for the treatment phase;
- Unacceptable toxicity deemed per investigator to be related to study drug, which may also be an adverse event;
- Significant protocol violation;
- Lost to follow-up;
- Subject withdraws consent for further treatment;
- Study terminated by the investigator or sponsor;
- Pregnancy;
- Death.

Reasons for subject withdrawal from study follow-up may include:

- Completed study follow-up;
- Study terminated by the investigator; sponsor; and/or pharmaceutical distributor(s);
- Lost to follow-up;
- Withdrawn consent for further follow-up;
- Death.

If a subject does not return for a scheduled visit, every effort should be made to contact the subject. In any circumstance, every effort should be made to document subject outcome, if possible. The investigator should inquire about the reason for withdrawal and follow-up with the subject regarding any unresolved adverse events. If the subject withdraws consent for

disclosure of future information, no further study specific evaluations should be performed, and no additional data should be collected. The investigators may retain and continue to use any data collected before such refusal for further follow-up.

4.3. Alternatives

Eligible patients who choose not to participate in this study will receive standard-of-care treatment for $ER^+/HER2^-$ breast cancer, to include surgery, radiation therapy as appropriate, chemotherapy as appropriate, and endocrine therapy. Participation in this study will not change this standard-of-care treatment after study end.

5. INVESTIGATIONAL AGENT INFORMATION

This umbrella trial has been designed to enable the addition or graduation of investigational agents during the course of the study. When an investigational agent is added or graduated from use during this trial, the treatment-specific appendices will be updated. At this time, this consists of **Appendix C** (REMOVED) and **Appendix D**. When a new investigational agent is added to the trial, an appendix for this agent will be added.

Adding an Investigational Agent During Trial

To add a new investigational agent or combination of agents while the trial is active, a protocol amendment will be prepared to include the new investigational agent's appendix and the corresponding informed consent.

Two steps must occur for each site to use a new investigational agent when the trial is active:

1. A protocol amendment to add the new investigational agent that must submitted for approval by the full IRB committee for each site. During this amendment process, accrual to the trial will continue for investigational agents that have already been approved and are under active randomization. New investigational agents will remain as pending randomization until all trial sites have received IRB approval.
2. A second protocol amendment to activate the new investigational agent for randomization must be approved by IRB review for each site (expedited review, if possible). Accrual to the trial will continue for investigational agents that have already been approved and are under active randomization. Participants will not be randomized to the new investigational agent until all sites have IRB approval for the new agent.

Graduating an Investigational Agent

When an investigational agent is graduated from the trial, a protocol amendment will be generated to update the status of the agent. The amendment will be submitted concurrently to all the study sites' IRB(s). The trial will not stop accruing to the other active treatment arms during this period.

5.1. Investigational Agent

Please refer to investigational agent-specific appendices (**Appendices C to D**) for details regarding the investigational agent. For complete details, see Investigator's Brochure, which is provided as a separate document.

5.2. Availability of Investigational Agent

The pharmaceutical supplier for each investigational agent will supply the drug product.

5.3. Agent Ordering of Investigational Agent

The investigational agent will be shipped by the pharmaceutical supplier to the Investigational Drug Service (IDS), or a pharmacy of equal function if different name at each participating site. The shipping address of the lead coordinating center is:

Stanford Health Care
Stanford Medicine at Stanford University
Investigational Drug Service
300 Pasteur Drive, Room H0302
Stanford, CA 94305

Please refer to **Appendix B** for the IDS pharmacy (or a equivalent pharmacy entity) address of each participating site.

5.4. Investigational Agent Compliance and Accountability

The investigational agents [ie, alpelisib tablets; zotatifin vials] will be received by designated personnel at the study site, handled and stored safely and properly, and kept in a secured location to which only the investigator and designated site personnel have access. Upon receipt, the investigational agent should be stored according to the instructions specified on the drug label and in the drug-specific Investigator's Brochure. Refer to the Pharmacy Manual for further details.

The investigator or designee must maintain an accurate record of the shipment and dispensing of the investigational agent in a drug accountability log. Records must include dates and amount of drug received, lot number, to whom dispensed (patient by patient accounting) and accounts of any study drug accidentally or deliberately destroyed. Drug dispensing will be documented by investigational drug pharmacy staff or other qualified staff member, and administration accountability will be documented via medical records by infusion or treatment staff, then later assessed by the investigator and/or study personnel. This information, including but not limited to the date; dose; strength; the numbers of capsules, tablets or vials; and expiry must be captured in the source documentation. For infused agents, the start and end time of each infusion of study drug, must be captured in the source document at each subject visit.

At study closeout and after accountability has been verified, the investigational agents can be returned or destroyed at the site if permitted by local regulations and as instructed by the specific drug manufacturer. Alternatively, the study drug can be destroyed at a third-party depot if prior approval by specific drug manufacturer. After such destruction, the site must notify the

specific drug manufacturer, in writing, of the method of destruction, the date of destruction, and the location of destruction.

6. DOSE MODIFICATIONS

Please also refer to the appendices that are specific to the study cohorts and treatments (**Appendix C** [REMOVED] and **Appendix D**).

7. ADVERSE EVENTS AND REPORTING PROCEDURES

7.1 Potential Adverse Events

The active control agents are well-defined, FDA-approved agents with well-established risk profiles. There is no other specific risk, ie, of the investigational agents, that is not defined in the respective Investigator Brochures.

7.2. Adverse Event Definitions

An adverse event is any untoward medical occurrence in humans during a clinical study, whether or not considered drug-related. An adverse event can be any unfavorable and unintended sign or symptom, including abnormal laboratory findings, or disease, that is temporally associated with the use of a drug, and does not imply any judgment about causality. An adverse reaction is any event that is caused by a drug or device, ie, possibly-, probably-, or definitely-related to the use of the study control or investigational agents specified by this protocol.

All events of clinical deterioration within the setting of the disease are considered adverse events. However, anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected (documented) at the start of the study that do not worsen over time will not be considered adverse events. Disease progression; tumor relapse or recurrence; upstaging; new cancers; or similar, will be preferentially reported as the preferred term for the actual clinical event.

Serious adverse events (SAEs) are defined per the FDA definition at 21CFR§312.32(a) and ICH GCP E6. An adverse event is considered "serious" if, in the opinion of the local Principal Investigator or IND-holder, it results in ANY of the following.

- Death
- Life-threatening adverse event, ie, with an **actual and immediate risk of death** [21CFR§312.32(a)]
- Requires inpatient hospitalization or prolongation of existing hospitalization, unless hospitalization is for:
  - Routine treatment or monitoring of the studied indication, not associated with any deterioration in condition
  - Elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since signing the informed consent
  - Treatment on an emergency outpatient basis for an event not fulfilling any of the definitions of a SAE given above and not resulting in hospital admission
  - Social reasons and respite care in the absence of any deterioration in the subject's general condition
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- Event jeopardizes the subject and may require medical or surgical intervention to prevent one of the outcomes listed here

Abnormal laboratory values or test results constitute AEs only if they induce clinical signs or symptoms, are considered clinically significant, require therapy (eg, hematologic abnormality that requires transfusion or hematological stem cell support), or require changes in study drug(s).

7.3 Classification of Adverse Events

7.3.1 Severity of Event

NCI CTCAE version 5.0 is used to assess the severity of adverse events in this study.

7.3.2 Adverse Event Attribution to Intervention or Study

For this study, all recorded adverse events will be assessed on the basis of whether or not the adverse event was caused by / due to (ie, related) to the study intervention(s). AEs, serious or otherwise, will be attributed by the Principal Investigator or qualified designate to study treatment in accordance with the definitions below.

• **Definitely Related**. There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study intervention (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary. • **Probably Related**. There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study intervention, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition. • **Potentially / Possibly Related**. There is some evidence to suggest a causal relationship (eg, the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (eg, the participant's clinical condition, other concomitant events). Although an adverse event may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related," as appropriate.	These are treated as **"Related"**
• **Unlikely to be related**. A clinical event, including an abnormal laboratory test result, whose temporal relationship to study intervention administration makes a causal relationship improbable (eg, the event did not occur within a reasonable time after administration of the study intervention) and in which other drugs or chemicals or underlying disease provides plausible explanations (eg, the participant's clinical condition, other concomitant treatments). • **Not Related**. The adverse event is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.	These are treated as **"Not Related"**

In addition, for adverse events determined "Not Related" to the study intervention(s), the Principal Investigator or qualified designate will attribute the event to the study or procedures according to the definitions above. Note that adverse events can be determined related to both the intervention(s) and/or the study / procedures.

7.3.3 Expectedness of Event

The Principal Investigator or qualified designate will also assess all recorded adverse events on basis of event severity, frequency (if applicable/assessable), and the established product risk information described within the product Investigator Brochure; the FDA-approved product labeling (if an approved agent); and/or this protocol document, as to whether the events are "expected" or "not expected" relative to the study interventions and/or the study / procedures.

Note that unexpected adverse events may have reporting requirements as described elsewhere in this section.

7.4. Adverse Event Reporting

For each phase of this trial, adverse events (AEs) will be documented and recorded at each scheduled or unscheduled visit, including end-of-treatment and during follow-up, using NCI CTCAE v5.0. AEs will be monitored until all drug-related toxicities have resolved or the Investigator determines in his/her clinical judgment that no further improvement is expected. For serious adverse events (SAEs), the active reporting period will be from the time that the subject provides initial informed consent, which is obtained prior to the subject's participation in the study, ie, prior to undergoing any study-related procedure and/or receiving investigational product, through and including 30 calendar days after the last administration of the investigational product. SAEs experienced by a subject after the active reporting period has ended will be reported if the Investigator becomes aware of them.

Conditions already present at the time of informed consent will be recorded in the Medical History CRF at the time of baseline screening/visit.

AEs (including lab abnormalities that constitute AEs) will be described using a diagnosis whenever possible, rather than individual underlying signs and symptoms. When a clear diagnosis cannot be identified, each sign or symptom will be reported as a separate AE.

The following information will be captured in the CRF for each AE: severity grade (CTCAE v5.0, Grade 1 to 5); duration (start and end dates); relationship to study drug (reasonable possibility that AE is related: No, Yes); action taken with respect to study drug (none, dose reduction, temporarily interrupted, permanently discontinued, unknown, not applicable); whether medication or therapy was given (no concomitant medication/non-drug therapy given, concomitant medication/non-drug therapy given); outcome (not recovered/not resolved, recovered/resolved, recovering/resolving, recovered/resolved with sequelae, fatal, unknown); and whether the event is serious and seriousness criteria.

Subjects who experience Grade 3 or 4 events will be monitored at least every 2 weeks (by phone or video, laboratory evaluation, or in-person evaluation as appropriate) until these drug-related toxicities have resolved or the Investigator determines in his/her clinical judgment that no further improvement is expected.

7.5. Reporting Serious Adverse Events

Reportable adverse events, based on relatedness (attribution) to the study agent; expectedness, severity (Grade 1 to 5), seriousness (Yes/No), or any other aspect of the investigation, will be reported as described below.

- Whenever possible, diagnoses should be given when signs and symptoms are due to a common etiology (eg, cough, runny nose, sneezing, sore throat; and head congestion should be reported as "upper respiratory infection").
- Serious adverse events (SAEs) per the definition at 21CFR§312.32 will be reported to the IND-holder, **within 24 hours of the knowledge of the event**.
- Every SAE, regardless of suspected causality, occurring after the subject has signed informed consent through 30 days after the subject has taken his/her last dose of study

drug, will be reported to the Stanford Cancer Institute (SCI) Data and Safety Monitoring Committee (DSMC) via the Clinical Trials Office (SCI-CTO) using the study-specific CRF **within 24 hours of learning of its occurrence**. Participating sites will report SAE and protocol deviations within 24 hours of observing or learning of event to the coordinating center to be reported to DSMC via SCI-CTO, as well as any governing committee per site's local institutional guidelines. Reports to SCI-CTO and the subsequent internal reporting actions fulfill sponsor requirements to report to the Data and Safety Monitoring Committee (DSMC). Events will be reported using the study-specific CRFs and will identify subjects by their unique code numbers.

- SAEs meeting the criteria for an IND safety report per 21CFR§312.32, ie, the event was serious, unexpected, and at least possibly-related to the study drug [a serious, unexpected, suspected adverse reaction (SUSAR)], will be reported by the IND-holder to the IND under which this study is being conducted within 7 days (for a life-threatening or fatal event) or 15 days (for other SAEs) of the IND-holder's determination that the event is reportable. Note that events that are common in the clinical setting will not be considered unexpected / not expected. Significant changes or updates regarding the SUSAR event will be submitted to the IND according to the original timeframe. IND Safety Reports will be submitted to the IND using the MedWatch Form FDA 3500A for mandatory reporting. IND Safety Reports and Follow-up (FU) reports will also be promptly distributed to all participating Principal Investigators.

  Do not send IND Safety Reports to the MedWatch fax number, ie, the number for voluntary post-marketing safety reporting.

- All **Unanticipated Problem (UPs)** associated with the study, including those related to the study intervention will be reported as follows.
  - Note that for the purposes of Stanford IRB Unanticipated Problem reporting, subjects are considered to be study participants when consented, ie, events during screening and/or pre-treatment through that subject's official end of study participation may qualify as reportable.
  - To the SCI Data and Safety Monitoring Committee (DSMC), by submission to SCI-CTO-Safety / OnCore as an SAE (see SAE reporting to DSMC above. See also the SCI DSMP).
  - To the IRB of record, if an adverse event meets that entity's current definition of an Unanticipated Problem. Note that for the purposes of Unanticipated Problem reporting, subjects are considered to be study participants when consented.

Subsequent follow-up reports, whether to SAEs or UPs, will be reported as required by the receiving entities.

7.5.1. Serious Adverse Event Reporting for Multi-site Studies

All serious adverse events occurring at the additional clinical sites will be reported in writing via email or fax to the Stanford Principal Investigator / IND-holder, or other designate within 24 hours of the determination that an event was serious, according to the procedures described for serious adverse events (SAEs). The Stanford Principal Investigator or designate will report

the event to the IRB of record and submit the report to SCI-CTO-Safety for entry into OnCore and subsequent reporting to the Stanford DSMC in accordance with their requirements.

Report all SAEs to:

Jennifer L Caswell-Jin, MD (Stanford Principal Investigator & IND-holder)
Assistant Professor of Medicine (Oncology)
Stanford University School of Medicine
269 Campus Drive West; CCSR 1145
Stanford, CA 94305
Telephone: 301-332-6541
E mail: caswell@stanford.edu

The local Principal Investigator will report SAEs to the local IRB of record (if applicable) and data safety monitoring committee/board in accordance with local standards.

Dr Caswell-Jin will, through the Stanford study team, will report SAEs as defined to the investigational agent manufacturers. See additional information within each cohort-specific appendix.

7.6 Pregnancy

Female subjects must be discontinued from study drug in the event of pregnancy.

To ensure subject safety, each pregnancy of a subject or a pregnancy as a result from a male subject occurring while the subject is on study drug must be reported to the Stanford DSMC via SCI-CTO within 24 hours of learning of its occurrence. Non-Stanford sites should report to the coordinating center and follow any local institutional requirements. The pregnancy should be followed up to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications. Consent to report information regarding pregnancy and pregnancy outcomes should be obtained from the partner of the male subject.

Pregnancy of a subject or partner of a male subject should be recorded on a Pregnancy Notification Form and entered on the Pregnancy eCRF. Pregnancy follow-up should be recorded and include an assessment of the possible relationship to the study drug of any pregnancy outcome. Pregnancy and pregnancy follow-up should be reported to the Stanford DSMC via SCI-CTO; or for non-Stanford sites, to the coordinating center and follow any local institutional requirements.

7.7. Adverse Event Records

For the main “umbrella” protocol and each of the sub-protocols, the investigator will retain adverse event source data supporting documentation of attribution and seriousness, and copies of official adverse event reports or SAE CRFs, as well as documentation of informal communications (such as telephone calls or emails) in accordance with the current version of Stanford School of Medicine standard operating procedure SOP-005 “Identifying and Reporting Adverse Events.”

8. LABORATORY CORRELATIVE/SPECIAL STUDIES

8.1. Tumor Tissue Collection and Shipment

In the pre-screening phase, a subset of subjects will undergo a pre-treatment biopsy (for example if tissue is inadequate from their existing diagnostic biopsy).

In the treatment phase, a subset of subjects will undergo an on-treatment biopsy, and another subset of subjects will have their on-treatment specimen collected at time of definitive surgery.

In a pre-treatment or on-treatment biopsy, the number of cores is left up to the discretion of the person performing the procedure. In guidance, with a larger sized needle (e.g. 14-18 gauge needle), 4 to 6 cores would be typical. With a smaller sized needle (e.g. 20-22 gauge needle), 6 to 10 cores would be typical.

At definitive surgery, it is preferred that within 30 minutes of the removal of the surgical specimen, an approximately 0.5 cm^3 piece of tissue be extracted from the tumor specimen. This extraction may be performed either in the operating room or in pathology. The person performing the extraction has the discretion to change the size of the piece of tissue extracted to ensure that adequate tumor tissue remains for clinical pathologic analysis, and if greater than 0.5 cm^3 is feasible, a larger specimen is desirable. It is also acceptable to submit a specimen from the specimen processed per routine clinical protocol; however, this method is not preferred because of varying time between extraction of the specimen and its placement in formalin. Whether the specimen was collected via the preferred method (extraction from tumor specimen within 30 minutes of its removal) or non-preferred method (submitted from specimen processed per routine clinical protocol) should be documented in the electronic CRF.

In some situations (i.e. after discussion with the Clinical Coordinating Center), viable cell processing may be performed as followed: Approximately one-half of the research specimen from surgery or 2 cores from core biopsy will be placed in formalin for paraffin embedding, and the remaining tissue cores will go through cryopreservation and shipping for viable cells.

Formalin-fixed paraffin-embedded (FFPE) tumor tissue along with its corresponding unredacted pathology report will be shipped to Caris using the tumor tissue collection kits provided by Caris to each site. For detailed shipping instructions, site staff will reference the study-provided Caris laboratory manual. Viable cells should be transferred on dry ice to Stanford University Curtis Laboratory within 24 hours of collection, using shipping labels provided by the study or by local site if appropriate.

8.2. Plasma Collection, Processing, and Shipment

Blood may be collected at specified times per the protocol using the study-provided lab kits from Caris. These tubes should be shipped (room air) to Caris per the timeline and packing instructions in the provided specimen kits and study lab manual.

8.3. Coding of Specimens for Privacy Protection

Study staff will create a unique anonymous identifier for each specimen at time of patient data collection and entry into the secure REDCap database. The unique anonymized identifier will

be assigned sequentially within each cohort and will thus not be linked in any way to the patient's identifying information. All tissue and plasma specimens will be labeled with this anonymous identifier and no patient-identifiable information. The link between anonymous identifier and patient information will be stored in the secure REDCap database.

9. STUDY CALENDAR

Pre-Screening Phase

Pre-screening protocol activities, as described below, will take place over up to 28 days, in any order with the exception that informed consent is the first activity. The collection of blood and tumor tissue samples may occur prior to eligibility sign-off given the timeline of this phase. Protocol activities that involve the subject may occur in-person or remotely (video or phone), when feasible.

- Pre-screening informed consent. This must be obtained prior to undergoing any study-specific procedure.
- Assessment of ECOG performance status, to ensure meeting inclusion criteria.

  ECOG score definition:

  0 Fully active, able to carry on all pre-disease activities without restriction

  1 Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, eg, light house work or office work

  2 Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours

  3 Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours

  4 Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair

  5 Dead
- Review of oncologic history and medical history to ensure the prospective subject meets inclusion/exclusion criteria.
- Collection of blood sample for matched normal (strongly recommended; if not feasible, subtype may be called without. However, subtyping without blood sample is not preferred).
- Collection of tumor tissue sample, to be sent to Caris for sequencing and to Stanford for Ki67 immunohistochemistry. In most cases, tumor tissue will be collected from existing archival samples. In some cases, subject will undergo a breast biopsy with up to 4 cores obtained.
- Determination of eligibility for one of the active protocols in the umbrella study to which these pre-screening procedures apply, based on classified integrative subtype and

centrally assessed Ki67 immunohistochemistry (must be at least 10%). Informing subject of this eligibility. Release of tumor sequencing results to subjects who have opted to receive them.

- Adverse event assessment for adverse events deemed to be caused by pre-screening procedures will occur at each scheduled and unscheduled study visit.

Treatment Phase

Please refer to the investigational agent-specific appendices (**Appendix C** [REMOVED] and **Appendix D**).

10. MEASUREMENTS

Treatment Phase

10.1. Primary Outcome

Primary outcome title: Change in Ki67

Primary outcome measure description: Tumor proliferation will be assessed as the percentage of tumor cells staining for Ki67 as assessed by immunohistochemistry using the MIB1 antibody. The tumor samples are collected at pre-treatment baseline and after 14 days of treatment. The outcome is reported as the difference of the mean Ki67 level between the baseline and Day 14 specimens, by treatment cohort, with standard deviation.

Time frame: 14 days

10.2. Secondary Outcome

Secondary outcome title: Number of participants with Ki67 < 10% in the post-treatment sample.

Outcome measure description: Tumor proliferation will be assessed as the percentage of tumor cells staining for Ki67 as assessed by immunohistochemistry using the MIB1 antibody. The tumor samples are collected at pre-treatment baseline and after 14 days of treatment. The outcome is reported as the number of participants by treatment cohort for whom Ki67 post-treatment is measured as < 10%, a number without dispersion.

Time frame: 14 days

10.3. Exploratory Outcome

Exploratory outcome title: Modified PEPI score

Outcome measure description: Preoperative endocrine prognostic index (PEPI) score is a predictive measurement for survival after endocrine therapy. Modified PEPI score will be derived by assigning a certain number of points to each of three factors (excluding ER status, which is part of the regular PEPI score):

Preoperative Prognostic Index (PEPI)				
Pathology, biomarker status	RFS		BCSS	
	HR	Points	HR	Points
Tumor Size				
T1/2	—	0	—	0
T3/4	2.8	3	4.4	3
Node status				
Negative	—	0	—	0
Positive	3.2	3	3.9	3
Ki67 level				
0–2.7%	—	0	—	0
>2.7–7.3%	1.3	1	1.4	1
>7.3–19.7%	1.7	1	2.0	2
>19.7–53.1%	2.2	2	2.7	3
>53.1%	2.9	3	3.8	3

The outcome will be expressed as the proportion of subjects who underwent surgery on study (not core biopsy) with a modified PEPI score of 0, after 14 days of treatment (-2/ +7 days).

Time frame: After 14 days of treatment

11. REGULATORY CONSIDERATIONS

11.1. Institutional Review of Protocol

Before implementing this study, the protocol, the informed consent form and other information to be provided to participants, must be reviewed by the central Institutional Review Board (WCG IRB) with individual Memoranda of Understanding (MOU) for approval and housing of documents negotiated between the central IRB and each individual participating site's local IRB. Any changes made to the protocol will be submitted by the CCC as a modification and will be approved by WCG IRB prior to implementation. The CCC will disseminate the WCG approved protocol amendment to all participating investigators. Site staff will follow local institutional guidelines for any applicable internal review board approvals.

11.2. Data and Safety Monitoring Plan

For this study, the Lead Principal Investigator (PI) and IND-holder will be responsible for the active oversight of the patient safety, including at the participating sites, including regular data review to determine any necessary changes to the study design or safety monitoring. These regular reviews will occur internally at Stanford on a monthly basis between study investigator and delegated clinical research staff. A monthly call with participating sites will be held to discuss enrollment, safety data, and other study matters. The Stanford PI/PD may delegate some of these aforementioned responsibilities to another entity, such as the CCC. Additionally, the Stanford Cancer Institute Data and Safety Monitoring Committee (DSMC) will provide support to the Stanford PI/PD on matters related to quality assurance monitoring, review of aggregated protocol deviations, and prompt reports submitted to the necessary regulatory authorities.

SCI DSMC, with the support from the CCC (if necessary) will conduct data monitoring for this study using risk-based monitoring. The Stanford Cancer Institute DSMC will also monitor Stanford sites per their routine protocol for Stanford investigator-sponsored trials.

An independent data safety and monitoring board (DSMB) has been established to ensure patient safety, assess protocol compliance, and monitor the progress of the study. The DSMB is made up of physician(s) with expertise in breast cancer and clinical trials as well as a statistician with experience in clinical trials. The DSMB will be supported by an independent statistician who will be a non-voting member of the board. Details of the composition, roles, responsibilities, and processes of the DSMB will be documented in the DSMB Charter. The DSMB will review safety data on an ongoing basis and may recommend stopping or amending the study based on safety findings. The DSMB will not be otherwise involved in the conduct of the study.

Stanford Cancer Institute (SCI) Data Safety Monitoring Committee (DSMC) will receive all reports of serious adverse events (SAEs) and monitoring reports, and will review those reports in conjunction with the deviations reported in OnCore and to the IRB to provide their oversight. Refer to the DSMC SOP for additional information on their oversight processes. When the DSMC identifies serious and/or persistent noncompliance on the part of an investigator/institution, the Investigator and/or DSMC may recommend corrective action or termination of the institution's participation in the trial and will submit a corrective action plan to WCG IRB if deemed necessary by the sponsor.

11.3. Data Management Plan

Pre-Screening Phase

The local Principal Investigator, or designee, will prepare and maintain adequate and accurate subject case histories with observations and data pertinent to the study. Study-specific Case Report Forms (CRFs) will document inclusion/exclusion criteria assessment, results of pre-screening procedures (integrative subtype classification, Ki67 immunohistochemistry, eligibility for active umbrella sub-protocol, and if subject enrolled on active umbrella sub-protocol), and adverse events deemed to be caused by pre-screening procedures, should they occur. Case report forms will be entered in a secure REDCap database and will be maintained by the local Principal Investigator and study staff. All subjects will be registered in the OnCore database, allowing the Stanford SRC and independent DSMB to judge the aggregate accrual and stopping rules for the study respectively.

Tissue sections that remain at Stanford after completion of pre-screening procedures will be labeled with a unique anonymous identifier generated by study staff and linked to protected health information in the secure REDCap database. The unique anonymized identifier will be assigned sequentially and will not be linked in any way to the subject's identifying information.

Treatment Phase

11.3.1. Case Report Form

All data for this study will be collected on the electronic Case Report Form (eCRF) developed for that purpose. For this study, REDCap will be the eCRF. Sites will be suitably trained on the use of the eCRF and appropriate site personnel will be provided electronic signatures, as required.

All site entries will be made in a secured web site and monitoring delegates will review the record for completeness using risk-based monitoring. The site investigator or designee will make necessary eCRF corrections. The site investigator must authorize the corrections to the entered data on eCRF. Specific instructions on use of the EDC system and guidelines for data entry and correction will be provided to the sites.

11.3.2. Study files and subject source documents

Subject confidentiality is strictly held in trust by the participating investigators, research staff, the Sponsor and their designees. This confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to subjects. Authorized representatives of the Sponsor may inspect all documents and records required to be maintained by the site investigator, including but not limited to, medical records (office, clinic or hospital) and pharmacy records for the subjects in this study. Any data, specimens, forms, reports, and other records that leave the site will be identified only by a subject identification number to maintain confidentiality.

The site investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. These documents include Investigators' Study Files and original subject clinical source documents generated at the study site. The term "original" means the first recording of the data. The site investigator will ensure the site master files are maintained, including the study protocol and its amendments, IRB and regulatory approvals with associated correspondence, informed consents, study drug records, staff curriculum vitae, all correspondence, and other appropriate documents.

Subject clinical source documents may include, but are not limited to, subject hospital/clinic records, physicians' and nurses' notes, appointment books, laboratory reports, ECGs, radiographs, and consultant letters. The site investigator must assure that all original source documents are available to support monitoring activities.

All subjects will be reported at the time of consent to the coordinating site (Stanford) to be registered in the OnCore database, allowing the Stanford SRC and independent DSMB to judge the aggregate accrual and stopping rules for the study respectively.

11.4 Protocol Deviation

Protocol deviation is a general term and includes protocol exceptions, changes made to avoid immediate harm to subjects, and protocol violations. Protocol deviations can be either major or minor. Protocol deviation may include unplanned instances or protocol noncompliance. For

example, situations in which the clinical investigator failed to perform tests or assessments as required by the protocol or failures on the part of participants to complete scheduled visits as required by the protocol, would be considered deviations.

11.4.1. Definitions

Protocol deviation (PD) is any change, divergence, or departure from the study design or procedures defined in the study protocol, consent document, recruitment process, or study materials (eg. questionnaires) originally approved by the IRB. Two classifications are used; Major Deviation and Minor Deviation.

Major deviation is any deviation that significantly affects the quality or completeness of data or impacts a participant's safety, rights, or welfare. Major deviations are study-specific and are determined by the protocol.

Minor deviation is a deviation that does not meet the criteria for major, but still represents a change, divergence or departure from the study design or procedures and does not have impact on data integrity or the final outcome defined in the protocol.

11.4.2. Deviation Management

All deviations from all participating sites must be entered into OnCore within 3 calendar days from the deviation awareness date. Classification of deviation (major vs. minor) can be performed by site with the help of site investigator, protocol director, and/or the Clinical Coordinating Center (CCC). Any deviation, particularly if classified as major, may be reviewed additionally at the CCC to ensure that deviation is addressed and closed with an appropriate Corrective and Preventive Action (CAPA) plan or system. While this study does not grant protocol waivers, deviations intended to eliminate a hazard to participants or to protect the life or well-being of participants should be used when necessary (eg. in an emergency) and discussed with the CCC as early as possible if known ahead of time. Research team members may not implement any deviations or changes from the protocol without agreement by Protocol Director.

12. STATISTICAL CONSIDERATIONS

Treatment Phase

12.1. Statistical Design

The primary objective of this study is to evaluate the safety of the investigational therapy compared with standard endocrine therapy in reducing Ki67 values from baseline to on-treatment biopsy after 14 days in estrogen receptor-positive, HER2-negative tumors (tumor size ≥ 1 cm) with Ki67 ≥ 10% that classify as specific integrative subtypes by integrative subtype screening.

Randomization

To address this objective, a randomized, open-label, multi-center study will be conducted in subjects with estrogen receptor-positive, HER2-negative tumors (tumor size ≥ 1 cm, Ki67 ≥ 10%) for each integrative subtype. Subjects will be randomly assigned to receive either

an IC-subtype-specific standard endocrine therapy alone or in combination an investigational therapy in a 1:1 ratio. Covariate-adaptive randomization will be done using a biased-coin minimization approach to ensure balance between the treatment arms of each cohort. The co-variates of interest are (1) prior endocrine therapy for breast cancer treatment or prevention (not hormone replacement therapy) vs no prior endocrine therapy; and (2) planned on-treatment core biopsy vs definitive surgery. For the IC2/IC6 cohort, an additional co-variate of interest is (3) integrative subtype classification (IC2 or IC6). The study will be divided into a pre-screening phase and treatment phase. In the pre-screening phase, subjects' who meet other eligibility criteria will be identified will undergo assessment to determine their integrative subtype. In the treatment phase, subjects will receive study drug for 14 days and receive a Ki67 post-treatment tumor assessment.

12.2. Interim Analysis and Stopping Rules

There will be ongoing safety monitoring with periodic safety assessments by the DSMB. There will also be two formal assessments by the DSMB upon certain events within each cohort.

1. For the purposes of the interim toxicity assessment, toxicity is defined as treatment-related SAEs Grade 3 or higher. Toxicity will be formally analyzed in each investigational therapy arm after 12 patients are randomized to investigational therapy. If at least 4 of the first 12 patients randomized to investigational therapy experience toxicity, the study will be stopped; in other words, the threshold for unacceptable toxicity is 30%. If the true toxicity rate is 30%, the probability of stopping is 28%. If the true toxicity rate is 40%, the probability of stopping is 56% and if the true toxicity rate is 20%, then probability of stopping is 7%.
2. If 2 or more subjects in any cohort have their surgery delayed past the scheduled day due to possibly-, probably- or definitely-related adverse effects, as assessed by the treating physician or local Principal Investigator, enrollment to that cohort of the study will be halted, and the trial data will be reviewed by the DSMB to determine whether permanent closure of that arm is appropriate. The occurrence of surgery delay must be documented in the study REDCap database within 24 hours, which will immediately notify the IND-holder and Data Coordinating Center.

12.3. Descriptive Statistics and Exploratory Data Analysis

Demographic and other baseline data including disease characteristics will be listed and summarized by study arm. Categorical data will be presented as frequencies and percentages. For continuous data, mean, standard deviation, median, 25^{th} and 75^{th} percentiles will be presented, as appropriate.

12.4. Primary Analysis

12.4.1. Analysis populations

The primary analysis will be conducted on a modified intent-to-treat (mITT) principle. The mITT population will consist of subjects that were randomized and had both baseline and

on-treatment Ki67; subjects will be analyzed by the treatment arm to which they were randomized.

Per-protocol analysis will also be conducted for subjects who do not have any protocol violations and who complete their treatment course. For oral drugs, completing the treatment course is defined as, per self-report, taking at least 80% of the doses of the study drugs including the day prior to surgery or biopsy. Medication compliance for oral drugs will be assessed using a daily drug diary kept by the subject. For injectable/infusion drugs, completing the treatment course is defined as receiving all protocol-mandated injections or infusions.

12.4.2. Analysis plan

For our primary analysis, we will compare the log proportional change of Ki67 between treatment arms using an analysis of covariance (ANCOVA) model adjusting for the log Ki67 at baseline. Geometric LS-means ratio of proportional change (and associated 95% CI) between treatment arms will be calculated from the estimate (and associated 95% CI) of the difference of least squared (LS)-means of the log-proportional change (and associated 95% CI) from the ANCOVA model and converted by antilog transformations. For each treatment arm, geometric LS-means of the proportional change from baseline (and associated 95% CI) will be obtained from the ANCOVA model after conversion by antilog transformations. Geometric LS-means of percentage reduction in Ki67 (and associated 95% confidence intervals) will be calculated with 1 minus geometric LS-means of proportional change (and associated 95% confidence intervals). We will adjust for stratification variables and any differences between treatment arms in baseline covariates, as necessary.

Significance will be determined at a one-sided alpha of 0.1 within each cohort. Because the cohorts are each drawn as separate samples, we consider each to be an independent trial, and no adjustments for multiplicity will be required.

12.5. Secondary Analyses

12.5.1. Analysis populations

For secondary efficacy outcomes, the analysis populations will be the same as the analysis populations for the primary efficacy analysis.

For the exploratory outcome of modified PEPI, the analysis population will be the population of subjects who undergo surgery on study to assess post-treatment Ki67 (excluding those who undergo core biopsy post-treatment instead).

12.5.2. Analysis plan

The proportion of subjects achieving Ki67 < 10% on-treatment will be reported by each treatment arm with Clopper-Pearson 95% confidence intervals. Similarly, the proportion of subjects who undergo surgery who achieve modified PEPI of 0 on treatment will by reported by each treatment arm with Clopper-Pearson 95% confidence intervals. For each of these outcomes, we will conduct a logistic regression model to assess the efficacy of the investigational treatment. We will also compare the log proportional change of Ki67 assessed

through digital pathology between treatment arms using an ANCOVA model adjusting for the log Ki67 at baseline, as in the primary analysis but with digital pathology software-assessed Ki67 rather than pathologist-assessed Ki67.

12.6. Sample Size

12.6.1. Sample size considerations

We assumed that reduction in log Ki67 in the endocrine therapy only arm (tamoxifen or fulvestrant, depending on group) will be 50%, based on historical data, [49] we are using the lower end of this historical range because of data suggesting that tumors that classify as high-risk integrative subtypes are resistant to endocrine therapy. [50] We assumed that log Ki67 reduction increases to 75% in the integrative subtype-targeted therapy arm based on historical data indicating that an increased reduction by ~25% is associated with clinical benefit (for example, in the IMPACT study, anastrozole induced a reduction of 75% change in proliferation and tamoxifen a reduction of 60% change, [49] and in neoMONARCH, anastrozole induced a reduction in change of 63% and anastrozole plus abemaciclib a reduction in change of 93% change [30]).

Assuming a geometric mean ratio of the proportional change of Ki67 of 0.5 (log transformed mean difference of 0.69), standard deviation (log scale) of 1.0, with a one-sided Type I error of 0.10 and a power of at least 0.8, we would need 20 subjects in each treatment arm (40 subjects total per cohort). Based on the drop-out rates seen in neoMONARCH, we have assumed that 80% of subjects in each arm will be eligible for analysis for the primary objective. With these assumptions, we would need to randomize 50 subjects per cohort in order to reach our planned accrual of 40 evaluable subjects (20 subjects per treatment arm, 2 arms per cohort) to reject the null hypothesis under the planned assumptions.

12.6.2. Accrual considerations

Please refer to the investigational agent-specific appendices (**Appendix C** [REMOVED] and **Appendix D**).

12.7. Criteria for Future Studies

The integrative subtype targeted therapy will be deemed appropriate for future studies in the integrative subtype-selected population if the primary objective of the study is met. Additionally, data from the per-protocol analysis and secondary analyses will be considered when making decisions about future studies.

13. APPENDICES

APPENDIX A: Pre-Screening General Participant Eligibility Checklist

A Participant General Eligibility Checklist must be completed in its entirety for each participant prior to registration. The completed, signed, and dated checklist must be retained in the patient's study file and the study's Regulatory Binder. In addition, a treatment assignment-specific checklist will be needed for each cohort (ie, **Appendix C-A** [REMOVED] or **Appendix D-A**).

D. The site PI or treating physician must verify that the participant's eligibility is accurate, complete, and legible in source records. A description of the eligibility verification process should be included in the EPIC or other Electronic Medical Record progress note. **Protocol Information:**

Protocol Title:	An Umbrella, Randomized, Controlled, Pre-Operative Trial Testing Integrative Subtype-Targeted Therapeutics in Estrogen Receptor-Positive, HER2-Negative Breast Cancer
Local Protocol Number (Stanford):	(Stanford BRS0124 / IRB-52869)
Local Institution:	
Local Principal Investigator:	

II. Participant Information:

Participant Name/ID:
Gender: ☐ Male ☐ Female

III. Study Information:

Stanford SRC-approved ☐ IRB-approved ☐ Contract signed ☐

IV. Inclusion Criteria:

Prospective Participant Must MATCH ALL these Inclusion Criteria to be Eligible	Yes	No	Supporting Documentation *
1. Biopsy-proven ER-positive, HER2-negative breast cancer. ER-positivity is defined as ≥ 1% cells staining positive by immunohistochemistry. HER2-negativity is defined by IHC or FISH, per ASCO-CAP 2018 guidelines. Breast tumor must be intact and tumor size must be ≥ 1 cm as measured by ultrasound, mammogram, MRI, or clinical exam. If tumor is locally recurrent, it must be in the breast (not skin, node, or chest wall recurrence). In the pre-screening phase, Ki67 may or may not have been done locally. If done locally, Ki67 score must be ≥ 5%. Any nodal status is allowed, as is M0 or M1 disease.	☐	☐	
2. Women or men, age ≥ 18 years old.	☐	☐	
3. Performance status 0 to 1 (by Eastern Cooperative Oncology Group [ECOG] scale).	☐	☐	
4. Ability to understand and the willingness to sign a written informed consent document.	☐	☐	

V. Exclusion Criteria:

Prospective Participant Must NOT MATCH ANY these Exclusion Criteria	Yes	No	Supporting Documentation *
1. Pregnant or nursing (lactating)	☐	☐	
2. Prior breast cancer-directed therapy (surgery, radiation, chemotherapy, or endocrine therapy to treat breast cancer) is not allowed, with the exception of people with in-breast recurrences. People with in-breast recurrences cannot have had breast cancer-directed therapy (radiation, chemotherapy, or endocrine therapy; surgery is acceptable) within the 6 months prior to signing the pre-screening consent. Previous endocrine therapy for breast cancer risk reduction and/or ovarian suppression for premenopausal women is allowed.	☐	☐	

* All participant files must include supporting documentation to confirm participant eligibility. The method of confirmation can include, but is not limited to, laboratory test results, radiology test results, participant self-report, and medical record review.

VI. Statement of Eligibility

By signing this form of this trial I verify that this participant is [☐ **eligible** / ☐ **ineligible**] for participation in the study. This study is approved by the Stanford Cancer Institute Scientific Review Committee, the Stanford IRB, and has finalized financial and contractual agreements as required by Stanford School of Medicine's Research Management Group.

Site PI or Treating Physician Signature:	Date:
Printed Name:	

APPENDIX B: IDS Pharmacy (or equivalent) Address of Each Participating Site

For Stanford Medicine

Stanford Medicine at Stanford University
Investigational Drug Service
300 Pasteur Drive, Room H0302
Stanford, CA 94305

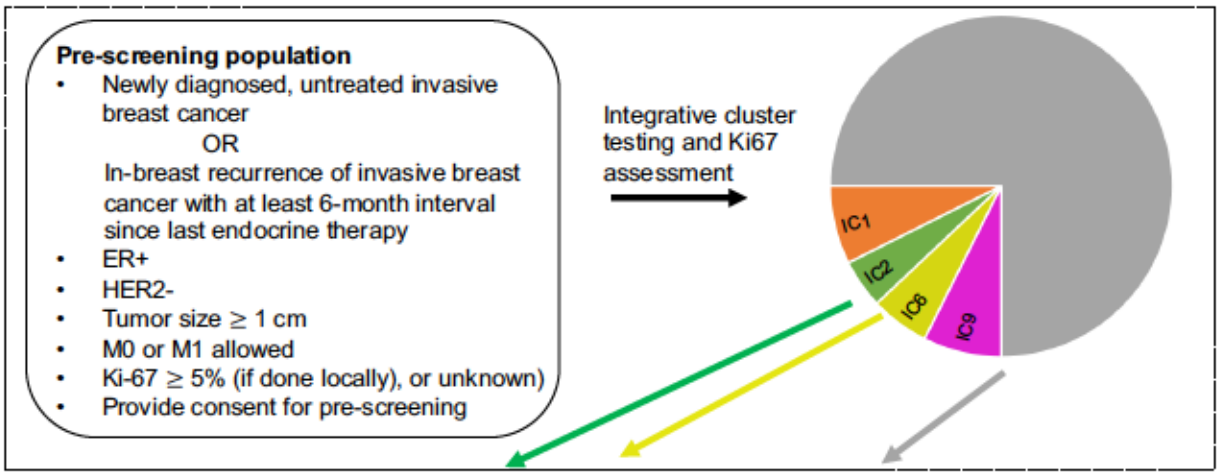
Other sites may use their local investigational pharmacy.

APPENDIX C: Study Details Specific to Integrative Subtype 1 Tumors (Cohort 1) -- REMOVED

APPENDIX D: Study Details Specific to Integrative Subtype 2 & 6 Tumors (Cohort 2 Zotatifin) and Typical risk (Cohort 3 Zotatifin)

Comparing fulvestrant to zotatifin in combination with fulvestrant

SCHEMA – Integrative Subtype 2 and 6 Tumors (Cohort 2) and Typical Risk (Cohort 3)



Cohort 2 (N=50)
Integrative cluster 2 (N = 25) or
Integrative cluster 6 (N = 25)

or

Cohort 3 (N=50)
Typical-risk

1: 1 randomization, based on the following co-variates:
(1) Prior endocrine therapy for breast cancer treatment or prevention (not hormone replacement therapy) vs. no prior endocrine therapy;
(2) Planned on-treatment core biopsy vs. definitive surgery;
(3) For Cohort 2 only: Integrative cluster assignment (IC2 vs. IC6)

Assigned therapy: Fulvestrant

Assigned therapy: Zotatifin + Fulvestrant

14 (-2, +7) days therapy → On-treatment core biopsy or On-treatment surgery → Assessment of percentage of tumors cells staining positive for Ki-67 before (diagnostic or research biopsy) and rate of PEPI 0 after treatment

Primary endpoint:
- Change in quantitative Ki67 after treatment

Secondary endpoint:
- Proportion with Ki67 < 10%; rate of PEPI 0 after treatment

D-1. BACKGROUND

D-1.1. Study Disease

Cohort 2 pertains to **integrative subtype 2,** characterized by gain and overexpression of the 11q13/14 locus, and **integrative subtype 6**, characterized by gain and overexpression of the 8p12 locus (**Figure D-1**). Integrative subtype 2 accounts for approximately 5% and integrative subtype 6 accounts for approximately 6% of all $ER^+/HER2^-$ breast tumors in METABRIC. [15] In addition to being associated with high risk of relapse in the METABRIC cohort, [15] the 11q13/14 and 8p12 loci have been associated with endocrine resistance in early-stage disease, based on a lack of Ki67 response to 10 to 21 days of aromatase inhibitor therapy. [50] The 11q13/14 locus harbors multiple putative driver genes, including the *FGF3* ligand and *CCND1*, and similarly the 8p12 locus harbors multiple putative driver genes, including the *FGFR1* receptor. Patients whose early-stage tumors classify as these subtypes are in need of more efficacious novel approaches to adjuvant endocrine therapy to prevent recurrence. Similarly, patients whose advanced-stage tumors classify as this subtype would also benefit from novel approaches that could overcome endocrine therapy resistance.

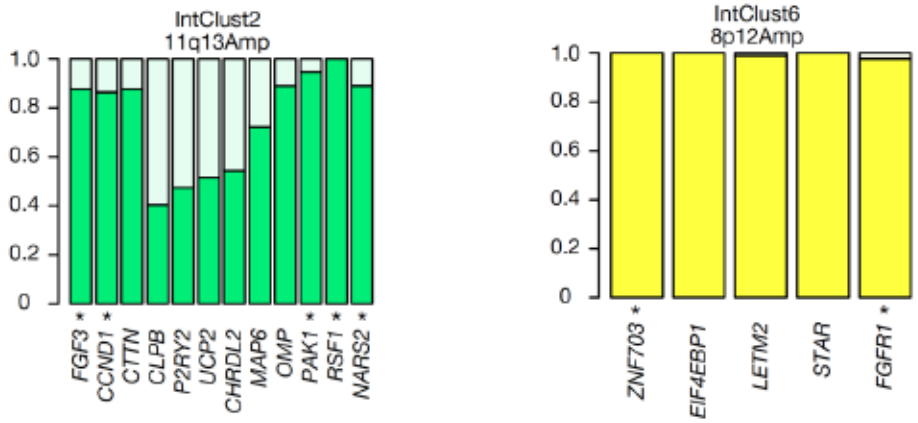


Figure D-1. Frequencies of copy number amplifications in integrative subtypes 2 and 6 (IC2 and IC6). Putative driver genes are indicated by asterisks. Adapted from Rueda, *et al* (2019) *Nature*.

Cohort 3 pertains to ER^+, $HER2^-$ **typical-risk integrative subtypes (IC3, IC4, IC7, IC8)**. There are a variety of putative drivers included in typical-risk subtypes, including a dependency on estrogen receptor signaling, which is believed to be inhibited by zotatifin.

D-1.2. Study Agents

D-1.2.1. Zotatifin (eFT226)

D-1.2.1.1. Regulatory status. Zotatifin is not currently FDA-approved for any indication.

D-1.2.1.2. Mechanism of action. Zotatifin is a potent and sequence-selective inhibitor of eIF4A1-mediated translation that imparts its regulation through a reversible enhancement of

eIF4A1 binding to RNAs with specific polypurine motifs within the 5'-UTR. Zotatifin mRNA sequence recognition motifs are located in the 5'UTR of several oncogenes, and of particular relevance to this study, has been shown to downregulate FGFR1 and CCND1 (other key oncogenes include KRAS; FGFR2; HER2; HER3; CDK4; c-MYC; BCL-2; and MCL-1).

D-1.2.1.3. Chemistry. The chemical name of zotatifin is 4-((5aR,6S,7S,8R,8aS)-7-((dimethylamino)methyl)-8,8adihydroxy-1,3-dimethoxy-6-phenyl-6,7,8,8a-tetrahydro-5aH-cyclopenta[4,5]furo[3,2-c]pyridin- 5a-yl)benzonitrile. The molecular formula is $C_{28}H_{29}N_3O_5$, the molecular weight is 487.56. The drug product is provided as a solution containing 0.5 mg/mL zotatifin, 20 mM acetate buffer and 5% w/w dextrose with pH of 5.0. The solution is sterile filtered and filled into vials and provided for administration as a 1-hour intravenous (IV) infusion.

D-1.2.1.4. Pharmacokinetics. Zotatifin PK are being evaluated in Study eFT226-0002, which is in progress. Interim PK data have been assessed for Part 1 dose escalations and includes partial data from expansion cohorts using the 0.07mg/kg dose. These data established that zotatifin appears to have linear PK with low plasma clearance at 4.45 mL/min/kg, high steady-state volume of distribution (Vss) at 33 L/kg, and a long elimination half-life ($T_{1/2}$) of approximately 4 days. Due to the high Vss and long $T_{1/2}$ zotatifin is determined to be appropriate for dosing once weekly or a less frequent dosing schedule.

D-1.2.1.5. Metabolism. In studies conducted with human recombinant cytochrome P450 isozymes, zotatifin was primarily a substrate for CYP3A4. In bile duct cannulated rats, clearance primarily occurred through biotransformation followed by biliary excretion and renal excretion of metabolites. Evaluation of urine from tolerance studies conducted with dogs and monkeys indicated that the contribution of biotransformation to zotatifin clearance may vary across species.

D-1.2.1.6. Potential for drug interactions. Based on metabolism and nonclinical drug interaction studies, there is low potential for zotatifin to perpetrate a drug-drug interaction via modulation of drug transporter and/or CYP enzyme activity. Zotatifin could be a victim of a drug-drug interaction caused by co-administered CYP3A modulating (inhibitors or inducers) drugs; zotatifin is primarily a substrate for CYP3A4. Concomitant use of strong or moderate inhibitors or inducers of CYP3A4 (**Appendix D-B**) with zotatifin is not allowed per protocol.

D-1.2.1.7. Non-clinical experience. Preclinical efficacy studies using zotatifin showed significant in vitro tumor growth inhibition and regression across multiple solid tumor cancer models; in particular, in vitro cell line screens identified that cell lines harboring KRAS activating mutations and/or receptor tyrosine kinase amplification (eg, FGFR or HER2) are especially sensitive to zotatifin-mediated apoptosis. These observations were extended in vivo with mouse xenograft models including for RTK-dependent (eg, HER2 or FGFR) breast cancer. Further details are available in the Investigator's Brochure, which is included with the IRB submission.

D-1.2.1.8. Safety. Clinical experience with zotatifin to date includes 1 ongoing, first-in-human, phase 1-2, open-label, sequential-group, dose-escalation and cohort-expansion study evaluating the safety, PK, pharmacodynamics, and antitumor activity of zotatifin in patients with

selected advanced solid tumor malignancies (Study eFT226-0002). The maximum tolerated dose (MTD) has not yet been defined, and dose escalation is in progress.

Investigations of zotatifin in combination with fulvestrant continue to explore dose escalation starting at zotatifin 0.1 mg/kg administered IV every other week in 14-day cycles (Part 1b cohort). As of 13 February 2023, the planned 3 participants have been enrolled in this cohort and completed their DLT-period.

Dose-limiting toxicities

As of 12 August 2022, there was 1 DLT (grade 3 thrombocytopenia) in the 0.035 mg/kg/week cohort, and 2 DLTs (grade 3 anemia and grade 3 GI hemorrhage) in the 0.1 mg/kg 2 weeks on / 1 week off cohort. In the Part 1B cohort utilizing zotatifin 0.1 mg/kg every other week in 14-day cycles, the dose planned for this study, all 3 planned DLT-evaluable were enrolled as of 13 February 2023 with no DLTs observed during the reportable period.

Treatment-emergent adverse events

Treatment with zotatifin has been generally well tolerated, with most treatment-emergent adverse events (TEAEs) recorded as Grade 1 or Grade 2. For patients treated in dose cohorts as of cut date of 12 August 2022, including patients treated in the expansion cohorts, the primary TEAEs reported have been fatigue, anemia, vomiting, diarrhea, and nausea (incidence in more than 10% of subjects). Table D-1 summarizes adverse events through 12 August 2022.

Table D-1: Study eFT226-0002 Treatment-Emergent Adverse Events Considered Related to Zotatifin Reported Across All Doses

Adverse Event	Zotatifin (N=72)[a] n (%)
Fatigue	15 (21%)
Anaemia	13 (18%)
Vomiting	13 (18%)
Diarrhoea	12 (17%)
Nausea	11 (15%)
Headache	5 (7%)
Back pain	3 (4%)
Platelet count decreased	3 (4%)
Abdominal pain	2 (3%)
Aspartate aminotransferase increased	2 (3%)

Blood creatine phosphokinase increased	2 (3%)
Constipation	2 (3%)
Dry mouth	2 (3%)
Dyspnoea	2 (3%)
Epistaxis	2 (3%)
Hypertension	2 (3%)
Hypokalaemia	2 (3%)
Rash maculo-papular	2 (3%)
Rhabdomyolysis	2 (3%)
Alanine aminotransferase increased	1 (1%)
Chills	1 (1%)
Confusional state	1 (1%)
Cough	1 (1%)
Dehydration	1 (1%)
Dizziness	1 (1%)
Dysgeusia	1 (1%)
Gastrointestinal haemorrhage	1 (1%)
Haematemesis	1 (1%)
Hypercalcaemia	1 (1%)
Hypomagnesaemia	1 (1%)
Hypotension	1 (1%)
Infusion related reaction	1 (1%)
Insomnia	1 (1%)
Muscular weakness	1 (1%)
Myalgia	1 (1%)
Oesophagitis	1 (1%)
Proteinuria	1 (1%)
Pyrexia	1 (1%)

Sinus bradycardia	1 (1%)
Stomatitis	1 (1%)
Tachycardia	1 (1%)
Thrombocytopenia	1 (1%)

a. Table includes data from Dose Escalation Cohort subjects for eFT226-0002 (n=37) and Cohort Expansion Cohort subjects for eFT226-0002 (n=32) through 12 August 2022.

Combination:

In Study eFT226-002, expansion cohorts have studied zotatifin in combination with fulvestrant. In these cohorts, zotatifin was administered IV for 60-minutes at a dose of 0.07mg/kg in 21-day cycles as non-continuous dosing (i.e. 2 weeks on, 1 week off). As of August 12, 2022, treatment has been generally well-tolerated, with treatment-emergent adverse events considered related to zotatifin summarized in Table D-2.

Table D-2. Study eFT226-0002:Treatment-Emergent Adverse Events Considered Related to Zotatifin Reported Across ECBF and ECBF+A Cohorts.

AE Listing for ECBF				
	All TRAEs (All Grades)		**CTCAE Grade 3 or 4**	
AE Preferred Term	**AE Count (n=18)**	**Subjects (n=17)**	**AE Count (n=18)**	**Subjects (n=17)**
Anaemia	2 (12%)	(2 (12%_	0	0
Aspartate aminotransferase increased	1 (6%)	1 (6%)	0	0
Diarrhoea	1 (6%)	1 (6%)	0	0
Haematemesis	1 (6%)	1 (6%)	0	0
Headache	2 (12%)	2 (12%)	0	0
Hypercalcaemia	1 (6%)	1 (6%)	0	0
Hypotension	1 (6%)	1 (6%)	1 (6%)	1 (6%)
Myalgia	1 (6%)	1 (6%)	0	0
Nausea	3 (17%)	1 (6%)	0	0

Pruritis	1 (6%)	1 (6%)	0	0
Sinus tachycardia	1 (6%)	1 (6%)	0	0
Vomiting	3 (17%)	1 (6%)	0	0
AE Listing for ECBF+A				
AE Preferred Term	**AE Count (n=16)**	**Subjects (n=8)**	**AE Count (n=16)**	**Subjects (n=8)**
Blood creatinine phosphokinase increased	1 (7%)	1 (13%)	1 (7%)	1 (13%)
Diarrhoea	1 (7%)	1 (13%)	0	0
Dry mouth	1 (7%)	1 (13%)	0	0
Dysgeusia	1 (7%)	1 (13%)	0	0
Epistaxis	1 (7%)	1 (13%)	0	0
Fatigue	1 (7%)	1 (13%)	0	0
Muscular weakness	1 (7%)	1 (13%)	0	0
Nausea	4 (25%)	3 (38%)	0	0
Oesophagitis	1 (7%)	1 (13%)	0	0
Rhabdomyolysis	1 (7%)	1 (13%)	1 (7%)	1 (13%)
Thrombocytopenia	1 (7%)	1 (13%)	0	0
Vomiting	2 (13%)	1 (13%)	0	0

Data as of 12 August 2022. AE = adverse event; CTCAE = Common Terminology Criteria for Adverse Events; ECBF = Expansion, Combination, Breast, Fulvestrant; ECBF+A = Expansion, Combination, Breast, Fulvestrant+Abemaciclib; TRAE = treatment-related adverse events.

Since August 2022, a new dose escalation cohort (Part 1b) was introduced under Study eFT226-0002 to study zotatifin in combination with fulvestrant. In the first 3 patients enrolled in this cohort, IV zotatifin was administered at a dose of 0.1mg/kg once every other week in 14-day cycles. As of 13 February 2023, one of the three subjects has gone off treatment due to disease progression. No grade 3 or 4 AEs have observed through 13 February 2023, and TEAEs in these 3 subjects are summarized here in table D-3:

Table D-3. Study eFT226-0002: All Treatment-Emergent Adverse Events Considered Related to Zotatifin's dose escalation.

Summary of All Treatment Emergent Adverse Events Listing for eFT226-002				
	All TEAEs (All Grades)		CTCAE Grade 3 or 4	
AE Listing for Cohort 1b (0.1mg/kg)				
AE Preferred Term	AE Count (n=12)	Subjects (n=3)	AE Count (n=12)	Subjects (n=3)
CONSTIPATION	1 (8%)	1 (33%)	0	0
EPISTAXIS	1 (8%)	1 (33%)	0	0
HEADACHE	1 (8%)	1 (33%)	0	0
LOWER GASTROINTESTINAL HEMMORRHAGE	1 (8%)	1 (33%)	0	0
MACULO-PAPULAR RASH	2 (17%)	1 (33%)	0	0
NAUSEA	2 (17%)	2 (67%)	0	0
URINARY TRACT INFECTION	1 (8%)	1 (33%)	0	0
VOMITING	3 (25%)	2 (67%)	0	0

Table D-4. Study eFT226-0002: Adverse Events Considered Possibly Related to Zotatifin's dose escalation.

Summary of Adverse Events Listing possibly related to eFT226-002				
	All TEAEs (All Grades)		CTCAE Grade 3 or 4	
AE Listing for Cohort 1b (0.1mg/kg)				
AE Preferred Term	AE Count (n=10)	Subjects (n=3)	AE Count (n=10)	Subjects (n=3)
CONSTIPATION	1 (10%)	1 (33%)	0	0
EPISTAXIS	1 (10%)	1 (33%)	0	0
HEADACHE	1 (10%)	1 (33%)	0	0
MACULO-PAPULAR RASH	2 (20%)	1 (33%)	0	0
NAUSEA	2 (20%)	2 (67%)	0	0

VOMITING	3 (30%)	2 (67%)	0	0

Serious adverse events

As of 12 August 2022, there were 5 subjects generating 6 serious adverse events (SAEs) that were reported as possibly related to zotatifin across all escalation and expansion cohorts in study eFT226-0002, 1 of which was Grade 1 sinus bradycardia, and the other 5 were Grade 3 anemia, GI hemorrhage, hypotension, blood creatine phosphokinase increased, and rhabdomyolysis.

D-1.2.1.9. Efficacy. As of data cut-off May 2 2022, of 7 subjects enrolled in the ECBF cohort (zotatifin in combination with fulvestrant), 1 had a partial response, 3 had stable disease, and 3 had progressive disease.

D-1.2.1.10. Additional information. Further details are available in the Investigator's Brochure, which is included with the IRB submission. This study will be conducted under IND 161462.

D-1.2.2. Fulvestrant

D-1.2.2.1. Regulatory status. Fulvestrant was first approved in 2002 in the United States and is indicated to treat hormone receptor-positive, HER2-negative advanced breast cancer in postmenopausal women not previously treated with endocrine therapy, and to treat HR-positive advanced breast cancer in postmenopausal women with disease progression following endocrine therapy.

D-1.2.2.2 Mechanism of action. Fulvestrant is an estrogen receptor antagonist.

D-1.2.2.3 Additional information. Further details are available in the FDA label.

D-1.3. Rationale

D-1.3.1. Rationale for use of zotatifin and fulvestrant in breast tumors classifying as integrative subtype 2

Integrative subtype 2 (IC2) tumors possess a characteristic 11q13/14 amplification, which includes *CCND1* as well as the FGFR ligands *FGF3*, *FGF4*, and *FGF19*. [15] This amplification has been identified as being associated with intrinsic endocrine therapy resistance, based on lack of suppression of Ki67 on aromatase inhibition in early-stage tumors. [50] While *CCND1* has been a leading candidate driver oncogene in this amplification, increasing data suggest that the *FGF3* ligand amplification may additionally or primarily serve a driver role via overactivation of the FGFR pathway. For example, a genome-scale gain-of-function lentiviral screen in an ER-positive breast cancer cell line identified *FGF3* as one of the key genes that conferred resistance to selective estrogen receptor degradation; *FGF3* overexpression indeed conferred fulvestrant resistance, which was blocked by the FGFR inhibitor PD173014. [56] We hypothesize that simultaneously downregulating both *CCND1* and *FGFR1* with zotatifin may overcome intrinsic endocrine therapy resistance in this high-risk subgroup.

D-1.3.2. Rationale for use of zotatifin and fulvestrant in breast tumors classifying as integrative subtype 6

Integrative subtype 6 (IC6) tumors possess a characteristic 8p12 amplification, which includes the receptor *FGFR1*. [15] This amplification has also been identified as being associated with intrinsic endocrine therapy resistance, based on lack of suppression of Ki67 on aromatase inhibition in early-stage tumors. [50] Further supporting the role of this amplicon in endocrine therapy resistance, in both MONALEESA-2 [57] and PALOMA-3, [58] *FGFR1* amplification and/or overexpression were associated with shorter progression-free survival in both the treatment (endocrine therapy with CDK4/6 inhibition) and control (endocrine therapy alone) arms. In pre-clinical models, FGFR inhibition could overcome this endocrine therapy resistance; for example, breast cancer cell line models overexpressing FGFR1 displayed resistance to endocrine therapy and CDK4/6 inhibition that was overcome with the addition of an FGFR inhibitor, and patient-derived xenografts with FGFR1 amplification experienced complete responses with this triplet combination. [57] Similarly, FGFR1 overexpression in a breast cancer cell line induced resistance to the combination treatment of fulvestrant and palbociclib, which was abrogated by adding the pan-FGFR inhibitor PD173074. [56] Pre-clinical data looking at addition of FGFR inhibition to endocrine therapy in breast cancer cell lines with *FGFR1* amplification and PDXs have also shown more potent inhibition of growth with the combination of endocrine therapy and FGFR inhibition than either agent alone. [59] It is hypothesized that downregulating FGFR1 expression with zotatifin may overcome intrinsic endocrine therapy resistance in this high-risk subgroup.

D-1.3.3. Rationale for the use of zotatifin and fulvestrant in breast tumors classifying as typical risk

There are a variety of putative drivers included in typical-risk subtypes, including a dependency on estrogen receptor signaling. Preclinical data on zotatifin suggest it may reduce translation of the *ESR1* transcript, as well as other drivers such as *c-MYC* and *RAS* proteins, which could indicate a role for zotatifin in combination with fulvestrant in halting activation of the estrogen receptor pathway.

D-1.3.4. Rationale for dose of zotatifin. The dose and schedule selected for zotatifin is 0.10 mg/kg administered by IV once on Day 1 of the 14-day treatment period. This dose and schedule is selected based on safety data for monotherapy with zotatifin as well as combination therapy with fulvestrant. The 0.10 mg/kg dose (given every 14 days in Study eFT226-0002) was chosen over the 0.07 mg/kg dose (given on day 1 and 8 of a 21-day cycle in Study eFT226-0002) to allow subjects to receive only one infusion on this study. When possible, preference should be to administer fulvestrant prior to zotatifin.

D-1.3.5. Rationale for dose of fulvestrant. The dose and schedule selected for fulvestrant (a total of two 250 mg/5 mL injections for intramuscular administration on Day 1 of this study's period) is selected based on the FDA-approval for the treatment of advanced $ER^+/HER2^-$ breast cancer.

D-1.3.6. Rationale for study design. In this study, subjects with either integrative subtype 2 or integrative subtype 6 tumors (collectively, Cohort 2) are randomized to 14 days of fulvestrant vs zotatifin plus fulvestrant in order to learn whether the addition of zotatifin improves endocrine therapy response based on the established endpoint of a reduction in Ki67.

Subjects with typical-risk tumors (Cohort 3) are randomized to 14 days of fulvestrant vs the combination (fulvestrant and zotatifin) in order to learn whether the combination of fulvestrant and zotatifin improves endocrine therapy response compared to fulvestrant alone, based on the established endpoint of a reduction in Ki67.

D-2. PARTICIPANT SELECTION AND ENROLLMENT PROCEDURES

*Refer to the Participant Eligibility Checklist in **Appendix D-A**.*

D-3. TREATMENT PLAN

D-3.1. Concomitant therapies, supportive care, and other considerations specific to Cohort 2 & 3

D-3.1.1. Prohibited medications: Strong or moderate CYP3A4 inhibitors or inducers (**Appendix D-B**) are prohibited during the treatment phase of the study.

- While no clinical drug-drug interaction data are available, in vitro data suggest that concomitant administration of strong or moderate inhibitors or inducers of CYP3A4 might alter zotatifin clearance and lead to clinically significant increases or decreases in zotatifin exposure. Consequently, the concomitant use of strong or moderate inhibitors or inducers of CYP3A4 (see **Appendix D-B**) is not permitted during the treatment period.
- Based on these considerations, protocol candidates who require therapy with strong or moderate CYP3A4 inhibitors or inducers listed in **Appendix D-B** should not be enrolled into the study.
- It is also recommended for those randomized to receive zotatifin to consider holding statin or fenofibrate for the treatment period (i.e. 14 days) due to possible CPK elevation.

D-3.1.2. Supportive care

D-3.1.2.1. *Anti-diarrheal*. Patients experiencing diarrhea (and/or abdominal cramping) following zotatifin administration may take loperamide. The recommended regimen is 4 mg at the first onset of diarrhea, then 2 mg with each succeeding diarrheal stool until the patient is diarrhea-free for at least 12 hours. Additional antidiarrheal measures may be implemented as needed. Patients should also be instructed to maintain oral fluid intake to help sustain fluid and electrolyte balance during episodes of diarrhea.

D-3.1.2.2. *Antiemetic*. It is recommended that patients on the zotatifin arm may be offered antiemetics, such as ondansetron and prochlorperazine, to have available at home as needed. Other classes of antiemetic medications that may be employed include dopamine antagonists or benzodiazepines. Aprepitant should not be used because it is a potent CYP3A4 inhibitor.

D-3.1.2.3. *Anti-inflammatory and antipyretic*. Patients may receive ibuprofen, 400 to 600 mg orally, as needed every 4 to 6 hours in response to constitutional symptoms or fever. Other NSAIDs may be substituted as medically necessary. Acetaminophen, 650 mg orally, as needed every 4 to 6 hours, may be used in response to constitutional or pyretic symptoms if medically necessary, but use of acetaminophen is not encouraged given its potential for adverse hepatic effects.

D-3.1.2.4. Hematopoietic Support

G-CSF (eg, filgrastim, filgrastim-snd, peg-filgrastim, lenograstim, etc) may be administered in response to Grade ≥ 3 neutropenia or neutropenic complications. Erythropoietic agents (eg, erythropoietin or darbepoetin) may be administered for Grade ≥ 3 anemia, at the discretion of the treating physician. Red blood cell or platelet transfusions may be administered as medically indicated.

D-3.1.3. Contraception

- Sexually-active females of childbearing potential must agree to use either an IUD or diaphragm AND male condom with spermicide during heterosexual intercourse from the start of screening until 30 days after the final dose of study therapy.
- For female subjects of childbearing potential, a negative serum pregnancy test is required within 7 days prior to initiation of study treatment).
- In the context of this protocol appendix, a female subject is considered to be of childbearing potential unless she has had a hysterectomy, bilateral tubal ligation, or bilateral oophorectomy; has medically documented ovarian failure (with serum estradiol and FSH levels within the institutional laboratory postmenopausal range and a negative serum or urine βHCG); or is menopausal (age ≥ 55 years with amenorrhea for ≥ 6 months).
- Sexually-active male subjects who can father a child and are having intercourse with females of childbearing potential who is using a hormonal method of contraception (oral contraceptive, transdermal patch, vaginal ring, injection, or implant) must agree to use a barrier method as well (diaphragm with spermicide or male condom with spermicide). If they are having intercourse with a female of childbearing potential who is not using contraception, they must agree to use both barrier methods. The period of these restrictions for a male patient are from the start of study therapy until ≥ 30 days after the final dose of the study therapy. A male subject must also agree to refrain from sperm donation from the start of study therapy until ≥ 90 days after administration of the final dose of study therapy.
- In the context of this protocol appendix, a male subject is considered able to father a child unless he has had a bilateral vasectomy with documented aspermia or a bilateral orchiectomy.

D-3.1.4. Diet

- Patients on the zotatifin arm should be advised to avoid ingestion of grapefruit, grapefruit juice, or Seville oranges (which contain a potent CYP3A4 inhibitor) and should not use St John's wort, which is a potent CYP3A4 inducer. No other specific dietary restrictions are required.

D-4. INVESTIGATIONAL AGENT INFORMATION

D-4.1. Zotatifin (eFT226)

For a summary of Zotatifin, see Section D-1.2.1. For complete details, see Investigator's Brochure, which is provided as a separate document.

D-4.1.1. Description

Zotatifin (eFT226) Injection is provided as 0.5 mg/mL, and is a clear, colorless, sterile solution of eFT226 Drug Substance in a 20 mM pH 5 acetate buffer containing 5% weight by weight (w/w) dextrose filled into 5 mL vials. Zotatifin Injection, 5.0 mL (final drug amount of 1.0 mg or 2.5 mg) is filled into single-use 5 mL clear Type I glass tubing vials, stoppered with a 13 mm serum stopper and 13 mm flip-off seal.

D-4.1.2. Availability

eFFECTOR Therapeutics, Inc. will supply zotatifin (eFT226) drug product.

D-4.1.3. Ordering

Zotatifin (eFT226) will be shipped by eFFECTOR Therapeutics, Inc to the Investigational Drug Service or similar pharmacy entities at Stanford University Medical Center and other sites (**Appendix B**).

D-4.1.4. Manufacturing, Packaging, and Labeling

Each carton will be labeled with a non-patient-specific, open, single-part investigational label bearing the protocol number, lot number, and a unique serial number (Med ID). Each 5 mL vial will be labeled with a non-patient-specific, 2-part, perforated investigational label bearing the protocol number, lot number, and matching unique serial number (Med ID) as the carton. Zotatifin (eFT226) is manufactured according to current Good Manufacturing Practices. Zotatifin will be packaged and labeled under the responsibility of the Manufacturer and will comply with all applicable federal and local laws and regulations. Zotatifin will be identified as an investigational product. No repackaging and/or relabeling activities at the study site are allowed. If the packaging is damaged, or if there is anything unusual about the appearance or attributes of the drug, it should not be used. The vial in question should be saved at the study site and any problems immediately reported to the IND-holder and manufacturer.

D-4.1.5. Storage

Store zotatifin (eFT226) at 20°C to 25°C (68°F to 77°F) and maintain in the original carton until preparation for infusion. Allowable excursions are permitted between 15°C to 30°C (59°F to 86°F). Do not freeze.

D-4.1.6. Administration

A pharmacist or other qualified staff member will prepare and dispense zotatifin (eFT226).

The dose amount required to prepare the zotatifin infusion solution will be based on the patient's weight in kilograms (kg). All patients should be weighed on the day of dosing, prior to the infusion. If the patient experiences either a weight loss or gain > 10% compared to the weight used for the last dose calculation, the amount of zotatifin must be recalculated using the most recent weight obtained.

Refer to the Study Pharmacy Manual for complete instructions on zotatifin preparation.

Given the nausea and vomiting that has been observed at the 0.1mg/kg dose of zotatifin, premedication with ondansetron (for example, 16mg PO x1 or 8mg IV x1) prior to zotatifin administration is recommended, but not mandated.

D-4.1.7. Investigational Agent Compliance and Accountability

See Section 5.4 Investigational Agent Compliance and Accountability of the main protocol for information on compliance and accountability of zotatifin.

D-5. STUDY DRUG ADMINISTRATION

D-5.1. Dosing Regimen

D-5.1.1. Zotatifin (eFT226)

Subjects in the investigational arm should receive Zotatifin via IV once (e.g., on Day 1 of the 14-day treatment period) by 1-hour infusion at the study site. However, given the variability of infusion pumps from site to site and patient tolerability of the infusion, time windows of minus 10 minutes and plus 60 minutes are permitted (ie, infusion time is 50 to 120 minutes); infusion time may be extended beyond 120 minutes if needed to manage infusion-related reactions.

D-5.1.2. Fulvestrant

Subjects in the investigational or standard of care arm should receive fulvestrant once intramuscularly via injection on Day 1 (up to 3 days late is permissible).

D-5.2. Infusion-related Reactions

It is unknown if a patient will experience an infusion-related reaction (IRR) with the administration of zotatifin (eFT226). Currently, premedication for IRR is not part of the treatment regimen for zotatifin.

Signs and symptoms of IRRs may include, but are not limited to; fever; chills; pruritus; headache; nausea; chest pain; palpitations; edema; rash; tachycardia; bradycardia; hypotension; hypertension; rigors; and shortness of breath.

In the case of an IRR, the IND-holder and manufacturer should be notified.

Recommended management for IRRs is provided in Table D-2. Institutional standards for the treatment of IRRs may also be used. The recommended management does not supersede Investigator judgment.

Table D-2: Management of Zotatifin Infusion-Related Reactions

IRR Severity	Recommended Management
Grade 1	1. No intervention 2. Continue infusion unless symptoms worsen, monitor closely
Grade 2	1. Interrupt infusion 2. Symptomatic treatment * 3. Resume infusion at half the previous rate when infusion related symptoms improve to Grade ≤ 1
Grade 3	1. Discontinue infusion 2. Symptomatic treatment* 3. Monitor patient until symptoms resolve, including hospitalization if necessary 4. Discontinue study therapy (if the patient is benefiting from study therapy the patient may be allowed to continue after discussion with the Sponsor and Investigator)
Grade 4	1. Discontinue infusion 2. Symptomatic treatment * 3. Hospitalize patient 4. Discontinue study therapy

* Symptomatic treatment may include, but not limited to, antihistamines, NSAIDs, narcotics, IV fluids, corticosteroids, oxygen, anti-emetics, and epinephrine.

Abbreviations: IV = intravenous, NSAIDS = nonsteroidal anti-inflammatory drug.

D-5.3 Dose Modifications

If a patient experiences an AE, appropriate supportive care (eg, antiemetics, antidiarrheals) should be instituted consistent with the nature of the event. Management for CPK elevation is provided in Table D-3.

If there is a grade 3 or 4 adverse event that occurs after registration but prior to the administration of study drug, investigators should consider holding treatment.

Study drug will be discontinued after any Grade 3 or 4 toxicity of clinical significance.

Table D-5. Management of CPK increased

CPK increased	
Grade 2 (> 2.5 x ULN) and above, or as clinically indicated	CPK elevation has been rarely reported with zotatifin. If grade 2 or higher is observed, **permanently discontinue zotatifin** and continue checking CPK at least weekly until grade 1 or lower. Recommend work-up for CPK elevation is CK isoenzymes, including CK-MM, CK-BB, and CK-MB, and urine myoglobin if available. If CK-MB is elevated, recommend checking troponin level (troponin-I if available) and EKG.

D-6. ADVERSE EVENTS AND REPORTING PROCEDURES

D-6.1. Potential Adverse Events

D-6.1.1. Related to Zotatifin (eFT226)

As of July 2021, the primary adverse events reported were back pain, diarrhea, fatigue, nausea, and vomiting.

Reported risks considered to be related to zotatifin include the following

• Abdominal pain	• Dyspnea	• Insomnia
• Anemia	• Epistaxis	• Intermittent tachycardia
• AST/ALT increase	• Fatigue	• Mucositis oral
• Back pain	• Fever	• Nausea
• Chills	• Gastrointestinal bleeding	• Platelet count decrease
• Cough	• Headache	• Proteinuria
• Creatine phosphokinase (CPK) increase	• Hypertension	• Rash
• Dehydration	• Hypokalemia	• Sinus bradycardia
• Diarrhea	• Hypomagnesemia	• Tachycardia
• Dizziness	• Infusion-related reactions	• Vomiting

For full information, please see the zotatifin Investigator's Brochure.

D-6.1.2. Related to Fulvestrant

Reported risks considered to be related to fulvestrant include the following

• Anorexia	• Cough	• Injection site pain
• Asthenia	• Dyspnea	• Musculoskeletal pain
• Arthralgia	• Fatigue	• Nausea
• Bone pain	• Headache	• Pain in extremity
• Back pain	• Hot flash	• Vomiting
• Constipation		

For full information, please see the current FDA-approved fulvestrant package insert.

D-6.2. Reporting Serious Adverse Events (SAEs)

In addition to the reporting procedure outlined in Section 7.4 of the main "umbrella" protocol, SAEs will also be reported to eFFECTOR Therapeutics for subjects enrolled in the zotatifin plus fulvestrant arm only using the provided CRFs within 24 hours of learning of their occurrence.

SAE reporting guidelines to other entities outlined in Section 7.4 of the main "umbrella" protocol should continue to be followed for both subjects enrolled in the treatment and SOC arms (see also Section D-7.13). Follow-up information is submitted in the same way as the original SAE Report within 24 hours of the treating site learning of the follow-up information. Each re-occurrence, complication, or progression of the original event should be reported as a

follow-up to that event regardless of when it occurs. The follow-up information should describe whether the event has resolved or continues, if and how it was treated, and whether the patient continued or withdrew from study participation.

D-6.3. Pregnancy

In addition to the reporting procedure outlined in Section 7.5 of the main "umbrella" protocol, pregnancy of a subject or a pregnancy as a result from a male subject on the zotatifin plus fulvestrant arm only must also be reported to eFFECTOR Therapeutics within 24 hours upon learning of its occurrence using the provided CRFs. The pregnancy should be followed up to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications, and should be reported within 5 days of the conclusion of the pregnancy. For male subjects, consent to report information regarding these pregnancy outcomes should be obtained from the mother.

D-7. STUDY CALENDAR – Cohorts 2 & 3 (Fulvestrant ± Zotatifin)

Cohorts 2 & 3 Treatment Phase

Protocol Activity	Screening	Treatment		Surgery/Biopsy	Follow-Up
	Within 14 days of Day 1 of treatment	Day 1 [14]	Day 8 (± 3 days)	Day 15 (-3, +6 days)	30 Days (+/-7 days) after Final Day of Treatment [15]
Informed Consent [1]	X				
Medical/Oncologic History [2]	X				
Physical Examination [3]	X	X			
ECOG Performance Status [4]	X				
Safety Labs/Measurements					
Vital Signs [5]	X	X		X	
Blood Laboratory Tests [6]					
• Complete Blood Count with Differential • Complete Metabolic Panel • Creatine phosphokinase (CPK)	X	X (for zotatifin arm only)	X (for zotatifin arm only)	X (for zotatifin arm only)	X (for zotatifin arm only)
• Calcium	X	X (for zotatifin arm only)	X (for zotatifin arm only)	X (for zotatifin arm only)	
• Prothrombin Time (PT) • Partial Thromboplastin Time (PTT)	X			X (for zotatifin arm only)	
• Magnesium • Serum pregnancy (WOCBP only)	X				
Electrocardiogram [7]	X				
Registration [1]	X				
Treatment					

Fulvestrant Administration [8]		X			
Zotatifin Administration [9]		X			
Tumor Assessment					
Post-treatment Tumor Tissue [10]				X	
Research Plasma Collection [11]				(X)	
Other Clinical Assessment					
Adverse Event Assessment [12]	X	X	X (for zotatifin arm only)	X	X
Concomitant Medications [13]	X	X	X (for zotatifin arm only)	X	X
Footnotes: See numbered sections below.					

D-7.1. Informed Consent: Must be obtained prior to undergoing any study specific procedure and may occur prior to the 14-day screening period. Registration in OnCore should have already occurred after signed informed consent for the main "umbrella" protocol. Subject assignment to a treatment cohort will be entered in OnCore when eligibility is documented. If not yet done, the subject's surgery or core biopsy should also be scheduled. Last day of screening can be as late as C1D1.

D-7.2. Medical History: Includes but not limited to history of other diseases (active or resolved), concomitant illnesses, prior endocrine therapy usage, menopausal status and OncotypeDX results (if available). Prior endocrine therapy includes tamoxifen, raloxifene, anastrozole, letrozole, exemestane, fulvestrant, or other anti-estrogen therapy as determined by investigator; note that ovarian suppression agents (e.g. leuprolide, goserelin) do not count as prior endocrine therapy. Prior endocrine therapy could have been for treatment of a previous breast cancer, treatment of this breast cancer (for those with in-breast recurrences), for breast cancer risk reduction, or for other reason determined by investigator. The exception is usage of these agents for reproductive endocrinology or fertility purposes, which do not count as "prior endocrine therapy".

D-7.3. Physical Examination: Includes an examination of major body systems and weight. Height from within the last 2 years should be recorded. On the day of zotatifin administration day (C1D1), the dose amount should be prepared based on the patient's weight in kilograms.

D-7.4. ECOG Performance Status

Score Definition

0	Fully active, able to carry on all pre-disease activities without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, eg, light house work or office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair
5	Dead

D-7.5. Vital Signs: Blood pressure, pulse rate, and body temperature to be recorded in the same position, either supine or sitting.

D-7.6. Blood Laboratory Tests: All subjects will have labs performed at screening, which includes complete blood count with differential, comprehensive metabolic panel, calcium, magnesium, and creatine phosphokinase (CPK).

Coagulation studies including PT and PTT will also be done at screening. For female subjects of childbearing potential, a serum pregnancy test must also be performed at screening. Subjects randomized to the zotatifin plus fulvestrant arm will have complete blood count with differential, comprehensive metabolic panel, calcium, and creatine phosphokinase checked on Day 1 prior to drug administration and again on Day 8. They will have complete blood count with differential, comprehensive metabolic panel, calcium, creatine phosphokinase, PT and PTT checked on the day of surgery or biopsy; and have complete blood count with differential, comprehensive metabolic panel at the 30-day follow-up. The Day 1 labs do not need to be repeated if the Screening labs were within 72 hours before start of study therapy.

D-7.7. Electrocardiogram: 12-lead ECG in triplicate to assess for exclusion criteria of significant screening ECG abnormalities. It is suggested that the three measurements be obtained within 1 hour.

D-7.8. Fulvestrant Administration: Fulvestrant will be administered on Day 1. A total of 500 mg Fulvestrant should be administered intramuscularly into the buttocks slowly (1 to 2 minutes per injection) as two 5 mL injections, 1 in each buttock. Instructions in the package insert should be followed for safety concerns.

D-7.9. Zotatifin Administration: Subjects will be administered zotatifin 0.10 mg/kg as a 60-minute IV infusion (a window of 50 minutes to 120 minutes is permitted, as needed) on Day 1. For the order of administration, fulvestrant should be given prior to zotatifin when possible.

D-7.10. Post-treatment Tumor Tissue: Subjects will either undergo core biopsy or definitive surgery on Day 15 (-2, +7 days). The collected tumor tissue will be sent to central laboratory, and Ki67 immunohistochemistry performed. For those participants who undergo surgery (not core biopsy), information from their surgical pathology report will also be collected as available, including but not limited to tumor size, T-stage, N-stage, and grade; T- and N-stage will be used to calculate the modified PEPI score centrally.

D-7.11. Research Plasma Collection: Research plasma collection for cell-free DNA/RNA and matched normal (if feasible), to be sent to central laboratory. The collection may occur (if feasible) on the day of surgery or core biopsy (or up to 2 days prior) and may be obtained at the same time as the blood laboratory tests for patients on the zotatifin arm. It is not a protocol deviation if this collection does not occur.

D-7.12. Adverse Event Assessment: Adverse events (AEs) will be documented and recorded using NCI CTCAE v5.0. For serious adverse events (SAEs), the active reporting period will be from the time that the subject provides informed consent, which is obtained prior to the subject's participation in the study, ie, prior to undergoing any study-related procedure and/or receiving investigational product, through and including 30 calendar days after the last administration of the investigational product. SAEs experienced by a subject after the active reporting period has ended should be reported if the Investigator becomes aware of them. The reporting period for non-serious AEs will be from the time the subject has taken at least one dose of study treatment throughout time on study. All events regardless of grade will be monitored until all drug-related toxicities have resolved or the Investigator determines in his/her clinical judgment that no further improvement is expected. Adverse events should be assessed at any scheduled or unscheduled visit.

D-7.13. Concomitant Medications: Concomitant medications will be recorded at screening. All concomitant medications should be recorded in the CRF including supportive care drugs (eg, anti-emetic treatment and prophylaxis), herbal supplements or vitamins, the drugs used to treat adverse events or chronic diseases, and non-drug supportive interventions (eg, transfusions).

D-7.14. Day 1 of Treatment: Day 1 of treatment should take place 14 days (-2, +7) before scheduled biopsy or surgery.

D-7.15. 30-Day Safety Follow-Up: To be performed 30 ± 7 days after last dose of study drug. Information related to concomitant medications and adverse events will be collected for 30 days after the last dose of the study drug, and the information may be obtained in person, by telephone or by e-mail contact.

D-8. STATISTICAL CONSIDERATIONS

D-8.1. Statistical Design

The primary objective of this study is to evaluate the efficacy of combination fulvestrant and zotatifin compared with fulvestrant alone in reducing Ki67 values from baseline to on-treatment biopsy after 14 days of treatment in estrogen receptor-positive, HER2-negative tumors (tumor size ≥ 1 cm) with Ki67 ≥ 10% that classify as integrative subtype 2 or 6 by integrative subtype screening.

Additional statistical considerations will follow the details provided in the main "umbrella" protocol.

Two exploratory analyses will be done. First, we will assess if there is a differential treatment effect by integrative subtype (IC2 vs IC6) by including an interaction term between treatment arm and integrative subtype in the main ANCOVA models. Second, we will pool the data from Cohorts 2 and 3 and fit the main ANCOVA models with two extra variables: a binary flag for cohort (cohort 2 vs. cohort 3) and an interaction between treatment arm and that flag. To avoid the risk of false discovery with multiple comparisons, we will not report p-values from these models, but only the point estimates and 95% CIs for the interaction terms.

D-8.2. Accrual Considerations

Each cohort of the study will need to accrue approximately 3 subjects per quarter to accrue the planned 50 subjects in 4 years for each cohort. Subjects will be recruited to participate in this study from an anticipated 10-15 sites, including Stanford Cancer Center. Similar sites have successfully completed accrual to TRIO 030 (NCT03004534), a window-of-opportunity study of an androgen receptor inhibitor in 36 early-stage breast cancer subjects. Early accrual to this study was predominantly patients with $ER^+/HER2^-$ breast cancer (the study also had a triple-negative breast cancer cohort), and during their first quarter, 7 patients were accrued to this study. As the study population in the present trial is anticipated to be 8% of the overall population of patients with $ER^+/HER2^-$ breast cancer, it will be necessary to screen approximately 40 patients per quarter to enroll 3 subjects. Thus, 6 sites performing similarly in the aforementioned window-of-opportunity trial would complete accrual on schedule, and it is anticipated that a total of 10-15 sites, recognizing that not all will have equivalent accrual capacity, should enable adequate accrual.

APPENDIX D-A: Eligibility Criteria Checklist Specific for Cohort 2 & 3 (Zotatifin)

I. Protocol Information:

Protocol Title:	An Umbrella, Randomized, Controlled, Pre-Operative Trial Testing Integrative Subtype-Targeted Therapeutics in Estrogen Receptor-Positive, HER2-Negative Breast Cancer
Cohort 2:	IC2 or IC6 (zotatifin and fulvestrant vs. fulvestrant only)
Cohort 3:	Typical Risk (zotatifin and fulvestrant vs. fulvestrant only)
Local Protocol Number (Stanford):	(Stanford BRS0124 / IRB-52869)
Local Institution:	
Local Principal Investigator:	

II. Participant Information:

Participant Name/ID:

III. Inclusion Criteria

Prospective Participant Must MATCH ALL these Inclusion Criteria to be Eligible	Yes	No	Supporting Documentation *
1. Biopsy-proven ER-positive, HER2-negative breast cancer. ER-positivity is defined as ≥ 1% cells staining positive by immunohistochemistry. HER2-negativity is defined by IHC or FISH, per ASCO-CAP 2018 guidelines. Breast tumor must be intact and tumor size must be ≥ 1 cm as measured by ultrasound, mammogram, MRI, or clinical exam. If tumor is locally recurrent, it must be in the breast (not skin, node, or chest wall recurrence). In the pre-screening phase, Ki67 may or may not have been done locally. If done locally, Ki67 score must be ≥ 5%. Any nodal status is allowed, as is M0 or M1 disease.			
2. ☐ **Cohort 2**: Integrative subtype tumor classification as integrative subtype 2 (IC2, N = 25) or as integrative subtype 6 (IC6, N = 25) per Dr Christina Curtis's laboratory. ☐ **Cohort 3:** Integrative subtype tumor classification as Typical Risk (N = 50) per Dr Christina Curtis's laboratory.	☐	☐	
3. Breast tumor Ki67 score ≥ 10% as assessed by central laboratory.	☐	☐	
4. Women or men, age ≥ 18 years old.	☐	☐	

Prospective Participant Must MATCH ALL these Inclusion Criteria to be Eligible	Yes	No	Supporting Documentation *
5. Performance status 0 to 1 (by Eastern Cooperative Oncology Group [ECOG] scale).	☐	☐	
6. Ability to understand and the willingness to sign a written informed consent document.	☐	☐	
7. Adequate bone marrow and organ function, defined as: 3.1. Absolute neutrophil count ≥ 1.5 x 10^9/L 3.2. Platelets ≥ 100 x 10^9/L 3.3. Hemoglobin ≥ 9.0 g/dL.	☐	☐	
8. Adequate hepatic function during Screening as defined as: 4.1. Serum alanine aminotransferase (ALT) ≤ 3 × upper limit of normal (ULN) 4.2. Serum aspartate aminotransferase (AST) ≤ 3 × ULN 4.3. Serum bilirubin ≤ 1.5 × ULN (unless due to Gilbert's syndrome or hemolysis, in which case ≤ ≤ 3 × ULN is permitted)	☐	☐	
9. Adequate kidney function during Screening as defined as measured or estimated creatinine clearance (eCl_{CR}) > 50 mL/min (eCl_{CR} to be calculated by the Cockcroft-Gault formula)	☐	☐	
10. Adequate electrolyte values within 72 hours before the start of study therapy: 6.1. Serum potassium within normal limits (WNL) 6.2. Serum calcium (adjusted for serum albumin concentration) WNL 6.3. Serum magnesium WNL ***Note: Oral or IV supplementation or medical therapy may be used to achieve normal values for serum potassium, calcium, and magnesium.*** ***Patients with abnormal serum chemistry values may still be considered for the trial if there is adequate assessment and documentation from the Principal Investigator that the patient is asymptomatic, and the values are not clinically significant. Patients who show clinical signs and symptoms related to their abnormal serum chemistry values as well as patients whose serum chemistry values are asymptomatic but clinically significant (eg, hypokalemia or hyponatremia) should be excluded.***	☐	☐	

Prospective Participant Must MATCH ALL these Inclusion Criteria to be Eligible	Yes	No	Supporting Documentation *
11. Adequate coagulation profile: 7.1. Prothrombin time (PT) ≤ 1.5 × ULN (Grade ≤ 1) ***Note: Does not apply if patient is stable and on therapeutic doses of an approved anticoagulant for deep vein thrombosis (DVT) or pulmonary embolism PE).*** 7.2. Activated partial thromboplastin time ≤ 1.5 × ULN (Grade ≤ 1)	☐	☐	
12. Creatine phosphokinase (CPK) ≤ 1.5 x ULN	☐	☐	
13. For female patients of childbearing potential, a negative serum pregnancy test within 7 days prior to start of study therapy.	☐	☐	
14. For female patients of childbearing potential, documented willingness to use a protocol-recommended method of contraception from the start of the screening period until ≥ 30 days after the final dose of study therapy. ***Note: A female patient is considered to be of childbearing potential unless she has had a hysterectomy, bilateral tubal ligation, or bilateral oophorectomy; has medically documented ovarian failure (with serum estradiol and follicle-stimulating hormone [FSH] levels within the institutional laboratory postmenopausal range and a negative serum or urine beta human chorionic gonadotropin [βHCG]); or is menopausal (age ≥ 55 years with amenorrhea for ≥ 6 months).***	☐	☐	
15. For male patients who can father a child and are having intercourse with females of childbearing potential who are not using adequate contraception, documented willingness to use a protocol-recommended method of contraception from the start of study therapy until ≥ 30 days after the final dose of study therapy and to refrain from sperm donation from the start of study therapy until ≥ 90 days after administration of the final dose of study therapy. ***Note: A male patient is considered able to father a child unless he has had a bilateral vasectomy with documented aspermia or a bilateral orchiectomy.***	☐	☐	

IV. Exclusion Criteria:

Prospective Participant Must NOT MATCH ANY these Exclusion Criteria	Yes	No	Supporting Documentation *
1. Significant cardiovascular disease (eg, myocardial infarction, arterial thromboembolism, cerebrovascular thromboembolism) within 6 months prior to start of study therapy; symptomatic dysrhythmias or unstable dysrhythmias requiring medical therapy; unstable angina; symptomatic peripheral vascular disease; New York Heart Association Class 3 or 4 congestive heart failure; uncontrolled Grade ≥ 3 hypertension (diastolic blood pressure ≥ 100 mmHg or systolic blood pressure ≥ 160 mmHg) despite antihypertensive therapy; or history of congenital prolonged QT syndrome	☐	☐	
2. Significant screening ECG abnormalities, including unstable cardiac arrhythmia requiring medication, left bundle-branch block, 2nd degree atrioventricular (AV) block type II, 3rd degree AV block, Grade ≥ 2 bradycardia, or corrected QT (QTcF) > 450 msec (based on the average of 3 measurements at 5-minute intervals).	☐	☐	
3. Evidence of an ongoing systemic bacterial, fungal, or viral infection (including upper respiratory tract infections) at the time of start of study therapy. ***Note: Patients with localized fungal infections of skin or nails are eligible.***	☐	☐	
4. Known active hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), or acquired immunodeficiency syndrome-related illness. ***Note: Patients with treated and inactive HBV, HCV, or HIV may enroll, following discussion with the Sponsor.***	☐	☐	
5. Major surgery within 4 weeks before the start of study therapy or not fully recovered from major surgery.	☐	☐	
6. Prior solid organ transplantation.	☐	☐	

Prospective Participant Must NOT MATCH ANY these Exclusion Criteria	Yes	No	Supporting Documentation *
7. Ongoing immunosuppressive therapy, including systemic or enteric corticosteroids. ***Note: At screening, patients may be using systemic corticosteroids (at doses of ≤ 10 mg of prednisone or equivalent) or topical or inhaled corticosteroids. During study therapy, patients may use systemic, enteric, topical or enteric corticosteroids as required for treatment-emergent conditions.***	☐	☐	
8. Use of a strong or moderate inhibitor or inducer of cytochrome P450 (CYP) 3A4 within 7 days prior to the start of study therapy or expected requirement for use of a strong or moderate inhibitor or inducer of CYP3A4 during study therapy.	☐	☐	
9. See **Appendix D-B** for a list of prohibited concomitant medications and supplements	N/A	N/A	N/A
10. Any other concurrent severe and/or uncontrolled medical condition that would, in the investigator's judgment, contraindicate subject participation in the clinical study (eg, chronic active hepatitis [testing not mandatory unless required by local regulations or requirements], severe hepatic impairment, etc)	☐	☐	If Yes, ineligible by investigator decision.
11. Pregnant or nursing (lactating)	☐	☐	
12. Prior breast cancer-directed therapy (surgery, radiation, chemotherapy, or endocrine therapy to treat breast cancer) is not allowed, with the exception of people with in-breast recurrences. People with in-breast recurrences cannot have had breast cancer-directed therapy (radiation, chemotherapy, or endocrine therapy; surgery is acceptable) within the 6 months prior to signing the pre-screening consent. Previous endocrine therapy for breast cancer risk reduction and/or ovarian suppression for premenopausal women is allowed.	☐	☐	

* All participant files must include supporting documentation to confirm participant eligibility. The method of confirmation can include, but is not limited to, laboratory test results, radiology test results, participant self-report, and medical record review.

VI. Statement of Eligibility

By signing this form of this trial I verify that this participant is [☐ **eligible** / ☐ **ineligible**] for participation in the study. This study is approved by the Stanford Cancer Institute Scientific Review Committee, the study's IRB of record, and has finalized financial and contractual agreements as required by Stanford School of Medicine's Research Management Group.

Site PI or Treating Physician Signature:	Date:
Printed Name:	

Secondary Reviewer Signature:	Date:
Printed Name:	

Study Coordinator Signature:	Date:
Printed Name:	

APPENDIX D-B: List of CYP3A4 Inducers and Inhibitors (Cohorts 2 & 3 - Zotatifin)

Effect on CYP3A		Drug Class	Medications
Inhibitors	Strong	**Antiarrhythmic**	Diltiazem
		Antibacterial	Clarithromycin, troleandomycin
		Antidepressant	Nefazodone
		Antifungals	Ketoconazole, itraconazole, posaconazole, Voriconazole
		Antihypertensive	Conivaptan
		Antineoplasic	Idelalisib
		Antiviral	Boceprevir, nelfinavir, telaprevir. Ritonavir alone or with any of: danoprevir, elvitegravir indinavir, lopinavir, saquinavir, tipranavir, paritaprevir with ombitasvir and/or asabuvir, nirmatrelvir
		Foods/herbs	Grapefruit juice, Seville orange juice
		Pharmacokinetic enhancer	Cobicistat
	Moderate	**Antiarrhythmic**	Dronedarone, verapamil
		Antibacterial	Ciprofloxacin, erythromycin
		Antidepressant	Fluvoxamine
		Antiemetic	Aprepitant
		Antifungal	Clotrimazole, fluconazole
		Antineoplastic	Crizotinib, imatinib
		Anxiolytic	Tofisopam
		Gastric acid reducing	Cimetidine
		Immunosuppressant	Cyclosporine
	Weak	**Antianginal**	Ranolazine
		Antiemetic	Fosaprepitant
		Anti-Parkinsonian	Istradefylline
		Antithrombotic	Cilostazol, Ticagrelor
		Gastric acid reducing	Ranitidine
		hypolipidemic	Lomitapide
		Immunosupressant	Tacrolimus
		Respiratory enhancer(cystic fibrosis)	Ivacaftor
		Spasmolytic	Chlorzoxazone

Inducers	**Strong**	**Antiandrogen (cancer treatment)**	Enzalutamide
		Antibacterial	Rifampin
		Anticonvulsant	Carbamazepine, Phenytoin
		Antineoplastic	Mitotane
		Foods/herbs	St John's wort
	Moderate	**Antihypertensive**	Bosentan
		Antiviral	Efavirenz, Etravirine
		Stimulant	Modafinil
	Weak	**Anticonvulsant**	Rufinamide
		Stimulant	Armodafinil

Reference: Food and Drug Administration. Drug Development and Drug Interactions: Table of Substrates, Inhibitors and Inducers. 9/27/2016. Available at: http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm093664.htm **#4** (accessed 03 October 2017)

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