

**LASOFOXIFENE COMBINATION TREATMENT OF ER⁺ BREAST CANCER
THAT HAS PROGRESSED ON A CDK4/6 INHIBITOR**

1. CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] This application claims benefit of and priority to U.S. Provisional Application No. 63/345,843, filed May 25, 2022; U.S. Provisional Application No. 63/411,633, filed September 30, 2022; U.S. Provisional Application No. 63/426,737, filed November 19, 2022; U.S. Provisional Application No. 63/430,194, filed December 5, 2022; and U.S. Provisional Application No. 63/446,760, filed February 17, 2023, the disclosure of each of which is incorporated herein by reference in its entirety.

2. BACKGROUND OF THE INVENTION

[0002] Estrogen receptor positive (ER⁺) breast cancers express estrogen receptor α (ER α), which is encoded by the *ESR1* gene. Approximately 70% of breast cancers are ER⁺ and are, therefore, treated with agents that deplete circulating estrogen levels or that block estrogen signaling in the cancer cell (collectively, endocrine therapy). Endocrine therapy has led to significant improvement in outcome of women with ER⁺ breast cancer. However, the effectiveness of endocrine therapy is limited by intrinsic and, importantly, acquired endocrine resistance. In response to the selective pressure imposed by endocrine therapies, in particular by aromatase inhibitors (AI), ER⁺ tumors evolve various escape mechanisms. Among these is acquisition of gain-of-function mutations in the *ESR1* gene that alter the ligand binding domain of the ER α receptor, rendering the receptor constitutively active at low levels, or in the absence, of estrogen. Despite the benefits of endocrine therapy, the majority of ER⁺ tumors will eventually acquire resistance and progress.

[0003] Lasofoxifene, a third generation selective estrogen receptor modulator (SERM), has been shown to reduce the risk of invasive ER⁺ breast cancer in women with wild type estrogen receptors: *i.e.*, post-menopausal women with no history of breast cancer being treated for osteoporosis. LaCroix *et al.*, *J. Natl. Cancer Inst.* 102:1706-1715 (2010). Lasofoxifene was later shown to retain the ability to inhibit progression of ER⁺ cancers that have developed gain-of-function mutations in the ligand binding domain of the ER α receptor (*ESR1* gene). US Pat. Nos. 10,258,605 and 10,905,659; WO 2019/199891; Lainé *et al.*, *Breast Cancer Res.* 23(1):54 (2021). The efficacy of lasofoxifene as a single agent in the treatment of premenopausal and postmenopausal women with locally advanced or metastatic

ER⁺ breast cancers that have acquired *ESR1* gain-of-function mutations is currently being confirmed in a phase 2 clinical trial, NCT03781063 (the ELAINE trial).

[0004] Over the last decade, a new class of drugs, cyclin dependent kinase 4/6 inhibitors (CDK4/6i), have become commercially available for the treatment of women with (ER⁺) breast cancer. Three CDK4/6i have been approved in a number of countries around the world, including the United States, for treatment of ER⁺ cancers in combination with endocrine therapy: palbociclib (IBRANCE, Pfizer), ribociclib (KISQALI, Novartis) and abemaciclib (VERZENIO, Eli Lilly). However, ER⁺ tumors have been shown to develop resistance to CDK4/6i and ultimately progress.

[0005] There remains a need for new therapies that are effective to treat ER⁺ tumors harboring mutations in *ESR1* and that have developed resistance to, and thus progressed on, endocrine therapies and CDK4/6 inhibitors.

3. SUMMARY OF THE INVENTION

[0006] The ongoing ELAINE 2 clinical trial is an open-label, multicenter, study evaluating the efficacy, safety and tolerability of the combination of the third generation SERM, lasofoxifene, and the CDK4/6 inhibitor, abemaciclib, for the treatment of pre-menopausal and post-menopausal women who have locally advanced or metastatic ER⁺/HER2⁻ breast cancer with an *ESR1* mutation and disease progression on first, second, or third lines of hormonal treatment for metastatic disease. To be eligible for enrollment, progression may have occurred after up to three of the following treatments for metastatic breast cancer: an aromatase inhibitor (AI) and/or fulvestrant, either as monotherapy or in combination with any commercially available CDK4/6i; and/or the combination of fulvestrant and alpelisib; and/or tamoxifen; and/or the combination of exemestane/everolimus; and up to one line of chemotherapy in the metastatic setting (48%). A majority of the patients enrolled in the trial (28/29) had progressed on prior CDK4/6 inhibitor combination therapy. We have now discovered that the combination of lasofoxifene with the CDK4/6 inhibitor (CDK4/6i) abemaciclib is well tolerated and demonstrates robust and meaningful efficacy in such patients, reducing progression of breast cancer in women with locally advanced or metastatic ER⁺ breast cancer harboring an *ESR1* gain-of-function mutation who had progressed on prior CDK4/6i therapies.

[0007] In addition, we have now discovered that the combination of lasofoxifene with the CDK4/6 inhibitor (CDK4/6i) abemaciclib reduces progression of breast cancer in women with locally advanced or metastatic ER+ breast cancer harboring an *ESR1* gain-of-function mutation and an oncogenic mutation in one or more genes other than the *ESR1* gene.

[0008] Accordingly, in a first aspect, provided herein is a method of reducing the progression of breast cancer in a patient, comprising:

administering to the patient an effective amount of lasofoxifene or a pharmaceutically acceptable salt thereof and an effective amount of a CDK4/6 inhibitor (CDK4/6i),

wherein the breast cancer:

- (i) is estrogen receptor positive (ER⁺);
- (ii) has at least one gain of function missense mutation within the ligand binding domain (LBD) of the Estrogen Receptor 1 (*ESR1*) gene; and
- (iii) has progressed during prior CDK4/6 inhibitor therapy.

[0009] In one aspect, provided herein is a method of reducing the progression of breast cancer in a patient, comprising:

administering to the patient an effective amount of lasofoxifene or a pharmaceutically acceptable salt thereof and an effective amount of a CDK4/6 inhibitor (CDK4/6i),

wherein the breast cancer:

- (i) is estrogen receptor positive (ER⁺);
- (ii) has at least one gain of function missense mutation within the ligand binding domain (LBD) of the Estrogen Receptor 1 (*ESR1*) gene; and
- (iii) has an oncogenic mutation in one or more genes other than the *ESR1* gene.

[0010] In one aspect, provided herein is a method of reducing the progression of breast cancer in a patient, comprising:

administering to the patient an effective amount of lasofoxifene or a pharmaceutically acceptable salt thereof and an effective amount of a CDK4/6 inhibitor (CDK4/6i),

wherein the breast cancer:

- (i) is estrogen receptor positive (ER⁺);
- (ii) has at least one gain of function missense mutation within the ligand binding domain (LBD) of the Estrogen Receptor 1 (*ESR1*) gene; and

- (iii) has increased expression of one or more genes other than the *ESR1* gene.

[0011] In one aspect, provided herein is a method of monitoring a patient on a breast cancer treatment, comprising:

- (a) determining quantitative measures of the mutant allele frequency (MAF) of circulating tumor DNA (ctDNA) of at least one gain of function missense mutation within the ligand binding domain (LBD) of the Estrogen Receptor 1 (*ESR1*) gene (*ESR1* ctDNA) in a biological sample of the patient, wherein the quantitative measures are performed over a period of time at determined intervals; and
- (b) determining a positive predictive value (PPV) for clinical benefit with stable disease of the cancer treatment,
 - wherein the PPV indicates responsiveness to the cancer treatment,
 - wherein the cancer treatment comprises an effective amount of lasofoxifene or a pharmaceutically acceptable salt thereof and an effective amount of a CDK4/6 inhibitor (CDK4/6i).

[0012] The method of any preceding embodiment, wherein the ER⁺ breast cancer is HER2⁻.

[0013] The method of any preceding embodiment, wherein the ER⁺ breast cancer is locally advanced or metastatic.

[0014] The method of any preceding embodiment, wherein lasofoxifene is administered as lasofoxifene tartrate.

[0015] The method of any preceding embodiment, wherein lasofoxifene is administered at 5 mg/day per os.

[0016] The method of any preceding embodiment, wherein the CDK4/6i administered to the patient is selected from palbociclib, ribociclib, and abemaciclib.

[0017] The method of any preceding embodiment, wherein the CDK4/6i administered to the patient is abemaciclib. In certain embodiments, abemaciclib is administered orally at 50 mg to 200 mg BID. In certain embodiments, abemaciclib is administered orally at 100 mg to 200 mg BID. In certain embodiments, abemaciclib is administered orally at 150 mg BID.

[0018] The method of any preceding embodiment, wherein the prior administered CDK4/6 inhibitor is selected from palbociclib, ribociclib, and abemaciclib. In certain of these embodiments, the prior administered CDK4/6 inhibitor is abemaciclib.

[0019] The method of any preceding embodiment, wherein the cancer has previously been determined to have at least one gain of function missense mutation within the ligand binding domain (LBD) of the Estrogen Receptor 1 (*ESR1*) gene.

[0020] The method of any preceding embodiment, further comprising an earlier step of:
determining that the patient has at least one gain of function missense mutation within the ligand binding domain (LBD) of the Estrogen Receptor 1 (*ESR1*) gene.

[0021] The method of any preceding embodiment, wherein the at least one of gain of function missense mutation is in any one of amino acids D538, Y537, L536, P535, V534, L469, S463, V392, and E380.

4. BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

[0022] These and other features, aspects, and advantages of the present invention will become better understood with regard to the following description, and accompanying drawings, in which:

[0023] FIG. 1 is a swimmer plot presenting data on individual patient response to treatment with lasofoxifene and abemaciclib in the ELAINE 2 clinical trial (NCT04432454) on a first interim date.

[0024] FIG. 2 illustrates patient maximum tumor response at the same timepoint as FIG. 1 (PD = progressive disease; SD = stable disease; PR = partial remission).

[0025] FIG. 3 is a swimmer plot presenting data on patient response to treatment with lasofoxifene and abemaciclib in the ELAINE 2 clinical trial (NCT04432454) at a later interim date than FIGS. 1 and 2, with additional information on each subject's pre-enrollment treatments.

[0026] FIG. 4 depicts, for each individual subject enrolled in the ELAINE 2 trial, the duration of the subject's pre-enrollment response on prior second-line and third-line cancer therapies versus on-trial duration of response to the combination of lasofoxifene and abemaciclib at the same timepoint as in FIG. 3.

[0027] FIG. 5 presents prevalence of oncogenic mutations in a panel of genes, including the *ESR1* gene and other genes known or suspected to contribute to neoplasia in enrolled ELAINE 2 subjects at baseline, and the subject's subsequent achievement of clinical benefit (CB) and the median progression-free survival (mPFS) on treatment with lasofoxifene and abemaciclib in the ELAINE 2 trial, as of the interim date of FIG. 3.

[0028] FIG. 6 presents individual MAF kinetics for the most commonly observed mutant *ESR1* variants. Shown are Y537C, Y537N, Y537S, and D538G variants. Variants with low baseline MAF (solid line with diamond ends) utilize the left vertical axis (y-axis) and those with high baseline MAF (broken line with circle ends) utilize the right vertical axis (y-axis). X-axis: timeline from baseline to week 4. BL: baseline; MAF: mutant allele fraction.

[0029] FIG. 7 is a swimmer plot presenting data on patient response to treatment with lasofoxifene and abemaciclib in the ELAINE 2 clinical trial (NCT04432454) at an interim date later than FIGS. 1-4. Also shown are additional information on each subject's pre-enrollment treatments.

[0030] FIGS. 8A and 8B illustrate exemplary types of copy number variations (CNVs) of various oncogenes, including *CCND1*, detected in circulating tumor DNA (ctDNA) of subjects in the ELAINE 2 clinical trial (NCT04432454), as of the date of FIG. 5. FIG. 8A shows CNV events detected per gene. FIG. 8B shows copy number distribution per gene for the CNV events shown on the left panel. CNVs were annotated as "focal" or "aneuploidy", or "amplification" where focal or aneuploidy status was indeterminate.

[0031] FIG. 9 presents prevalence of oncogenic mutations in a panel of genes, including the *ESR1* gene and other genes known or suspected to contribute to neoplasia in enrolled ELAINE 2 subjects at baseline, and the subject's subsequent achievement of clinical benefit (CB) and the median progression-free survival (mPFS) on treatment with lasofoxifene and abemaciclib in the ELAINE 2 trial, at an interim date later than FIG. 5.

[0032] FIGS. 10A-10B are swimmer plots presenting data on patient response to treatment with lasofoxifene and abemaciclib in the ELAINE 2 clinical trial (NCT04432454) at an interim date later than FIGS. 1-4 and 7. FIG. 10B is a duplicate of FIG. 10A including individual patient (albeit de-identified) designation numbers. Also shown are additional information on each subject's pre-enrollment treatments.

5. DETAILED DESCRIPTION OF THE INVENTION

[0033] The ongoing ELAINE 2 clinical trial is an open-label, multicenter, study evaluating the efficacy, safety and tolerability of the combination of the third generation SERM, lasofoxifene, and the CDK4/6 inhibitor, abemaciclib, for the treatment of premenopausal and postmenopausal women who have locally advanced or metastatic ER⁺/HER2⁻ breast cancer with an *ESR1* mutation and who have had disease progression on first, second or third lines of hormonal treatment for metastatic disease. To be eligible for enrollment, progression may have occurred on no more than three of the following treatments for metastatic breast cancer: an aromatase inhibitor (AI) and/or fulvestrant, either as monotherapy or in combination with any commercially available CDK4/6i; and/or the combination of fulvestrant and alpelisib; and/or tamoxifen; and/or the combination of exemestane/everolimus.

[0034] All but one (28/29) of the patients enrolled in the trial had progressed on prior CDK4/6 inhibitor combination therapy. We have now discovered that the combination of lasofoxifene with the CDK4/6 inhibitor (CDK4/6i) abemaciclib is well tolerated and demonstrates robust and meaningful efficacy in such patients, reducing progression of breast cancer in women with advanced ER⁺ breast cancer harboring a gain of function mutation in the ligand binding domain of *ESR1* and who had progressed on prior CDK4/6i therapies.

[0035] In addition, we have now discovered that the combination of lasofoxifene with the CDK4/6 inhibitor (CDK4/6i) abemaciclib reduces progression of breast cancer in women with locally advanced or metastatic ER⁺ breast cancer harboring an *ESR1* gain-of-function mutation and an oncogenic mutation in one or more genes other than the *ESR1* gene.

5.1. Methods of Treatment

[0036] Accordingly, in a first aspect, disclosed herein are methods of reducing (or slowing) the progression of breast cancer in a patient by administering to the patient an effective amount of lasofoxifene or a pharmaceutically acceptable salt thereof and an effective amount of a CDK4/6 inhibitor (CDK4/6i), wherein the breast cancer: (i) is estrogen receptor positive (ER⁺); (ii) has at least one gain-of-function missense mutation within the ligand binding domain (LBD) of the Estrogen Receptor 1 (*ESR1*) gene; and (iii) has progressed during prior CDK4/6 inhibitor therapy.

[0037] In certain embodiments, the patient's breast cancer is human epidermal growth factor receptor 2 negative (HER2⁻). In certain embodiments, the patient's breast cancer is locally advanced. In certain embodiments, the patient's breast cancer is metastatic.

[0038] Also disclosed herein are methods of treating breast cancer in a patient by administering to the patient an effective amount of lasofoxifene or a pharmaceutically acceptable salt thereof and an effective amount of a CDK4/6 inhibitor (CDK4/6i), wherein the breast cancer: (i) is estrogen receptor positive (ER⁺); (ii) has at least one gain-of-function missense mutation within the ligand binding domain (LBD) of the Estrogen Receptor 1 (*ESR1*) gene; and (iii) has an oncogenic mutation in one or more genes other than the *ESR1* gene. In certain embodiments, the patient's cancer is human epidermal growth factor receptor 2 negative (HER2⁻). In certain embodiments, the patient's ER⁺ breast cancer is locally advanced. In certain embodiments, the patient's ER⁺ breast cancer is metastatic.

5.1.1. Patient with ER⁺ Cancer

[0039] In accordance with the provided methods of treatment, the patient has ER⁺ breast cancer. In various embodiments, the patient has been diagnosed with ER⁺ breast cancer by immunohistochemistry (IHC) performed on a sample of the patient's cancer.

[0040] In some embodiments, the patient is premenopausal, perimenopausal or postmenopausal. In some embodiments, the patient is premenopausal and has locally advanced or metastatic ER⁺ breast cancer. In some embodiments, the patient is perimenopausal and has locally advanced or metastatic ER⁺ breast cancer. In some embodiments, the patient is postmenopausal and has locally advanced or metastatic ER⁺ breast cancer.

[0041] In some embodiments of the provided methods of treatment, the patient's breast cancer is HER2⁻ (ER⁺/HER2⁻). In particular embodiments, the patient has locally advanced or metastatic ER⁺/HER2⁻ breast cancer.

5.1.2. Mutations in *ESR1* Gene

[0042] In accordance with the provided methods of treatment, cells of the patient's cancer have acquired at least one gain-of-function missense mutation within the ligand binding domain (LBD) of the Estrogen Receptor 1 (*ESR1*) gene.

[0043] In some embodiments, the mutation leads to the ligand-independent activity of the estrogen receptor. In some embodiments, the mutation leads to enhanced ligand-stimulated activity of the estrogen receptor. In some embodiments, the mutation leads to resistance to endocrine therapy. In some embodiments, the mutation promotes tumor growth. In some embodiments, the mutation enhances metastatic activity of the cancer. In some embodiments, the mutation enhances further metastatic activity of ER⁺ metastatic breast cancer.

[0044] In various embodiments, the mutation arises from a rare and undetectable pre-existing clone. In some embodiments, the mutation is acquired *de novo* during the course of endocrine therapy treatment. In some embodiments, the mutation is acquired *de novo* after multiple lines of endocrine therapy treatment. In some embodiments, the mutation is acquired *de novo* after multiple lines of endocrine therapy treatment of metastatic breast cancer. In various embodiments, the mutant clone expands to become a more dominant clone over the course of successive lines of endocrine therapy.

[0045] In some embodiments, the mutation in the *ESR1* gene is a missense point mutation. In some embodiments, the mutation in the *ESR1* gene is a truncating mutation. In some embodiments, the mutation in the *ESR1* gene is a gene amplification. In some embodiments, the mutation in the *ESR1* gene is a genomic rearrangement.

[0046] In some embodiments, the patient has an ER⁺ breast cancer that has at least one gain-of-function missense mutation within the ligand binding domain (LBD) of the ESR1 gene. In various embodiments, at least one of the mutations is in an amino acid selected from D538, Y537, L536, P535, V534, L469, S463, V392, and E380, wherein the amino acids are numbered according to the ESR1 protein with NCBI accession number NP_000116.2.

[0047] In particular embodiments, the mutation increases the stability of the agonist conformation of Helix 12 of the ER α protein. In some of these embodiments, the mutation increases the binding of the estrogen receptor to its co-activators. In some of these embodiments, the mutation leads to hormone independent activity of estrogen receptor. In some of these embodiments, the mutation leads to resistance to tamoxifen, fulvestrant, and/or aromatase inhibitors.

[0048] In certain embodiments, the mutation is in amino acid D538. In certain preferred embodiments, the mutation is D538G. In certain embodiments, the ER⁺ breast cancer has at least a D538G mutation and at least one mutation in an amino acid selected from Y537,

L536, P535, V534, L469, S463, V392, and/or E380. In certain embodiments, the ER⁺ breast cancer has at least a D538 mutation and at least a Y537 mutation.

[0049] In certain embodiments, the mutation is in amino acid Y537. In certain embodiments, the ER⁺ breast cancer has at least one mutation in amino acid Y537 and at least one mutation in an amino acid selected from D538, L536, P535, V534, L469, S463, V392, and/or E380. In some of these embodiments, the mutation is Y537S, Y537N, Y537C, or Y537Q. In certain preferred embodiments, the mutation is Y537S. In certain preferred embodiments, the mutation is Y537C. In certain preferred embodiments, the mutation is Y537N. In certain preferred embodiments, the mutation is Y537Q.

[0050] In some embodiments, the mutation is in amino acid L469. In certain embodiments, the ER⁺ breast cancer has at least one mutation in amino acid L469 and at least one mutation in an amino acid selected from D538, L536, Y537, P535, V534, S463, V392, and/or E380. In certain preferred embodiments, the mutation is L469V.

[0051] In some embodiments, the mutation is in amino acid L536. In certain embodiments, the ER⁺ breast cancer has at least one mutation in amino acid L536 and at least one mutation in an amino acid selected from D538, Y537, P535, V534, L469, S463, V392, and/or E380. In certain embodiments, the mutation is L536R or L536Q. In certain embodiments, the mutation is L536R. In certain embodiments, the mutation is L536Q. In certain embodiments, the mutation is L536P. In certain embodiments, the mutation is L536H.

[0052] In some embodiments, the mutation is in amino acid P535. In certain embodiments, the ER⁺ breast cancer has at least one mutation in amino acid P535 and at least one mutation in an amino acid selected from D538, Y537, L536, V534, L469, S463, V392, and/or E380. In certain embodiments, the mutation is P535H.

[0053] In some embodiments, the mutation is in amino acid V534. In certain embodiments, the ER⁺ breast cancer has at least one mutation in amino acid V534 and at least one mutation in an amino acid selected from D538, Y537, L536, P535, L469, S463, V392, and/or E380. In certain embodiments, the mutation is V534E.

[0054] In some embodiments, the mutation is in amino acid S463. In certain embodiments, the ER⁺ breast cancer has at least one mutation in amino acid S463 and at least one mutation

in an amino acid selected from D538, Y537, L536, P535, V534, L469, V392, and/or E380. In certain embodiments, the mutation is S463P.

[0055] In some embodiments, the mutation is in amino acid V392. In certain embodiments, the ER⁺ breast cancer has at least one mutation in amino acid V392 and at least one mutation in an amino acid selected from D538, Y537, L536, P535, V534, L469, S463, and/or E380. In certain embodiments, the mutation is V392I.

[0056] In some embodiments, the mutation is in amino acid E380. In certain embodiments, the ER⁺ breast cancer has at least one mutation in amino acid E380 and at least one mutation in an amino acid selected from D538, Y537, L536, P535, V534, L469, S463, and/or S463. In certain embodiments, the mutation is E380Q.

5.1.2.1 Detection of the *ESR1* Gene Mutations

[0057] In various embodiments, the patient's ER⁺ breast cancer has been previously determined to have at least one mutation in the *ESR1* gene. Some embodiments of the methods described herein further include the earlier step of detecting the mutations in the *ESR1* gene.

[0058] In some embodiments, massively parallel next generation sequencing (NGS) is used for detecting the estrogen receptor mutations in the patient's cancer. In certain embodiments, the entire genome is sequenced. In certain embodiments, selected gene panels of cancer-related genes are sequenced. In certain embodiments, all coding exons within a given set of genes are sequenced. In certain embodiments, known "hotspot" regions within a given set of genes are sequenced. However, the inherent error rate of current next generation sequencing techniques is up to 1%, limiting the sensitivity and specificity of detection. In some embodiments, targeted sequencing is used for detecting the presence of the *ESR1* mutations. Although targeted sequencing allows deeper sequencing, it is also currently limited by the 1% error rate. In some embodiments, methods with reduced sequencing error rate are used. In a particular embodiment, Safe-Sequencing System (Safe-SeqS) is used, which tags each template molecule to allow for confident identification of rare variants. See Kinde *et al.*, *Proceedings of the National Academy of Sciences* 108(23): 9530-9535 (2011). In particular embodiments, ultrasensitive Duplex sequencing is used, which independently tags and sequences each of the two strands of a DNA duplex. See Schmitt *et al.*, *Proceedings of the National Academy of Sciences* 109(36): 14508-14513 (2012). In some embodiments, digital

droplet PCR is used, which emulsifies DNA in thousands to millions of droplets to encapsulate single DNA molecules, designed with mutant specific primers. See Vogelstein and Kinzler, *Proceedings of the National Academy of Sciences* 96(16): 2322-2326 (1999) and Huggett *et al.*, *Clinical Chemistry* 61(1): 79-88 (2014).

[0059] In some embodiments, the detection of the *ESRI* mutations occurs along with the initial diagnosis. In some embodiments, the detection of the mutations occurs at the time of evaluating disease progression, relapse, or recurrence. In some embodiments, the detection of the mutations occurs at the time of disease progression. In some embodiments, the detection of the mutations takes place at the time when the disease is stable.

[0060] In some embodiments, one or more biologic specimens are obtained from the patient for detection of the mutations. In certain embodiments, the biologic specimen is a tissue specimen. In certain embodiments, the tissue specimen is a tumor biopsy. In certain embodiments, the tissue specimen is a biopsy of metastases. In some other embodiments, the biologic specimen is a body fluid, e.g., obtained from peripheral blood (liquid biopsy). In certain embodiments, the liquid biopsy comprises circulating tumor cells (CTCs). In certain embodiments, the liquid biopsy comprises cell free DNA.

[0061] In specific embodiments of the methods provided herein, the *ESRI* mutations are monitored by analysis of circulating tumor DNA (ctDNA). In some embodiments, the ctDNA analysis is performed, e.g., intermittently or regularly, throughout the duration of treatment. In some of these embodiments, the ctDNA is extracted from patient blood samples. In certain embodiments, the ctDNA is evaluated by digital PCR analysis of the *ESRI* mutations.

[0062] In some embodiments, ctDNA analysis is performed by a liquid biopsy assay as a companion diagnostic device to identify patients with breast cancer lacking or having *ESRI* mutations. Exemplary liquid biopsy assays are Guardant360® CDx (2021, FDA approved panel or professional services panel [guardant360cdx.com/gene-list/]), Guardant360 Response™ (2021, [ncbi.nlm.nih.gov/gtr/tests/593444/]), and FoundationOne® Liquid CDx (2021, [assets.ctfassets.net/w98cd481qyp0/wVEm7VtICYR0sT5C1VbU7/fd055e0476183a6acd4eae6b583e3a00/F1LCDx_Technical_Specs_072021.pdf]), each of which is incorporated herein by reference in its entirety.

5.1.3. Patient with oncogenic mutations other than *ESR1* mutations

[0063] In one aspect, disclosed herein are methods of reducing (or slowing) the progression of breast cancer in a patient by administering to the patient an effective amount of lasofoxifene or a pharmaceutically acceptable salt thereof and an effective amount of a CDK4/6 inhibitor (CDK4/6i), wherein the breast cancer: (i) is estrogen receptor positive (ER⁺); (ii) has at least one gain of function missense mutation within the ligand binding domain (LBD) of the Estrogen Receptor 1 (*ESR1*) gene; and (iii) has an oncogenic mutation in one or more genes other than the *ESR1* gene. In some embodiments, the oncogenic mutation is detected in the circulating tumor DNA (ctDNA) in a biological sample obtained from the patient. In some embodiments, the biological sample is blood, plasma, serum, or bodily fluids (such as saliva, tear, seminal fluid, cervical fluid, urine, cerebrospinal fluid, peritoneal fluid, pleural fluid, amniotic fluid, or extracellular fluid). In some embodiments, the biological sample is plasma.

[0064] In certain embodiments, the patient's ER⁺ breast cancer is locally advanced. In certain embodiments, the patient's ER⁺ breast cancer is metastatic.

[0065] In some embodiments, at least one of the one or more genes with an oncogenic mutation is selected from HNF1A, TERT, GATA3, CDK12, MAPK3, GNAS, RAF1, RHEB, NTRK3, TP53, PIK3CA, APC, ATM, MET, CCND1, EGFR, ROS1, BRCA2, STK11, FGFR1, ARID1A, CCNE1, AR, ALK, ERBB2, KIT, CDK4, SMAD4, NOTCH1, RB1, BRAF, PTEN, AKT1, CDH1, BRCA1, MYC, CDKN2A, IDH2, MTOR, or PDGFRA. In some embodiments, each of the one or more genes with an oncogenic mutation is selected from HNF1A, TERT, GATA3, CDK12, MAPK3, GNAS, RAF1, RHEB, NTRK3, TP53, PIK3CA, APC, ATM, MET, CCND1, EGFR, ROS1, BRCA2, STK11, FGFR1, ARID1A, CCNE1, AR, ALK, ERBB2, KIT, CDK4, SMAD4, NOTCH1, RB1, BRAF, PTEN, AKT1, CDH1, BRCA1, MYC, CDKN2A, IDH2, MTOR, or PDGFRA.

[0066] In certain embodiments, treatment with lasofoxifene in combination with a CDK4/6i (e.g., palbociclib, ribociclib, abemaciclib) in patients having the at least one or more oncogenic mutations provides a median progression-free survival (mPFS) of at least 4 weeks, 8 weeks, 12 weeks, 16 weeks, 20 weeks, 24 weeks, 28 weeks, 32 weeks, 36 weeks, 40 weeks, 44 weeks, 48 weeks, 52 weeks, 56 weeks, 60 weeks, 64 weeks, 68 weeks, 72 weeks, 76 weeks, 80 weeks, 84 weeks, 88 weeks, 92 weeks, 96 weeks, 100 weeks, or longer. In some embodiments, treatment with lasofoxifene in combination with abemaciclib provides the

patient a mPFS of at least 24 weeks. In some embodiments, the individual patient has one or more genes with an oncogenic mutation detected in the patient's ctDNA or cancer and the one or more genes is selected from HNF1A, TERT, GATA3, CDK12, MAPK3, TP53, PIK3CA, APC, ATM, MET, CCND1, EGFR, ROS1, STK11, FGFR1, ARID1A, CCNE1, AR, ALK, ERBB2, KIT, CDK4, SMAD4, NOTCH1, RB1, BRAF, RAF1, PTEN, AKT1, CDH1, BRCA1, MYC, CDKN2A, or PDGFRA.

[0067] In certain embodiments, treatment with lasofoxifene in combination with a CDK4/6i (e.g., palbociclib, ribociclib, abemaciclib) in patients having the at least one or more oncogenic mutations provides clinical benefit (CB; defined as stable disease $\geq$ 24 weeks, or confirmed partial or complete response) in at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% of the patient population. In some embodiments, treatment with lasofoxifene in combination with abemaciclib provides CB (defined as stable disease $\geq$ 24 weeks, or confirmed partial or complete response) to about 90%, about 95%, or about 100% of the patient population. In some embodiments, the individual patient has one or more genes with an oncogenic mutation detected in the patient's ctDNA or cancer and the one or more genes is selected from CCND1, FGFR1, CCNE1, AR, ALK, MAPK3, KIT, SMAD4, NOTCH1, RB1, BRAF, RAF1, PTEN, AKT1, CDH1, BRCA1, MYC, CDKN2A, or PDGFRA.

[0068] In some embodiments, the individual patient has one or more genes with an oncogenic mutation detected in the patient's ctDNA or cancer and the one or more genes are selected from TP53, PIK3CA, CCND1, ARID1A, FGFR1, CCNE1 and ERBB2, and combinations thereof. In some embodiments, one or more oncogenic mutations in each of the genes TP53, PIK3CA, CCND1, ARID1A, FGFR1, CCNE1 and ERBB2, is detected in the individual patient's ctDNA or cancer. In some embodiments, the oncogenic mutations are selected from single nucleotide variants (SNVs), insertions and deletions (indels), copy number variations (CNV) (focal, aneuploidy, amplification), fusions, and combinations thereof.

[0069] In some embodiments, the one or more genes with an oncogenic mutation have at least 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% prevalence in the patient population who respond positively to the treatment with lasofoxifene in combination with a CDK4/6i (e.g., palbociclib, ribociclib, abemaciclib) (e.g., achieve CB, defined as stable disease $\geq$ 24 weeks, or confirmed partial or complete response). In some embodiments, the one or more genes

with an oncogenic mutation have at least 17% prevalence in the patient population who respond positively (e.g., achieve CB) to the treatment with lasofoxifene in combination with a CDK4/6i (e.g., palbociclib, ribociclib, abemaciclib). In some embodiments, the individual patient has one or more genes with an oncogenic mutation detected in the patient's ctDNA or cancer and the one or more genes is selected from HNF1A, TERT, GATA3, CDK12, MAPK3, TP53, PIK3CA, APC, ATM, MET, CCND1, EGFR, ROS1, BRCA2, STK11, FGFR1, ARID1A, CCNE1, AR, ALK, ERBB2, KIT, BRAF, or CDK4.

[0070] In some embodiments, the one or more genes with an oncogenic mutation is detected in the patient's ctDNA or cancer and the one or more genes is selected from HNF1A, TERT, GATA3, CDK12, MAPK3, GNAS, RAF1, RHEB, or NTRK3. In some embodiments, the ctDNA or cancer does not have an oncogenic mutation in one or more genes selected from GNAS, RHEB, NTRK3, IDH2, or mTOR. In some embodiments, the ctDNA or cancer does not have an oncogenic mutation in IDH2 or mTOR.

[0071] In one aspect, disclosed herein are methods of reducing (or slowing) the progression of breast cancer in a patient by administering to the patient an effective amount of lasofoxifene or a pharmaceutically acceptable salt thereof and an effective amount of a CDK4/6 inhibitor (CDK4/6i), wherein the breast cancer: (i) is estrogen receptor positive (ER⁺); (ii) has at least one gain of function missense mutation within the ligand binding domain (LBD) of the Estrogen Receptor 1 (*ESR1*) gene; and (iii) has increased expression of one or more genes other than the *ESR1* gene as compared to the expression in non-cancerous breast cells in the subject or the expression level in a subject who does not have breast cancer. In certain embodiments, the patient's ER⁺ breast cancer is locally advanced. In certain embodiments, the patient's ER⁺ breast cancer is metastatic. In some embodiments, at least one of the one or more genes with an increased level of expression detected in the patient's ctDNA or cancer is selected from ABL1, AKT1, AKT2, ALK, APC, AR, ARID1A, ASXL1, ATM, AURKA, BAP, BAP1, BCL2L11, BCR, BRAF, BRCA1, BRCA2, CCND1, CCND2, CCND3, CCNE1, CDH1, CDK4, CDK6, CDK8, CDKN1A, CDKN1B, CDKN2A, CDKN2B, CEBPA, CTNNB1, DDR2, DNMT3A, E2F3, EGFR, EML4, EPHB2, ERBB2, ERBB3, ESR1, EWSR1, FBXW7, FGF4, FGFR1, FGFR2, FGFR3, FLT3, FRS2, HIF1A, HRAS, IDH1, IDH2, IGF1R, JAK2, KDM6A, KDR, KIF5B, KIT, KRAS, LRP1B, MAP2K1, MAP2K4, MCL1, MDM2, MDM4, MET, MGMT, MLL, MPL, MSH6, MTOR, MYC, NF1, NF2, NKX2-1, NOTCH1, NPM, NRAS, PDGFRA, PIK3CA, PIK3R1, PML,

PTEN, PTPRD, RARA, RB1, RET, RICTOR, ROS1, RPTOR, RUNX1, SMAD4, SMARCA4, SOX2, STK11, TET2, TP53, TSC1, TSC2, or VHL. In some embodiments, each of the one or more genes with an increased expression is selected from ABL1, AKT1, AKT2, ALK, APC, AR, ARID1A, ASXL1, ATM, AURKA, BAP, BAP1, BCL2L11, BCR, BRAF, BRCA1, BRCA2, CCND1, CCND2, CCND3, CCNE1, CDH1, CDK4, CDK6, CDK8, CDKN1A, CDKN1B, CDKN2A, CDKN2B, CEBPA, CTNNB1, DDR2, DNMT3A, E2F3, EGFR, EML4, EPHB2, ERBB2, ERBB3, ESR1, EWSR1, FBXW7, FGF4, FGFR1, FGFR2, FGFR3, FLT3, FRS2, HIF1A, HRAS, IDH1, IDH2, IGF1R, JAK2, KDM6A, KDR, KIF5B, KIT, KRAS, LRP1B, MAP2K1, MAP2K4, MCL1, MDM2, MDM4, MET, MGMT, MLL, MPL, MSH6, MTOR, MYC, NF1, NF2, NKX2-1, NOTCH1, NPM, NRAS, PDGFRA, PIK3CA, PIK3R1, PML, PTEN, PTPRD, RARA, RB1, RET, RICTOR, ROS1, RPTOR, RUNX1, SMAD4, SMARCA4, SOX2, STK11, TET2, TP53, TSC1, TSC2, or VHL. In some embodiments, the one or more genes with an increased expression is detected in the patient's ctDNA or cancer and the one or more genes is selected from AKT1, AKT2, BRAF, CDK4, CDK6, PIK3CA, PIK3R1, or mTOR.

[0072] In one aspect, disclosed herein are methods of monitoring a patient on a breast cancer treatment by (a) determining quantitative measures of the mutant allele frequency (MAF) of circulating tumor DNA (ctDNA) of at least one gain of function missense mutation within the ligand binding domain (LBD) of the Estrogen Receptor 1 (*ESR1*) gene (*ESR1* ctDNA) in a biological sample of the patient, wherein the quantitative measures are performed over a period of time at predetermined intervals; and (b) determining a positive predictive value (PPV) for clinical benefit (CB, defined as stable disease $\geq$ 24 weeks, or confirmed partial or complete response) wherein the PPV indicates responsiveness to the cancer treatment, wherein the cancer treatment comprises an effective amount of lasofoxifene or a pharmaceutically acceptable salt thereof and an effective amount of a CDK4/6 inhibitor (CDK4/6i). In some embodiments, the method further comprises determining that the patient has at least one gain of function missense mutation within the ligand binding domain (LBD) of the Estrogen Receptor 1 (*ESR1*) gene. In certain embodiments, the at least one gain of function missense mutation is selected from D538, Y537, L536, P535, V534, L469, S463, V392, and E380. In some embodiments, the gain of function mutation is D538G, Y537N, or Y537S. In some embodiments, the patient has decreased MAF post-treatment as determined at 4 weeks. In some embodiments, the patient has decreased MAF post-treatment as determined at 24 weeks.

[0073] In some embodiments, the patient is a postmenopausal woman. In some embodiments, the patient is a premenopausal woman. In some embodiments, the patient has osteoporosis or a higher risk of osteoporosis.

[0074] In various embodiments, the patient derives clinical benefit (CB, defined as stable disease $\geq$ 24 weeks, or confirmed partial or complete response) with stable disease for at least 24 weeks, 28 weeks, 32 weeks, 36 weeks, 40 weeks, 44 weeks, 48 weeks, 52 weeks, 56 weeks, 60 weeks, 64 weeks, 68 weeks, 72 weeks, 74 weeks, 78 weeks, 82 weeks, 86 weeks, 88 weeks, or longer in response to treatment with lasofoxifene in combination with a CDK4/6i (e.g., palbociclib, ribociclib, abemaciclib). In various embodiments, the patient derives an average longer duration of stable disease than on preceding second-line or third-line therapies by at least about 20%, 15%, or 10% in response to treatment with lasofoxifene in combination with a CDK4/6i (e.g., palbociclib, ribociclib, abemaciclib). In some embodiments, the patient has had prior treatment of one or more CDK4/6i (e.g., palbociclib, ribociclib, abemaciclib). In some embodiments, the breast cancer has progressed on one or more prior endocrine therapies selected from a selective ER degrader (SERD), a selective ER modulator (SERM), optionally a SERM other than lasofoxifene, an aromatase inhibitor (AI), a mTOR inhibitor, and/or a PI3K inhibitor. In some embodiments, the breast cancer has progressed on a prior treatment with fulvestrant. In some embodiments, the breast cancer has progressed on a prior treatment with sirolimus, temsirolimus, everolimus, or ridaforolimus. In some embodiments, the breast cancer has progressed on a prior treatment with everolimus. In some embodiments, the breast cancer has progressed on a prior treatment with an mTOR inhibitor and a CDK4/6i (e.g., palbociclib, ribociclib, abemaciclib). In some embodiments, the breast cancer has progressed on a prior treatment with everolimus and palbociclib.

5.1.4. Prior Therapy

5.1.4.1 Prior Endocrine Therapy

[0075] In various embodiments of the methods provided herein, the patient has previously been treated with one or more lines of endocrine therapy. In certain embodiments, the patient's cancer has relapsed or progressed after the previous therapy.

[0076] In some embodiments, the prior endocrine therapy is administration of a selective ER modulator (SERM) other than lasofoxifene. In various embodiments, the SERM is selected from tamoxifen, raloxifene, toremifene, ospemifene, broparestrol, bazedoxifene, and

ormeloxifene. In certain embodiments, the prior endocrine therapy is administration of tamoxifen.

[0077] In some embodiments, the prior endocrine therapy is administration of a selective ER degrader (SERD). In some embodiments, the selective ER degrader is selected from fulvestrant, elacestrant (RAD1901), ARN-810 (GDC-0810), giredestrant (GDC-9545), amcenestrant (SAR439859), rintodestrant (G1T48), LSZ102, imlunestrant (LY3484356), zN-c5, D-0502, SHR9549, camizestrant (AZD9833), and AZD9496. In certain embodiments, the prior endocrine therapy is administration of fulvestrant.

[0078] In some embodiments, the prior endocrine therapy is administration of an aromatase inhibitor (AI). In some embodiments, the aromatase inhibitor is selected from exemestane (Aromasin[®]), letrozole (Femara[®]), and anastrozole (Arimidex[®]).

[0079] In some embodiments, the prior endocrine therapy is ovarian suppression. In various embodiments, the ovarian suppression is achieved by oophorectomy or administration of a GnRH antagonist.

[0080] In some embodiments, the patient's cancer has relapsed or progressed after tamoxifen treatment. In some embodiments, the patient's cancer has relapsed or progressed after fulvestrant treatment. In some embodiments, the patient's cancer has relapsed or progressed after aromatase inhibitor (AI) treatment. In some embodiments, the patient's cancer has relapsed or progressed after AI treatment in combination with a CDK4/6i. In certain embodiments, the patient's cancer has relapsed or progressed after AI treatment in combination with palbociclib or ribociclib. In some of these embodiments, the patient's cancer has relapsed or progressed after multiple lines of endocrine therapy treatment.

5.1.4.2 Prior Endocrine Treatment for Metastatic Disease

[0081] In certain embodiments, the patient's ER⁺ breast cancer is metastatic and has progressed on a first (1L), second line (2L), or third line (3L) of treatment for metastatic disease. In some embodiments, the metastatic disease is local metastasis or metastasis to lymph nodes, or metastasis to visceral organs (*e.g.*, lung, pleural effusion, liver, ascites, CNS). In some embodiments, the patient's cancer has relapsed or progressed after one or more second line or third line (3L) cancer therapies, as illustrated in FIG. 4. In some embodiments, the patient's cancer has relapsed or progressed after treatment of metastatic

disease with at least one CDK 4/6 inhibitor and at least one of an endocrine therapy, a mammalian target of rapamycin (mTOR) inhibitor, a phosphatidylinositol-3-kinase (PI3K) inhibitor, a heat shock protein 90 (HSP90) inhibitor, a Poly (ADP-ribose) polymerase (PARP) inhibitor, an AKT inhibitor, or a histone deacetylase (HDAC) inhibitor. In some embodiments, the patient's metastatic breast cancer has relapsed or progressed after treatments of at least one of tamoxifen, fulvestrant, capecitabine, everolimus, alpelisib, talazoparib, palbociclib, ribociclib, or abemaciclib, alone or in combination.

[0082] In certain embodiments, progression has occurred on one or two of the following prior treatments for metastatic breast cancer: aromatase inhibitor (AI) and/or fulvestrant either as monotherapy or in combination with any commercially approved CDK 4/6 inhibitor (CDKi); and/or the combination of fulvestrant and alpelisib; and/or tamoxifen; and/or the combination of exemestane/everolimus. In some embodiments, the prior-administered CDK 4/6 inhibitor is abemaciclib, ribociclib, or palbociclib. In some embodiments, progression of metastatic cancer has occurred on at least a prior treatment of abemaciclib, palbociclib, or ribociclib. In some embodiments, progression of metastatic cancer has occurred on a prior abemaciclib treatment. In some embodiments, progression for metastatic cancer has occurred on a prior palbociclib treatment. In some embodiments, progression for metastatic cancer has occurred on a prior ribociclib treatment. In some embodiments, the cancer has metastasized to visceral organs.

[0083] In certain embodiments, the metastatic cancer has progressed while on a non-steroid aromatase inhibitor (AI); a SERD (e.g., fulvestrant); AI in combination with a CDK4/6 inhibitor; or a SERD (e.g., fulvestrant) in combination with a CDK4/6 inhibitor.

[0084] In certain embodiments, progression has occurred on a CDK 4/6 inhibitor either as monotherapy or combination therapy.

5.2. Pharmaceutical Compositions

[0085] Methods for treatment of estrogen receptor positive (ER⁺) breast cancer described herein comprise administering to the patient an effective amount of lasofoxifene or a pharmaceutically acceptable salt thereof and an effective amount of a CDK4/6 inhibitor (CDK4/6i).

[0086] In some embodiments, lasofoxifene is administered as lasofoxifene tartrate.

[0087] In some embodiments the CDK4/6i is selected from palbociclib, abemaciclib, and ribociclib. In certain embodiments, the CDK4/6i is abemaciclib.

[0088] The term “pharmaceutically acceptable salt” refers to non-toxic pharmaceutically acceptable salts. Other salts well known to those in the art may, however, be used. Representative organic or inorganic acids include, but are not limited to, hydrochloric, hydrobromic, hydriodic, perchloric, sulfuric, nitric, phosphoric, acetic, propionic, glycolic, lactic, succinic, maleic, fumaric, malic, tartaric, citric, benzoic, mandelic, methanesulfonic, hydroxyethanesulfonic, benzenesulfonic, oxalic, pamoic, 2-naphthalenesulfonic, p-toluenesulfonic, cyclohexanesulfamic, salicylic, saccharinic or trifluoroacetic acid. Representative organic or inorganic bases include, but are not limited to, basic or cationic salts such as benzathine, chloroprocaine, choline, diethanolamine, ethylenediamine, meglumine, procaine, aluminum, calcium, lithium, magnesium, potassium, sodium and zinc.

[0089] Embodiments also include prodrugs of the active compounds disclosed herein. In general, such prodrugs will be functional derivatives of the compounds which are readily convertible *in vivo* into the required compound. Thus, in the methods of treatment of the present invention, the term “administering” shall encompass the treatment of the various disorders described with the compound specifically disclosed or with a compound which may not be specifically disclosed, but which converts to the specified compound *in vivo* after administration to the subject. Conventional procedures for the selection and preparation of suitable prodrug derivatives are described, for example, in “*Design of Prodrugs*”, H. Bundgaard, Elsevier, 1985.

[0090] Some of the crystalline forms for the compounds exist as polymorphs and as such are intended to be included in the present invention. In addition, some of the solid forms of the active compounds exist as solvates e.g., with water (i.e., hydrates) or common organic solvents, and such solvates are encompassed by embodiments of the present invention.

[0091] Where the processes for the preparation of the active compounds administered in the presently provided methods give rise to mixtures of stereoisomers, in some embodiments, these isomers are separated by conventional techniques such as preparative chromatography. In some embodiments, the compounds are prepared in racemic form or as individual enantiomers or diastereomers by either stereospecific synthesis or by resolution. In some embodiments, the compounds are resolved into their component enantiomers or

diastereomers by standard techniques, such as the formation of stereoisomeric pairs by salt formation with an optically active base, followed by fractional crystallization and regeneration of the free acid. In some embodiments, the compounds are resolved by formation of stereoisomeric esters or amides, followed by chromatographic separation and removal of the chiral auxiliary. Alternatively, the compounds are resolved using a chiral HPLC column. It is to be understood that compositions comprising all stereoisomers, racemic mixtures, diastereomers, cis-trans isomers, and enantiomers thereof are encompassed by embodiments herein.

[0092] In certain embodiments, the active compounds are formulated in separate pharmaceutical compositions. In addition to the active compound, the pharmaceutical formulation or composition further comprises one or more of a pharmaceutically acceptable excipient, carrier, buffer, stabilizer or other material well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material depends on the route of administration, e.g. oral, intravenous, transdermal, vaginal topical, or vaginal ring.

[0093] In certain embodiments, pharmaceutical compositions for oral administration are in tablet, capsule, powder or liquid form. In certain embodiments, the tablet includes a solid carrier such as gelatin or an adjuvant. In certain embodiments, the liquid pharmaceutical composition comprises a liquid carrier such as water, petroleum, animal oil, vegetable oil, mineral oil or synthetic oil. Physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol can also be included.

[0094] For parenteral administration, the composition is in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilizers, buffers, antioxidants and/or other additives can be included, as required.

5.3. Treatment Regimens

[0095] In the methods described herein, the terms "treating", "treatment", and grammatical variations thereof are used in the broadest sense understood in the clinical arts. Accordingly,

the terms do not require cure or complete remission of disease, and encompass obtaining any clinically desired pharmacologic and/or physiologic effect. In certain embodiments, the effect is partial or complete response of the breast cancer, slowing or inhibiting the progression of the cancer; or causing regression of the cancer.

[0096] The term "effective amount" as used herein with respect to the combination therapy means the individual dosages of each of lasofoxifene and CDK4/6i that, in combination, produce the desired effect for which they are administered.

5.3.1. Combination administration

[0097] In some embodiments of the provided methods, lasofoxifene or pharmaceutically acceptable salt thereof and the CDK4/6i (e.g., abemaciclib, palbociclib, ribociclib) are administered as separate dosage forms. In certain of these embodiments, lasofoxifene or salt thereof and the CDK4/6i are separately administered at the same time (simultaneously). In some other embodiments, lasofoxifene or salt thereof and the CDK4/6i are administered as separate dosage forms at separate times (e.g., sequentially, or on unrelated schedules).

[0098] In certain embodiments, lasofoxifene is administered in a single dosage form comprising both lasofoxifene or salt thereof and a CDK4/6i. In certain embodiments, the CDK4/6i is abemaciclib.

5.3.1.1 Administration of Lasofoxifene

[0099] In various embodiments of the methods of treatment described above, lasofoxifene or pharmaceutically acceptable salt thereof is administered by oral administration.

[0100] In some embodiments, lasofoxifene or pharmaceutically acceptable salt thereof is administered to the patient by oral administration (*per os*; p.o.) at a lasofoxifene dosage of about 0.5 mg/day per os to about 10 mg/day per os, such as about 0.5 mg/day per os to about 5 mg/day per os, about 1 mg/day per os to about 5 mg/day per os, about 2 mg/day per os to about 5 mg/day per os, about 3 mg/day per os to about 5 mg/day per os, about 4 mg/day per os to about 5 mg/day per os, about 0.5 mg/day per os to about 4 mg/day per os, about 1 mg/day per os to about 4 mg/day per os, about 2 mg/day per os to about 4 mg/day per os, about 3 mg/day per os to about 4 mg/day per os, about 0.5 mg/day per os to about 3 mg/day per os, about 1 mg/day per os to about 3 mg/day per os, about 2 mg/day per os to about 3

mg/day per os, about 0.5 mg/day per os to about 2 mg/day per os, about 1 mg/day per os to about 2 mg/day per os, or about 0.5 mg/day per os to about 1 mg/day per os.

[0101] In some embodiments, lasofoxifene or pharmaceutically acceptable salt thereof is administered at about 0.5 mg lasofoxifene/day per os. In some embodiments, lasofoxifene or pharmaceutically acceptable salt thereof is administered at about 1 mg lasofoxifene/day per os. In some embodiments, lasofoxifene or pharmaceutically acceptable salt thereof at about 1.5 mg lasofoxifene/day per os, about 2 mg lasofoxifene/day per os, 2.5 mg lasofoxifene/day per os, about 3 mg lasofoxifene/day per os, about 3.5 mg lasofoxifene/day per os, about 4 mg lasofoxifene/day per os, about 4.5 mg lasofoxifene/day per os, about 5 mg lasofoxifene/day per os, about 6 mg lasofoxifene/day per os, about 7 mg lasofoxifene/day per os, about 8 mg lasofoxifene/day per os, about 9 mg lasofoxifene /day per os, or about 10 mg lasofoxifene /day per os. In some other embodiments, lasofoxifene or pharmaceutically acceptable salt thereof is administered at a dose of more than 10 mg lasofoxifene /day per os.

[0102] In some embodiments, lasofoxifene or pharmaceutically acceptable salt thereof is administered at 0.5 mg/day lasofoxifene to 10 mg/day. In some embodiments, lasofoxifene or pharmaceutically acceptable salt thereof is administered at 0.5 mg lasofoxifene/day, 1 mg lasofoxifene/day, 1.5 mg lasofoxifene/day, 2 mg lasofoxifene/day, 2.5 mg lasofoxifene/day, 3 mg lasofoxifene/day, 3.5 mg lasofoxifene/day, 4 mg lasofoxifene/day, 5 mg lasofoxifene/day, 5.5 mg lasofoxifene/day, 6 mg lasofoxifene/day, 6.5 mg lasofoxifene/day, 7 mg lasofoxifene/day, 7.5 mg lasofoxifene/day, 8 mg lasofoxifene/day, 8.5 mg lasofoxifene/day, 9 mg lasofoxifene/day, 9.5 mg lasofoxifene/day, or 10 mg lasofoxifene/day. In currently preferred embodiments, lasofoxifene or pharmaceutically acceptable salt thereof is administered orally at 5 mg lasofoxifene/day. In particular embodiments, lasofoxifene tartrate is administered orally at 5 mg lasofoxifene/day.

[0103] In certain embodiments, lasofoxifene is administered once every day. In certain embodiments, lasofoxifene is administered once every two days. In certain embodiments, lasofoxifene is administered once every three days. In certain embodiments, lasofoxifene is administered once every four days. In certain embodiments, lasofoxifene is administered once every five days. In certain embodiments, lasofoxifene is administered once every six days. In certain embodiments, lasofoxifene is administered once every week. In certain embodiments, lasofoxifene is administered once every two weeks. In certain embodiments,

lasofoxifene is administered once every three weeks. In certain embodiments, lasofoxifene is administered once every month.

[0104] In some embodiments, lasofoxifene is administered to the patient by vaginal ring administration. In some of these embodiments, lasofoxifene is administered once every two weeks. In some of these embodiments, lasofoxifene is administered once every three weeks. In some of these embodiments, lasofoxifene is administered once every month. In some of these embodiments, lasofoxifene is administered once every two months. In some of these embodiments, lasofoxifene is administered once every three months. In some of these embodiments, lasofoxifene is administered once every four months.

[0105] In some embodiments, lasofoxifene is administered to the ER⁺ breast cancer patient until the patient's cancer progresses on therapy, is in full remission, or until the side effects are intolerable.

5.3.1.2 Administration of Abemaciclib

[0106] In each of the methods of treatment described above, the patient is administered lasofoxifene (e.g., lasofoxifene tartrate) in combination with a CDK4/6 inhibitor. In typical embodiments, the CDK4/6 inhibitor is selected from palbociclib, ribociclib, and abemaciclib. In currently preferred embodiments, the patient is administered lasofoxifene tartrate orally at 5 mg lasofoxifene/day and abemaciclib at 50 mg to 200 mg BID, 100 mg to 200 mg BID, or 150 mg BID.

[0107] In various embodiments, abemaciclib is administered orally.

[0108] In some embodiments, abemaciclib is administered at about 25 mg/day per os to about 600 mg/day per os, such as about 50 mg/day per os to about 200 mg/day per os, e.g., about 25 mg/day per os, about 50 mg/day per os, about 100 mg/day per os, about 150 mg/day per os, about 200 mg/day per os, about 250 mg/day per os, about 300 mg/day per os, about 350 mg/day per os, about 400 mg/day per os, about 450 mg/day per os, or about 600 mg/day per os.

[0109] In some embodiments, abemaciclib is administered once every day. In certain embodiments, abemaciclib is administered twice a day.

[0110] In certain embodiments, abemaciclib is administered twice daily (BID). In typical embodiments, the daily dose is administered in two equally divided doses. In some embodiments, abemaciclib 300 mg/day per os is administered as two separate 150mg doses orally (e.g., one 150 mg tablet in the morning and one 150 mg tablet in the evening).

[0111] In some embodiments, abemaciclib is administered in a starting dose that is subsequently adjusted downward according to a first dose reduction schedule. In certain embodiments, the abemaciclib dose is subsequently reduced according to a second dose reduction schedule. In certain embodiments, the abemaciclib dose is subsequently reduced according to a third dose reduction schedule. In certain of these embodiments, each dose reduction is by 50 mg per dose. For example, a 300 mg/day dose (150 mg per os administered twice daily) is reduced according to a first dose reduction such that 200 mg/day dose is administered twice daily (100 mg per os administered twice daily).

5.4. Clinical Endpoints

[0112] In various embodiments, the methods comprise administering the combination of lasofoxifene and a CDK4/6i, wherein the combination is effective to treat ER⁺ breast cancer having at least one ESR1 mutation. In certain embodiments, the method is effective to reduce progression of ER⁺ breast cancer having at least one ESR1 mutation.

[0113] In certain embodiments, the combination is effective to increase the disease-free survival of the ER⁺ breast cancer patient. In certain embodiments, the combination is effective to reduce recurrence of ER⁺ breast cancer. In certain embodiments, the combination is effective to increase time until recurrence of ER⁺ breast cancer. In certain embodiments, the combination is effective to reduce metastasis of ER⁺ breast cancer. In certain embodiments, the combination is effective to increase duration of progression-free survival of the ER⁺ breast cancer patient. In certain embodiments, the comparison to determine efficacy is made against the standard of care.

[0114] In some embodiments, the method provided herein increases disease-free survival, reduces recurrence, increases time to recurrence, reduces metastasis, and/or increases duration of progression-free survival in patients with ER⁺ locally advanced or metastatic breast cancer that has one or more of the ESR1 mutations discussed herein. In some embodiments, the method reduces the selective pressure and prevents the expansion of the

endocrine resistant clones in ER⁺ locally advanced or metastatic breast cancer during treatment.

[0115] In some embodiments, lasofoxifene or salt thereof and abemaciclib are administered to the patient until the patient's cancer is in full remission, progresses on therapy, or until the side effects are intolerable.

[0116] In some embodiments, lasofoxifene or pharmaceutically acceptable salt thereof and a CDK 4/6 inhibitor (*e.g.*, abemaciclib, ribociclib, palbociclib) are administered at doses and for a time effective to provide clinical benefit (CB, defined as stable disease $\geq$ 24 weeks, or confirmed partial or complete response) in patients whose cancer has relapsed or progressed after one or more 2L or 3L cancer therapies, as illustrated in FIGS. 1 and 3 and in Examples 2 and 3. In some embodiments, lasofoxifene and a CDK 4/6 inhibitor (*e.g.*, abemaciclib, ribociclib, palbociclib) are administered at doses and for a time effective to provide greater duration of response than any preceding 2L or 3L therapies. In some embodiments, lasofoxifene and a CDK 4/6 inhibitor (*e.g.*, lasofoxifene and abemaciclib) confers CB with stable disease for at least 24 weeks, at least 28 weeks, at least 32 weeks, at least 36 weeks, at least 40 weeks, at least 44 weeks, at least 48 weeks, at least 52 weeks, at least 56 weeks, at least 60 weeks, at least 64 weeks, at least 68 weeks, at least 72 weeks, at least 76 weeks, at least 80 weeks, at least 84 weeks, at least 88 weeks, or longer. In some embodiments, lasofoxifene plus abemaciclib treatment confers stable disease for at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11 months, at least 12 months, or longer. In some embodiments, lasofoxifene and a CDK 4/6 inhibitor (*e.g.*, lasofoxifene/abemaciclib) treatment confers CB (defined as stable disease $\geq$ 24 weeks, or confirmed partial or complete response) for at least 1 year, at least 2 years, at least 3 years, at least 4 years, at least 5 years, at least 6 years, at least 7 years, at least 8 years, at least 9 years, at least 10 years, at least 11 years, at least 12 years, or longer. In some embodiments, lasofoxifene and CDK 4/6 inhibitor (*e.g.*, lasofoxifene/abemaciclib) treatment confers CB with complete or partial response where the patient's tumor is significantly reduced as illustrated in FIG. 2.

6. EXAMPLES

6.1. Example 1: Phase 2 Clinical Study (ELAINE 2)

[0117] A phase 2 clinical study, NCT04432454 (ELAINE 2), is currently underway to evaluate the efficacy, safety and tolerability of the combination of lasofoxifene and abemaciclib for the treatment of postmenopausal women and certain premenopausal women with locally advanced or metastatic estrogen receptor positive (ER⁺) / human epidermal growth factor 2 negative (HER2⁻) (ER⁺/HER2⁻) breast cancer having an *ESR1* mutation. As discussed in Section 5.1.1 below, the study has enrolled patients whose tumors had progressed on prior CDK4/6 inhibitor treatment.

6.1.1. Summary of Experimental Observations

[0118] ELAINE 2 is an open-label, phase 2, multicenter trial evaluating the safety and efficacy of LAS combined with the CDK4/6i, abemaciclib (Abema). Study participants were pre- and postmenopausal women with ER⁺/HER2⁻ mBC (metastatic breast cancer) with acquired *ESR1* mutation (identified by ctDNA testing), whose disease had progressed on one or two lines of hormonal therapy for metastatic disease with or without a CDK4/6i (including Abema). Patients took oral lasofoxifene (LAS) 5 mg/day and abemaciclib 150 mg BID. Treatment continued until evidence of disease progression, death, unacceptable toxicity, or withdrawal from the study. The primary endpoint was safety, and secondary endpoints were progression free survival (PFS), objective response rate (ORR), and clinical benefit rate (CBR).

[0119] Twenty-nine (29) patients were enrolled at 16 US sites (Oct 2020 to June 2021). Mean age was 58.3 y (35-79 y); 86% were Caucasian. Most had progressed with at least 2 previous hormonal treatments (80%). All except 1 patient had received a prior CDK4/6i and 72% had received prior fulvestrant (FVT); 48% had had chemotherapy in the metastatic setting. Four patients discontinued the trial due to adverse events (AEs, n=2), consent withdrawal (n=1), or investigator withdrawal (n=1). No deaths occurred during the study and few Grade 3/4 AEs were observed. Most common AEs were diarrhea, nausea, and leukopenia. Five patients had an abemaciclib dose reduction from 150 mg to 100 mg BID. As of the first interim date, 11 patients have progressed and 14 continue treatment. The censored median PFS was 13.9 mos (95% CI, 8.0–NE), the ORR 33.3% (95% CI, 16.3–56.3) with 6 confirmed partial responses, and the CBR 62.1% (95% CI, 44.0–77.3).

[0120] As shown in FIGS. 1-4, patients derived clinical benefit (CB, defined as stable disease ≥ 24 weeks, or confirmed partial or complete response) from lasofoxifene in combination with abemaciclib even after progression on prior cyclin-dependent kinase 4/6 inhibitor (CDK4/6i) treatment, including prior treatment with ribociclib, palbociclib, and/or abemaciclib.

[0121] Conclusion: lasofoxifene combined with abemaciclib was well tolerated and demonstrated robust and meaningful efficacy in women with ER⁺/HER2⁻ metastatic breast cancer (mBC) and an *ESR1* mutation who had progressed on previous CDK4/6i therapies.

6.1.2. Study Design

[0122] This is an open-label, multicenter, study evaluating the efficacy, safety and tolerability of the combination of lasofoxifene and abemaciclib for the treatment of pre- and postmenopausal women who have locally advanced or metastatic ER⁺/HER2⁻ breast cancer with an *ESR1* mutation and disease progression on first, second or third lines of hormonal treatment for metastatic disease. To be eligible for enrollment, progression may have occurred on no more than three of the following treatments for metastatic breast cancer: an AI and/or fulvestrant, either as monotherapy or in combination with any commercially available CDK4/6i; and/or the combination of fulvestrant and alpelisib; and/or tamoxifen; and/or the combination of exemestane/everolimus.

[0123] On the day of enrollment (Day 1), subjects received 5 mg of oral lasofoxifene once a day and 150 mg of abemaciclib twice a day. Study medication is continued until documented breast cancer progression or withdrawal from the study for any reason.

[0124] Treatment continues until radiographic or clinical evidence of disease progression, death, unacceptable toxicity, or until withdrawal from the study for any reason. Enrolled subjects are seen every 2 weeks for the first two months of treatment and then monthly until progression. Efficacy assessments are done every 8 weeks. Safety assessments are done at weeks 2, 4, 6, and 8 after enrollment and then every month until disease progression.

6.1.3. Drug Schedules

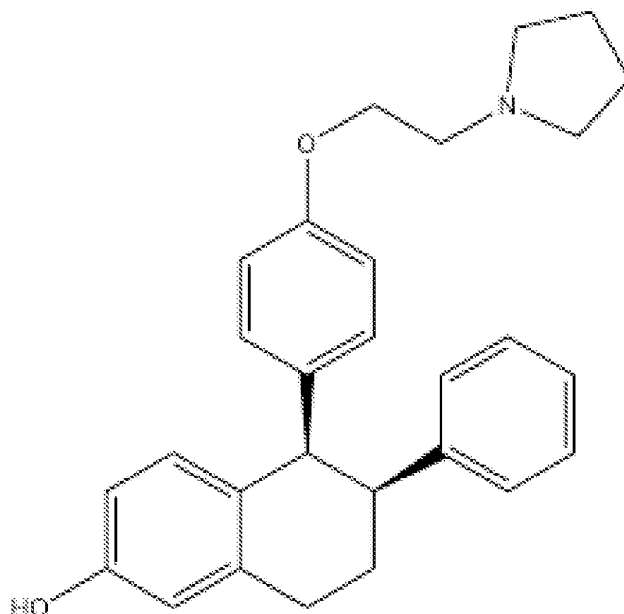
[0125] Subjects receive oral lasofoxifene 5 mg once a day (one tablet) and oral abemaciclib 150 mg (3 tablets) twice daily with or without food. Study medication will continue to be

given until documented breast cancer progression or withdrawal from the study for any reason.

[0126] Lasofloxifene

[0127] The active pharmaceutical ingredient is lasofloxifene. The chemical name is 6S-Phenyl-5R-[4-(2- pyrrolidin-1-yl-ethoxy)-phenyl]-5,6,7,8-tetrahydro-naphthalen-ol,2S,3S-Dihydroxy-succinic acid.

Chemical structure:



Molecular formula:
Molecular weight:
Physical appearance:

$C_{28}H_{31}NO_2 \cdot C_4H_6O_6$
563.64 Daltons
white to off-white solid

Lasofloxifene is provided as a white to off-white solid 5 mg tablet using the D-(-)-tartrate salt.

[0128] Abemaciclib

[0129] Abemaciclib is a kinase inhibitor and the chemical name is 2-Pyrimidinamine, N-[5-[(4-ethyl-1- piperazinyl)methyl]-2-pyridinyl]-5-fluoro-4-[4-fluoro-2-methyl-1-(1-methylethyl)-1H- benzimidazol-6-yl].

Molecular formula: $C_{27}H_{32}F_2N_8$
Molecular weight: 506.59 Daltons
Physical appearance: white to yellow powder

6.1.3.1 Dose Modifications for Treatment Toxicity

[0130] Lasofoxifene

[0131] There are no dose reductions for any toxicity associated with lasofoxifene. If a lasofoxifene related Grade 3 or 4 AE event occurs, hold therapy until the toxicity resolves to <Grade 2 or baseline and resume lasofoxifene at the assigned dose. Should a patient need to be NPO, hold lasofoxifene and abemaciclib until the subject can take fluids by mouth. If a subject cannot tolerate lasofoxifene or has not taken lasofoxifene for 3 consecutive weeks, the subject should be withdrawn from the study.

[0132] Physical appearance: white to yellow powder

[0133] Abemaciclib

[0134] Two abemaciclib dose modifications for adverse reactions are permitted:

- Starting dose – 150 mg twice a day (3 tablets twice a day)
- First dose reduction – 100 mg twice a day (2 tablets twice a day)
- Second dose reduction – 50 mg twice a day (1 tablet twice a day)
- Third dose reduction – not allowed

If subjects are unable to tolerate 50 mg of abemaciclib twice daily, this treatment should be stopped; however, lasofoxifene can be continued until disease progression is documented. However, if lasofoxifene is discontinued, abemaciclib cannot be continued as monotherapy and the subject needs to be withdrawn from the study. Dose modifications for treatment toxicity are summarize in Table 1 below.

Table 1	
Criteria for abemaciclib dose reductions	
CTCAE Grade	Hematological
Grade 1 & 2	No dose modification is required
Grade 3	Suspend dose until toxicity resolves to ≤Grade 2; Dose reduction is not required
Grade 3 recurrent, or Grade 4	Suspend dose until toxicity resolves to ≤Grade 2; Resume dosing at the next lower dose
CTCAE Grade	Diarrhea
Grade 1	No dose modification is required
Grade 2	If toxicity does not resolve within 24 hours to ≤Grade 1, suspend dose until toxicity resolves. No dose reduction Suspend dose until toxicity resolves to ≤Grade 1 Resume at next lower dose

Table 1	
Criteria for abemaciclib dose reductions	
Grade 2 that persists or recurs after resuming the same dose despite maximal supportive measures Grade 3 or 4 or requires hospitalization	Suspend dose until toxicity resolves to $\leq$ Grade 1 Resume at next lower dose
CTCAE Grade for ALT and AST	Hepatotoxicity
Grade 1 ($>ULN-3.0 \times ULN$) Grade 2 ($>3.0-5.0 \times ULN$), WITHOUT increase in total bilirubin above $2 \times ULN$	No dose modification is required
Persistent or Recurrent Grade 2 or Grade 3 ($>5.0-20.0 \times ULN$), WITHOUT increase in total bilirubin above $2 \times ULN$	Suspend dose until toxicity resolves to baseline or Grade 1, Resume at next lower dose .
Elevation in AST and/or ALT $>3 \times ULN$ WITH total bilirubin $>2 \times ULN$, in the absence of cholestasis	Discontinue abemaciclib
Grade 4 ($>20.0 \times ULN$)	Discontinue abemaciclib
CTCAE Grade	Interstitial lung disease/pneumonitis
Grade 1 or 2	No dose modification is required
Persistent or recurrent Grade 2 toxicity that does not resolve with maximal supportive measures within 7 days to baseline or Grade 1	Suspend dose until toxicity resolves to $\leq$ Grade 1 Resume at next lower dose
Grade 3 or 4	Discontinue abemaciclib
CTCAE Grade	Other Toxicities
Grade 1 or 2	No dose modification is required
Persistent or recurrent Grade 2 toxicity that does not resolve with maximal supportive measures within 7 days to baseline or Grade 1	Suspend dose until toxicity resolves to baseline or $\leq$ Grade 1. Resume at next lower dose
Grade 3 or 4	Suspend dose until toxicity resolves to baseline or $\leq$ Grade 1. Resume at next lower dose

6.1.4. Primary and Secondary Endpoints

[0135] The primary endpoint is evaluation of the safety and tolerability of the combination of lasofoxifene and abemaciclib for the treatment of postmenopausal women with locally advanced or metastatic ER⁺/HER2⁻ breast cancer and have an *ESR1* mutation based on the incidence of adverse events, severity of the adverse events and mortality due to adverse events. The pharmacokinetic endpoint is to determine if there are any drug-drug interactions between lasofoxifene and abemaciclib as compared with steady-state drug concentrations obtained in previous clinical trials.

[0136] The secondary endpoints include: progression free survival (PFS); clinical benefit (CB, defined as stable disease ≥ 24 weeks, or confirmed partial or complete response) rate

(CBR), duration of response, objective response rate (ORR), quality of life (QoL), and time to response. For subjects with measurable disease at baseline, progression is determined according to the RECIST criteria.

6.1.5. Inclusion and Exclusion Criteria

[0137] The inclusion criteria include:

1. Pre- or postmenopausal.

Postmenopausal women are

defined as:

- a. ≥ 60 years of age with no vaginal bleeding over the prior year, or
- b. < 60 years with "premature menopause" or "premature ovarian failure" manifest itself with secondary amenorrhea for at least 1 year and follicle stimulating hormone (FSH) and estradiol levels in the postmenopausal range according to institutional standards, or
- c. surgical menopause with bilateral oophorectomy.

Note: Premenopausal women who meet all of the other entry criteria must be maintained on ovarian suppression (such as Lupron) during the study and subjects counseled to use appropriate contraception to prevent pregnancy.

2. If possible, a biopsy of metastatic breast cancer tissue will be obtained to provide histological or cytological confirmation of ER+ and HER2– disease as assessed by a local laboratory, according to American Society of Clinical Oncology/College of American Pathologists guidelines, using slides, paraffin blocks, or paraffin samples. If a biopsy is not possible, the ER and HER2 status from the tissue obtained at the time of the original diagnosis must confirm that the subject is ER+ and HER2–.
3. Locally advanced or metastatic breast cancer with radiological or clinical evidence of progression on first and/or second line of hormonal therapy for metastatic disease. Progression may have occurred on no more than 2 of the following endocrine treatments for metastatic breast cancer: an aromatase inhibitor (AI) and/or fulvestrant either as monotherapy or in combination with any commercially approved CDK4/6i; and/or the combination of fulvestrant and alpelisib; and/or tamoxifen; and/or the combination of exemestane/everolimus. (Note: Before starting study treatment, subjects should have stopped any CDK4/6i for at least 21 days)
4. Subjects must have had no evidence of progression for at least 6 months during their first hormonal treatment for advanced breast cancer.
5. At least one or more of the following ESR1 point mutations as assessed in cell-free circulating tumor DNA (ctDNA) obtained from a blood or tissue sample: Y537S, Y537C, D538G, E380Q, S463P, V534E, P535H, L536H, L536P, L536R, L536Q, or Y537N. Note: the Sponsor's blood ctDNA assay must be used but

tissue sequencing (if done) may be done by a validated commercial assay.

Note: A positive ESR1 mutation in tissue or ctDNA using a validated commercial assay if done prior or at the time of disease progression is acceptable to meet this entry criteria. However, blood for ctDNA must still be obtained for genomic analyses using the sponsor's ctDNA assay.

6. Locally advanced or metastatic breast cancer with either measurable (according to RECIST 1.1 [Eisenhauer et al. New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). European Journal of Cancer. 2009; 45:228-47]) or non-measurable lesions.
7. Subjects who may have received one cytotoxic chemotherapy regime for metastatic disease, as well as those who received one cytotoxic chemotherapy regimen in the neo-adjuvant or adjuvant setting prior to entry into the trial, can be enrolled but must be free of all chemotherapy acute toxicity, excluding alopecia and Grade 2 peripheral neuropathy, before study entry. A washout period of at least 21 days is required between last chemotherapy dose and entry into the study.
8. Stable breast cancer metastasis to the brain is allowed as long as the subject has received radiotherapy and not demonstrated any evidence of brain metastasis progression for at least 3 months after the completion of radiotherapy.
9. ECOG performance score of 0 or 1.
10. Adequate organ function as shown by:
 - a. absolute neutrophil count (ANC) $\geq 1,500$ cells/mm³
 - b. platelet count $\geq 100,000$ cells/mm³
 - c. hemoglobin ≥ 8.0 g/dl
 - d. ALT and AST levels ≤ 3 upper limit of normal (ULN) or ≤ 5 in the presence of liver metastasis
 - e. total serum bilirubin $\leq 0.5 \times \text{ULN}$ ($\leq 3.0 \times \text{ULN}$ for subjects known to have Gilbert Syndrome)
 - f. alkaline phosphatase level $\leq 3 \times \text{ULN}$
 - g. creatinine clearance of 40 ml/min or greater as calculated by the Cockcroft-Gault formula
 - h. international normalized ratio (INR) and activated partial thromboplastin time (aPTT) $< 2.0 \times \text{ULN}$
11. Able to swallow tablets.
12. Able to understand and voluntarily sign a written informed consent before any screening procedures.

[0138] Exclusion criteria. Subjects who meet any of the following criteria are excluded from entering the trial.

1. Lymphangitic carcinomatosis involving the lung.
2. Visceral crisis in need of cytotoxic chemotherapy as assessed by the investigator.
3. Radiotherapy within 30 days prior to entry into the trial except in case of localized radiotherapy for analgesic purposes or for lytic lesions at risk of fracture, which can then be completed within 7 days prior to entry into the trial. Subjects must have recovered from radiotherapy toxicities prior entry.
4. Subjects with known inactivating RB1 mutations or deletions (Screening for RB1 mutation is not required for entry).
5. History of long QTC syndrome or a QTC of >480 msec.
6. History of a pulmonary embolus (PE) or deep vein thrombosis (DVT) within the last 6 months or any known thrombophilia. Subjects stable on anti-coagulants for maintenance are eligible as long as the DVT and/or PE occurred >6 months prior to enrollment and there is no evidence for active thrombosis. The use of low-dose ASA is permitted.
7. Subjects on concomitant strong CYP3A4 inhibitors such as clarithromycin, telithromycin, nefazodone, itraconazole, ketoconazole, atazanavir, darunavir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir, tipranavir.
8. Subjects on strong and moderate CYP3A4 inducers such as amprenavir, barbituates, carbamazepine, clotrimazole, dexamethasone, efavirenz, ethosuximide, griseofulvin, modafinil, nevirapine, oxcarbazepine, phenobarbital, phenytoin, chronic prednisone treatment, primidone, rifabutin, rifampin, rifapentine, ritonavir, topiramate.
9. Any significant co-morbidity that would impact the study or the subject's safety. Since CDK4/6i have reported the occurrence of interstitial lung disease (ILD), subjects who have a history of ILD or have severe dyspnea at rest or require oxygen therapy should not enter the study.
10. Subject has an active systemic bacterial or fungal infection (requiring intravenous [IV] antibiotics at the time of initiating treatment).
11. History of a positive human immunodeficiency virus (HIV) or hepatitis B virus (HBV) test [Screening is not required for enrollment].
12. Subjects with hepatitis C virus (HCV) at Screening who still have a viral load. Subjects previously treated and achieved a HCV cure (no viral load) can be entered into the study.
13. History of malignancy within the past 5 years (excluding breast cancer), except basal cell or squamous cell carcinoma of the skin curatively treated by surgery, or early-stage cervical cancer.
14. A positive pregnancy test (only if premenopausal).
15. History of non-compliance to medical regimens.
16. Unwilling or unable to comply with the protocol.
17. Current participation in any clinical research trial involving an investigational drug or device within the last 30 days.

6.1.6. Efficacy Analysis

[0139] A Kaplan-Meier curve is presented for PFS with an estimated median PFS. The clinical benefit rate (CBR), defined as the percentage of subjects with a complete or partial response or stable disease for ≥ 24 weeks, is presented with a 95% confidence interval. The ORR, defined as the percentage of subjects with a complete or partial response, is similarly summarized. Time to response and duration of response (DoR) is presented for each individual responder.

6.1.7. Safety Analysis

[0140] For the Safety Population, descriptive summaries of AEs, clinical laboratory data, vital signs, and ECGs are presented.

[0141] Verbatim descriptions of AEs reported during the study period are mapped to the appropriate system organ class and preferred term using the Medical Dictionary for Regulatory Activities (MedDRA). All reported AEs are tabulated and graded according to the CTCAE version 5.0. AEs are summarized by worst grade per subject and by grade. All treatment-emergent adverse events (TEAEs) (i.e., occurring during or after the first dose of study drug) are summarized in frequency tables. Treatment-emergent serious AEs and TEAEs that resulted in early study discontinuation are listed and summarized in frequency tables. The numbers and proportions of subjects who discontinue either or both study treatments early due to AEs are reported with 95% confidence intervals. Subjects experiencing key adverse events of interest are similarly summarized. With 24 evaluable subjects, the upper half width of the Wilson score 95% confidence interval is estimated to within 20%. An adverse event with a 7% incidence rate has a 0.82 chance of being seen at least once.

[0142] All AEs that led to death are listed by subject with narratives.

[0143] Clinical laboratory test results are summarized using descriptive statistics for absolute values and change-from-baseline values summarized as cumulative shift tables.

6.1.8. Analysis of Pharmacokinetic Variables

[0144] Pharmacokinetic sampling for lasofoxifene and abemaciclib concentrations are done pre-dose at every visit starting at Visit 0 (Day 1) through Final/ET visit. Pharmacokinetic concentrations of lasofoxifene, abemaciclib and 3 abemaciclib metabolites

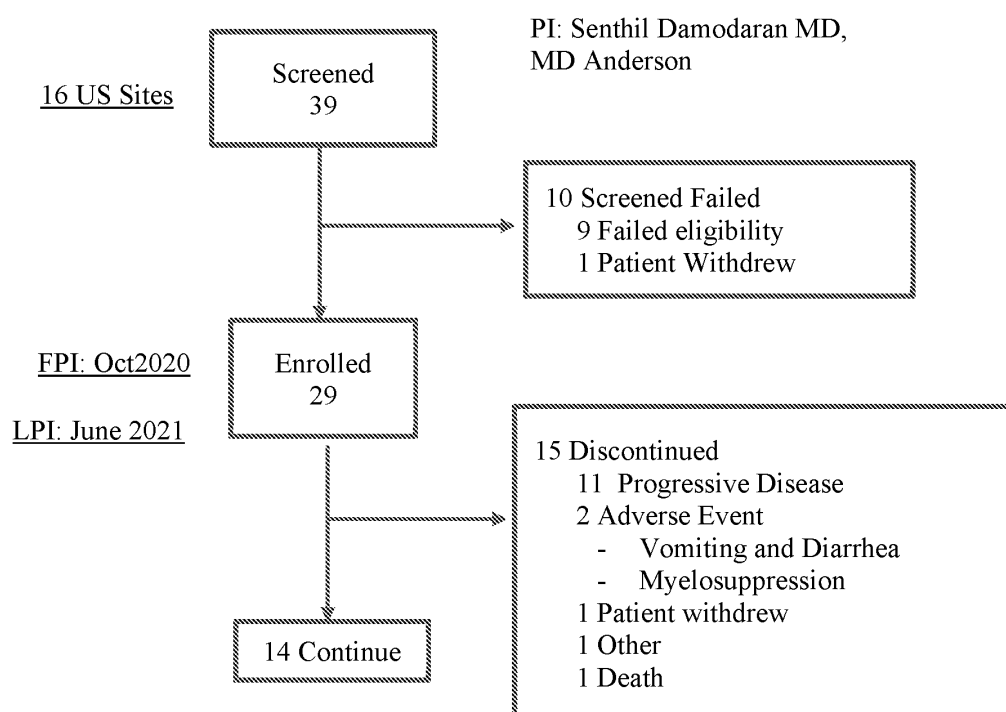
(LSN2839567, LSN3106726, and LSN3106729) are summarized and mean, median, SD, and range at each time are presented and compared with previous PK obtained results.

6.2. Example 2: First Interim Results of ELAINE 2 Clinical Trial

[0145] Twenty-nine (29) patients total were enrolled. Patient disposition is summarized in Table 2. Patient demography and baseline characteristics are summarized in Table 3, and patient prior cancer therapies are summarized in Table 4.

6.2.1. Patient Disposition

Table 2



6.2.2. Demography and Baseline Characteristics n = 29

Table 3		
N = 29		
Age (yrs)	Mean	58.3
	Median	60
	Range	35-79
Menopause	Pre n (%)	4 (13.8%)

Table 3		
	Post n (%)	25 (86.2%)
Ethnicity	Non-Hispanic	26 (89.7%)
	Hispanic	3 (10.3%)
Race	White	25 (86.2%)
	Black	2 (6.9%)
	Not Reported	2 (6.9%)
Measurable Disease		18 (62.1%)
Visceral Disease		16 (55.2%)
Bone Only		10 (34.5%)

6.2.3. Prior Breast Cancer Therapy

Table 4	
	N = 29
Prior breast cancer therapy	
Chemotherapy (total)	25 (86.2)
Chemotherapy in metastatic setting	14 (48.3)
CDK4/6i	28 (96.6)
Palbociclib	25 (86.2)
Abemaciclib	4 (13.8)
Ribociclib	2 (6.9)
Unknown	1 (3.4)
Endocrine therapy	29 (100)
Aromatase inhibitor	28 (96.6)
Fulvestrant	23 (79.3)
Tamoxifen	12 (41.4)
Everolimus	4 (13.8)
Alpelisib	3 (10.3)

* Data expressed as n (%), unless stated otherwise. CDK4/6i, cyclin-dependent kinase 4/6 inhibitor. 26 (89.7%) patients had prior radiotherapy.

6.2.4. ELAINE 2 swimmer plot

[0146] Patient response to lasofoxifene and CDK 4/6 inhibitor therapy as of a first interim date is summarized in FIG. 1.

[0147] Referring to FIG. 1, as of the first interim date, about 68.9% (20/29) of subjects receiving lasofoxifene and abemaciclib (laso/abema) treatment had clinical benefit (CB) with stable disease and showed complete or partial response for at least 24 weeks (vertical dotted line) post treatment. Among these patients, about 65% (13/20) had continued response (arrow) as of the first interim date. Patients 29, 21, 16, 6 (patients marked with an asterisk in FIG. 1) had had progression on abemaciclib prior to enrollment. Notably, 75% (3/4) of these

patients had CB and stable disease to 40 weeks (patient 16), 48 weeks (patient 21), and 68 weeks (patient 29). Patients 16 and 6 progressed and withdrew at 40 weeks and 8 weeks, respectively.

[0148] Approximately 48% (14/29) of patients had progressed on prior, pre-enrollment, fulvestrant treatment (square). These patients generally responded well to laso/abema treatment. Approximately 64% (9/14) achieved CB and had complete or partial response to laso/abema treatment for up to 62 weeks as of the first interim date. For example, Patients 28, 27, 24, 19, and 14 had stable disease and continued response to laso/abema (arrow) to 62 weeks, 58 weeks, 54 weeks, 50 weeks, 42 weeks, and 38 weeks, respectively. Patients 15 and 13 had stable disease and progressed at 36 weeks and 32 weeks, respectively.

[0149] All patients had at least one missense mutation in *ESR1* at enrollment. Approximately 69% (20/29) of patients had a Y537S mutation, and approximately 65% (13/20) of these patients achieved CB, and had complete or partial response and stable disease to 62 weeks. Patients 28, 27, 25, 24, 23, 21, 20, 19, 18, 17, 11, and 10 had stable disease and continued response to 62 weeks, 58 weeks, 54 weeks, 48 weeks, 48 weeks, 48 weeks, 48 weeks, 40 weeks, 40 weeks, 40 weeks, 32 weeks, and 32 weeks, respectively. Patients 15 and 13 had stable disease and progressed at 32 weeks.

[0150] Notably, 75% (12/16) of patients with visceral metastasis benefitted from laso/abema treatment and achieved CB, even if their cancers had metastasized to visceral organs prior to enrollment.

6.2.5. Progression Free Survival

[0151] Abemaciclib/Laso combination. Table 5 summarizes patient progression free survival (PFS).

Table 5		
		PFS
# of Progression Events	Progression	11
	Death	0
Kaplan Meier PFS	Median	56.0 weeks (13 mos)
	95% CI	31.9-NE

6.2.6. Tumor Response Waterfall Plot

[0152] Preliminary data of maximum tumor response evaluated as of the first interim date is illustrated in FIG. 2. Patients having complete or partial response to laso/abema treatment had maximum % change in the sum of dimensions of target lesion of up to 80%. Nine (9/18) patients with measurable lesions had a partial response yielding an objective response rate (ORR) of 50% (95% confidence, 29.0-71.0). Table 6 summarizes patient ORR (ORR=50%), and Table 7 summarizes patient DOR and TTR.

6.2.7. ORR, DOR and TTR

[0153] Table 6 summarizes patient ORR (ORR=50%), and Table 7 summarizes patient DOR.

Table 6		
	N = 18 ¹	95% CI
CR	0 (0%)	
PR	9 (50%)	12.5-50.9

Table 7				
	Mean $\pm$ SD	Median	Min-Max	No Censored
DOR ² (Days)	134.8 $\pm$ 49.97	164	57-173	2
TTR ³ (Days)	92.8 $\pm$ 53.60	69	54-176	0

¹ Number of Subjects with measurable target lesions

² Event or censoring date – first PR date

³ First PR date – date of randomization + 1

6.2.8. Clinical Benefit Rate (CBR) N = 29

[0154] Table 8 summarizes patient clinical benefit rate (CBR).

Table 8	
	Data as of first interim date
CR (n)	0
PR (n)	9
$\geq$ 24 wks (n)	11*
CBR (%)	69%

*Number with SD excluding those with PR

6.2.9. Number of Subjects with Most Common Adverse Events (AEs)

[0155] Subjects with maximum Grade counts are summarized in Table 9.

Table 9								
Adverse Events by Grade	Grade 1		Grade 2		Grade 3		Grade 4	
	# Subjects	%	# Subjects	#	# Subjects	#	# Subjects	#
Diarrhea	20	69.0%	4	13.8%		0.0%		0.00%
Nausea	10	34.5%	4	13.8%		0.0%		0.00%
Fatigue	6	20.7%	3	10.3%	1	3.4%		0.00%
WBC Decreased	3	10.3%	6	20.7%		0.0%		0.00%
Vomiting	5	17.2%	2	6.9%	1	3.4%		0.00%
Dyspnea	4	13.8%	2	6.9%		0.0%		0.00%
Muscle Spasm	6	20.7%		0.0%		0.0%		0.00%
Lymph Decreased	1	3.4%	2	6.9%	3	10.3%		0.00%
Anemia	4	13.8%	1	3.4%	1	3.4%		0.00%
Myalgia	5	17.2%		0.0%		0.0%		0.00%
Cough	4	13.8%	1	3.4%		0.0%		0.00%
Constipation	5	17.2%		0.0%		0.0%		0.00%
Dec appetite	3	10.3%	1	3.4%		0.0%		0.00%
Hyperglycemia	3	10.3%	1	3.4%		0.0%		0.00%
Hypokalemia	1	3.4%	1	3.4%	2	6.9%		0.00%
Dehydration	2	6.9%	2	6.9%		0.0%		0.00%
Incr Creatnine	3	1.3%	2	6.9%		0.0%		0.00%

6.2.10. Number of Subjects with Hematologic Adverse Events (AEs)

[0156] Number of subjects with hematologic adverse events (AEs) are summarized in Table 10.

Table 10								
Adverse Events by Grade	Grade 1		Grade 2		Grade 3		Grade 4	
	# Subjects	%	# Subjects	#	# Subjects	#	# Subjects	#
Dec Neutrophils	1	3.4%	2	6.9%		0.0%	1	3.4%

Table 10								
Adverse Events by Grade	Grade 1		Grade 2		Grade 3		Grade 4	
	# Subjects	%	# Subjects	#	# Subjects	#	# Subjects	#
Neutropenia		0.0%	0	0.0%	1	3.4%	1	3.4%
WBC Decreased	3	10.3%	6	20.7%		0.0%		0.0%
Lymph Decreased	1	3.4%	2	6.9%	3	10.3%		0.0%
Anemia	4	13.8%	1	3.4%	1	3.4%		0.0%
Dec PLTs	3	10.3%		0.0%		0.0%		0.0%
Thrombocytopenia	1	3.4%		0.0%		0.0%		0.0%
Bone Marrow Failure		0.0%		0.0%	1	3.4%		0.0%

6.2.11. Number of Subjects with Liver Adverse Events (AEs)

[0157] Number of subjects with liver AEs are summarized in Table 11.

Table 11								
Adverse Events by Grade	Grade 1		Grade 2		Grade 3		Grade 4	
	# Subjects	%	# Subjects	#	# Subjects	#	# Subjects	#
Hypoalbuminemia	4	13.8%		0.0%		0.0%		0.0%
AlkPhos Incr	1	3.4%	1	3.4%		0.0%		0.0%
Bilirubin Incr	0	0.0%	1	3.4%		0.0%		0.0%
ALT	2	6.9%		0.0%		0.0%		0.0%
AST	2	6.9%		0.0%		0.0%		0.0%

6.2.12. Grade 3 and 4 Toxicities (n=29)

[0158] Table 12 summarizes Grade 4 and 4 toxicities.

Table 12				
Adverse Events by SOC and Grade	Grade 3 – Severe		Grade 4 – Life Threatening	
	# Subjects	%	# Subjects	#
Anemia	1	3.4%		0.0%
Bone Marrow Failure	1	3.4%		0.0%
Neutropenia	1	3.4%	1	3.4%
Neutrophil count decr	0	0.0%	1	3.4%
Lymph count decr	3	10.3%	1	3.4%
Hypokalemia	2	6.9%		0.0%
Hyponatremia	1	3.4%		0.0%
Hypoglycemia	1	3.4%		0.0%
DVT/PE	2	6.9%		0.0%
Vomiting	1	3.4%		0.0%
Fatigue	1	3.4%		0.0%
Fall	1	3.4%		0.0%
Maculopapular rash	1	3.4%		0.0%
Grand Total	16		3	

6.2.13. Adverse Events (AEs) of Special Interest

[0159] Table 13 summarizes adverse events (AEs) of special interest.

Table 13								
Adverse Events by Grade	Grade 1		Grade 2		Grade 3		Grade 4	
	# Subjects	%	# Subjects	#	# Subjects	#	# Subjects	#
Dec Neutrophils	1	3.4%	2	6.9%		0.0%	1	3.4%
Neutropenia		0.0%	0	0.0%	1	3.4%	1	3.4%
WBC Decreased	3	10.3%	6	20.7%		0.0%		0.0%
Lymph Decreased	1	3.4%	2	6.9%	3	10.3%		0.0%
Anemia	4	13.8%	1	3.4%	1	3.4%		0.0%
Dec PLts	3	10.3%		0.0%		0.0%		0.0%
Thrombocytopenia	1	3.4%		0.0%		0.0%		0.0%
Bone Marrow Failure		0.0%		0.0%	1	3.4%		0.0%
Diarrhea	20	69.0%	4	13.8%		0.0%		0.0%
Fatigue	6	20.7%	3	10.3%	1	3.4%		0.0%
Muscle Spasm	6	20.7%		0.0%		0.0%		0.0%
Myalgia	5	17.2%		0.0%		0.0%		0.0%
ALT	2	6.9%		0.0%		0.0%		0.0%
AST	2	6.9%		0.0%		0.0%		0.0%
Hot Flashes	3	10.3%	1	3.4%		0.0%		0.0%
DVT/PE		0.0%		0.0%	2	6.9%		0.0%
UTI		0.0%	3	10.3%		0.0%		0.0%
Vaginal Discharge	1	3.4%		0.0%		0.0%		0.0%

6.2.14. Summary of Dose Reduction

- * Lasofoxifene dose was not reduced per protocol
- * Abemaciclib
 - o No Patient was reduced to 50 mg BID
 - o Reduction from 150 mg BID to 100 mg BID
 - * 4 due to AE
 - o Hyponatremia
 - o Dizziness, Fatigue, Vomiting, Wt loss
 - o Inc Creatinine
 - o Anorexia, Fatigue, Nausea, Gen Muscle Weakness
 - * 1 Due to Investigator discretion

6.2.15. ELAINE 2 interim PFS data comparison with other trials

[0160] As shown in Table 14 below, lasofoxifene in combination with abemaciclib provides greater median PFS compared to published and publicly presented data in patients who have had prior administration of CDK4/6i, and comparable efficacy to CDK4/6i- naïve patients; in greater detail, as compared to abema alone, either in CDK naïve patients (Monarch 1 trial) or in a post-CDK population (Abema trial); Piqray/fulvestrant in patients with PIK3CA mutations (Bylieve trial); camizestrant plus palbociclib (Serena-1 trial),

amcenestran/CDK4/6i (Ameera-1 trial); fulvestrant plus abemaciclib (Monarch 2 trial), and fulvestrant plus palbociclib (Paloma-3 trial)

Table 14										
	LAS Elaine 2 ¹	Abema Monarch 1 ²	Abema ³			Fulvestrant/ Piqray Bylieve ⁴	Camizes/ Palbo Serena-1 ⁵	Amcenes/C DKi Ameera-1 ⁶	Fulvestrant/ abema Monarch 2 ⁷	Fulv/Palbo Paloma-3 ⁸
N	29	132	Total 87	Combo 70	Mono 17	121	25	35	446	347
CDK4/6I status	Post	Naïve	Post			Post	Post	Naïve	Naïve	Naïve
PFS (mos) (95% CI)	13.9 (7.4- NE)	6.0 (4.2-7.5)	5.3 (3.5-7.8)	5.1 (3.2-7.6)	5.4 (1.9-NR)	7.3 (5.6-8.3)	NA -4.3 mo.s*	14.7 (11-22.3)	16.4	9.5 (9.2-11)
ORR	50%	19.7%	NR	NR	NR	21%	5.9%	32.4%	48.1%	25%
CBR (≥24 wks)	69%	41%	36.8%	NR	NR	45.5%	28%	74%	72.2%	67%

¹Damodaran et al ASCO 2022;
²Dickler et al Clin Cancer Res 2017 Sep 1;23(17):5219-5224;
³Wander et al, JNCCN, 10:6004, March 24, 2021;
⁴Rugo et al Lancet Onc 2021;
⁵Oliveria ASCO 2022;
⁶Chandarlapaty ASCO 2021
⁷Sledge et al JCO 2017
⁸Cristofanilli et al Lancet Onc 2016
*Calculated from reported swimmer plot

6.2.16. Efficacy in patients whose tumors have progressed on prior CDK4/6i therapy

[0161] A number of patients have been enrolled whose tumors had progressed on prior CDK4/6 inhibitor treatment. The combination of lasofoxifene and abemaciclib reduced progression of breast cancer in patients who had previously advanced on abemaciclib therapy.

- Patient 29
 - o 40 yr old with bone metastases: letrozole 3 yrs; letrozole/palbociclib 3 yrs; fulvestrant/abemaciclib 12 weeks; capecitabine 7 mos
 - o D538G 6.855% mutant allele fraction (MAF)
 - o Stable disease at 68 weeks
- Patient 21
 - o 42 yr old: Chemo/Herceptin; tamoxifen 10 yrs; letrozole/palbociclib 2 yrs 8 mos; abemaciclib 16 weeks
 - o 24mm liver mass
 - o Y537S 0.248% MAF
 - o Confirmed partial response at 48 weeks with liver lesion decreased 71% at 40 weeks
- Patient 16
 - o 78 yr old on letrozole/palbo for 2 yrs 2 mos; fulvestrant/abemaciclib 1 yr 3 mos; capecitabine 1 mos
 - o 18 mm target liver lesion, pleural, LN and bone mets
 - o D538G 0.3% MAF
 - o Progressed at 40 weeks with stable disease (target lesion decreased 6%)
- Patient 6
 - o 59 yr old fulvestant/abemaciclib for 2 years; capecitabine 1 mos
 - o 35 mm liver met
 - o D538G 1.28 MAF
 - o Progressed at 8 wks (liver lesion stable, but with new lesion)

6.3. Example 3: Second Interim Results of ELAINE 2 clinical trial

[0162] As of a second, later, interim date, two out of the four patients (50%) who had progressed on prior abemaciclib treatment continued to benefit from the combination of

lasofoxifene and abemaciclib (laso/abema) treatment. Referring to FIG. 3, Patient 29 (2011-01), who had a D538G mutation, and prior treatments with palbociclib and fulvestrant, had continued response and stable disease to 88 weeks. Patient 21 (2005-02), who had a Y537S missense mutation, visceral metastases, and who had progressed on prior abemaciclib and palbociclib treatments, had stable disease in response to laso/abema until 56 weeks, at which time her cancer progressed. As shown in FIG. 3, patients who had had progression on other CDK 4/6 inhibitors (*e.g.*, palbociclib, ribociclib) prior to enrollment also achieved clinical benefit (CB). Most had received palbociclib, and 70% (17/24) of such patients achieved CB, while 100% (2 of 2) who had been previously treated with ribociclib achieved CB. The data demonstrate that regardless of the prior CDK 4/6i, patients with an *ESR1* mutation who had progressed on a CDK4/6i have a high probability of getting clinical benefit with combination treatment with lasofoxifene and abemaciclib. Patient 2004-03, who had visceral metastasis and had received prior palbociclib treatment, achieved CB and stable disease but withdrew early at 56 weeks for non-compliance (diamond). Table 15 summarizes second interim results of patients enrolled after previous abemaciclib progression.

Table 15				
Second interim results of patients enrolled after previous abemaciclib progression				
Patient age	ESR1 Mut, MAF baseline/wk 4	Baseline disease status	Prior mBC treatment	Current disease status
40 y	D538G, 6.855%/ND	Bone metastases	LTZ/Palbo (3 yrs) Fulv/Abema (12 wks) CAPE (7 mos)	At 88 wks with SD
42 y	Y537S, 0.248%/ND	24 mm liver lesion	LTZ/Palbo (2.7 yrs) Abema (16 wks)	Stable disease until 56 wks with confirmed PR (liver lesion decreased 71% at 40 wks)
78 y	D538G, 0.3%/ND	18 mm liver lesion, pleural, and bone metastases	LTZ/Palbo (2.2 yrs) Fulv/Abema (1.3 yrs) CAPE (1 mo)	SD up to 40 wks (target lesion decreased 6%)
59 y	D538G, 1.28%/1.926%	35 mm liver metastases	Fulv/Abema (2 yrs) CAPE (1 mo)	Progressed at 8 wks (liver lesion stable, but new lesion noted)

[0163] Patients who had had other relevant treatments prior to on-study laso/abema treatment also benefitted from the combination treatment. Referring to FIG. 3, Patient 2001-01, who had had prior alpelisib treatment, achieved CB with stable disease to 32 weeks. Patient 2009-02, who had had prior alpelisib, achieved CB with stable disease on the combination of lasofoxifene and abemaciclib to 36 weeks. Patient 2016-01, who had had prior PARP inhibitor (talzenna) treatment achieved CB and had confirmed partial response to 32 weeks on the combination of lasofoxifene and abemaciclib. Patient 2018-01, who had had prior ribociclib, achieved CB with stable disease at 60 weeks on combination treatment with lasofoxifene and abemaciclib. Patient 2004-06, who had previously been treated with ribociclib/fulvestrant for 9 months, showed confirmed partial response on lasofoxifene and abemaciclib, which was ongoing at 64 weeks.

[0164] Patients in this study are routinely achieving greater response on the combination of lasofoxifene and abemaciclib than on their preceding 2L and 3L therapies, which is surprising since, historically, subsequent lines of treatment often provide shorter duration benefit than was provided by the patient's earlier lines of treatment. As shown in FIG. 4, patients with laso/abema had longer duration of stable disease than on their respective preceding 2L and 3L therapies. As of the second interim date, subjects treated with laso/abema had an average of about 8.7 months duration of stable disease. All 29 patients had had preceding 2L therapies, which provided an average of about 4.2 months duration of stable disease. Nine of these subjects had also received 3L therapies prior to enrollment, providing an average of about 7.2 months of stable disease. Patients treated with the combination of lasofoxifene and abemaciclib have had, on average, an increase in duration of stable disease of about 51.2% as compared to their preceding 2L therapies, and an increase in duration of stable disease of about 16.9% as compared to their preceding 3L therapies. For example, Patient 2011-00001 (Patient 29) had stable disease to 22 months with laso/abema, as compared to 7 months of stable disease on preceding 2L therapy and 3 months of stable disease on preceding 3L therapy. Patient 2005-00002 (Patient 21) had stable disease to 14 months on lasofoxifene and abemaciclib, as compared to 4 months of stable disease on preceding 3L therapies. Patient 2015-00001 had stable disease to 19 months with laso/abema as compared to 10 months and 2 months of stable disease on preceding 2L and 3L therapies, respectively.

[0165] The results demonstrate acceptable tolerability, safety, and efficacy with combination of lasofoxifene and abemaciclib treatment in metastatic breast cancer patients harboring at

least one *ESR1* mutation who had progressed on one or more CDK4/6 inhibitors and endocrine therapies.

6.4. Example 4: *ESR1* mutations in circulating tumor DNA (ctDNA) from ELAINE 2 clinical trial patients

[0166] This example investigates *ESR1* mutations in circulating tumor DNA (ctDNA) in patients with ER⁺/HER2⁻ metastatic breast cancer (mBC) treated with lasofoxifene plus abemaciclib in the ELAINE 2 clinical trial. Data in this example demonstrate correlations of *ESR1* mutant allele frequency (MAF) changes with clinical benefit (CB).

[0167] Use of long-term endocrine therapy (ET) for ER⁺ breast cancer often leads to acquired *ESR1* mutations (*mutESR1*), causing endocrine resistance, tumor progression, and poor prognosis. An unmet clinical need exists for treating ER⁺ mBC patients with *mutESR1*, particularly after progression on CDK4/6 inhibitors (CDK4/6i). ELAINE 2 is an open-label, phase 2, multicenter trial evaluating safety and efficacy of lasofoxifene (LAS [selective estrogen receptor modulator]) plus abemaciclib (Abema [CDK4/6i], provided by Eli Lilly) in patients with ER⁺/HER2⁻ and *mutESR1* mBC who progressed after prior ET. Preliminary data with LAS plus Abema showed median progression-free survival of 55.7 weeks, objective response rate of 50%, and 24-week clinical benefit (CB) rate of 69%, with an acceptable safety and tolerability profile.

[0168] ELAINE 2 clinical trial patients with detectable ctDNA *mutESR1* at baseline (BL) were analyzed. Oral LAS 5 mg/day and Abema 150 mg BID were taken until disease progression, death, unacceptable toxicity, or withdrawal from the study. ctDNA was assessed by the Sysmex- Inostics SafeSeq assay, which detects *mutESR1* at low allele fractions at BL, every 4 weeks and end of treatment. MAF changes from BL to week 4 were characterized as decreased (decrease in *ESR1* MAF or none detected [ND]), increased (increase in MAF), or equivocal (in polyclonal patients [>1 *mutESR1*] with some MAF increasing and decreasing trends). Correlations of MAF change at 4 weeks and with CB at 24 weeks were explored.

[0169] A total of 29 patients (median of 2 prior metastatic therapies: 97% CDK4/6i, 79% fulvestrant, 48% chemotherapy) had BL *mutESR1* of Y537S (66%), D538G (45%), Y537N (28%), Y537C (10%), and other less frequently detected mutations; 14 (48.3%) patients were polyclonal. Twenty-six (26) of 29 patients had evaluable BL and week-4 ctDNA results: 21

patients had decreased MAF (81%, clearance in 14 [54% with ND]), 3 (12%) had increased, and 2 (8%) had equivocal *ESRI* MAF changes as summarized in the Table 16 below.

Table 16			
Change from baseline to week 4 in <i>ESRI</i> MAF and clinical benefit at 24 weeks			
Clinical benefit at 24 weeks	MAF change at 4 weeks (n=26)		
	Decreased/ND	Increased	Equivocal
Yes	17 (65%)	2 (8%)	1 (4%)
No	4 (15%)	1 (4%)	1 (4%)
	Decreased/ND/Increased (n=24)		
Sensitivity*	89.5% (95% CI, 65.5–98.2)		
Specificity*	20.0% (95% CI, 1.05–70.1)		
PPV	81.0% (95% CI, 57.4–93.7)		
NPV	33.3% (95% CI, 1.80–87.5)		
	ND only (n=14)		
Sensitivity*	86.7% (95% CI, 58.4–97.7)		
Specificity*	50.0% (95% CI, 9.50–90.5)		
PPV	92.9% (95% CI, 64.2–99.6)		
NPV	33.3% (95% CI, 1.80–87.5)		

CI, confidence interval; MAF, mutant allele fraction; ND, none detected; NPV, negative predictive value; PPV, positive predictive value.

*Sensitivity and specificity analyses do not include equivocal results.

[0170] *mESRI* clearance at week 4 was observed in 3 of the 4 patients who had previously progressed while taking prior Abema-based therapies with all 3 achieving CB.

Decreased/cleared MAF was frequently observed for all the commonly detected *mESRI* variants, including the Y537S, D538G, Y537N, and Y537C variants, after 4 weeks of LAS plus abema (FIG. 6). CB at 24 weeks was seen in 17 patients with decreased *ESRI* MAF, 2 with an increase, and 1 with equivocal MAF change. A sensitivity of 89.5% and specificity of 20%, and a positive likelihood rPBatio (LR+) of 1.1 were calculated for predicting CB based on

direction of *ESR1* MAF change. The positive predictive value (PPV) for CB with decreased MAF was 81% and the negative predictive value (NPV) for an increased MAF was 33%. Of the 14 (54%) patients with ND *ESR1* MAF, 13 had CB resulting in about 87% sensitivity, 50% specificity, 93% PPV, and 33% NPV with increased *ESR* MAF. *mutESR1* clearance at week 4 had a similar sensitivity (about 87%) for CB prediction and a higher PPV (about 93%) compared with decreased MAF, with a LR+ of 1.7. All 9 patients with an objective response (OR) showed complete *mESR1* clearance (n=5) or 50%-93% decreases in *ESR1* MAF (n=4) at week 4.

[0171] In the ELAINE 2 clinical trial, 81% of patients had decrease/cleared (ND) *mutESR1* after four (4) weeks of LAS plus Abema, which correlated with clinical benefit. All *mutESR1* detected appear targeted with this therapy. High sensitivity and favorable PPV were observed in patients with decreased MAF, and even more so in those with ND MAF; however, increased MAF was less specific and not as predictive of treatment failure.

[0172] In summary, analyses of ctDNA data in ELAINE 2 demonstrated that *mutESR1* variants, including the difficult-to-treat Y537S, were decreased/cleared in most (about 81%) patients after 4 weeks of LAS plus Abema. Decreased/cleared *ESR1* MAF was associated with CB and OR, with a high sensitivity (89%) and favorable PPV (81%) for predicting CB.

[0173] PPV was higher with *mutESR1* clearance (93%). An increase in MAF was less specific and not as predictive of treatment failure. The results indicate robust target engagement of LAS plus Abema with *mutESR1*. Overall, the results demonstrate that *ESR1* liquid biopsy evaluation is an appropriate non-invasive surrogate marker for monitoring patients' treatment response or resistance to this novel LAS-Abema combination.

6.5. Example 5: Oncogenic mutations in circulating tumor DNA (ctDNA) from ELAINE 2 clinical trial patients

[0174] This example investigates oncogenic mutations of genes other than *ESR1* in circulating tumor DNA (ctDNA) in patients with ER+/HER2– metastatic breast cancer (mBC) treated with lasofoxifene plus abemaciclib in the ELAINE 2 clinical trial. FIG. 5 summarizes the panel of genes being tested and that were present in the ELAINE 2 patient population. *ESR1* gain of function mutation (*mutESR1*; top row) is included as a positive control. Data in this example demonstrate correlations of prevalence of oncogenic mutations

of genes other than *mutESR1* with clinical benefit (CB) and median progression-free survival (mPFS).

[0175] Approximately 5 mL whole blood samples were collected from individual ELAINE 2 clinical trial patient at baseline (BL) in Streck Cell-Free DNA Blood Collection Tubes (BCTs). The individual patient had previously been diagnosed by an oncologist as having ER+ mBC. To qualify for enrollment, the patient must have had either prior medical history indicating presence of *mutESR1* or had been detected to have *mutESR1*, either intrinsic or acquired, using the assay as described in Example 4. Oral lasofoxifene 5 mg/day and abemaciclib 150 mg BID were taken until disease progression, death, unacceptable toxicity, or withdrawal from the study. Samples were processed for plasma isolation and cell free DNA (cfDNA) extraction, which may contain circulating tumor DNA. About 5-30 ng of cfDNA was used to prepare sequencing libraries which were enriched by hybridization capture. The enriched libraries were then sequenced using next generation sequencing, for example on the Illumina NextSeq 550 platform. Sequencing data were analyzed using a bioinformatics pipeline designed to detect single nucleotide variants (SNVs), insertions and deletions (indels), copy number amplifications (CNAs), and fusions. Both pathogenic (e.g., oncogenic) germline alteration and somatic alteration were detected.

[0176] As shown in FIG. 5, a total of 41 genes with one or more oncogenic mutations in the blood samples of the ELAINE 2 patient population were detected. Each gene mutation had a prevalence of at least about 3% of the patient population. The mutations were either germline, somatic or both. The most prevalent genes with one or more oncogenic mutations other than *ESR1* were HNF1A (62%), TERT (59%), TP53 (41%), APC (28%), PIK3CA (28%), ATM (24%), CCND1 (21%), MET (17%), EGFR (17%), FGFR1 (17%), GATA3 (17%), and BRCA1 (17%). At least 43% of patients having oncogenic mutations in one or more of these genes achieved clinical benefit (CB) – stable disease and mPFS for at least 24 weeks – in response to the lasofoxifene and abemaciclib treatment. For example, about 100% of patients having an oncogenic mutation in CCND1 achieved CB and mPFS for at least 56 weeks, while 76% of patients having an oncogenic mutation in TERT achieved CB and mPFS for at least 44 weeks. In general, patients having an oncogenic mutation in at least one of the genes selected from HNF1A, TERT, GATA3, CDK12, MAPK3, TP53, PIK3CA, APC, ATM, MET, CCND1, EGFR, ROS1, STK11, FGFR1, ARID1A, CCNE1, AR, ALK, ERBB2, KIT, CDK4, SMAD4, NOTCH1, RB1, BRAF, RAF1, PTEN, AKT1, CDH1, BRCA1, MYC,

CDKN2A, or PDGFRA achieved mPFS for at least 24 weeks. Patients having one or more mutations in at least one of the genes selected from CCND1, FGFR1, CCNE1, AR, ALK, MAPK3, KIT, SMAD4, NOTCH1, RB1, BRAF, RAF1, PTEN, AKT1, CDH1, BRCA1, MYC, CDKN2A, or PDGFRA achieved 100% clinical benefit and mPFS for at least 32 weeks. It is noted that patients having oncogenic mutations in one or more of the genes selected from GNAS, RHEB, NTRK3, IDH2, and/or mTOR had no CB and had mPFS for 8 weeks or less.

[0177] Interestingly, in these baseline measurements, an *ESR1* mutation was detected in only about 90% of the enrolled subjects. In at least some of the subjects whose enrollment in the clinical trial was based on prior medical history of *ESR1* mutation rather than detection of *ESR1*mut at enrollment, this likely can be attributed to the patient's response to prior treatments. This would be consistent with our observations in the ELAINE 2 clinical trial itself, where approximately 68.9% (20 out of 29) of subjects displayed decreased or clear (ND) *ESR1* mutation at week 4 on LAS + abema treatment.

[0178] For example, Patient 29 (2011-00001) had an *ESR1* D538G mutation at baseline, following prior (pre-enrollment) abemaciclib treatment and other first line or second line endocrine therapies (FIGS. 1 and 3). Patient 29 became ND for *ESR1* mutation in ctDNA at week 4 on lasofoxifene and abemaciclib combination treatment. Patient 29 continues to respond to the treatment and is having clinical benefit with stable disease for at least 88 weeks (FIG. 3).

[0179] Patient 16 (2003-00001) had prior medical history indicating presence of the *ESR1* gain of function mutation D538G. Patient 16 also had received prior abemaciclib treatment and other first line or second line endocrine therapies (FIGS. 1 and 3). At baseline, Patient 16 had no detectable mutESR1 (ND for *ESR1* mutation) using the assay described in Example 4. At baseline, Patient 16 was detected to have oncogenic mutations in TERT, ATM, and MAPK3 (data not shown). Patient 16 maintained ND for *ESR1* mutation in ctDNA until end of treatment at week 40.

[0180] The data in this example demonstrate that lasofoxifene in combination with abemaciclib is effective in treating metastatic ER+ breast cancers having an *ESR1* gene mutation and an oncogenic mutation in one or more genes other than *ESR1*; and that lasofoxifene in combination with abemaciclib is effective in treating metastatic ER+ breast

cancers that do not have a detectable *ESR1* mutation and have one or more oncogenic mutations in a gene other than *ESR1*.

6.6. Example 6: Third Interim Results of ELAINE 2 clinical trial

[0181] As of a third, later, interim date, 20 out of 29 patients (about 69%) had CB from treatment with the combination of lasofoxifene and abemaciclib (laso/abema). Referring to FIG. 7, which is an updated Swimmer Plot, 11 out of 20 patients (55%) who had CB had updated status as compared to FIG. 3. Among of these patients, 8 out of 11 patients (about 72.7%) had continued or partial response and stable disease at week 68 to 100 since the last update. For example, Patient 29 (2011-01) continued to have continued response and stable disease to 100 weeks, while Patient 2005-01 had continued response and stable disease to 84 weeks, Patient 2002-01 had continued response and stable disease to 80 weeks, Patients 2001-05 and 2016-03 had continued response and stable disease to 72 weeks, Patient 2018-01 had continued response and stable disease to 68 weeks, and Patient 2017-01 had partial response and stable disease to 80 weeks. Patient 2015-01 had partial response and stable disease to 88 weeks. Two (2) out of 20 patients (10%) had progressed at week 72 (Patient 2001-02 and Patient 2014-01), and one patient (5%) had an early withdrawal (Patient 2004-06). As shown in Table 17 below, 7 out of 11 patients (about 63.6%) had the difficult-to-treat Y537S mutation at baseline. The data show that all 7 patients had continued or partial response to the combination of laso/abema treatment, and had stable disease up to at least 72 weeks. Laso/abema efficacy is observed even in patients who had visceral metastases at baseline (about 42.8%, 3 out of 7). At least 7 out of 11 patients (about 63.6%) had oncogenic mutations in one or more genes other than *ESR1*, and at least one of the oncogenic mutations had a prevalence of over 20% in the patient population (e.g., HNF1A, TERT, TP53, APC, PIK3CA, ATM, CCND1, see also FIG. 5).

Table 17								
Efficacy of combination of laso/abema treatment on patients with <i>ESR1</i> , other oncogenic mutations and prior treatments								
Patient	ESR1 (ctDNA)	Other oncogenic mutations (ctDNA)	Continued response/stable (week)	Partial response/stable (week)	Progressed (week)	Early withdrawal (week)	Prior CDK4/6i treatment	Visceral metastases
2011-01	D538G	TBD	100	n/a	n/a	n/a	palbociclib, abemaciclib	No
2015-01	Y537S	TBD		88	n/a	n/a	palbociclib	Yes

Table 17								
Efficacy of combination of laso/abema treatment on patients with <i>ESR1</i> , other oncogenic mutations and prior treatments								
Patient	ESR1 (ctDNA)	Other oncogenic mutations (ctDNA)	Continued response/stable (week)	Partial response/stable (week)	Progressed (week)	Early withdrawal (week)	Prior CDK4/6i treatment	Visceral metastases
2005-01	Y537S , Y537N, D538G	HNF1A, TERT, TP53, ATM, CCND1, MET, EGFR1, GATA3, CCNE1, CDK4	84	n/a	n/a	n/a	palbociclib	No
2002-01	Y537S , Y537N, D538G	HNF1A, AKT1, CDH1	80	n/a	n/a	n/a	palbociclib	No
2017-01	E380Q, L469V	TERT, APC, CCNE1, ALK	n/a	80	n/a	n/a	palbociclib	No
2001-02	Y537S , Y537N, Y537D, D538G	PIK3CA, CDK12, BRCA1	n/a	n/a	72	n/a	palbociclib	Yes
2001-05	Y537S	APC, PIK3CA, BRCA2, AR	72	n/a	n/a	n/a	palbociclib	No
2004-06	Y537S	HNF1A, TERT, GATA3, ROS1, KIT	n/a	n/a	n/a	72	palbociclib	Yes
2014-01	Y537S , Y537N, D538G	TP53, CCND1, MET, ARID1A, BRAF	n/a	n/a	72	n/a	palbociclib	No
2016-03	D538G	TBD	n/a	72	n/a	n/a	palbociclib	Yes
2018-01	Y537C	TBD	68	n/a	n/a	n/a	palbociclib, ribociclib	No

*TBD: oncogenic mutations to be determined

[0182] Referring to Table 17, it is noted that patients who have mutations that have previously been associated with endocrine resistance or CDK4/6i resistance achieved clinical benefit (CB) with stable disease and showed complete or partial response for at least 72 weeks. For example, patients who have at least one of FGFR1, ERBB2, CCND1, CCNE1, ARD1A, PIK3CA and TP53, had consistent robust clinical response to the

lasofoxifene/abemaciclib combination in Elaine 2. Patient 2005-01, who had TP53, CCND1 and CCNE1 mutations or CNV, had complete response and stable disease to 84 weeks. Patient 2014-01, who had TP53, CCND1 and ARID1A mutations or CNV, had partial response and progressed at 72 weeks. Patient 2001-02, who had PIK3CA mutation or CNV, had partial response and progressed at 72 weeks. Patient 2001-05, who had PIK3CA mutation or CNV, had complete response and stable disease to 72 weeks. It is noted that these four patients also have the difficult-to-treat Y537S mut*ESR1* variant.

[0183] These results are unexpected and demonstrate that combination treatment with lasofoxifene and abemaciclib is efficient in reducing or preventing tumor progression in patients who have higher risk of developing resistance to endocrine therapy or CDK4/6i treatment, or who have developed resistance to endocrine therapy or CDK4/6i treatment.

[0184] The results are consistent with our observations presented in Example 3 and further demonstrate acceptable tolerability, safety, and efficacy with combination treatment with lasofoxifene and abemaciclib in metastatic breast cancer patients harboring at least one *ESR1* mutation and often at least one or more mutations in other genes, who had progressed on one or more CDK4/6 inhibitors, and/or one or more endocrine therapies.

6.7. Example 7: Copy number variations of *ESR1* mutations and oncogenic mutations in circulating tumor DNA (ctDNA) from ELAINE 2 clinical trial patients

[0185] This example investigates copy number variations (CNVs) of *ESR1* mutations and oncogenic mutations in circulating tumor DNA (ctDNA) in patients with ER+/HER2– metastatic breast cancer (mBC) treated with lasofoxifene plus abemaciclib in the ELAINE 2 clinical trial as described in Example 5. Data in this example demonstrate correlations of presence of *ESR1* mutation variants and/or oncogenic mutation variants with clinical benefit (CB).

[0186] A total of 29 samples received from 29 patients (Batch 1: 25 samples/patients; Batch 2: 4 samples/patients) were collected from the ELAINE 2 clinical trial. Patients were administered oral lasofoxifene 5 mg/day and abemaciclib 150 mg BID until disease progression, death, unacceptable toxicity, or withdrawal from the study. Samples were processed for plasma isolation and cell free DNA (ctDNA) extraction and sequencing as described in Example 5. Sequencing data were analyzed using a bioinformatics pipeline designed to detect single nucleotide variants (SNVs), insertions and deletions (indels), copy

number variations (CNVs), and fusions. In Batch 1, copy number amplifications (CNAs) were detected in 19 genes, including *CCND1*. Amplification type was annotated as focal, aneuploidy, or amplification where focal/aneuploidy status is indeterminate. The results are provided in Table 18.

Table 18 Copy number variations of <i>ESR1</i> mutations and oncogenic mutations				
Mutation	Batch 1		Batch 2	
	Alteration	Number of samples (n=25)	Alteration	Number of samples (n=4)
<i>ESR1</i>	L536P, L536H, L536R, Y537S, Y537N, Y537C, D538G, E380Q	22 (88%)	Y537S, Y537C, D538G	4 (100%)
<i>PIK3CA</i>	E542K, E545K, H1047R, M1043I, Q546K, G118D	8 (32%)	n/a	n/a
<i>ERBB2</i>	S310F	1 (4%)	M1014R	1 (4%)
<i>CCND1</i>	Focal amplification	5 (20%)	Focal amplification	1 (25%)
<i>FGFR1</i>	n/a	n/a	Amplification	2 (50%)

[0187] FIG. 8 illustrates an example of copy number variation events detected per gene. FIG. 8 left panel shows copy number variation (CNV) events detected per gene, including *CCND1*, *CCNE1*, *CDK4*, *EGFR*, *FGFR1*, *MYC*. FIG. 8 right panel shows copy number distribution per gene for the CNV events shown on the left panel. Five CNV events and over eight copy numbers were detected in *CCND1*, three CNV events and over seven copy numbers were detected in *FGFR1*, while two CNV events and about five copy numbers were detected in *CDK4*.

6.8. Example 8: Interim Results of oncogenic mutations in circulating tumor DNA (ctDNA) from ELAINE 2 clinical trial patients

[0188] This example provides, as of an interim date later than the date of Example 5, oncogenic mutations of genes other than *ESR1* present in circulating tumor DNA (ctDNA) in patients with ER+/HER2– metastatic breast cancer (mBC) treated with lasofoxifene plus abemaciclib in the ELAINE 2 clinical trial as described in Example 5. The results are summarized in FIG. 9. *ESR1* gain of function mutation (mut*ESR1*; top row) is included for completeness.

[0189] As shown in FIG. 9, a total of 41 genes with one or more oncogenic mutations in the blood samples of the ELAINE 2 patient population were detected. At least 87.8% (36 out of 41) of patients having oncogenic mutations in one or more of these genes achieved clinical benefit (CB) – defined as stable disease $\geq$ 24 weeks, or confirmed partial or complete

response – in response to the lasofoxifene and abemaciclib treatment. Patients having one or more mutations in TERT, APC, ATM, CCND1, MET, EGFR, FGFR1, GATA3, STK11, ROS1, ERBB2, CCNE1, AR, SMAD4, ALK BRAF, KIT, CDK4, AKT1, CDH1, BRCA1, MYC, and PDGFRA continued to achieve CB and mPFS. For example, about 100% of patients having an oncogenic mutation in CCND1 continued to achieve CB and mPFS for at least 72 weeks, 100% of patients having an oncogenic mutation in FGFR1 continued to achieve CB and mPFS for at least 72 weeks, while 76% of patients having an oncogenic mutation in TERT continued to achieve CB and mPFS for at least 56 weeks. Patients having one or more mutations in TP53 or PIK3CA achieved CB and mPFS for at least 36 weeks and 34 weeks, respectively. Notably, patients having co-existing copy number variant (CNV) in CCND1 and FGFR1 and alterations in PIK3CA and TP53 responded to lasofoxifene and abemaciclib.

[0190] The data in this example demonstrate that lasofoxifene in combination with abemaciclib is effective in treating metastatic ER+ breast cancers having an *ESR1* gene mutation and an oncogenic mutation in one or more genes other than *ESR1*, and in maintaining CB and mPFS in this subset of patients, and in patients who have metastatic ER+ breast cancers that do not have a detectable *ESR1* mutation and have one or more oncogenic mutations in a gene other than *ESR1*.

6.9. Example 9: Fourth Interim Results of ELAINE 2 clinical trial

[0191] As of a fourth, later date than the date of Example 6, 20 out of 29 patients (about 68.9%) continued to have CB from treatment with the combination of lasofoxifene and abemaciclib (laso/abema). Referring to FIGS. 10A-10B, which is an updated Swimmer Plot, 8 out of 20 patients (40%) who had CB had updated status as compared to FIG. 7. Among of these patients, 6 out of 8 patients (75%) had continued or partial response and stable disease at week 96 to 128 since the last update, while 2 out of 8 patients (25%) had continued or partial response and progressive disease. For example, Patient 29 (2011-01) continued to have continued response and stable disease to 128 weeks, while Patient 2002-01 had continued response and stable disease to 100 weeks, Patients 2001-05 and 2018-01 had continued response and stable disease to 96 weeks. Patient 2015-01 had partial response and stable disease to 120 weeks. Patient 2016-03 had partial response and stable disease to 96 weeks. Patient 2005-01 had continued response but progressed at 104 weeks. Patient 2017-01 had partial response and progressed at 100 weeks. Table 19 summarizes efficacy of

lasofoxifene/abemaciclib combination in patients who achieved clinical benefit, including patients described in Table 17. As shown, 13 out of 20 patients (65%) had the difficult-to-treat Y537S mutation at baseline but had continued or partial response and stable disease up to 120 weeks (e.g., Patient 2015-01). Lasofoxifene/abemaciclib efficacy is observed even in patients who had visceral metastases at baseline (60%, 12 out of 20). All 20 patients (100%) who had clinical benefit also had oncogenic mutations in one or more genes other than *ESR1*, and at least one of the oncogenic mutations had a prevalence of over 17% in the patient population (e.g., HNF1A, TERT, TP53, APC, PIK3CA, ATM, CCND1, FGFR1, see also FIG. 9).

Table 19								
Efficacy of combination of laso/abema treatment on patients with <i>ESR1</i> , other oncogenic mutations and prior treatments								
Patient	ESR1 (ctDNA)	Other oncogenic mutations (ctDNA)	Continued response/stable (week)	Partial response/stable (week)	Progressed (week)	Early withdrawal (week)	Prior CDK4/6i treatment	Visceral metastases
2011-01	D538G	CCND1, TP53, TERT, HNF1A	128	n/a	n/a	n/a	palbociclib, abemaciclib	No
2015-01	Y537S	FGFR1, TP53	n/a	120	n/a	n/a	palbociclib	Yes
2005-01	Y537S , Y537N, D538G	HNF1A, TERT, TP53, ATM, CCND1, MET, EGFR1, GATA3, CCNE1, CDK4, KIT	n/a	n/a	104	n/a	palbociclib	No
2002-01	Y537S , Y537N, D538G	HNF1A, AKT1, CDH1	100	n/a	n/a	n/a	palbociclib	No
2017-01	E380Q, L469V	TERT, APC, CCNE1, ALK	n/a	n/a	100	n/a	palbociclib	No
2001-02	Y537S , Y537N, Y537D, D538G	PIK3CA, CDK12, BRCA1, FGFR1, CCND1	n/a	n/a	72	n/a	palbociclib	Yes
2001-05	Y537S	APC, PIK3CA, BRCA2, AR	96	n/a	n/a	n/a	palbociclib	No

Table 19								
Efficacy of combination of laso/abema treatment on patients with <i>ESR1</i> , other oncogenic mutations and prior treatments								
Patient	ESR1 (ctDNA)	Other oncogenic mutations (ctDNA)	Continued response/stable (week)	Partial response/stable (week)	Progressed (week)	Early withdrawal (week)	Prior CDK4/6i treatment	Visceral metastases
2004-06	Y537S	HNF1A, TERT, GATA3, ROS1, KIT	n/a	n/a	n/a	72	palbociclib	Yes
2014-01	Y537S, Y537N, D538G	TP53, CCND1, MET, ARID1A, BRAF	n/a	n/a	72	n/a	palbociclib	No
2016-03	D538G	TERT, BRAF, ATM, EGFR, BRCA2, ERBB2, STK11, PDGFRA, APC, TP53	n/a	96	n/a	n/a	palbociclib	Yes
2018-01	Y537C	FGFR1, TERT, ATM, HNF1A, SMAD4	96	n/a	n/a	n/a	palbociclib, ribociclib	No
2001-01	Y537S, D538G	PIK3CA, RAF1, PTEN, APC	n/a	n/a	32	n/a	palbociclib	Yes
2002-02	Y537N, L536H, D538G, E380Q	TERT, CCNE1, EGFR	n/a	n/a	32	n/a	palbociclib	Yes
2004-03	Y537S	CDK12, TERT, ROS1, ERBB2, HNF1A, MAPK3, RB1	n/a	n/a	n/a	56	palbociclib	Yes
2005-02	Y537S	TERT, STK11, PIK3CA, AR, ARID1A	n/a	n/a	56	n/a	abemaciclib, palbociclib	Yes
2008-01	Y537S, D538G	HNF1A, BRCA2, CDKN2A, AR, CCND1, FGFR1	n/a	n/a	32	n/a	palbociclib	Yes

Table 19								
Efficacy of combination of laso/abema treatment on patients with <i>ESR1</i> , other oncogenic mutations and prior treatments								
Patient	ESR1 (ctDNA)	Other oncogenic mutations (ctDNA)	Continued response/stable (week)	Partial response/stable (week)	Progressed (week)	Early withdrawal (week)	Prior CDK4/6i treatment	Visceral metastases
2009-02	Y537S	PIK3CA, TP53, TERT, MET	n/a	n/a	36	n/a	palbociclib	No
2013-01	D538G	ATM, SMAD4	n/a	n/a	56	n/a	palbociclib	Yes
2016-01	Y537S, Y537C, L536P, D538G	TERT, TP53, HNF1A, NOTCH1, ALK, CCND1, FGFR1	n/a	n/a	32	n/a	palbociclib	Yes
2003-01	D538G	TERT, MAPK3, ATM	n/a	n/a	Progressed at 14 weeks and remained on study with stable disease until 40 weeks	n/a	abemaciclib, palbociclib	Yes

[0192] Referring to Table 19, it is noted that patients who have mutations that have previously been associated with endocrine resistance or CDK4/6i resistance, such as FGFR1, ERBB2, CCND1, CCNE1, ARD1A, PIK3CA and TP53, continued to have consistent robust clinical response to the lasofoxifene/abemaciclib combination in Elaine 2. Subject 2011-01, who has D538G mut*ESR1* variant and TP53 and CCND1 mutations or copy number variant (CNV), continued to have complete response and stable disease to 128 weeks. Patient 2017-01, who has E380Q and L469V mut*ESR1* variants and CCNE1 mutation or CNV, continued to have partial response and progressed at 100 weeks. Patient 2016-03, who has D538G mut*ESR1* variant and ERBB2 mutation or CNV, continued to have partial response and stable disease at 96 weeks. Patient 2002-02, despite having multiple mut*ESR1* variants, including Y537N, L536H, D538G, and E380Q, and who had CCNE mutation or CNV, had partial response until progression at 32 weeks.

[0193] It is noted that patients who have the difficult-to-treat Y537S mut*ESR1* variant continued to have consistent robust clinical efficacy to the lasofoxifene/abemaciclib

combination. Patient 2015-01, who has Y537S mut*ESR1* variant and FGFR1 and TP53 mutations or CNV, continued to have partial response and stable disease to 120 weeks. Patient 2005-01, who has Y537S, Y537N, and D538G mut*ESR1* variants and TP53, CCND1 and CCNE1 mutations or CNV, continued to have complete response and stable disease and progressed at 104 weeks. Patient 2001-02, who has Y537S, Y537N, Y537D, and D538G mut*ESR1* variants and PIK3CA, FGFR1 and CCND1 mutations or CNV, had partial response and progressed at 72 weeks. Patient 2014-01, who has Y537S, Y537N, and D538G mut*ESR1* variants and TP53, CCND1 and ARID1A mutations or CNV, had partial response and progressed at 72 weeks. Patient 2004-03, who has Y537S mut*ESR1* variant and ERBB2 mutation or CNV, had complete response and stable disease at 56 weeks, but had early withdrawal due to non-compliance. Patient 2005-02, who has a Y537S mut*ESR1* variant and PIK3CA and ARID1A mutations or CNV, had partial response and progressed at 56 weeks. Patient 2008-01, who has Y537S and D538G mut*ESR1* variants and CCND1 and FGFR1 mutations or CNV, had partial response and progressed at 32 weeks. Patient 2016-01, who has Y537S, Y537C, L536P and D538G mut*ESR1* variants and TP53, CCND1 and FGFR1 mutations or CNV, had partial response and progressed at 32 weeks. Patient 2001-05, who has Y537S mut*ESR1* variant and PIK3CA mutation or CNV, continued to have complete response and stable disease to 96 weeks. Patient 2001-01, who has Y537S and D538G mut*ESR1* variants and PIK3CA mutation or CNV, had partial response and progressed at 32 weeks. Patient 2009-02, who has Y537S mut*ESR1* variant and TP53 and PIK3CA mutations or CNV, had complete response and stable disease and progressed at 36 weeks.

[0194] Patients who had target lesion at baseline were monitored for change on study. It is noted that 10 out of 20 (50%) had confirmed partial response and achieved clinical benefit. Patient 2001-01 had target lesion in the lung (11 mm) at baseline and the lesion decreased by 55% at 64 weeks. Patient 2002-02 had target lesions in the liver (20 mm), lungs (23 mm, 29 mm) at baseline (sum diameter of 72 mm) and the lesion decreased by 74% at 32 weeks. Patient 2004-06 had target lesions in liver, bone, and pleural cavity (sum diameter of 79 mm) at baseline and the lesion decreased by 47% at 64 weeks. Patient 2005-02 had target lesions in liver (sum diameter of 24 mm) at baseline and the lesion decreased by 33% at 56 weeks. Patient 2008-01 had target lesions in liver (sum diameter of 56 mm) at baseline and the lesion decreased by 50% at 32 weeks. Patient 2014-01 had target lesions in liver and spleen (41 mm) and had the lesions decreased by 22% at 64 weeks. Patient 2015-01 had target lesions in the liver (sum diameter of 56 mm) and the lesion decreased by 52% at 104 weeks. Patient

2016-01 had target lesions in the liver (47 mm) and the lesion decreased by 47% at 32 weeks. Patient 2016-03 had target lesion in the liver (sum diameter of 67 mm) and the lesion decreased by 67% at 88 weeks. Patient 2017-01 had target lesion in left paraceliac (sum diameter 15 mm) and the lesion decreased by 40% at 96 weeks. The data demonstrate that combination treatment with lasofoxifene/abemaciclib is effective in reducing tumor progression and/or inhibiting tumor growth.

[0195] The results are consistent with our observations in Examples 3 and 7 and further demonstrate acceptable tolerability, safety, and efficacy with combination treatment with lasofoxifene and abemaciclib in metastatic breast cancer patients harboring at least one *ESR1* mutation and often at least one or more mutations in other genes, who had progressed on one or more CDK4/6 inhibitors, and/or one or more endocrine therapies. Additionally, the results further demonstrate that combination treatment with lasofoxifene and abemaciclib is effective in patients harboring the difficult-to-treat mut*ESR1* variants. The results are unexpected in patients who also have one or more oncogenic mutations or CNV of biomarkers associated with endocrine therapy or CDK4/6i resistance.

7. EQUIVALENTS AND INCORPORATION BY REFERENCE

[0196] While the invention has been particularly shown and described with reference to a preferred embodiment and various alternate embodiments, it will be understood by persons skilled in the relevant art that various changes in form and details can be made therein without departing from the spirit and scope of the invention.

[0197] All references, issued patents and patent applications cited within the body of the instant specification are hereby incorporated by reference in their entirety, for all purposes.

WHAT IS CLAIMED IS:

1. A method of reducing the progression of breast cancer in a patient, comprising:
administering to the patient an effective amount of lasofoxifene or a pharmaceutically acceptable salt thereof and an effective amount of a CDK4/6 inhibitor (CDK4/6i),
wherein the breast cancer:
 - (i) is estrogen receptor positive (ER⁺);
 - (ii) has at least one gain of function missense mutation within the ligand binding domain (LBD) of the Estrogen Receptor 1 (*ESR1*) gene; and
 - (iii) has progressed during prior CDK4/6 inhibitor therapy.
2. The method of claim 1, wherein the ER⁺ breast cancer is HER2⁻.
3. The method of claim 1 or claim 2, wherein the ER⁺ breast cancer is locally advanced.
4. The method of claim 1 or claim 2, wherein the ER⁺ breast cancer is metastatic.
5. The method of any one of claims 1-4, wherein lasofoxifene is administered as lasofoxifene tartrate.
6. The method of any one of claims 1-5, wherein lasofoxifene is administered orally at 5 mg/day.
7. The method of any one of claims 1-6, wherein the CDK4/6i administered to the patient is selected from palbociclib, ribociclib, and abemaciclib.
8. The method of claim 7, wherein the CDK4/6i administered to the patient is abemaciclib.
9. The method of claim 8, wherein abemaciclib is administered orally at 50 mg to 200 mg BID.
10. The method of claim 9, wherein abemaciclib is administered orally at 100 mg to 200 mg BID.
11. The method of claim 10, wherein abemaciclib is administered orally at 150 mg BID.

12. The method of claim 1, wherein the prior administered CDK4/6 inhibitor is selected from palbociclib, ribociclib, and abemaciclib.
13. The method of claim 12, wherein the prior administered CDK4/6 inhibitor is abemaciclib.
14. The method of any one of claims 1-13, wherein the cancer has previously been determined to have at least one gain of function missense mutation within the ligand binding domain (LBD) of the Estrogen Receptor 1 (*ESR1*) gene.
15. The method of any one of claims 1-14, further comprising the earlier step of:
determining that the patient has at least one gain of function missense mutation within the ligand binding domain (LBD) of the Estrogen Receptor 1 (*ESR1*) gene.
16. The method of any one of claims 1-15, wherein the at least one of gain of function missense mutation is in any one of amino acids D538, Y537, L469, L536, P535, V534, S463, V392, and E380.
17. The method of claim 16, wherein the at least one gain of function missense mutation is in amino acid D538.
18. The method of claim 17, wherein the mutation is D538G.
19. The method of claim 16, wherein the at least one gain of function missense mutation is in the amino acid Y537.
20. The method of claim 19, wherein the mutation is Y537S, Y537N, Y537C, or Y537Q.
21. The method of claim 20, wherein the mutation is Y537C.
22. The method of claim 16, wherein the at least one gain of function missense mutation is in the amino acid L469.
23. The method of claim 22, wherein the mutation is L469V.
24. The method of claim 16, wherein the at least one gain of function missense mutation is in the amino acid L536.

25. The method of claim 24, wherein the mutation is L536R or L536Q.
26. The method of claim 16, wherein the at least one gain of function missense mutation is in the amino acid P535.
27. The method of claim 26, wherein the mutation is P535H.
28. The method of claim 16, wherein the at least one gain of function missense mutation is in the amino acid V534.
29. The method of claim 28, wherein the mutation is V534E.
30. The method of claim 16, wherein the at least one gain of function missense mutation is in the amino acid S463.
31. The method of claim 30, wherein the mutation is S463P.
32. The method of claim 16, wherein the at least one gain of function missense mutation is in the amino acid V392.
33. The method of claim 32, wherein the mutation is V392I.
34. The method of claim 16, wherein the at least one gain of function missense mutation is in the amino acid E380.
35. The method of claim 34, wherein the mutation is E380Q.
36. The method of claim 4, wherein the ER⁺ breast cancer is visceral metastatic.
37. The method of any one of claims 1-36, wherein the breast cancer had progressed on one or more prior endocrine therapies.
38. The method of claim 37, wherein the prior endocrine therapy is a selective ER degrader (SERD), a selective ER modulator (SERM), optionally a SERM other than lasofoxifene, an aromatase inhibitor (AI), a mTOR inhibitor, and/or a PI3K inhibitor.
39. The method of claim 38, wherein the SERD is fulvestrant.

40. The method of any one of claims 1-39, wherein the patient derives clinical benefit with stable disease for at least 24 weeks, 28 weeks, 32 weeks, 36 weeks, 40 weeks, 44 weeks, 48 weeks, 52 weeks, 56 weeks, 60 weeks, 64 weeks, 68 weeks, 72 weeks, 74 weeks, 78 weeks, 82 weeks, 86 weeks, or longer following commencement of treatment with lasofoxifene or a pharmaceutically acceptable salt thereof and a CDK4/6i.
41. The method of claim 40, wherein the patient has complete or partial response to the treatment with lasofoxifene or a pharmaceutically acceptable salt thereof and a CDK4/6i.
42. The method of any one of claims 1-41, wherein the patient derives a longer duration of stable disease than on the patient's preceding second-line or third-line therapies by at least about 20%, 15%, or 10%.
43. A method of reducing the progression of breast cancer in a patient, comprising:
administering to the patient an effective amount of lasofoxifene or a pharmaceutically acceptable salt thereof and an effective amount of a CDK4/6 inhibitor (CDK4/6i),
wherein the breast cancer:
- (i) is estrogen receptor positive (ER⁺);
 - (ii) has at least one gain of function missense mutation within the ligand binding domain (LBD) of the Estrogen Receptor 1 (*ESR1*) gene; and
 - (iii) has an oncogenic mutation in one or more genes other than the *ESR1* gene.
44. The method of claim 43, wherein the breast cancer has progressed during prior CDK4/6 inhibitor therapy.
45. The method of claim 43 or 44, wherein the ER⁺ breast cancer is HER2⁻.
46. The method of claim 45, wherein the ER⁺ breast cancer is locally advanced.
47. The method of claim 45, wherein the ER⁺ breast cancer is metastatic.
48. The method of any one of claims 43-47, wherein lasofoxifene is administered as lasofoxifene tartrate.

49. The method of any one of claims 43-48, wherein lasofoxifene is administered orally at 5 mg/day.
50. The method of any one of claims 43-49, wherein the CDK4/6i administered to the patient is selected from palbociclib, ribociclib, and abemaciclib.
51. The method of claim 50, wherein the CDK4/6i administered to the patient is abemaciclib.
52. The method of claim 51, wherein abemaciclib is administered orally at 50 mg to 200 mg BID.
53. The method of claim 52, wherein abemaciclib is administered orally at 100 mg to 200 mg BID.
54. The method of claim 53, wherein abemaciclib is administered orally at 150 mg BID.
55. The method of claim 44, or of any one of claims 45-54 as dependent from claim 44, wherein the prior administered CDK4/6 inhibitor is selected from palbociclib, ribociclib, and abemaciclib.
56. The method of claim 55, wherein the prior administered CDK4/6 inhibitor is abemaciclib.
57. The method of any one of claims 43-56, further comprising the preceding step of detecting oncogenic mutations in the one or more genes with an oncogenic mutation in circulating tumor DNA (ctDNA).
58. The method of claim 57, wherein at least one of the one or more genes with an oncogenic mutation is selected from HNF1A, TERT, GATA3, CDK12, MAPK3, GNAS, RAF1, RHEB, NTRK3, TP53, PIK3CA, APC, ATM, MET, CCND1, EGFR, ROS1, BRCA2, STK11, FGFR1, ARID1A, CCNE1, AR, ALK, ERBB2, KIT, CDK4, SMAD4, NOTCH1, RB1, BRAF, PTEN, AKT1, CDH1, BRCA1, MYC, CDKN2A, IDH2, MTOR, or PDGFRA.
59. The method of claim 58, wherein each of the one or more genes with an oncogenic mutation is selected from HNF1A, TERT, GATA3, CDK12, MAPK3, GNAS, RAF1, RHEB, NTRK3, TP53, PIK3CA, APC, ATM, MET, CCND1, EGFR, ROS1, BRCA2, STK11,

FGFR1, ARID1A, CCNE1, AR, ALK, ERBB2, KIT, CDK4, SMAD4, NOTCH1, RB1, BRAF, PTEN, AKT1, CDH1, BRCA1, MYC, CDKN2A, IDH2, MTOR, or PDGFRA.

60. The method of any one of claims 43-59, wherein the one or more genes with an oncogenic mutation is selected from HNF1A, TERT, GATA3, CDK12, MAPK3, TP53, PIK3CA, APC, ATM, MET, CCND1, EGFR, ROS1, STK11, FGFR1, ARID1A, CCNE1, AR, ALK, ERBB2, KIT, CDK4, SMAD4, NOTCH1, RB1, BRAF, RAF1, PTEN, AKT1, CDH1, BRCA1, MYC, CDKN2A, or PDGFRA.

61. The method of any one of claims 43-59, wherein the one or more genes with an oncogenic mutation is selected from CCND1, FGFR1, CCNE1, AR, ALK, MAPK3, KIT, SMAD4, NOTCH1, RB1, BRAF, RAF1, PTEN, AKT1, CDH1, BRCA1, MYC, CDKN2A, or PDGFRA.

62. The method of any one of claims 43-59, wherein the one or more genes with an oncogenic mutation is selected from HNF1A, TERT, GATA3, CDK12, MAPK3, TP53, PIK3CA, APC, ATM, MET, CCND1, EGFR, ROS1, BRCA2, STK11, FGFR1, ARID1A, CCNE1, AR, ALK, ERBB2, KIT, BRAF, or CDK4.

63. The method of claim 62, wherein the one or more genes with an oncogenic mutation is selected from TP53, PIK3CA, CCND1, ARID1A, FGFR1, CCNE1 and ERBB2.

64. The method of claim 63, wherein the oncogenic mutation is PIK3CA.

65. The method of any one of claims 43-59, wherein the one or more genes with an oncogenic mutation is selected from HNF1A, TERT, GATA3, CDK12, MAPK3, GNAS, RAF1, RHEB, or NTRK3.

66. The method of any one of claims 43-65, wherein the cancer has previously been determined to have at least one gain of function missense mutation within the ligand binding domain (LBD) of the Estrogen Receptor 1 (*ESR1*) gene.

67. The method of any one of claims 43-66, further comprising the earlier step of: determining that the patient has at least one gain of function missense mutation within the ligand binding domain (LBD) of the Estrogen Receptor 1 (*ESR1*) gene.

68. The method of any one of claims 43-67, wherein the at least one of gain of function missense mutation is in any one of amino acids D538, Y537, L469, L536, P535, V534, S463, V392, and E380.
69. The method of claim 68, wherein the at least one gain of function missense mutation is in amino acid D538.
70. The method of claim 69, wherein the mutation is D538G.
71. The method of claim 68, wherein the at least one gain of function missense mutation is in the amino acid Y537.
72. The method of claim 71, wherein the mutation is Y537S, Y537N, Y537C, or Y537Q.
73. The method of claim 72, wherein the mutation is Y537C.
74. The method of claim 72, wherein the mutation is Y537S.
75. The method of claim 68, wherein the at least one gain of function missense mutation is in the amino acid L469.
76. The method of claim 75, wherein the mutation is L469V.
77. The method of claim 68, wherein the at least one gain of function missense mutation is in the amino acid L536.
78. The method of claim 77, wherein the mutation is L536R or L536Q.
79. The method of claim 68, wherein the at least one gain of function missense mutation is in the amino acid P535.
80. The method of claim 79, wherein the mutation is P535H.
81. The method of claim 68, wherein the at least one gain of function missense mutation is in the amino acid V534.
82. The method of claim 81, wherein the mutation is V534E.

83. The method of claim 68, wherein the at least one gain of function missense mutation is in the amino acid S463.
84. The method of claim 83, wherein the mutation is S463P.
85. The method of claim 68, wherein the at least one gain of function missense mutation is in the amino acid V392.
86. The method of claim 85, wherein the mutation is V392I.
87. The method of claim 68, wherein the at least one gain of function missense mutation is in the amino acid E380.
88. The method of claim 87, wherein the mutation is E380Q.
89. The method of claim 47, wherein the ER⁺ breast cancer is visceral metastatic.
90. The method of any one of claims 43-89, wherein the breast cancer had progressed on one or more prior endocrine therapies.
91. The method of claim 90, wherein the prior endocrine therapy is a selective ER degrader (SERD), a selective ER modulator (SERM), optionally a SERM other than lasofoxifene, an aromatase inhibitor (AI), a mTOR inhibitor, and/or a PI3K inhibitor.
92. The method of claim 91, wherein the SERD is fulvestrant.
93. The method of any one of claims 43-92, wherein the patient derives clinical benefit with stable disease for at least 24 weeks, 28 weeks, 32 weeks, 36 weeks, 40 weeks, 44 weeks, 48 weeks, 52 weeks, 56 weeks, 60 weeks, 64 weeks, 68 weeks, 72 weeks, 74 weeks, 78 weeks, 82 weeks, 86 weeks, or longer following commencement of treatment with lasofoxifene or a pharmaceutically acceptable salt thereof and a CDK4/6i.
94. The method of claim 93, wherein the patient has complete or partial response to the treatment with lasofoxifene or a pharmaceutically acceptable salt thereof and a CDK4/6i.
95. The method of any one of claims 43-94, wherein the patient derives a longer duration of stable disease on with lasofoxifene or a pharmaceutically acceptable salt thereof and a

CDK4/6i than on the patient's preceding second-line or third-line therapies by at least about 20%, 15%, or 10%.

96. A method of reducing the progression of breast cancer in a patient, comprising:
administering to the patient an effective amount of lasofoxifene or a pharmaceutically acceptable salt thereof and an effective amount of a CDK4/6 inhibitor (CDK4/6i),
wherein the breast cancer:

- (i) is estrogen receptor positive (ER⁺);
- (ii) has at least one gain of function missense mutation within the ligand binding domain (LBD) of the Estrogen Receptor 1 (*ESR1*) gene; and
- (iii) has increased expression of one or more genes other than the *ESR1* gene.

97. The method of claim 96, wherein the breast cancer has progressed during prior CDK4/6 inhibitor therapy.

98. The method of claim 96 or 97, wherein the ER⁺ breast cancer is HER2⁻.

99. The method of claim 98, wherein the ER⁺ breast cancer is locally advanced.

100. The method of claim 98, wherein the ER⁺ breast cancer is metastatic.

101. The method of any one of claims 96-100, wherein lasofoxifene is administered as lasofoxifene tartrate.

102. The method of any one of claims 96-101, wherein lasofoxifene is administered orally at 5 mg/day.

103. The method of any one of claims 96-102, wherein the CDK4/6i administered to the patient is selected from palbociclib, ribociclib, and abemaciclib.

104. The method of claim 103, wherein the CDK4/6i administered to the patient is abemaciclib.

105. The method of claim 104, wherein abemaciclib is administered orally at 50 mg to 200 mg BID.

106. The method of claim 105, wherein abemaciclib is administered orally at 100 mg to 200 mg BID.
107. The method of claim 106, wherein abemaciclib is administered orally at 150 mg BID.
108. The method of any one of claims 96-107, wherein the prior administered CDK4/6 inhibitor is selected from palbociclib, ribociclib, and abemaciclib.
109. The method of claim 108, wherein the prior administered CDK4/6 inhibitor is abemaciclib.
110. The method of any one of claims 96-109, further comprising the earlier step of detecting increased expression of the one or more genes other than ESR1 in circulating tumor DNA (ctDNA).
111. The method of claim 110, wherein at least one of the one or more genes with increased expression is selected from ABL1, AKT1, AKT2, ALK, APC, AR, ARID1A, ASXL1, ATM, AURKA, BAP, BAP1, BCL2L11, BCR, BRAF, BRCA1, BRCA2, CCND1, CCND2, CCND3, CCNE1, CDH1, CDK4, CDK6, CDK8, CDKN1A, CDKN1B, CDKN2A, CDKN2B, CEBPA, CTNNB1, DDR2, DNMT3A, E2F3, EGFR, EML4, EPHB2, ERBB2, ERBB3, ESR1, EWSR1, FBXW7, FGF4, FGFR1, FGFR2, FGFR3, FLT3, FRS2, HIF1A, HRAS, IDH1, IDH2, IGF1R, JAK2, KDM6A, KDR, KIF5B, KIT, KRAS, LRP1B, MAP2K1, MAP2K4, MCL1, MDM2, MDM4, MET, MGMT, MLL, MPL, MSH6, MTOR, MYC, NF1, NF2, NKX2-1, NOTCH1, NPM, NRAS, PDGFRA, PIK3CA, PIK3R1, PML, PTEN, PTPRD, RARA, RB1, RET, RICTOR, ROS1, RPTOR, RUNX1, SMAD4, SMARCA4, SOX2, STK11, TET2, TP53, TSC1, TSC2, or VHL.
112. The method of claim 111, wherein each of the one or more genes with increased expression is selected from ABL1, AKT1, AKT2, ALK, APC, AR, ARID1A, ASXL1, ATM, AURKA, BAP, BAP1, BCL2L11, BCR, BRAF, BRCA1, BRCA2, CCND1, CCND2, CCND3, CCNE1, CDH1, CDK4, CDK6, CDK8, CDKN1A, CDKN1B, CDKN2A, CDKN2B, CEBPA, CTNNB1, DDR2, DNMT3A, E2F3, EGFR, EML4, EPHB2, ERBB2, ERBB3, ESR1, EWSR1, FBXW7, FGF4, FGFR1, FGFR2, FGFR3, FLT3, FRS2, HIF1A, HRAS, IDH1, IDH2, IGF1R, JAK2, KDM6A, KDR, KIF5B, KIT, KRAS, LRP1B, MAP2K1, MAP2K4, MCL1, MDM2, MDM4, MET, MGMT, MLL, MPL, MSH6, MTOR,

MYC, NF1, NF2, NKX2-1, NOTCH1, NPM, NRAS, PDGFRA, PIK3CA, PIK3R1, PML, PTEN, PTPRD, RARA, RB1, RET, RICTOR, ROS1, RPTOR, RUNX1, SMAD4, SMARCA4, SOX2, STK11, TET2, TP53, TSC1, TSC2, or VHL.

113. The method of any one of claims 96-112, wherein the one or more genes with increased expression is selected from AKT1, AKT2, BRAF, CDK4, CDK6, PIK3CA, PIK3R1, or mTOR.

114. The method of any one of claims 96-113, wherein the cancer has previously been determined to have at least one gain of function missense mutation within the ligand binding domain (LBD) of the Estrogen Receptor 1 (*ESR1*) gene.

115. The method of any one of claims 96-114, further comprising the earlier step of: determining that the patient has at least one gain of function missense mutation within the ligand binding domain (LBD) of the Estrogen Receptor 1 (*ESR1*) gene.

116. The method of any one of claims 96-115, wherein the at least one of gain of function missense mutation is in any one of amino acids D538, Y537, L469, L536, P535, V534, S463, V392, and E380.

117. The method of claim 116, wherein the at least one gain of function missense mutation is in amino acid D538.

118. The method of claim 117, wherein the mutation is D538G.

119. The method of claim 116, wherein the at least one gain of function missense mutation is in the amino acid Y537.

120. The method of claim 119, wherein the mutation is Y537S, Y537N, Y537C, or Y537Q.

121. The method of claim 120, wherein the mutation is Y537C.

122. The method of claim 120, wherein the mutation is Y537S.

123. The method of claim 116, wherein the at least one gain of function missense mutation is in the amino acid L469.

124. The method of claim 123, wherein the mutation is L469V.
125. The method of claim 116, wherein the at least one gain of function missense mutation is in the amino acid L536.
126. The method of claim 125, wherein the mutation is L536R or L536Q.
127. The method of claim 116, wherein the at least one gain of function missense mutation is in the amino acid P535.
128. The method of claim 127, wherein the mutation is P535H.
129. The method of claim 116, wherein the at least one gain of function missense mutation is in the amino acid V534.
130. The method of claim 129, wherein the mutation is V534E.
131. The method of claim 116, wherein the at least one gain of function missense mutation is in the amino acid S463.
132. The method of claim 131, wherein the mutation is S463P.
133. The method of claim 116, wherein the at least one gain of function missense mutation is in the amino acid V392.
134. The method of claim 133, wherein the mutation is V392I.
135. The method of claim 116, wherein the at least one gain of function missense mutation is in the amino acid E380.
136. The method of claim 135, wherein the mutation is E380Q.
137. The method of claim 100, wherein the ER⁺ breast cancer is visceral metastatic.
138. The method of any one of claims 96-137, wherein the breast cancer had progressed on one or more prior endocrine therapies.

139. The method of claim 138, wherein the prior endocrine therapy is a selective ER degrader (SERD), a selective ER modulator (SERM), optionally a SERM other than lasofoxifene, an aromatase inhibitor (AI), a mTOR inhibitor, and/or a PI3K inhibitor.
140. The method of claim 139, wherein the SERD is fulvestrant.
141. The method of claim 139, wherein the mTOR inhibitor is selected from sirolimus, temsirolimus, everolimus, or ridaforolimus.
142. The method of claim 141, wherein the mTOR inhibitor is everolimus.
143. The method of any one of claims 96-142, wherein the patient derives clinical benefit with stable disease for at least 24 weeks, 28 weeks, 32 weeks, 36 weeks, 40 weeks, 44 weeks, 48 weeks, 52 weeks, 56 weeks, 60 weeks, 64 weeks, 68 weeks, 72 weeks, 74 weeks, 78 weeks, 82 weeks, 86 weeks, or longer following commencement of treatment with lasofoxifene or a pharmaceutically acceptable salt thereof and a CDK4/6i.
144. The method of claim 143, wherein the patient has complete or partial response to the treatment with lasofoxifene or a pharmaceutically acceptable salt thereof and a CDK4/6i.
145. The method of any one of claims 96-144, wherein the patient derives a longer duration of stable disease on treatment with lasofoxifene or a pharmaceutically acceptable salt thereof and a CDK4/6i than on the patient's preceding second-line or third-line therapies by at least about 20%, 15%, or 10%.
146. A method of monitoring a patient on a breast cancer treatment, comprising:
- (a) determining quantitative measures of the mutant allele frequency (MAF) in circulating tumor DNA (ctDNA) of at least one gain of function missense mutation within the ligand binding domain (LBD) of the Estrogen Receptor 1 (*ESR1*) gene (*ESR1* ctDNA) in a biological sample of the patient, wherein the quantitative measures are performed over a period of time at determined intervals; and
 - (b) determining a positive predictive value (PPV) for clinical benefit with stable disease of the cancer treatment,
 - wherein the PPV indicates responsiveness to the cancer treatment, and
 - wherein the cancer treatment comprises an effective amount of lasofoxifene or a

pharmaceutically acceptable salt thereof and an effective amount of a CDK4/6 inhibitor (CDK4/6i).

147. The method of claim 146, wherein the patient has decreased MAF post treatment.

148. The method of any one of claims 146-147, wherein the quantitative measures of MAF are determined at 0 week, 4 weeks, 8 weeks, 12 weeks, 16 weeks, 20 weeks, and/or 24 weeks.

149. The method of claim 148, wherein the quantitative measures of MAF are determined at every 4 weeks.

150. The method of any one of claims 146-149, wherein the patient derives clinical benefit with stable disease for at least 24 weeks, 28 weeks, 32 weeks, 36 weeks, 40 weeks, 44 weeks, 48 weeks, 52 weeks, 56 weeks, 60 weeks, 64 weeks, 68 weeks, 72 weeks, 74 weeks, 78 weeks, 82 weeks, 86 weeks, or longer.

151. The method of any one of claims 146-150, wherein the biological sample is blood, plasma, or serum.

152. The method of any one of claims 146-151, further comprising the earlier step of: determining that the patient has at least one gain of function missense mutation within the ligand binding domain (LBD) of the Estrogen Receptor 1 (*ESR1*) gene.

153. The method of claim 152, wherein the at least one of gain of function missense mutation is in any one of amino acids D538, Y537, L469, L536, P535, V534, S463, V392, and E380.

154. The method of claim 153, wherein the at least one gain of function missense mutation is in amino acid D538.

155. The method of claim 154, wherein the mutation is D538G.

156. The method of claim 152, wherein the at least one gain of function missense mutation is in the amino acid Y537.

157. The method of claim 152, wherein the mutation is Y537S, Y537N, Y537C, or Y537Q.
158. The method of claim 157, wherein the mutation is Y537C.
159. The method of claim 157, wherein the mutation is Y537S.
160. The method of claim 152, wherein the at least one gain of function missense mutation is in the amino acid L469.
161. The method of claim 160, wherein the mutation is L469V.
162. The method of claim 152, wherein the at least one gain of function missense mutation is in the amino acid L536.
163. The method of claim 162, wherein the mutation is L536R or L536Q.
164. The method of claim 152, wherein the at least one gain of function missense mutation is in the amino acid P535.
165. The method of claim 164, wherein the mutation is P535H.
166. The method of claim 152, wherein the at least one gain of function missense mutation is in the amino acid V534.
167. The method of claim 166, wherein the mutation is V534E.
168. The method of claim 152, wherein the at least one gain of function missense mutation is in the amino acid S463.
169. The method of claim 168, wherein the mutation is S463P.
170. The method of claim 152, wherein the at least one gain of function missense mutation is in the amino acid V392.
171. The method of claim 170, wherein the mutation is V392I.
172. The method of claim 152, wherein the at least one gain of function missense mutation is in the amino acid E380.

173. The method of claim 172, wherein the mutation is E380Q.
174. The method of claim any one of claims 146-173, wherein the breast cancer is ER⁺ breast cancer.
175. The method of claim 174, wherein the ER⁺ breast cancer is HER2⁻.
176. The method of claim 174 or 175, wherein the ER⁺ breast cancer is locally advanced.
177. The method of claim 174 or 175, wherein the ER⁺ breast cancer is metastatic.
178. The method of any one of claims 146-177, wherein lasofoxifene is administered as lasofoxifene tartrate.
179. The method of any one of claims 146-178, wherein lasofoxifene is administered at 5 mg/day per os.
180. The method of any one of claims 146-179, wherein the CDK4/6i administered to the patient is selected from palbociclib, ribociclib, and abemaciclib.
181. The method of claim 180, wherein the CDK4/6i administered to the patient is abemaciclib.
182. The method of claim 181, wherein abemaciclib is administered orally at 50 mg to 200 mg BID.
183. The method of claim 182, wherein abemaciclib is administered orally at 100 mg to 200 mg BID.
184. The method of claim 183, wherein abemaciclib is administered orally at 150 mg BID.
185. The method of any one of claims 146-184, wherein the subject has progressed during prior CDK4/6 inhibitor therapy.
186. The method of claim 185, wherein the CDK4/6i administered to the patient is selected from palbociclib, ribociclib, and abemaciclib.

187. The method of claim 186, wherein the CDK4/6i administered to the patient is abemaciclib.

188. The method of any one of claims 146-187, wherein the subject has an oncogenic mutation in one or more genes other than *ESR1*.

189. The method of claim 188, wherein at least one of the one or more genes with an oncogenic mutation is selected from HNF1A, TERT, GATA3, CDK12, MAPK3, GNAS, RAF1, RHEB, NTRK3, TP53, PIK3CA, APC, ATM, MET, CCND1, EGFR, ROS1, BRCA2, STK11, FGFR1, ARID1A, CCNE1, AR, ALK, ERBB2, KIT, CDK4, SMAD4, NOTCH1, RB1, BRAF, PTEN, AKT1, CDH1, BRCA1, MYC, CDKN2A, IDH2, MTOR, or PDGFRA.

190. The method of claim 189, wherein each of the one or more genes with an oncogenic mutation is selected from HNF1A, TERT, GATA3, CDK12, MAPK3, GNAS, RAF1, RHEB, NTRK3, TP53, PIK3CA, APC, ATM, MET, CCND1, EGFR, ROS1, BRCA2, STK11, FGFR1, ARID1A, CCNE1, AR, ALK, ERBB2, KIT, CDK4, SMAD4, NOTCH1, RB1, BRAF, PTEN, AKT1, CDH1, BRCA1, MYC, CDKN2A, IDH2, MTOR, or PDGFRA.

191. The method of any one of claims 146-190, wherein the one or more genes with an oncogenic mutation is selected from HNF1A, TERT, GATA3, CDK12, MAPK3, TP53, PIK3CA, APC, ATM, MET, CCND1, EGFR, ROS1, STK11, FGFR1, ARID1A, CCNE1, AR, ALK, ERBB2, KIT, CDK4, SMAD4, NOTCH1, RB1, BRAF, RAF1, PTEN, AKT1, CDH1, BRCA1, MYC, CDKN2A, or PDGFRA.

192. The method of any one of claims 146-190, wherein the one or more genes with an oncogenic mutation is selected from CCND1, FGFR1, CCNE1, AR, ALK, MAPK3, KIT, SMAD4, NOTCH1, RB1, BRAF, RAF1, PTEN, AKT1, CDH1, BRCA1, MYC, CDKN2A, or PDGFRA.

193. The method of any one of claims 146-190, wherein the one or more genes with an oncogenic mutation is selected from HNF1A, TERT, GATA3, CDK12, MAPK3, TP53, PIK3CA, APC, ATM, MET, CCND1, EGFR, ROS1, BRCA2, STK11, FGFR1, ARID1A, CCNE1, AR, ALK, ERBB2, KIT, BRAF, or CDK4.

194. The method of claim 193, wherein the one or more genes with an oncogenic mutation is selected from TP53, PIK3CA, CCND1, ARID1A, FGFR1, CCNE1 and ERBB2.

195. The method of claim 194, wherein the oncogenic mutation is PIK3CA.

196. The method of any one of claims 146-190, wherein the one or more genes with an oncogenic mutation is selected from HNF1A, TERT, GATA3, CDK12, MAPK3, GNAS, RAF1, RHEB, or NTRK3.

197. The method of any one of claims 146-196, wherein the subject has increased expression of one or more genes other than *ESR1*.

198. The method of claim 197, wherein the at least one of the one or more genes with an increased expression is selected from ABL1, AKT1, AKT2, ALK, APC, AR, ARID1A, ASXL1, ATM, AURKA, BAP, BAP1, BCL2L11, BCR, BRAF, BRCA1, BRCA2, CCND1, CCND2, CCND3, CCNE1, CDH1, CDK4, CDK6, CDK8, CDKN1A, CDKN1B, CDKN2A, CDKN2B, CEBPA, CTNNB1, DDR2, DNMT3A, E2F3, EGFR, EML4, EPHB2, ERBB2, ERBB3, ESR1, EWSR1, FBXW7, FGF4, FGFR1, FGFR2, FGFR3, FLT3, FRS2, HIF1A, HRAS, IDH1, IDH2, IGF1R, JAK2, KDM6A, KDR, KIF5B, KIT, KRAS, LRP1B, MAP2K1, MAP2K4, MCL1, MDM2, MDM4, MET, MGMT, MLL, MPL, MSH6, MTOR, MYC, NF1, NF2, NKX2-1, NOTCH1, NPM, NRAS, PDGFRA, PIK3CA, PIK3R1, PML, PTEN, PTPRD, RARA, RB1, RET, RICTOR, ROS1, RPTOR, RUNX1, SMAD4, SMARCA4, SOX2, STK11, TET2, TP53, TSC1, TSC2, or VHL.

199. The method of claim 198, wherein each of the one or more genes with an increased expression is selected from ABL1, AKT1, AKT2, ALK, APC, AR, ARID1A, ASXL1, ATM, AURKA, BAP, BAP1, BCL2L11, BCR, BRAF, BRCA1, BRCA2, CCND1, CCND2, CCND3, CCNE1, CDH1, CDK4, CDK6, CDK8, CDKN1A, CDKN1B, CDKN2A, CDKN2B, CEBPA, CTNNB1, DDR2, DNMT3A, E2F3, EGFR, EML4, EPHB2, ERBB2, ERBB3, ESR1, EWSR1, FBXW7, FGF4, FGFR1, FGFR2, FGFR3, FLT3, FRS2, HIF1A, HRAS, IDH1, IDH2, IGF1R, JAK2, KDM6A, KDR, KIF5B, KIT, KRAS, LRP1B, MAP2K1, MAP2K4, MCL1, MDM2, MDM4, MET, MGMT, MLL, MPL, MSH6, MTOR, MYC, NF1, NF2, NKX2-1, NOTCH1, NPM, NRAS, PDGFRA, PIK3CA, PIK3R1, PML, PTEN, PTPRD, RARA, RB1, RET, RICTOR, ROS1, RPTOR, RUNX1, SMAD4, SMARCA4, SOX2, STK11, TET2, TP53, TSC1, TSC2, or VHL.

200. The method of any one of claims 197-199, wherein the one or more genes with an increased expression is selected from AKT1, AKT2, BRAF, CDK4, CDK6, PIK3CA, PIK3R1, or mTOR.
201. The method of claim 177, wherein the ER⁺ breast cancer is visceral metastatic.
202. The method of any one of claims 146-201, wherein the breast cancer had progressed on one or more prior endocrine therapies.
203. The method of claim 202, wherein the prior endocrine therapy is a selective ER degrader (SERD), a selective ER modulator (SERM), an aromatase inhibitor (AI), a mTOR inhibitor, and/or a PI3K inhibitor.
204. The method of claim 203, wherein the SERD is fulvestrant.
205. The method of claim 203, wherein the mTOR inhibitor is selected from sirolimus, temsirolimus, everolimus, and ridaforolimus.
206. The method of claim 205, wherein the mTOR inhibitor is everolimus.