ALLIANCE FOR CLINICAL TRIALS IN ONCOLOGY

ALLIANCE A211102

TESTING FOR ATYPIA IN RANDOM PERIAREOLAR FINE NEEDLE ASPIRATION (RPFNA) CYTOLOGY AFTER 12 MONTHS METFORMIN (1, 1-DIMETHYLBIGUANIDE HYDROCHLORIDE) CHEMOPREVENTION VERSUS PLACEBO CONTROL IN PREMENOPAUSAL WOMEN

For any communications regarding this protocol, please call the protocol resource person on the following page.

Study Chairs:

[redacted]

Study Co-chairs:

[redacted]

Primary Statistician

[redacted]

Secondary Statistician

[redacted]

Drug Availability IND Exempt (IND #122334)
Supplied Investigational Agents: Placebo for metformin supplied and compounded by Pharm Ops, Inc., in Phillipsburg, NJ.
Commercial Agents: Metformin will be purchased through the McKesson Clinical Research Services.

***Investigator having NCI responsibility for this protocol**
√Study contributor(s) not responsible for patient care.

Participating NCTN Groups: Alliance/Alliance for Clinical Trials in Oncology (lead); ECOG-ACRIN/ECOG-ACRIN Cancer Research Group; NRG/NRG Oncology; SWOG/SWOG

Data Manager

[redacted]

Protocol Coordinator

[redacted]

Study Resources:

Expedited Adverse Event Reporting

[redacted]

Medidata Rave® iMedidata portal

[redacted]

OPEN (Oncology Patient Enrollment Network)

[redacted]

Biospecimen Management System

[redacted]

Protocol Contacts:

A211102 Nursing Contacts

[redacted]

A211102 Pharmacy Contact

[redacted]

Alliance Biorepository at Ohio State

[redacted]

Protocol-related questions may be directed as follows:	
Questions	**Contact (via email)**
Questions regarding patient eligibility, treatment, and dose modification:	Study Chair, Nursing Contact, Protocol Coordinator, and (where applicable) Data Manager
Questions related to data submission, RAVE or patient follow-up:	Data Manager
Questions regarding the protocol document and model informed consent:	Protocol Coordinator
Questions related to IRB review	Alliance Regulatory Inbox [redacted]
Questions regarding CTEP-AERS reporting:	Alliance Pharmacovigilance Inbox [redacted]
Questions regarding specimens/specimen submissions:	Appropriate Alliance Biorepository

CANCER TRIALS SUPPORT UNIT (CTSU) ADDRESS AND CONTACT INFORMATION

For regulatory requirements:	**For patient enrollments:**	**For data submission:**
Regulatory documentation must be submitted to the Cancer Trials Support Unit (CTSU) via the Regulatory Submission Portal. (Sign in at [redacted], and select the Regulatory > Regulatory Submission.) Institutions with patients waiting that are unable to use the Portal should alert the CTSU Regulatory Office immediately by phone or email: [redacted] to receive further instruction and support. Contact the CTSU Regulatory Help Desk at [redacted] for regulatory assistance.	Refer to the patient enrollment section of the protocol for instructions on using the Oncology Patient Enrollment Network (OPEN). OPEN is accessed at [redacted] Contact the CTSU Help Desk with any OPEN related questions by phone or email : [redacted]	Data collection for this study will be done exclusively through Medidata Rave. Refer to the data submission section of the protocol for further instructions.

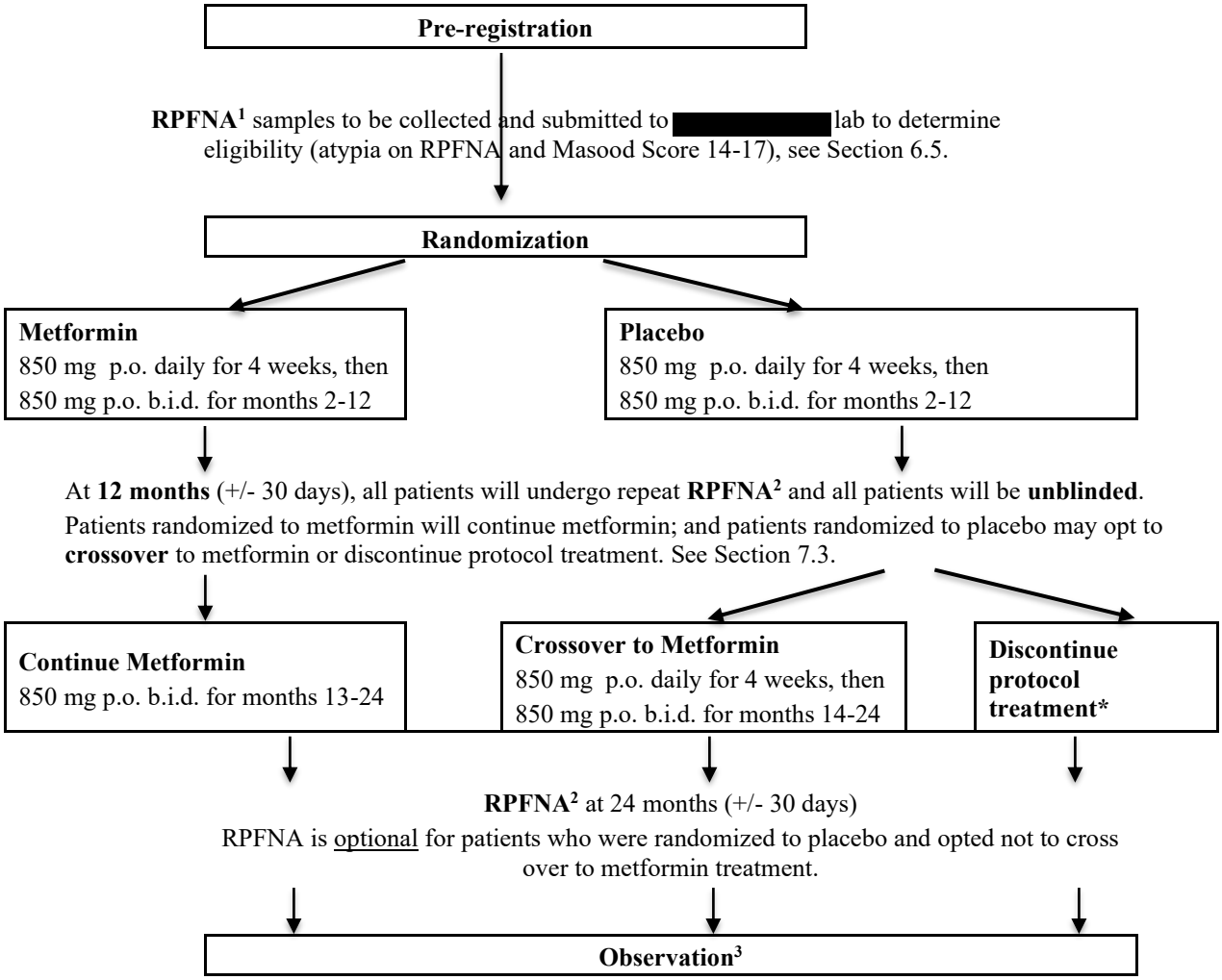
The most current version of the **study protocol and all supporting documents** must be downloaded from the protocol-specific page located on the CTSU members' website ([redacted]). Access to the CTSU members' website is managed through the Cancer Therapy and Evaluation Program - Identity and Access Management (CTEP-IAM) registration system and requires log in with a CTEP-IAM username and password.

Permission to view and download this protocol and its supporting documents is restricted and is based on person and site roster assignment housed in the CTSU Regulatory Support System (RSS).

For clinical questions (i.e. patient eligibility or treatment-related) see the Protocol Contacts, Page 2.

For non-clinical questions (i.e. unrelated to patient eligibility, treatment, or clinical data submission) Contact the CTSU Help Desk by phone or email:
CTSU General Information Line – [redacted] All calls and correspondence will be triaged to the appropriate CTSU representative.

SCHEMA



* Placebo is discontinued; however, assessments continue per protocol.

1 For patients undergoing initial RPFNA at surgery, only the unaffected/non-operated breast should be aspirated. This applies to patients undergoing surgery for DCIS and/or any atypical lesions. Only the contralateral breast can be aspirated in patients with DCIS. Aspiration of the unaffected breast is at the discretion of the subject's treating surgeon.

2 Only the breast(s) originally aspirated prior to randomization will undergo follow-up RPFNA.

3 Follow up visits will be performed at 36 and 48 months after the start of treatment.

Serum and whole blood samples for mandatory correlative science studies will be collected prior to treatment, and at 6, 12, and 24 months (see Section 14.0).

Site RPFNA Credentialing: Sites must either have been previously approved for participation in CALGB 70806 or routinely perform RPFNA (> 10 RPFNA per year); OR undergo RPFNA training (see Section 6.2.5).

Please refer to the full protocol text for eligibility criteria and a complete description of the treatment plan.

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1.0 BACKGROUND

1.1 Background

There is a need for alternative prevention strategies with acceptable toxicities. Tamoxifen (Tam) is currently standard of care for breast cancer prevention in women with atypia. While there are clear benefits to Tam, Tam does not prevent estrogen receptor-negative (ER-) breast cancer and only prevents half of ER (+) breast cancer[1, 2]. Additional interventions are needed to prevent ER (-) breast cancer.

We have performed over 3,000 RPFNA and have not found a RPFNA that was suspicious for cancer (unless we already knew that the woman was being evaluated via a surgical work up for suspected cancer). We include a detailed description of information that is communicated to women undergoing RPFNA regarding their cytology results (see Section 14.6.1.2). Women are told that this RPFNA is an investigational protocol. If findings are found that are suspicious for cancer the woman will have a clinical breast examination, mammogram, and, if clinically appropriate, breast magnetic resonance imaging.

1.2 Rationale

1.2.1 Evidence for Metformin from Population and Clinical Studies

Metformin (1,1-dimethylbiguanide hydrochloride) is a well-tolerated biguanide currently undergoing testing for secondary breast cancer prevention in women with prior breast cancer. Metformin belongs to a biguanide class of oral hypoglycemic agents and prescribed to over 120 million Type II diabetic patients worldwide, with an excellent safety profile. Recently published population studies suggest that metformin decreases the incidence of cancer and cancer-related mortality in diabetic patients[3-17]. Other clinical and epidemiologic evidence links hyperinsulinemia and insulin resistance to increased mitogenic effects and thus to increased risk of several cancers, in addition to leading to poor breast cancer outcomes. In addition, it is also hypothesized that insulin can promote tumorigenesis via a direct effect on epithelial tissues, or indirectly by affecting the levels of other modulators, such as insulin-like growth factors, sex hormones, and adipokines. In a limited retrospective study of patients who received neoadjuvant chemotherapy for breast cancer showed that diabetic cancer patients receiving metformin during neoadjuvant chemotherapy had a higher pathological complete response rate than diabetic patients not receiving metformin (24% versus 8%, p = 0.007) providing evidence for the potential role of metformin as an antineoplastic agent for breast cancer[10].

1.2.2 Evidence for Metformin from in vitro and preclinical studies

The antineoplastic effects of metformin in breast cancer are supported by its modulation of signaling keynodes associated with breast cancer[9-17]. Several lines of evidence demonstrate that metformin inhibits the growth of tumor cells, including breast cancer cells. In the liver, Metformin inhibits transcription of key gluconeogenesis genes and increases glucose uptake in skeletal muscle. It reduces levels of circulating glucose, increases insulin sensitivity, and reduces insulin resistance-associated hyperinsulinemia. There is evidence that metformin exerts its anti-diabetic effects through inhibition of complex 1 of the mitochondrial respiratory chain[16]. At the level of cell signaling, several mechanisms of metformin action have been proposed; the most important one relates to the activation of AMPK. Metformin regulates the AMPK/mTOR pathway. Work by Zakikhani, et al. demonstrated that Metformin inhibits the growth of breast cancer cells in an AMPK-dependent manner[17]. Several tumor suppressors are involved in the AMPK signaling network and activated AMPK results in suppression of cell proliferation in normal and tumor cells[9-17]. The growth

inhibition was associated with decreased mTOR activation and a general decrease in mRNA translation. Given the potential complexity of metformin, it is important to design biomarkers to evaluate the full range of metformin's downstream signaling targets.

1.3 Feasibility and Preliminary Studies

1.3.1 Random Periareolar Fine Needle Aspiration (RPFNA)

RPFNA is a research technique developed to test for early cytological changes in women who are at high-risk for developing breast cancer[18-20]. RPFNA has been prospectively validated in high-risk women to test for 1) short-term breast cancer risk and 2) response to chemoprevention[18-20]. The presence of atypia in RPFNA predicts a 5-6-fold independent increase in breast cancer risk in high-risk women. These observations support the use of cellular atypia in RPFNA as an independent surrogate marker of short-term breast cancer risk in high-risk women[19].

1.3.2 CALGB RPFNA Study

There is increasing acceptance of RPFNA. The Cancer and Leukemia Group B (CALGB) Prevention Group tested the reproducibility of RPFNA in a multi-institutional cross-sectional study[21]. Sixty-three high-risk women from five CALGB institutions (Duke, Ohio State, Roswell Park, Dana Farber, and Vermont) underwent RPFNA. Duplicate bilateral RPFNA was performed on each woman by a single investigator on a single day. Masood Cytology Index score was assessed by a single blinded cytopathologist. We observed a high degree of statistical agreement in the Masood Cytology Index scores of duplicate RPFNA samples from the same breast, with a Spearman correlation coefficient of 0.8312 ($p<0.0001$)[21]. Our multi-institutional study demonstrates that RPFNA is a reproducible measure of breast cytology in a cooperative group cross-sectional trial. RPFNA did not demonstrate a high degree of agreement between breasts, suggesting that breast cancer risk and progression may occur at different rates in individual breasts from a single woman. These studies provide proof-of-principle for future RPFNA-based studies.

1.3.3 RPFNA and Response to Tam Prevention

In a single institution Pilot and Feasibility analysis at Duke University we tested for persistent atypia in a heterogeneous collection of 40 high-risk women who took Tam prevention. Women underwent RPFNA before and after Tam chemoprevention administered at 20 mg/day. The presence or absence of atypia was measured by a single blinded cytopathologist using a 1) five point qualitative descriptor (normal, hyperplasia, atypia, suspicious, cancer) and 2) a numeric scoring system, the Masood Cytology Score Index. After 12 months of Tam, approximately half of the women had persistent cytological atypia in their RPFNA aspirate and half had disappearance of cytological atypia. RPFNA was performed at 18 and 24 months; no woman who had persistent atypia at 12 months had disappearance of atypia at 18 and 24 months.

1.3.4 High Through-Put Reverse Phase Protein Microarray of RPFNA

Reverse Phase Protein Microarray (RPPM) profiling of RPFNA cytology is able to detect protein phosphorylation patterning in 10,000 micro-dissected epithelial cells[22-31]. In our published studies[31,32], RPPM profiling of RPFNA cytology was tested in 5,000 micro dissected epithelial cells in duplicate. Each array was scanned; spot intensity was analyzed; data were normalized; and a standardized, single data value was generated for each sample on the array (Image Quant v5.2). Spot intensity was integrated over a fixed area. Local area background intensity was calculated for each spot with the unprinted adjacent slide background.

1.3.5 RPPM analysis of RPFNA cytology

In a single institution study at Duke University, we tested for the reproducibility of RPPM measurements for 53 individual proteins in quadruplicate. RPFNA samples from two independent test sets of 38 and 39 high-risk women. Importantly, there was a high level of community participation in this trial. Of the 77 women enrolled in this trial, 45% (35/77) of women in this trial were African American. Five thousand epithelial cells were Microdissected, solubilized, and tested by RPPM. Duplicate samples were prepared from micro dissection of paired RPFNA aspirates. Fifty of 53 proteins tested by RPPM had an interclass correlation coefficient of agreement of >0.80. These 50 proteins included EGFR, EGFR-pTyr992, EGFR-pTyr1148, ErbB2, ErbB2-pTyr1248, ErbB3, ErbB3-pTyr1289, IGF1R, ERα, ERα-pSer118, IRS1-pSer612, MEK-pSer217/221, ERK-pThr202/Tyr204, HIF1α, VEGF, GSK3β, AKT-pSer473, mTOR-Ser2448, 4EBP1-pThr37/46, eIF4G-pSer209, 70S6K-pThr412, FKHR-pSer256, IKB-pSer32-36, NFκB(p65)-pSer536, Bad-pSer136, Bcl2-pSer70, 14-3-3-ζ, p38MAPK, p90RSK, PTEN-pSer380, p53, p21, Stat3, Stat5, Src-pTyr416, Fak-pTyr576, paxillin, βcatenin-pThr42/Ser41, Snail-1, vimentin, E-cadherin, pACC-pSer79, and IRS1-pSer612[31].

Three high-intensity ER(-) clusters were identified: 1) Cluster #1 activated Akt/mTOR/insulin/Stat3/vimentin: AKTpSer473, mTORpSer2448, 4EBP1pThr37/46, eIF4GpSer209, 70S6KpThr412, p38MAPK, p90RSK, 14-3-3, Src-pTyr416, Fak-pTyr576, Stat3-pTyr694, Stat3-pSer727, vimentin, ACC-pSer79, and IRS1-pSer612. **2) Cluster #2** activated EGFR/MEK/ERK:EGFR, EGFRpTyr1068, MEKpSer217/221, ERKpThr202/Tyr204, HIF1α, VEGF, GSK3β-pTyr279/216, βcatenin-pThr42/Ser41. **3) Cluster #3** mitochondrial survival proteins Bad, Bad-pSer137, Bcl2-pSer270, Bax, Bcl-xl, PTEN-Ser137.

These data demonstrate our ability to test for phosphoprotein expression in mammary cytology from high-risk women. We also provide evidence for our ability to recruit African American women to RPFNA trials. The ability to associate RPFNA cytology with RPPM in a longitudinal study will lead to increased understanding of signaling pathways activated during breast cancer initiation.

1.3.6 Rationale for Metformin Dose

Our rationale for dose selection was to maximize bioavailability and the opportunity to observe efficacy for this new indication, while minimizing toxicity and adverse events in this cohort, based on earlier studies where metformin was used in other indications and age groups.

There is evidence that the use of metformin in individuals with "normal" insulin levels appears to be safe and it reduces insulin levels. Studies by Goodwin *et al*. show that in breast cancer patients given metformin (1500 mg/day), the mean baseline fasting insulin level was in the "normal" range (75.7 pmol/L) and only one woman had an insulin level above 140 pmol/L (the level was 150 pmol/L)[10]. Insulin levels were reduced by 22% without the occurrence of hypoglycemia[10]. Similar safety and insulin-lowering effects of metformin have been reported in non-diabetic, non-breast cancer subjects with normal insulin levels.

In a recent study of polycystic ovary syndrome (PCOS)[53], metformin (2250 mg/day) lowered fasting insulin levels by 18% from a mean baseline insulin of 64.5 pmol/L in women in the lowest quartile of baseline insulin (greater reductions were seen in women with insulin levels markedly above normal). Similar results in PCOS subjects were reported by Roumaldi[54] (mean baseline fasting insulin 47.0 pmol/L, 15% reduction after metformin at a low dose of 1000 mg/day for 6 months). Based on these observations, we anticipate that metformin will safely lower insulin levels but not promote significant hypoglycemia.

There is clinical evidence that fasting insulin levels are associated with poor breast cancer outcomes[4,6]. We have chosen a dose of metformin for breast cancer prevention that mirrors MA-32, an ongoing adjuvant multicenter trial of metformin vs. placebo in women with breast cancer. At the dose we propose Metformin safely lowers insulin levels by about 20% in 1) non-diabetic women with breast cancer and 2) women without cancer who have baseline fasting insulin levels in the normal range[10,53,54]. Evidence in observational, non-interventional trials suggests that metformin use in diabetics may reduce cancer risk and mortality in women receiving chemotherapy for breast cancer[7,10,11]. We hypothesize that the dose of metformin we propose will reduce breast cancer risk through its secondary targeting of insulin/Complex 1 and primary targeting of AMPK/Akt/mTOR.

There is growing evidence that metformin exerts an insulin independent effect on breast cancer via activation of an insulin independent 1) AMPK-dependent energy stress response[7,17] and 2) PI3K/Akt/mTOR. The existence of this direct (non-insulin dependent) metformin's effect on AMPK/mTOR[14] provides justification for inclusion of women with a broad range of insulin levels in rather than limiting enrolment to those with insulin levels above an arbitrarily chosen cut-point. Because metformin is a readily available, inexpensive oral agent, with known (and easily manageable) toxicities, we believe evaluation of its ability to prevent breast cancer will be feasible as well as tolerated.

1.3.7 Summary of Feasibility Data and Significance

Ultimately, the most effective way to reduce breast cancer mortality is to prevent breast cancer. Here we aim to conduct prevention trials using biomarker-guided efficacy evaluation with an agent that targets signaling pathway that is known to be activated in ER- breast cancer. Metformin is a well-tolerated, inexpensive, oral hypoglycemic agent and prescribed to over 120 million Type II diabetic patients worldwide, and is safe in pregnancy. While metformin is undergoing pilot clinical testing in women with breast cancer, metformin's mechanism of action is poorly understood. It is known that metformin targets Akt/mTor, AMPK, and promotes a secondary reduction in mitochondrial complex 1 and insulin signaling[9-17]. However, there is also evidence that metformin may also target NFκB and IL6. Given the potential complexity of metformin, we need to be able to precisely track signaling pathways targeted by metformin prevention.

We have developed the scientific framework, biomarkers, and strategies to test the ability of targeted agents to eliminate cytologic atypia in high-risk women. Using our combined proteomic/tracking tools we can directly test in an individual woman 1) for activated signaling pathways, 2) whether the prevention agent eliminates cytologic atypia, and 3) if not, we can then identify additional signaling pathways that can be targeted. The ability to test whether a prevention drug is working in an individual woman allows us to rapidly deliver targeted prevention tailored to an individual woman.

1.3.8 Proposed Study Overview

Premenopausal women at elevated risk for the development of breast cancer by either family history, a Gail risk greater than or equal to 1.66%, or a prior biopsy containing atypical lobular hyperplasia, atypical ductal hyperplasia, lobular carcinoma in situ, (LCIS) or ductal carcinoma in situ (DCIS) will be considered for RPFNA. RPFNA can be performed in the operating room at the time of surgical excision. Women are required to have atypia on RPFNA of non-radiated breast tissue (Masood Cytology Index Score 14-17). Women who are eligible to take tamoxifen must be offered tamoxifen prevention as part of their clinical care and have refused tamoxifen chemoprevention. Women will be randomized to receive RPFNA and metformin or RPFNA and placebo control.

We propose to test RPFNA at 0, 12, and 24 months (RPFNA at 24 months for the placebo-only group is optional for patients who remain on placebo arm and will not receive metformin) on both right and left breasts after metformin 850 mg p.o. b.i.d. versus placebo control for the 1) presence or absence of atypia or 2) changes in the Masood Cytology Index Score. These determinations will test for the presence or absence of cytological atypia in RPFNA before and after metformin administration and be compared with women who take placebo (control subjects). These studies will also serve as proof of principle for the 1) use of RPFNA to test for the presence or absence of atypia before, during, and after the administration of a prevention agent and 2) utility of cytological atypia in RPFNA to serve as a surrogate biological endpoint in a multi-institutional cross sectional trial. As a secondary objective, we will test the reproducibility of RPPM in duplicate RPPM determinations in a single RPFNA specimen. We will test for the presence of atypia after metformin or placebo; the presence of atypia means any atypia on RPFNA.

2.0 OBJECTIVES

2.1 Primary objective

Test for the presence or absence of cytological atypia (as measured by a Masood Cytology Index Score of 14-17) in unilateral or bilateral RPFNA aspirates after 12 and 24 months (24 month is optional for placebo-only group for patients who remain on placebo arm and will not receive metformin) for women receiving metformin versus placebo control. The presence of cytological atypia means any atypia in any RPFNA specimen.

2.2 Secondary objectives

2.2.1 Use the Masood Cytology Index Score to test for the presence of cytological atypia or disappearance of cytological atypia in RPFNA aspirates after 12 months for both arms, and 24 months (24 month is optional for placebo-only group for patients who remain on placebo arm and will not receive metformin, and mandatory for crossover patients) for women receiving metformin 850 mg p.o. bid (metformin group).

2.2.2 Compare Masood Cytology Score values at 0 and 12 months in right and left breasts (if both breasts were aspirated) from the same individual in the metformin and placebo group.

2.2.3 Test the reproducibility of RPPM in duplicate RPPM determinations from individual RPFNA specimens.

2.2.4 Correlate baseline RPPM values with presence of atypia (as measured by Masood Cytology Index Score) at month 12 and month 24 (month 24 optional for placebo-only group; for patients who remain on placebo arm and will not receive metformin) RPFNA.

2.3 Correlative Research

2.3.1 Test whether metformin alters RPFNA or blood biomarkers associated with breast cancer risk. Surrogate biomarkers of breast cancer risk include the following:

- Atypia - surrogate endpoint biomarker and risk biomarker
- Phosphoprotein expression – exploratory biomarker
- Circulating insulin growth factor (IGF) - surrogate biomarker and risk biomarker

2.3.2 Test whether metformin alters markers associated with obesity and insulin resistance. Markers of obesity and insulin resistance include the following:

- Waist to hip ratio
- Fasting serum insulin, fasting serum glucose
- Hemoglobin A1C (HgbA1c)

2.3.3 Test other exploratory measures in RPFNA and serum including the following:

- Metformin levels
- Estrogen/Progesterone.

2.3.4 Banking

As part of ongoing research for Alliance Cancer Control studies, we are banking residual blood and RPFNA products for future studies.

3.0 PATIENT ELIGIBILITY

3.1 Pre-registration - Inclusion Criteria

Use the spaces provided to confirm a patient's eligibility by indicating Yes or No as appropriate. It is not required to complete or submit the following page(s).

___ **3.1.1 Must be at increased risk for breast cancer, defined as <u>at least one</u> of the following four criteria:**

a. Having had a prior biopsy demonstrating atypical hyperplasia, lobular carcinoma *in situ* (LCIS), or ductal carcinoma *in situ* (DCIS).
b. A Gail Model Risk of ≥1.66% over 5 years.
c. A strong family history of breast and/or ovarian cancer which is defined as at least one of the following:
   - One first-degree relative with breast cancer before the age of 50 years
   - One first degree relative with bilateral breast cancer
   - Two or more first-degree relatives with breast cancer
   - One first degree relative and two or more second or third degree relatives with breast cancer
   - One first-degree relative with breast cancer and one or more relatives with ovarian cancer
   - Two second or third degree relatives with either breast cancer and one or more with ovarian cancer
   - One second or third degree relative with breast cancer and two or more with ovarian cancer
   - Three or more second or third degree relatives with breast cancer
d. Known BRCA1 or BRCA2 mutation carrier providing that the woman has 1) met with a Genetic Counselor to review genetic testing results, and 2) has been offered the opportunity to undergo prophylactic mastectomy and oophorectomy.

___ **3.1.2 Age 25-55 years.**

___ **3.1.3 Pre-menopausal women** as defined as four menstrual cycles within the last six months prior to pre-registration. Women with less than 4 menses within 6 months prior to pre-registration, or women who have had a hysterectomy with ovaries intact will be considered premenopausal if FSH level is < 20. Women who are using hormonal contraceptives that cause amenorrhea (e.g. injectable and extended oral contraceptives, hormone containing contraceptive ring, or hormone containing intrauterine device) will be considered eligible if they had a minimum of 4 menstrual cycles within the last six months prior to starting on the contraceptive.

___ **3.1.4 Digital mammogram within 365 days prior to pre-registration.**

___ **3.1.5 Mammograms must be read as not suspicious for breast cancer (ACR Class I-III).** Subjects with a class IV mammogram may be enrolled once they have been evaluated by a breast surgeon and there is no evidence of invasive malignancy.

___ **3.1.6 Must be non-lactating for at least one year prior to pre-registration.**

___ **3.1.7 If currently menstruating, subjects must use a reliable method of birth control.**

___ **3.1.8 Willing to provide RPFNA and blood samples for correlative research purposes** (see Section 6.0 and 14.0).

3.2 Pre-registration Exclusion Criteria

___ **3.2.1 Other active malignancy ≤5 years prior to pre-registration.** EXCEPTIONS: Non-melanotic skin cancer or carcinoma-in-situ of the cervix. NOTE: If there is a history or prior malignancy, they must not be receiving other specific treatment, i.e., other hormonal therapy, for their cancer.

___ **3.2.2 BMI < 25.**

___ **3.2.3 Receiving warfarin.**

___ **3.2.4 Bilateral breast implants or autologous breast flap reconstruction.**

___ **3.2.5 Active diagnosis of alcoholism**

___ **3.2.6 Contraindication to metformin prevention such as acute hypersensitivity or allergic reaction to metformin.**

___ **3.2.7 Currently receiving tamoxifen or raloxifene.**

___ **3.2.8 Administration of any investigational agent ≤ 30 days prior to pre-registration**.

___ **3.2.9 Previous radiation to both breasts.**

___ **3.2.10 Co-morbid systemic illnesses or other severe concurrent disease** which, in the judgment of the investigator, would make the patient inappropriate for entry into this study or interfere significantly with the proper assessment of safety and toxicity of the prescribed regimens.

___ **3.2.11 Uncontrolled intercurrent illness** including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

___ **3.2.12 Receiving pyrimethamine, cimetidine, rifampin or cephalexin.**

___ **3.2.13 Women who have a core biopsy or excisional biopsy containing invasive cancer.**

___ **3.2.14 Women who have taken metformin within the past 90 days.**

___ **3.2.15 Patients with hemoglobin a1c > 6.3 or who are being actively treated for diabetes.**

3.3 Registration/Randomization Inclusion Criteria

___ **3.3.1 Qualifying cytological atypia in RPFNA, Masood score of 14-17.**

The qualifying RPFNA (of one or both breasts) must be sent to [redacted] laboratory for cytological scoring and proteomic analysis. **Score results must be received from [redacted] lab prior to patient registration/randomization**. Test must be done **≤ 120 days prior to registration/randomization.**

Note: Only the contralateral breast can be aspirated in women with DCIS and those undergoing surgery for an atypical lesion. The decision to aspirate the contralateral breast is at the discretion of the woman's surgeon.

___ **3.3.2 The following laboratory values obtained ≤ 30 days prior to registration/randomization:**

- Hemoglobin ≥ 9 g/dL.
- Absolute neutrophil count (ANC) ≥1500/mm^3
- Platelet count ≥ 75,000/mm^3
- Creatinine ≤ 1.4 mg/dL
- Total bilirubin ≤ 3.0 mg/dL
- Aspartate transaminase (AST) ≤ 3 x upper limit of normal (ULN)
- Alanine transaminase (ALT) ≤ 3 x ULN

___ **3.3.3 Negative pregnancy test** done ≤ 7 days prior to registration/randomization, for women of childbearing potential only.

A female of childbearing potential is a sexually mature female who: 1) has not undergone a hysterectomy or bilateral oophorectomy; or 2) has not been naturally postmenopausal for at least 12 consecutive months (i.e., has had menses at any time in the preceding 12 consecutive months).

___ **3.3.4 Women eligible to take tamoxifen must be offered tamoxifen prevention as part of their clinical care and have refused tamoxifen treatment.**

4.0 TEST SCHEDULE

Tests and Procedures	Active-Monitoring Phase				
	≤ 60 days prior to Pre-registration	**≤ 30 days prior to registration/ randomization**	**During 4 week ramp up***	**During treatment at 6, 12, and 24 months**†**	**Observation at 36 and 48 months†**
History and exam, performance status	X			X	X
Height	X				
Weight/BMI	X			X	X
Waist/Hip ratio	X			X	X
Pregnancy test		X[1]			
Breast Exam	X			X	X
Mammogram	X[2]				
RPFNA[R]		X[3, 8, 9]		X[3, 4, 9]	
Hematocrit, CBC with Diff		X			
Na, K, BUN, Cr, AST, ALT, LDH, Bili		X		X	X
Hemoglobin a1c	X			X	
Glucose (fasting), insulin (fasting),		X		X	X
Adverse event assessment		X	X[5]	X	X
Concomitant Medication	X			X	X
Patient Medication Diary (Appendix III)			X[6]		
Nurse/CRA Phone Contact Guide (Appendix IV)			X[6]		
Mandatory Research Blood Draw (see Section 14.0)[R]		X[7]		X[7]	
Patient-reported knowledge of the arm to which they were randomized				X[10]	

* The ramp-up period occurs during the first 4 weeks of blinded treatment and during the first 4 weeks of open-label treatment for patients who cross over from placebo to metformin.

** Prior to the 12-month clinic visit, the CRA must call the Alliance Registration Office (507-284-4130) to unblind the patient's treatment assignment. Patients who were receiving placebo are eligible to crossover to metformin treatment (see Section 6.8).

† +/- 3 weeks for Month 6 visit; +/- 30 days for Month 12, 24, and 36 visits.

1. For women of childbearing potential only. Must be done ≤7 days prior to registration/randomization.
2. Baseline mammogram must have been done within 365 days of pre-registration. Mammographic screening should be continued as part of the patient's regular medical care.
3. See Section 14.0 for information regarding available RPFNA kits and RPFNA sample submission instructions. See Appendix II for RPFNA collection instructions. Following RPFNA, submit the RPFNA Procedure Documentation Form, available at the Alliance and CTSU A211102 study-specific web pages.
4. RPFNA at 12 months and 24 months ONLY. RPFNA at 24 months is optional for patients who were randomized to placebo and opted not to continue protocol treatment after 12 months.
5. At completion of 4 week ramp-up (phone).
6. The Patient Medication Diary will be provided for each patient for the ramp-up period. Telephone contact will be made with the patient at the end of the ramp up period to assess compliance and adverse events.
7. Blood collections (EDTA whole blood and serum samples) must be collected **Monday-Thursday ONLY.**
8. Pre-treatment RPFNA specimens must be submitted immediately following pre-registration (see Sections 6.6 and 14.0).

9. CRA or provider will make a follow-up phone call the day following the RPFNA to check in with the participant and review any side effects of the procedure (see Appendix V).
10. The single question is to be completed at month 12, i.e. after the 1-year study intervention, but before the patient is unblinded to the study agents and before they crossover to metformin. Patients who are beyond the 12-month intervention or off study at the time of this amendment should still be reached by the study coordinator – despite the patient already being unblinded to the study agents - via telephone to answer the single question or to coordinate a time for the patient to come in to complete the single question.

R Research funded (see the A211102 funding sheet, available on the Alliance and CTSU web sites).

5.0 DATA SUBMISSION

5.1 Data Collection and Submission

Medidata Rave is a clinical data management system being used for data collection for this trial/study. Access to the trial in Rave is controlled through the CTEP-IAM system and role assignments.

Requirements to access Rave via iMedidata:

- A valid CTEP-IAM account; and
- Assigned a Rave role on the LPO or PO roster at the enrolling site of: Rave CRA, Rave Read Only, Rave CRA (LabAdmin), Rave SLA, or Rave Investigator.

Rave role requirements:

- Rave CRA or Rave CRA (Lab Admin) role must have a minimum of an Associate Plus (AP) registration type;
- Rave Investigator role must be registered as an Non-Physician Investigator (NPIVR) or Investigator (IVR); and
- Rave Read Only role must have at a minimum an Associates (A) registration type.

Refer to [redacted] for registration types and documentation required.

Upon initial site registration approval for the study in Regulatory Support System (RSS), all persons with Rave roles assigned on the appropriate roster will be sent a study invitation email from iMedidata. To accept the invitation, site staff must either click on the link in the email or log in to iMedidata via the CTSU members' website under *Data Management > Rave Home* and click to *accept* the invitation in the *Tasks* pane located in the upper right corner of the iMedidata screen. Site staff will not be able to access the study in Rave until all required Medidata and study specific trainings are completed. Trainings will be in the form of electronic learnings (eLearnings) and can be accessed by clicking on the eLearning link in the *Tasks* pane located in the upper right corner of the iMedidata screen. If an eLearning is required for a study and has not yet been taken, the link to the eLearning will appear under the study name in the *Studies* pane located in the center of the iMedidata screen; once the successful completion of the eLearning has been recorded, access to the study in Rave will be granted, and a *Rave EDC* link will replace the eLearning link under the study name.

Site staff that have not previously activated their iMedidata/Rave account at the time of initial site registration approval for the study in RSS will receive a separate invitation from iMedidata to activate their account. Account activation instructions are located on the CTSU website in the Data Management section under the Rave resource materials (Medidata Account Activation and Study Invitation Acceptance). Additional information on iMedidata/Rave is available on the CTSU members' website in the Data Management > Rave section at [redacted] or by contacting the CTSU Help Desk at [redacted]

5.1.1 Data Quality Portal

The Data Quality Portal (DQP) provides a central location for site staff to manage unanswered queries and form delinquencies, monitor data quality and timeliness, generate reports, and review metrics.

The DQP is located on the CTSU members' website under Data Management. The Rave Home section displays a table providing summary counts of Total Delinquencies and Total Queries. DQP Queries, DQP Delinquent Forms, DQP Form Status and the DQP Reports modules are available to access details and reports of unanswered queries, delinquent forms, forms with current status and timeliness reports. Review the DQP modules on a regular basis to manage specified queries and delinquent forms.

The DQP is accessible by site staff that are rostered to a site and have access to the CTSU website. Staff that have Rave study access can access the Rave study data using a direct link on the DQP.

To learn more about DQP use and access, click on the Help icon displayed on the Rave Home, DQP Queries, DQP Delinquent Forms, DQP Form Status, and DQP Reports modules.

6.0 PRE-REGISTRATION AND REGISTRATION/RANDOMIZATION PROCEDURES

6.1 CTEP Investigator Registration

Food and Drug Administration (FDA) regulations and National Cancer Institute (NCI) policy require all individuals contributing to NCI-sponsored trials to register and renew their registration annually. To register, all individuals must obtain a Cancer Therapy Evaluation Program (CTEP) Identity and Access Management (IAM) account at [redacted] In addition, persons with a registration type of Investigator (IVR), Non-Physician Investigator (NPIVR), or Associate Plus (AP) must complete their annual registration using CTEP's web-based Registration and Credential Repository (RCR) at [redacted]

RCR utilizes five person registration types.

- IVR — MD, DO, or international equivalent;
- NPIVR — advanced practice providers (e.g., NP or PA) or graduate level researchers (e.g., PhD);
- AP — clinical site staff (e.g., RN or CRA) with data entry access to CTSU applications such as the Roster Update Management System [RUMS], OPEN, Rave, acting as a primary site contact, or with consenting privileges;
- Associate (A) — other clinical site staff involved in the conduct of NCI-sponsored trials; and
- Associate Basic (AB) — individuals (e.g., pharmaceutical company employees) with limited access to NCI-supported systems.

RCR requires the following registration documents:

Documentation Required	IVR	NPIVR	AP	A	AB
FDA Form 1572	✔	✔			
Financial Disclosure Form	✔	✔	✔		
NCI Biosketch (education, training, employment, license, and certification)	✔	✔	✔		
GCP training	✔	✔	✔		
Agent Shipment Form (if applicable)	✔				
CV (optional)	✔	✔	✔		

An active CTEP-IAM user account and appropriate RCR registration is required to access all CTEP and Cancer Trials Support Unit (CTSU) websites and applications. In addition, IVRs and NPIVRs must list all clinical practice sites and Institutional Review Boards (IRBs) covering their practice sites on the FDA Form 1572 in RCR to allow the following:

- Addition to a site roster;
- Assign the treating, credit, consenting, or drug shipment (IVR only) tasks in OPEN;
- Act as the site-protocol Principal Investigator (PI) on the IRB approval; and

In addition, all investigators acting as the Site-Protocol PI (investigator listed on the IRB approval), consenting/treating/drug shipment investigator in OPEN, or as the CI on the DTL must be rostered at the enrolling site with a participating organization.

Additional information is located on the CTEP website at [redacted] For questions, please contact the **RCR Help Desk** by email at [redacted]

6.2 Cancer Trials Support Unit Site Registration Procedures

Permission to view and download this protocol and its supporting documents is restricted and is based on person and site roster assignment housed in the CTSU Regulatory Support System (RSS).

This study is supported by the NCI Cancer Trials Support Unit (CTSU).

IRB Approval

For CTEP and Division of Cancer Prevention (DCP) studies open to the National Clinical Trials Network (NCTN) and NCI Community Oncology Research Program (NCORP) Research Bases after March 1, 2019, all U.S.-based sites must be members of the NCI Central Institutional Review Board (NCI CIRB). In addition, U.S.-based sites must accept the NCI CIRB review to activate new studies at the site after March 1, 2019. Local IRB review will continue to be accepted for studies that are not reviewed by the CIRB, or if the study was previously open at the site under the local IRB. International sites should continue to submit Research Ethics Board (REB) approval to the CTSU Regulatory Office following country-specific regulations.

Sites using their local IRB or REB, must submit their approval to the CTSU Regulatory Office using the Regulatory Submission Portal located in the Regulatory section of the CTSU website. Acceptable documentation of local IRB/REB approval includes:

- Local IRB documentation;
- IRB-signed CTSU IRB Certification Form; and/or
- Protocol of Human Subjects Assurance Identification/IRB Certification/Declaration of Exemption Form.

In addition, the Site-Protocol Principal Investigator (PI) (i.e. the investigator on the IRB/REB approval) must meet the following criteria in order for the processing of the IRB/REB approval record to be completed:

- Holds an active CTEP status;
- Active status at the site(s) on the IRB/REB approval (applies to US and Canadian sites only) on at least one participating organization's roster;
- If using NCI CIRB, active on the NCI CIRB roster under the applicable CIRB Signatory Institution(s) record;
- Includes the IRB number of the IRB providing approval in the Form FDA 1572 in the RCR profile;
- Lists all sites on the IRB/REB approval as Practice Sites in the Form FDA 1572 in the RCR profile; and
- Holds the appropriate CTEP registration type for the protocol.

Additional Requirements

Additional site requirements to obtain an approved site registration status include:

- An active Federal Wide Assurance (FWA) number;
- An active roster affiliation with the Lead Protocol Organization (LPO) or a Participating Organization (PO);
- Compliance with all protocol-specific requirements (PSRs).

6.2.1 Downloading Site Registration Documents

Download the site registration forms from the protocol-specific page located on the CTSU members' website. Permission to view and download this protocol and its supporting documents is restricted to institutions and its associated investigators and staff on a participating roster. To view/download site registration forms:

- Log in to the CTSU members' website ([redacted]) using your CTEP-IAM username and password;

- Click on *Protocols* in the upper left of the screen:
    - Enter the protocol number in the search field at the top of the protocol tree; or
    - Click on the By Lead Organization folder to expand, then select *Alliance*, and protocol number *A211102*.
- Click on *Documents*, *Protocol Related Documents*, and use the *Document Type* filter and select Site Registration to download and complete the forms provided. (Note: For sites under the CIRB, IRB data will load automatically to the CTSU.)

6.2.2 Requirements for A211102 Site Registration

- IRB approval (For sites not participating via the NCI CIRB; local IRB documentation, an IRB-signed CTSU IRB Certification Form, Protocol of Human Subjects Assurance Identification/IRB Certification/Declaration of Exemption Form, or combination is accepted)
- Completed and signed [redacted] RPFNA Training Form (see Section 6.3.4)

6.2.3 Submitting Regulatory Documents

Submit required forms and documents to the CTSU Regulatory Office using the Regulatory Submission Portal on the CTSU members' website.

To access the Regulatory Submission Portal log in to the CTSU members' website, go to the *Regulatory* section and select *Regulatory Submission*.

Institutions with patients waiting that are unable to use the Regulatory Submission Portal should alert the CTSU Regulatory Office immediately by phone or email: [redacted] in order to receive further instruction and support.

6.2.4 Checking Site's Registration Status

Site registration status may be verified on the CTSU members' website.

- Click on *Regulatory* at the top of the screen;
- Click on *Site Registration*; and
- Enter the sites 5-character CTEP Institution Code and click on Go:
    - Additional filters are available to sort by Protocol, Registration Status, Protocol Status, and/or IRB Type.

Note: The status shown only reflects institutional compliance with site registration requirements as outlined within the protocol. It does not reflect compliance with protocol requirements for individuals participating on the protocol or the enrolling investigator's status with NCI or their affiliated networks.

6.2.5 Site credentialing for RPFNA requirement

6.2.5.1 Participating institutions must have a RPFNA Training Form (RTF) on file with CTSU. If this form has been previously submitted to CTSU it does not need to be resubmitted unless personnel updates have occurred at the enrolling site. This training form will be available on the Alliance and CTSU websites as a supporting document to the protocol.

The completed RTF is to be sent by email to [redacted] After receiving documentation of either previous approval to perform RPFNA (see

Section 6.2.5.2) or completion of training, [redacted] will complete the signature line of the form and return it to the site. Site staff will then submit the form to the CTSU Regulatory Portal per Section 6.2.3.

6.2.5.2 Sites that either have been approved for participation in CALGB 70806 or routinely perform RPFNA (> 10 RPFNA per year) do not need to undergo RPFNA training and can indicate this on the RTF.

6.2.5.3 RPFNA training

For sites that wish to undergo RPFNA training, this can consist of either 1) video training and phone conferencing with [redacted] or, 2) on-site training at [redacted] Contact information for training is:

[redacted]

For sites that desire electronic training, there is a training video that can be sent and [redacted] can be available either by phone or email to answer questions and provide suggestions and feedback

We have an on-site training program for any institution that is interested in performing RPFNA in the operating room and in the clinic. In our prior CALGB study testing of the reproducibility of RPFNA, all interested sites sent a team to Duke University for RPFNA training. Typically, teams sent for training included either a surgeon or a physician assistant/midlevel provider who was well skilled in breast procedures.

6.3 Patient Registration Requirements

Informed consent: The patient must be aware of the neoplastic nature of her disease and willingly consent after being informed of the procedure to be followed, the experimental nature of the therapy, alternatives, potential benefits, side-effects, risks, and discomforts. Current human protection committee approval of this protocol and a consent form is required prior to patient consent and registration.

Protected Health Information: RPFNA and whole blood collected for this study will be sent directly to City of Hope Hospital in California. These samples will be labeled with patient initials, study ID, and collection date.

6.4 Patient pre-registration (Step 0) procedures

The Oncology Patient Enrollment Network (OPEN) is a web-based registration system available on a 24/7 basis. OPEN is integrated with CTSU regulatory and roster data and with the LPOs registration/randomization systems or the Theradex Interactive Web Response System (IWRS) for retrieval of patient registration/randomization assignment. OPEN will populate the patient enrollment data in NCI's clinical data management system, Medidata Rave.

Requirements for OPEN access:

- A valid CTEP-IAM account;
- To perform enrollments or request slot reservations: Must be on an LPO roster, ETCTN corresponding roster, or participating organization roster with the role of Registrar. Registrars must hold a minimum of an Associate Plus (AP) registration type;
- If a Delegation of Tasks Log (DTL) is required for the study, the registrars must hold the OPEN Registrar task on the DTL for the site; and
- Have an approved site registration for the protocol prior to patient enrollment.

To assign an Investigator (IVR) or Non-Physician Investigator (NPIVR) as the treating, crediting, consenting, drug shipment (IVR only), or receiving investigator for a patient transfer in OPEN, the IVR or NPIVR must list the IRB number used on the site's IRB approval on their Form FDA 1572 in RCR. If a DTL is required for the study, the IVR or NPIVR must be assigned the appropriate OPEN-related tasks on the DTL.

Prior to accessing OPEN, site staff should verify the following:

- Patient has met all eligibility criteria within the protocol stated timeframes; and
- All patients have signed an appropriate consent form and Health Insurance Portability and Accountability Act (HIPAA) authorization form (if applicable).

Note: The OPEN system will provide the site with a printable confirmation of registration and treatment information. You may print this confirmation for your records.

Access OPEN at [redacted] or from the OPEN link on the CTSU members' website. Further instructional information is in the OPEN section of the CTSU website at [redacted] For any additional questions, contact the CTSU Help Desk at [redacted]

6.5 Central review of RPFNA samples

After pre-registration and prior to registration/randomization, RPFNA samples must be collected and shipped to [redacted] laboratory per Section 14.0 for determination of eligibility (cytological atypia defined as Masood score of 14-17). [redacted] laboratory will report the results within 30 days (20 business days) from receipt of RPFNA specimens. The results, along with the cytology report, will be sent to the registering institution and the Alliance Pathology Coordinators at Mayo Clinic Rochester. The Alliance Pathology Coordinator will then go into the pre-approval application, mark the patient as approved or not approved. See Section 14.6.1 for an explanation of the cytology report.

If approved, the patient may be registered and randomized to the study.

Email Template (to be completed by [redacted]

Study Number: A211102
Patient ID:
Approved or Not approved:
Site contact information:
CRA/Study Coordinator's name:
Phone number:
E-mail address:

6.6 Registration/Randomization (step 1) procedures

6.6.1 All site staff will use OPEN to enroll patients to this study. OPEN can be accessed at [redacted] or from the OPEN tab on the CTSU members' side of the website at [redacted] Note: The OPEN system will provide the site with a printable confirmation of registration. Please print this confirmation for your records.

6.6.2 Treatment on this protocol must commence at the accruing institution under the supervision of an Alliance member physician.

6.6.3 Study treatment cannot begin prior to registration/randomization and must begin ≤14 days after registration/randomization.

6.6.4 Pre-treatment tests/procedures (see Section 4.0) must be completed within the guidelines specified on the test schedule.

6.6.5 All pre-treatment symptoms/conditions must be evaluated and documented at baseline (see Section 10.5.1).

6.6.6 RPFNA results are available on site.

Note: Once the above conditions have been met, access the OPEN website and follow the instructions for registration/randomization.

6.6.7 Correlative Research:

6.6.7.1 A mandatory correlative research component (i.e., RPFNA and blood samples) is part of this study for all patients, the patient will be automatically registered onto this component (see Sections 3, 4, and 14 of the protocol).

6.6.7.2 Patient has/has not given permission for coded samples and related coded information to be kept for use in future research to learn about, prevent, or treat cancer. This may also include research on inherited traits (genes passed on in families).

6.6.7.3 Patient has/has not given permission for coded samples and related coded information to be kept for use in future research to learn about, prevent, find or treat other health problems (for example: diabetes, Alzheimer's disease, or heart disease). This may also include research on inherited traits (genes passed on in families).

6.6.7.4 Patient has/has not given permission for someone from their hospital or the Alliance to contact them in the future to ask to take part in more research.

6.7 Stratification factors

The stratification factors defined below will be used at the time of patient registration:

- BRCA mutation (Yes vs. No mutation or unknown).
- Prior abnormal excisional biopsy results (Yes vs. No or unknown).
- Fasting baseline insulin: (Fasting insulin > 2x ULN vs. Fasting Insulin ≤ 2x ULN)

After the patient has been registered to the study, the values of the stratification factors will be recorded. The patient then will be assigned to one of the following treatment groups using the Pocock and Simon[60] dynamic allocation procedure which balances the marginal distributions of the stratification factors between the treatment groups:

- Metformin
- Placebo

6.8 Procedures for Double-Blinding the Treatment Assignment

After the treatment assignment has been ascertained in the OPEN application, the patient's study medication code number will be displayed on the confirmation of registration screen. If the code number is not displayed, contact the Registration Office at [redacted]

The number of the treatment bottle assigned to the patient will be recorded on the Treatment Form by institutional staff.

6.9 Open-label and crossover re-registration (step 2) procedures

At the time of the 12-month RPFNA visit, all patients will be unblinded per Section 7.3.

All patients who were randomized to receive metformin will be re-registered and begin receiving open-label metformin as described below.

Patients who were randomized to receive placebo and elect to cross over to open-label metformin therapy will be re-registered to receive open-label metformin as follows:

Patient re-registration steps:

- Log into the OPEN registration system and select the appropriate patient.
- Select the next registration step.
- Complete OPEN Enrollment Form for the patient to be registered to the open-label arm.
- Enter Cycle # of the last treatment received during blinded treatment, as well as the stratification factors, on the OPEN Enrollment Form.
- Once the open-label registration has been successfully completed confirmation will be displayed in OPEN.

If assistance is needed with this process, contact the Alliance Registration Office.

Following re-registration, open-label metformin should be ordered per Section 15.1.8.

7.0 PROTOCOL TREATMENT

7.1 Treatment Schedule

7.1.1 Months 1-12

Agent	Dose and Duration
Metformin /Placebo	850 mg (1 tablet) p.o. one a day for 4 weeks (ramp-up period), then 850 mg (1 tablet) p.o. twice a day, (pending gastrointestinal toxicity evaluation) for months 2-12.

7.1.2 Months 13-24 (See Section 7.3)

For unblinded patients originally randomized to metformin:	
Agent	**Dose and Duration**
Metformin	850mg (1 tablet) p.o. twice a day for months 13-24

For unblinded patients originally randomized to placebo who choose to crossover to metformin:	
Agent	**Dose and Duration**
Metformin	850 mg (1 tablet) p.o. one a day for 4 weeks (ramp-up period), then 850 mg (1 tablet) p.o. twice a day, (pending gastrointestinal toxicity evaluation) for months 2-12.

For unblinded patients originally randomized to placebo who choose <u>not</u> to crossover to metformin:	
Agent	**Dose and Duration**
None	Months 13-24

7.2 Patient Monitoring

A Patient Medication Diary will be provided for each patient for the ramp-up period and telephone contact will be made with the patient one month +/- 3 days after enrollment to assess compliance, toxicity, and to ensure the patient has increased medication to twice daily. (The diary is intended only as a tool to assist subjects in adjusting to full dose at the correct time after starting study treatment.) A record of the telephone contact will be made on the Nurse/CRA Phone Contact Guide (see Appendix IV). Any problems identified will be communicated to the responsible Investigator.

Optimal medication compliance is defined as ≥ 80%. Patients found to be non-adherent with their medication will receive additional interventions by the treatment team to improve adherence (i.e., registered nurse phone calls as a first-line intervention and extra study visits will be scheduled if non-adherence is still noted at the next follow-up time point).

7.3 Crossover

During the first 12 months of treatment, patients will not be unblinded except in cases of emergencies (see Section 7.5)

Prior to the patient's 12 month clinic visit, the CRA must call the Alliance Registration Office [redacted] to find out which study agent the patient is receiving.

7.3.1 Patients who were receiving metformin will be re-registered to receive open-label metformin.

7.3.2 Patients who were receiving placebo are eligible to cross over to metformin therapy.

If the patient was receiving placebo, the CRA must contact her and ask her if she wishes to

1) receive metformin 850 p.o. bid for 12 mos. (including 4-week ramp-up of 850 mg p.o. qd) or
2) discontinue protocol therapy.

If the patient wishes to crossover to metformin therapy, the CRA will then re-register the patient per Section 6.9 and to receive open label metformin, and the patient will be administered the drug as outlined in Section 7.1.2.

Patient Monitoring: The Patient Medication Diary will be provided for each patient who crosses over from placebo to open-label metformin to be completed during the ramp-up period and telephone contact will be made with the patient one month +/- 3 days after crossover to assess compliance, toxicity, and to ensure the patient has increased medication to twice daily. (The diary is intended only as a tool to assist patients in adjusting to full dose at the correct time after starting study treatment.) A record of the telephone contact will be made on the Nurse/CRA Phone Contact Guide (see Appendix IV). Any problems identified will be communicated to the responsible Investigator.

7.4 Emergency unblinding

Before the crossover at 12 months, unblinding may be done only in cases of an emergency. Follow the directions below to unblind patient treatment. Please note that if a treatment assignment is unblinded, the patient must discontinue protocol therapy.

Emergency Unblinding Procedures:

Examples of emergencies include 1) a life-threatening unexpected adverse event that is at least possibly related to the investigational agent and for which unblinding would influence treatment decisions; or 2) medication error, such as accidental overdose. Expected adverse events are listed in the "Toxicities" section below.

Contact the Alliance Executive Officer on call by calling [redacted]

The institution must provide the following information to the Alliance Executive Officer:

- Alliance study ID ("A211102")
- Alliance patient ID number (e.g., "999999")
- Patient initials (e.g., "L,FM")
- Institution name
- Name and telephone number of treating physician
- Name and contact information of person requesting the unblinding procedure
- Name and contact information of person to inform of treatment assignment
- Reason for emergency unblinding

Please remember that emergency unblinding request may be authorized only by an Alliance Executive Officer, and emergency unblinding applies only if unblinding would influence management of the medical situation.

After the Executive Officer deems unblinding is warranted, the treatment assignment will be provided to the contact person at the treating site.

8.0 DOSAGE MODIFICATION BASED ON ADVERSE EVENTS

8.1 Summary

The major toxic effects of metformin which limit dose are gastrointestinal (GI) (nausea, abdominal bloating, and diarrhea). Dose adjustments, for reasons of GI side effects, will be as in the tables below. (Please note that none of the recommended dose adjustments requires splitting of study medication pills. Pills are not to be split or crushed, prior to taking, for any reason.) Breaks of up to 4 weeks consecutively or 8 weeks overall are allowed to ascertain the cause of symptoms. Beyond this, treatment is at the discretion of the treating physician.

8.2 Dose modifications

Strictly follow the modifications in this table, until individual treatment tolerance can be ascertained. Thereafter, these modifications should be regarded as guidelines to define mild-to-moderate, but not debilitating, side effects. If multiple adverse events are seen, administer dose based on greatest reduction required for any single adverse event observed. Reductions or increases apply to treatment given in the preceding cycle and are based on adverse events observed since the prior dose.

8.2.1 If the patient has grade 3 or 4 diarrhea, metformin/placebo should be discontinued.

8.2.2 Dose modification tables

→ → *Use the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0* unless otherwise specified* ← ←			
CTCAE System/Organ/Class (SOC)	**ADVERSE EVENT**	**AGENT**	**ACTION**
BASED ON INTERVAL ADVERSE EVENT			
Gastrointestinal disorders	Grade 1 Abdominal distension Bloating Diarrhea Nausea Vomiting Gastrointestinal disorders – Other, specify	Metformin	Patient should be encouraged to remain on full dose. If patient is unwilling, delay study treatment for one week. For subjects who were unwilling to remain on full dose, after one week without study drug, follow the ramp-up procedure. If Grade 1 symptoms persist or recur during the ramp-up or at full dose and the patient is unwilling to continue, follow instructions for Grade 2 toxicity.
Gastrointestinal disorders	≥ Grade 2 or higher Abdominal distension Bloating Diarrhea Nausea Vomiting Gastrointestinal disorders – Other, specify	Metformin	Reduce to one tablet per day for one week. If symptoms resolve or are Grade 1 only, after one week, resume full dose (2 tablets per day). If symptoms persist or recur at Grade 2 or higher, delay study treatment for 4 weeks, then resume study treatment as follows: Re-start study drug according to original ramp-up schedule (one tablet per day for 4 weeks then one tablet twice a day if tolerated). If toxicity persists or recurs, the study drug should be adjusted to the maximum dose that is tolerated with Grade 1 toxicity or lower. A second attempt to increase to full dose should be made 3 months later and if the full dose is not tolerated at that time, the maximum tolerated dose should be used for the remainder of the 2-year (metformin group) or 1-year (Placebo crossover group) intervention.

* Located at [redacted]

9.0 ANCILLARY TREATMENT/SUPPORTIVE CARE

This is a prevention trial. If the patient clinically requires hydration and hospitalization, all appropriate supportive measures should be used.

10.0 ADVERSE EVENT (AE) REPORTING AND MONITORING

10.1 Adverse Event Characteristics

CTCAE term (AE description) and grade: The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for expedited AE reporting. However, CTCAE v5.0 must be used for serious AE reporting through CTEP-AERS as of April 1, 2018. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site: [redacted]

10.1.1 Adverse event monitoring and reporting is a routine part of every clinical trial. First, identify and grade the severity of the event using the CTCAE. Next, determine whether the event is expected or unexpected (see Section 10.2) and if the adverse event is related to the medical treatment or procedure (see Section 10.3). With this information, determine whether the event must be reported as an expedited report (see Section 10.4). Important: Expedited adverse event reporting requires submission of an Adverse Event Reporting System (CTEP-AERS) report(s). Expedited reports are to be completed within the timeframes and via the mechanisms specified in Sections 10.4 and 10.5. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for expedited AE reporting beginning April 1, 2018. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP website: [redacted] All AEs reported via expedited mechanisms must also be reported via the routine data reporting mechanisms defined by the protocol (see Sections 10.5 and the schedule of forms).

10.1.2 Each CTCAE term in the current version is a unique representation of a specific event used for medical documentation and scientific analysis and is a single MedDRA Lowest Level Term (LLT). Grade is an essential element of the guidelines and, in general, relates to **severity** for the purposes of regulatory reporting to NCI.

- **NOTE:** A severe AE, as defined by the above grading scale, is **NOT** the same as serious AE which is defined in the table in Section 10.4.

10.2 Expected vs. Unexpected Adverse Events (AEs)

The determination of whether an AE is expected is based on agent-specific information provided in Section 15.0 of the protocol.

Unexpected AEs are those not listed in the agent-specific information provided in Section 15.0 of the protocol.

NOTE: "Unexpected adverse events" means any adverse event that is identified in nature, severity, or frequency of risk in the information neither provided for IRB review nor mentioned in the consent form.

10.3 Assessment of Attribution

10.3.1 When assessing whether an adverse event is related to a medical treatment or procedure, the following attribution categories are utilized:

Definite - The adverse event is clearly related to the agent(s).

Probable - The adverse event is likely related to the agent(s).

Possible - The adverse event may be related to the agent(s).

Unlikely - The adverse event is doubtfully related to the agent(s).

Unrelated - The adverse event is clearly NOT related to the agent(s).

Events determined to be possibly, probably or definitely attributed to a medical treatment suggest there is evidence to indicate a causal relationship between the drug and the adverse event.

10.3.2 Persistent or Significant Disabilities/Incapacities

Any AE that results in persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions (formerly referred to as disabilities), congenital anomalies or birth defects, must be reported immediately if they occur at any time following treatment with an agent under an IND/IDE since they are considered to be a serious AE and must be reported to the sponsor as specified in 21 CFR 312.64(b).

10.3.3 Death

Note: A death on study requires both routine and expedited reporting, regardless of causality. Attribution to treatment or other cause must be provided.

- Death due to progressive disease should be reported as **Grade 5 "Disease progression"** in the system organ class (SOC) "General disorders and administration site conditions." Evidence that the death was a manifestation of underlying disease (e.g., radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted.
- Any death occurring within 30 days of the last dose, regardless of attribution to an agent/intervention under an IND/IDE requires expedited reporting within 24-hours.
- Any death occurring greater than 30 days with an attribution of possible, probable, or definite to an agent/intervention under an IND/IDE requires expedited reporting within 24-hours.
- Pregnancy loss: Pregnancy loss is defined in CTCAE as "Death in utero." Any Pregnancy loss should be reported expeditiously, as Grade 4 "Pregnancy loss" under the Pregnancy, puerperium and perinatal conditions SOC. A Pregnancy loss should NOT be reported as a Grade 5 event under the Pregnancy, puerperium and perinatal conditions SOC, as currently CTEP-AERS recognizes this event as a patient death.
- Death Neonatal: A neonatal death should be reported expeditiously as Grade 4, "Death neonatal" under the General disorders and administration SOC.

10.4 Expedited Reporting Requirements for Studies using Commercial Agent(s) ONLY:

10.4.1 Expedited Reporting Requirements for Adverse Events that Occur in a Non-IND/IDE trial within 30 Days of the Last Administration of a Commercial Agent [1, 2].

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)

NOTE: Investigators **MUST** immediately report to the sponsor (NCI) **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)

An adverse event is considered serious if it results in **ANY** of the following outcomes:

1) Death
2) A life-threatening adverse event
3) An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

ALL SERIOUS adverse events that meet the above criteria **MUST** be immediately reported to the NCI via CTEP-AERS within the timeframes detailed in the table below.

<table>
<tr><th>Hospitalization</th><th>• Grade 1 Timeframes</th><th>• Grade 2 Timeframes</th><th>• Grade 3 Timeframes</th><th>Grade 4 & 5 Timeframes</th></tr>
<tr><td>Resulting in Hospitalization ≥ 24 hrs</td><td colspan="3">10 Calendar Days</td><td rowspan="2">24-Hour;
5 Calendar Days</td></tr>
<tr><td>Not resulting in Hospitalization ≥ 24 hrs</td><td colspan="2">Not required</td><td>10 Calendar Days</td></tr>
</table>

NOTE: Protocol specific exceptions to expedited reporting of serious adverse events are found in the Specific Protocol Exceptions to Expedited Reporting (SPEER) portion of the CAEPR

Expedited AE reporting timelines are defined as:

- "24-Hour; 5 Calendar Days" - The AE must initially be reported via CTEP-AERS ≤ 24 hours of learning of the AE, followed by a complete expedited report ≤ 5 calendar days of the initial 24-hour report.
- "10 Calendar Days" - A complete expedited report on the AE must be submitted ≤ 10 calendar days of learning of the AE.

[1] Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:

Expedited 24-hour notification followed by complete report ≤ 5 calendar days for:

- All Grade 4, and Grade 5 AEs

Expedited 10 calendar day reports for:

- Grade 2 adverse events resulting in hospitalization or prolongation of hospitalization

- Grade 3 adverse events

[2] For studies using PET or SPECT IND agents, the AE reporting period is limited to 10 radioactive half lives, rounded UP to the nearest whole day, after the agent/intervention was last administered. Footnote "1" above applies after this reporting period.

Refer to Section 10.4.3 for NCI Contact Information or Technical Help regarding CTEP-AERS reporting.

10.4.2 Additional Instructions or Exceptions to Adverse Event Reporting System (CTEP-AERS) Reporting Requirements:

The Alliance SAE Coordinator will forward a copy of all CTEP-AERS reports to the Alliance IND Coordinator who will notify the FDA as warranted by the event and stipulated in the U.S. Code of Federal Regulations.

In the rare event when internet connectivity is disrupted, a 24 hour notification must be made to NCI by telephone. An electronic report must be submitted immediately upon establishment of internet re-connection.

10.4.3 Contact Information for NCI Safety Reporting:

Website for submitting Adverse events (CTEP-AERS)	[redacted]
CTEP-AERS MD Help Phone (for CTEP)*	[redacted]Monday through Friday, 7:00 AM to 7:00 PM (US Eastern Time)
CIP Help Phone for SAE reporting*	[redacted]Monday through Friday, 7:00 AM to 7:00 PM (US Eastern Time)
Fax for expedited report supporting Medical Documentation for CTEP trials	[redacted]
Fax for expedited report supporting Medical Documentation for CIP trials	[redacted]
CTEP-AERS MD Help Email:	[redacted]
CIP SAE Reporting Email	[redacted]
Technical (e.g., IT or computer issues ONLY) Help Phone*	[redacted]
Practice application	[redacted]
CTEP-AERS Technical Help Email	[redacted]
CTCAE v5.0 Help/Questions Email	[redacted]
CTEP-AERS Computer Based Training link	[redacted]

* Office phone and fax are accessible 24 hrs per day 7 days a week (The CTEP-AERS MD phone line is staffed from Monday through Friday, 7:00 AM to 7:00 PM ET. Any phone call after these hours will go to voicemail. Please leave contact information and the phone call will be returned the following business day.

10.5 Other Required Event Reporting

Event Type	REPORTING PROCEDURE
Other Grade 4 or 5 Events and/or Any Hospitalizations During Treatment Not Otherwise Warranting an Expedited Report	Complete the Grade 4 or 5 Non-AER Reportable Events/Hospitalization Form electronically via the Remote Data Entry System within 5 working days of the date the Clinical Research Associate (CRA) is aware of the event(s) necessitating the form. If an CTEP-AERS report has been submitted, this form does not need to be submitted.

10.5.1 Adverse events to be graded at each evaluation and pretreatment symptoms/conditions to be evaluated at baseline per CTCAE v4.0 grading unless otherwise stated in the table below:

System Organ Class (SOC)	Adverse event	Baseline	Each evaluation
Gastrointestinal disorders	Abdominal distension	X	X
	Bloating	X	X
	Diarrhea	X	X
	Nausea	X	X
	Vomiting	X	X
	Gastrointestinal disorders – Other, specify	X	X
	Gastrointestinal disorders – Other, # of stools / day	X	

10.5.2 Submit via appropriate Alliance Case Report Forms (i.e., paper or electronic, as applicable) the following AEs experienced by a patient and not specified in Section 10.5.1.

10.5.2.1 Grade 2 AEs deemed *possibly, probably, or definitely* related to the study treatment or procedure.

10.5.2.2 Grade 3 and 4 AEs regardless of attribution to the study treatment or procedure.

10.5.2.3 Grade 5 AEs (Deaths)

- Any death within 30 days of the patient's last study treatment or procedure regardless of attribution to the study treatment or procedure.
- Any death more than 30 days after the patient's last study treatment or procedure that is felt to be at least possibly treatment related must also be submitted as a Grade 5 AE, with a CTCAE type and attribution assigned.

10.5.3 Refer to the instructions in the Forms Packet (or electronic data entry screens, as applicable) regarding the submission of late occurring AEs following completion of the Active Monitoring Phase (i.e., compliance with Test Schedule in Section 4.0).

11.0 TREATMENT EVALUATION

Study outcomes are outlined in Section 16.0.

11.1 Primary endpoint

As a primary endpoint we will test for the presence or absence of cytological atypia in RPFNA bilateral aspirates after 12 and 24 months (24 month is optional for placebo-only group for patients who remain on placebo arm and will not receive metformin) for women receiving metformin versus placebo control. The presence of cytological atypia means any atypia in any RPFNA specimen.

11.2 Secondary endpoints

As secondary endpoints we will compare Masood Cytology Score values at 0 and 12 months in right and left breasts from the same individual in the Metformin and placebo group, test the reproducibility of RPPM in duplicate RPPM determinations from individual RPFNA specimens.

12.0 DESCRIPTIVE FACTORS

None

13.0 TREATMENT/FOLLOW–UP DECISION AT EVALUATION OF PATIENT

13.1 Discontinuation due to side effects

Patients who discontinue study medication before the completion of metformin due to side effects should still be followed per the test schedule for data collection time points. If the patient refuses, then the patient should be removed from study. Off Treatment Form must be submitted per the schedule of forms.

13.2 Diagnosis of breast cancer

Upon the diagnosis of breast cancer the patient should undergo standard therapy for the treatment and removal of the cancer. The Off Treatment Form must be submitted per the schedule of forms and the patient will go directly to off treatment.

13.3 Pregnancy

If a patient should become pregnant, the study medication will be discontinued and the patient should still be followed per the test schedule for data collection time points. The Off Treatment Form must be submitted per the schedule of forms.

13.4 Ineligible patients

A patient is deemed *ineligible* if after registration/randomization, it is determined that at the time of registration/randomization, the patient did not satisfy each and every eligibility criteria for study entry.

- If the patient received treatment, all data up until the point of confirmation of ineligibility must be submitted. The Off Treatment Form must be submitted per the schedule of forms and the patient will go directly to off treatment.
- If the patient never received treatment, on-study material must be submitted.

13.5 Protocol deviations

A protocol deviation is deemed a *major violation*, if protocol requirements during the first month of therapy are so severely violated that evaluability for the primary end point is questionable. All data up until the point of confirmation of a major violation must be submitted. The Off Treatment Form must be submitted per the schedule of forms and the patient would be off treatment.

13.6 Cancellations

A patient is deemed a *cancel* if she is removed from the study for any reason before any study treatment is given. On-study material and the Off Treatment Form must be submitted and patient would be off treatment. The patient should be followed as required.

13.7 Tamoxifen, raloxifene, pyrimethamine, cimetidine, rifampin and cephalexin

If the patient is found at any time during the study to be taking tamoxifen, raloxifene, pyrimethamine, cimetidine, rifampin or cephalexin the patient would go off treatment immediately.

14.0 CORRELATIVE STUDIES

14.1 Summary

For all patients registered to Alliance A211102: The submission of the biomarkers and biospecimens listed in Table 14.1 is required for all patients registered/randomized to this study. Rationale and methods for the scientific components of these studies are described in Section 14.6.

Table 14.1
Summary of Mandatory A211102 Biomarker/Biospecimen Collections for Correlative Studies

Biomarker/ Biospecimen	Mandatory Collection time point	Correlative Studies
Random Periareolar Fine Needle Aspiration (RPFNA) on both breasts[1]	Prior to treatment[*] 12 months 24 months[2]	• Atypia - surrogate endpoint biomarker and risk biomarker • Estrogen/Progesterone – exploratory biomarker • Phosphoprotein expression – exploratory biomarker (See Section 14.6.1 for additional information.)
Research Serum	Prior to treatment 6 months 12 months 24 months	• Insulin growth factor (IGF), estrogen, progesterone – Surrogate markers of breast cancer risk • Metformin levels (banked serum) – exploratory biomarker (See Section 14.6.2 for additional information.)
Whole blood (for DNA/plasma White blood cells)	Prior to treatment	• White Blood Cell Complex 1 • DNA imprinting • Pharmacogenomic studies (See Section 14.6.3 for additional information.)
Measures of Obesity and Insulin Resistance	Prior to treatment 6 months 12 months 24 months 36 months 48 months	• BMI • Waist to hip ratio • Serum glucose (fasting) [3], insulin (fasting) [3]– markers of obesity and insulin resistance (See Section 14.6.4 for additional information.)

[*] Pretreatment RPFNA must be submitted immediately following pre-registration.
[1] Note: Only the contralateral breast can be aspirated in women with DCIS and those undergoing surgery for an atypical lesion.
[2] RPFNA at 24 months for patients who were randomized to placebo and opted not to continue protocol treatment after 12 months is optional.
[3] These tests will be performed in the participating site's clinical lab.

14.2 Biospecimen Kits

14.2.1 Participating institutions may obtain kits for RPFNA sample collection and submission by completing the RPFNA Supply Order Form (found on the A211102 page of the Alliance and CTSU Web sites) and emailing it to the email address listed on the form. Fill out the site address to which the kits will be shipped on the form. Because the Alliance is charged for all outgoing kits, a small, but sufficient supply of the specimen collection kits should be ordered prior to patient entry.

14.2.2 Kits will be sent via FedEx® Ground at no additional cost to the participating institutions. Allow at least two weeks to receive the kits. Kits will not be sent via rush delivery service unless the participating institution provides their own FedEx® account number or alternate billing number for express service. The Alliance will not cover the cost for rush delivery of kits.

14.2.3 For sites that routinely perform RPFNAs and have materials available at their own institution, kit contents and instructions are provided in Appendix II. If you have any questions please call [redacted] at the number(s) listed on the cover page of the protocol.

14.2.4 No kits will be provided for the blood collections for this study.

14.3 Research Biospecimen Collection and Processing

RPFNA and all blood collections (serum and EDTA whole blood samples) must be collected **Monday-Thursday ONLY. All samples** (refrigerated RPFNA, frozen serum, and EDTA whole blood) **must be shipped the same day they are collected**.

Research biospecimens will be collected and processed at the following time points for these studies:

Table 14.3: Mandatory Research Biospecimens to Be Collected and Submitted for this Protocol

Specimen to be submitted by participating site	Processing required at site after specimen collection?	Collection tube and color	Prior to treatment	Months			Shipping conditions
				6	12	24	
RPFNA (both right and left breasts)[3]	No	10 mL modified Cytolyte in 15 mL conical tube (1 tube per breast)	X*		X	X[1]	Styrofoam insulated box with 2-3 blue ice packs to [redacted]
Research Serum[2]	Yes	SST 1 x 8.5 mL	X	X	X	X	Frozen, Styrofoam insulated box with dry ice to **OSU**
Research Serum[2]	Yes	Red top (no additive) 2 x 10 mL 1 x 5 mL	X	X	X	X	Frozen, Styrofoam insulated box with dry ice to **OSU**
Whole Blood[2]	No	EDTA (purple top) 2 x 10 mL	X				Styrofoam insulated box with 2-3 blue ice packs to [redacted]

* Pretreatment RPFNA must be submitted immediately following pre-registration.

1 This RPFNA collection is optional for patients who were randomized to placebo and opted not to continue protocol treatment after 12 months.

2 Order of blood draw: 1) SST tube, 2) 3 red top no-additive tubes, and 3) two EDTA tubes last.

3 Only contralateral breast is to be aspirated for patients with DCIS or surgery for atypical lesion.

14.3.1 RPFNA collection and submission

Kits are available for this collection (see Section 14.2.1). The RPFNA kit contains all necessary supplies and instructions for collecting, processing, and shipping RPFNA specimens. For sites that routinely perform RPFNAs and have materials available at their own institution, kit contents and instructions are provided in Appendix II. **Please include the study ID (A211102), patient initials, collection date, and Alliance patient ID number on all specimen containers and paperwork submitted with the specimens.**

Please note on the shipping form whether the RPFNA specimen was obtained in the operating room or in the clinic.

RPFNA has been performed at Duke University for 9 years with over 400 women participating. Bruising at the site is expected. Otherwise the number of complications is very low. The only reported complication was a single skin infection in a woman who had a prior history of skin infection. This infection resolved rapidly with oral antibiotics. Average pain score is 0-1 out of 10 with 0 being no discomfort and 10 being labor pain. Ativan is offered to all participants. The aspiration takes 15 minutes.

Briefly, RPFNA is performed as follows: Approximately 10^5 cells and 0.5-cc breast fluid will be withdrawn twice from each anaesthetized breast. Two areas in each breast (10:00 and 2:00 position) will be infiltrated with lidocaine and epinephrine. Two 15 mL tubes containing 10 mL modified Cytolyte (Cytolyte 9 mL + 1 mL 10% formalin freshly prepared on the morning of aspiration). The tubes will be provided by [redacted] lab and labeled Right and Left. For each breast, eight (8) 10 cc syringes with 21 gauge 1.5 inch needles will be pre-wetted with about 0.2 mL sterile tissue culture media or saline. Multiple passes will be made through the breast tissue with each needle, with the goal of covering as much of the breast tissue as possible. A total of 8 needles will be used per breast. Half of the aspirations will be made through the 2:00 skin site (4 needles) and half through the 10:00 skin site (4 needles).

14.3.2 Blood collection and serum and whole blood processing

Draw blood sequentially into 1 x 8.5 mL SST tube, 2 x 10 mL and 1 x 5 mL red top (no additive) tubes, and 2 x 10 mL EDTA tubes. Gently invert each tube 4-5 times.

Serum Processing: Allow the blood to clot in the red top and SST tubes for 15-30 minutes at room temperature. Centrifuge at 3000 rpm for 10 minutes. After centrifugation, aspirate the top clear serum layer (being careful to avoid the transfer of cells) and place 1 mL aliquots into 2.0 ml cryogenic vials*. Do NOT mix the serum collected from the SST tube with the serum collected from the red top no-additive tubes.

* Cryovial Choices: Some examples of acceptable 2.0 mL cryovials are: Nalgene (Cat #5012-0020), Fisher (Cat #05-669-57), Corning (Cat #430488), VWR (Cat#16001-102).

Label all serum cryovials with the following information:

1) Alliance study number (i.e., A211102)
2) Alliance patient number
3) Patient's initials (last, first, middle)
4) Procurement date and time
5) Red top-serum or SST-serum (as applicable).

Freeze serum samples at –70°C and keep frozen until ready to ship on dry ice to the Alliance Biorepository at the Ohio State University. Frozen samples should be shipped on dry ice on

the day of collection. Dry ice should be replenished as needed to ensure samples stay frozen and there is enough to last throughout shipment.

Whole blood processing: Label and refrigerate the EDTA tubes until ready to ship with the RPFNA specimen (see Section 14.5.1). There is no further processing required for the EDTA tubes.

Label EDTA tubes with the following information:

1) Alliance study number (i.e., A211102)
2) Alliance patient number
3) Patient's initials (last, first, middle)
4) Procurement date and time
5) Whole blood.

14.4 Biospecimen Submission Using the Alliance Specimen Tracking System

USE OF THE ALLIANCE BIOSPECIMEN MANAGEMENT SYSTEM (BioMS) IS MANDATORY AND ALL SPECIMENS MUST BE LOGGED AND SHIPPED VIA THIS SYSTEM.

BioMS is a web-based system for logging and tracking all biospecimens collected on Alliance trials. Authorized individuals may access BioMS at the following URL: [redacted] using most standard web browsers (Safari, Firefox, Internet Explorer). For information on using the BioMS system, please refer to the 'Help' links on the BioMS web page to access the on-line user manual, FAQs, and training videos. To report technical problems, such as login issues or application errors, please contact: [redacted] For assistance in using the application or questions or problems related to specific specimen logging, please contact: [redacted]

After logging collected specimens in BioMS, the system will create a shipping manifest. This shipping manifest must be printed and placed in the shipment container with the specimens.

Instructions for the collection of samples are provided above in Section 14.3. Please be sure to use a method of shipping that is secure and traceable. Extreme heat precautions should be taken when necessary.

14.5 Biospecimen Shipment

All shipments should follow appropriate International Air Transport Association (IATA) guidelines including shipment in a biohazard bag ([redacted]). At a minimum, all samples must be packaged within two containers to control any spill or leakage. The outer container must be puncture resistant (e.g. cardboard mail tube, corrugated cardboard box). A biohazard sticker must be affixed to both the inner and outer containers.

Collection of multiple specimen types will require shipment in independent packaging (e.g. dry ice shipment for frozen serum, cold packs for RPFNA and whole blood). ALL SPECIMENS should be sent by a reliable courier service with a tracking number (such as FedEx or UPS). Shipment of specimens must comply with appropriate regulations as specified by the carrier. All specimens must be shipped the same day they are collected, **Monday through Thursday**, by overnight service for next day delivery to assure receipt to the appropriate Alliance bio repository or designated non-Alliance laboratory (see below). Do not send specimens Thursday-Saturday or the day before a federal holiday.

14.5.1 **Refrigerated RPFNA and refrigerated EDTA whole blood specimen** shipment on Monday through Thursday by overnight service for next day delivery to assure receipt is encouraged. Do not ship specimens Friday-Saturday or just prior to federal holidays. RPFNA and one EDTA specimens should be placed in a Styrofoam box with a minimum of two cold packs (preferred three cold packs if between the months of May to September). Please call [redacted] if you have questions regarding the shipment. **RPFNA and EDTA whole blood samples** should be sent to the following address:

[redacted]

14.5.2 **Frozen serum specimens** shipment on Monday through Thursday by overnight service for next day delivery to assure receipt is encouraged. Do not ship specimens Friday-Saturday or just prior to federal holidays. Frozen serum samples should be shipped in a Styrofoam box with sufficient dry ice for 48 hours. Please call [redacted] if you have questions regarding the shipment. **Frozen serum samples** should be sent to the following address:

[redacted]

14.6 Correlative Study Methodology and Biospecimen Use/Storage Information

14.6.1 RPFNA Analyses

[redacted] laboratory (City of Hope, Duarte California) will process the specimens and perform ER/PR analyses. [redacted] (Miami, FL) will perform cytology analysis. [redacted] laboratory (George Mason, Manassas, VA) will perform proteomic analysis.

14.6.1.1 **Masood Cytology Index:** Cytology slides will be scored by [redacted] (Miami, FL) utilizing the Masood Cytology Index scoring system. [redacted] was part of the original team that developed RPFNA and has served as the cytopathologist for our prior studies and our published CALGB study. [redacted] will be joined by an additional cytologist, [redacted] at University of Pittsburg. The Masood Cytology Index allows for reproducible identification of early cytological changes in mammary epithelial cells and has been used to evaluate RPFNA cytology in risk assessment trials at Duke University, University of Kansas, and in our prior CALGB trial. For reliability of RPFNA, see Section 1.3 above for discussion of our published CALGB studies testing for reproducibility of RPFNA in our CALGB cross sectional cohort[22].

Cellular morphology	Cellular pleomorphism	Myoepithelial cells	Aniso-nucleosis	Nucleoi	Chromatin clumping	Score*
Monolayer	Absent	Many	Absent	Absent	Absent	**1**

Cellular morphology	Cellular pleomorphism	Myoepithelial cells	Aniso-nucleosis	Nucleoi	Chromatin clumping	Score*
Nucl. overlap	Mild	Moderate	Mild	Micro-nucleoli	Rare	**2**
Clustering	Moderate	Few	Moderate	Micro-nucleoli	Occasional	**3**
Loss cohesion	Conspicuous	Absent	Frequent	Macro-nucleoli	Frequent	**4**

***Sum of Masood scores: 6-10** Normal; **11-13** Hyperplasia; **14-17** Hyperplasia with Atypia; **>17** Suspicious for cancer. Women with scores of ≥17 will be referred to a breast surgeon for evaluation.

14.6.1.2 All women will be informed of the RPFNA results. Following are the details of the RPFNA information that will be provided to the patient:

- Participants are told that RPFNA is a research technique.
- Participants are told that RPFNA is ***not*** a diagnostic (cancer finding) technique. Examples of diagnostic techniques are provided to subjects and include mammography and breast MRI.
- Participants are told that the presence of atypia in high-risk women increases a woman's chance of subsequently developing breast cancer. It does not matter what technique is used to find atypia (excisional biopsy, core needle biopsy, FNA, or RPFNA), but that the presence of atypia increases the subsequent risk of breast cancer in high-risk women. The article Fabian et al. JNCI, 2000 may be provided to women to provide evidence that the presence of atypia in RPFNA increases breast cancer risk. This article is available on request.
- Participants are told that atypia is not cancer and that not all women with atypia will develop breast cancer. Sometimes atypia will go away of its own accord, sometimes atypia will stay atypia, and in some situations atypia will progress to become cancer. Currently, we do not have the ability to determine which specific woman with atypia will go on to develop breast cancer. This is the long-term goal of our research as well as the research of many other investigators.
- Participants are told that we may find atypia in RPFNA (about 30% of samples, common), and we may find cells that are suspicious for cancer (<1%, rare). All participants are given a copy of their RPFNA cytology report that includes cell count and cytology score. The report is presented to the participant, and all questions answered.

  It is standard of care that if cells are found in the breast of high-risk women that are suspicious for cancer, that these women undergo a standard diagnostic work up for cancer with a breast surgeon. In accordance with standard-of-care practices, if cells are found in RPFNA that are suspicious for cancer, the participant is referred to a breast surgeon for a standard diagnostic work-up. Further clinical decision-making is determined based on the findings of this standard diagnostic work up and not based on the RPFNA results. This course of action is stated in our NCI grants funding this trial and is standard-of-care. Participants are also told that if we fail to find atypia, suspicious, or cancerous cells in their RPFNA, this does not mean that these cells are not present in their breast. Again, participants are told that RPFNA is not diagnostic.
- Women with a known BRCA1 or BRCA2 mutation will be re-offered the opportunity to undergo prophylactic oophorectomy and mastectomy.

14.6.1.3 RPPM Analysis: RPPM will be performed (as described in Section 1.3) through funding provided by a Susan G. Komen Promise grant. The Promise grant provides funding for RPPM analysis only. RPPM will be performed at Theranostics under the guidance of [redacted] (George Mason) as was done in the study described in the Background Section. Theranostics provides CLIA-CAP certified RPPM analysis.

The sample will be immediately placed into fixative. The sample is sealed and sent by overnight priority Fed-EX to [redacted] lab per Section 14.5, micro-dissected within 24 hours of receipt, and "micro-dissection caps" placed at -80°C until analysis. Using our published methods[30,31], 5,000 micro-dissected epithelial cells will be spun onto a glass slide and isolated. Serial dilution of protein lysate will be arrayed onto nitrocellulose-coated slides. Samples will be spotted at 150 uM. In parallel with antibody staining, total protein concentrations will be determined by Spyro Ruby fluorescent staining (Mol. Probes) as a final normalization step. Each array will be scanned, spot intensity will be normalized, data will be normalized, and standard single value will be generated for each sample on the array (Image Quant v5.2). Local background intensity will be calculated for each spot with the unprinted adjacent slide background (JMP v5.0, SAS).

We will test 50 endpoints. These include: 1) AKT/mTor [pAKT-S473, mTor, p4EBP1-S65, p4EBP1-T37-46, peIF4E-S209, p70S6Kinase, pp70S6Kinase-T412, pIKB-S32-36, pNFKappaB-S536], 2) Receptor Tyrosine Kinase/Ras/RAF [EGFR, pEGFR-Y992, ErbB2, ErbB2-Y1248, ErbB3-Y1289, pERK1-2-T202-Y204, pMEK-S217, Ras, Raf, Ras-GRF1-S916], 3) IGF/Insulin [IGF1R-IR-Y1131-46, IRS1-pS612, pACC-S79, AMPKbeta-S108, ATPCL-S454, CREB-S133], 4) Apoptosis/mitochondrial signaling: [caspase-9/7/3, pBad-S136, Bad, Bax, Bcl-xL, pBcl2-S70, pBad-S136], 5) IL6/Stat: [IL6, pStat3-T705, Stat1, Stat6, Stat-5, Jak2-Y1007, vimentin], 6) Proliferation: [Ki67], and 7) Miscellaneous: [VEGFR2-Y951, pGSK3alpha/beta-Y279-Y216, E-Cadherin, HIF1alpha, p38MAPKinase-T180-Y182].

14.6.1.4 Reliability of RPPM Measurements (signal to noise, dynamic range, and linearity of RPPM measurements):

- ***Signal to Noise Ratio:*** For a given protein target (e.g. AKT-pSer473, mTOR, etc.), we scanned the array and the spot intensity normalized relative to total protein calculated using Spyro Ruby fluorescent staining. A standard single value was generated for each sample on the array using Image Quant v5.2 software. Spot intensity was integrated over a fixed area (signal). Local background intensity was calculated for each spot with the unprinted adjacent slide background using JMP v5.0 software (noise). The limit of detection was defined as 2 standard deviations above the background while the functional sensitivity was defined as the lowest signal giving a CV% of less than or equal to 20% within an array; 10 replicates of three defined cell lines were used to estimate the coefficient of variation in the average intensity of each protein. Individual RPPM protein targets that did not fall above 2 standard deviations above background in at least one cell condition were not carried forward in our analysis of RPFNA cytology.
- ***Defining the Dynamic Range and Linearity in RPPM:*** We tested for the dynamic range and linearity of RPPM for key nodal signaling proteins, through (a) internal serial dilution and (b) a mixture experiment. The range of and

linearity of RPPM protein expression was compared to western analysis. Twenty replicates of a defined standard titration curve [1:0, 1:1; 1:2; 1:5; 1:9; 0:1] were arrayed on each slide. Analysis was performed by taking the mean intensity of 20 individual applications for a given concentration within a slide and comparing it to the mean intensity of the remaining six slides. Analysis of inter- and intra-spot reproducibility and linearity showed good correlation (correlation coefficient r2=0.989). There was agreement for the 5 proteins tested AKT-pSer473 (r2=0.999), EGRF (r2=0.982), ERK-pThr204 (r2=0.991), MEK-pSer217 (r2=0.977), and mTor (r2=0.992). These data show strong correlation between RPPM and western analysis.

14.6.1.5 Exploratory Biomarker: RPFNA samples will be banked for future determination of metformin tissue levels. This endpoint will help support a direct insulin-independent mechanism. Tissue will be analyzed from the fluid associated with RPFNA using a protocol adapted from Wang *et al*.[58]. Therefore, there will be no additional risk or discomfort to the patient. This analysis will be performed in the laboratory of [redacted] as an exploratory study.

14.6.2 Research Serum

14.6.2.1 The risk of these serum studies is the loss of about 33.5 mL of blood. The blood collected will yield approximately 10-12 mL red top serum and 4 mL SST serum.

14.6.2.2 Measures of Obesity: Serum IGF and other cytokines such as IL6, IL8, MCP-1, TGFbeta, and leptin will be analyzed by ELISA. It is unclear whether the benefit from metformin is due to a direct effect on Akt/mTor or an indirect reduction on serum insulin. There is also evidence for the ability of metformin to affect IGF levels. Serum IGF as well as the other cytokines listed above will be measured by a commercially-available ELISA (ENZO kit number ADI-900150) according to manufacturer's instructions in the laboratory of [redacted] Approximately 2.0 ml serum will be required for these assays.

14.6.2.3 Measures of Breast Cancer Risk: Serum samples will be tested for estrogen and progesterone by commercially available ELISA assays according to manufacturer's instructions (Cayman Chemical Item Numbers 582251 and 582601) in the laboratory of [redacted] Approximately 500 microliters of serum will be required for this assay.

14.6.2.4 Exploratory Biomarker: Serum samples will be banked for future determination of metformin levels. This endpoint will help support a direct insulin-independent mechanism.

14.6.3 EDTA Whole Blood

14.6.3.1 The risk of these EDTA whole blood studies is the loss of about 20 mL of blood. DNA will be extracted from 10 mL and stored by the Alliance PCO. From the second 10 mL tube of whole blood, DNA will be extracted and white blood cells will be isolated from the collected blood in the laboratory of [redacted]

14.6.3.2 White Blood Cell Complex 1: Samples will be measured from isolated white blood cell mitochondria using the protocol outlined by in Methods in Enzymology[59]. This assay will be performed in the laboratory of [redacted]

14.6.3.3 DNA Imprinting: [redacted] laboratory will extract DNA from the EDTA whole blood and initially analyze a portion of the DNA for the presence of imprinting using standard laboratory protocols.

14.6.3.4 Pharmacogenomic studies: DNA extracted by [redacted] laboratory will be used for future risk/response assays (e.g., for genetic polymorphisms such as DNA repair proteins that may correlate with efficacy and tolerability).

14.6.4 Measures of Obesity and Insulin Resistance

BMI, waist to hip ratio, fasting serum glucose, fasting serum insulin, serum HOMA, serum IGF, hemoglobin A1C, and other inflammatory cytokines (as described in 14.6.2.2) will be analyzed. It is unclear whether the benefit from metformin is due to a direct effect on Akt/mTor, indirect reduction on serum insulin, or due to an indirect reduction in weight. These measures will help to support a direct insulin-independent versus an indirect insulin-dependent mechanism. BMI and waist to hip ratio will be measured by standard clinical criteria. We plan to examine whether baseline glucose predicts metformin benefit independent of insulin or whether glucose modifies effects of insulin as a predictor of metformin benefit. We will also investigate HOMA, an empirically derived estimate of insulin sensitivity calculated from a single measure of fasting insulin and glucose that correlates well with the gold standard frequently sampled intravenous glucose tolerance test[57], as a predictor of metformin benefit, focusing on whether it provides additional prediction beyond insulin alone. The blood collection for fasting glucose and insulin is a mandatory part of the baseline, 6-month, and yearly evaluations. Serum fasting glucose and fasting insulin will be determined by standard clinical criteria at the participating sites.

14.6.5 Residual Biospecimens

All residual specimens remaining after correlative studies are completed will be shipped to the Alliance Biorepository at Ohio State University, where they will be permanently stored at -80ºC according to patient consent information, until specific analyses are identified. As protocols are developed, they will be presented for Alliance and IRB review and approval. (This collection is part of a general strategy of investigation for the majority of Alliance studies.)

14.7 Return of Genetic Testing Research Results

14.7.1 This study will only test for low penetrance alleles that confer minimal risk. Because the results generated by the genetic testing described above are not currently anticipated to have clinical relevance to the patient or their family members and will be conducted in a research facility, the genetic results will not be disclosed to the patients or their physicians.

14.7.2 If, at any time, genetic results are obtained that may have clinical relevance, IRB review and approval will be sought regarding the most appropriate manner of disclosure and whether or not validation in a CLIA-certified setting will be required prior to reporting any finding. Sharing of research data with individual patients should only occur when data have been validated by multiple studies and testing has been done in CLIA-approved laboratories or in a setting where a clinical trial is available based on a CLIA laboratory validated sequencing result and the patient has consented to re-contact for additional studies.

15.0 DRUG INFORMATION

15.1 Metformin hydrochloride tablets

15.1.1 Background: Metformin hydrochloride is an oral antihyperglycemic agent used in the management of type 2 diabetes. It lowers both basal and postprandial glucose levels in type 2 diabetes patients through several mechanisms: decreases hepatic glucose production, decreases intestinal absorption and increases peripheral glucose uptake and improves insulin sensitivity. Metformin hydrochloride is not chemically or pharmacologically related to any other classes of oral antihyperglycemic agents.

The Alliance considers this study to be IND exempt. Consistent with the FDA Guidance "IND Exemptions for Studies of Lawfully Marketed Drugs or Biological Products for the Treatment of Cancer", the Alliance is not planning to use the results of A211102 to get a new indication for metformin. Metformin has been and is currently being investigated for treatment and prevention of cancer. It is not anticipated that the toxicity profile of metformin in A211102 is different from that already known. The FDA has confirmed this study to be IND exempt (IND 122334).

15.1.2 Formulation: Metformin hydrochloride tablets, containing 500 mg, 850 mg, or 1000 mg metformin hydrochloride per tablet, are manufactured and distributed by various pharmaceutical companies. The metformin hydrochloride 850 mg tablets used in this trial are available commercially from Heritage Pharmaceuticals. McKesson Clinical Research Services will use study-provided funds to purchase the metformin tablets. Each tablet also contains the inactive ingredients malto dextrin, hypromellose, magnesium stearate, polyethylene glycol, povidone, corn starch, and blackberry. The tablets will be bottled and labeled by Pharm Ops, Inc. Each bottle packaged by Pharm Ops, Inc. will contain 175 tablets.

15.1.3 Preparation and storage: The tablets should be stored at a controlled room temperature, with excursions to 15-30°C (59-86°F) permitted. All tablets should be kept in a secured storage area.

15.1.4 Administration: Metformin hydrochloride tablets should be taken with meals.

15.1.5 Pharmacokinetic information: The time to peak serum level for the immediate release product is 2 to 3 hours. The onset of action is within days, with maximum effects up to two weeks. Metformin is negligibly bound to plasma proteins. Metformin is excreted in the urine, with 90% as unchanged drug. It does not undergo hepatic metabolism. Renal clearance is approximately 3.5 times greater than creatinine clearance, which indicates that tubular secretion is the major route of elimination. Bioavailability while fasting is 50% to 60%. Half-life elimination in plasma is 4 to 9 hours.

15.1.6 Potential Drug Interactions: Concomitant use of the following agents may cause hyperglycemia: thiazides, other diuretics, corticosteroids, phenothiazines, thyroid products, estrogens, oral contraceptives, phenytoin, nicotinic acid, sympathomimetics, calcium channel blockers, and isoniazid. Metformin may interact with highly protein bound drugs (salicylates, sulfonamides, chloramphenicol, probenecid). Concurrent use of other glucose-lowering agents may cause hypoglycemia. Fluoroquinolones and antidiabetic agents have been reported to cause blood glucose changes. Beta blocking agents present a minimal risk of altering glucose control in nondiabetic individuals, although severe hypoglycemia has been reported with at least one beta blocking agent. Concurrent use of metformin is contraindicated in participants receiving intravascular iodinated contrast media due to an association with lactic acidosis and acute renal failure.

15.1.7 Known potential toxicities: Metformin is considered to have a favorable safety profile in diabetes mellitus patients. In a US double-blind clinical trial for patient with type 2 diabetes, gastrointestinal symptoms such as diarrhea, nausea and vomiting, abdominal discomfort, flatulence, indigestion, and asthenia were the most common reactions to the drug. These symptoms were dose-dependent, transient, and resolved spontaneously with continued use. The drug may cause GI upset so should be taken with food. Additional adverse reactions reported in 1 to 5% of subjects were abnormal stools, hypoglycemia, myalgia, lightheadedness, dyspnea, nail disorder, rash, increased sweating, taste disorder, chest discomfort, chills, flu syndrome, flushing and palpitation. A recently published trial of patients with type 2 diabetes mellitus reported a vitamin B_{12} deficiency with long term treatment. Lactic acidosis is a rare, but serious, metabolic complication that can occur due to metformin accumulation during treatment. Reported incidence of lactic acidosis in patients receiving metformin is very low. Reported cases have occurred primarily in diabetic patients with significant renal insufficiency.

15.1.8 Drug procurement: Metformin Hydrochloride 850 mg tablets will be purchased for use in the trial. Placebo tablets that are similar in size and color will also be purchased. The metformin and placebo tablets will be bottled and labeled by Pharm Ops, Inc. The bottles will be labeled so that study participants, caregivers, and investigators are blinded to the contents.

Blinded metformin/placebo: Patient-specific supplies of metformin/placebo will be ordered by the registering institution after each patient is enrolled to the study.

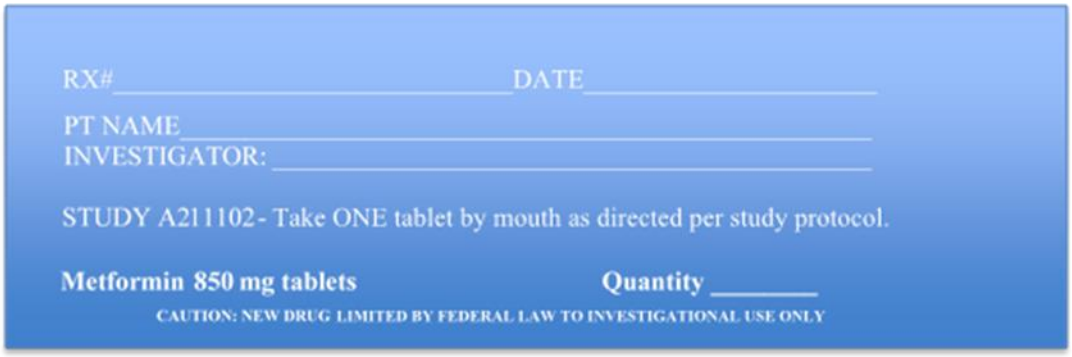
Fax or email the Alliance A211102 Drug Order Form to:

[redacted]

Patients will need to return to the clinic to receive additional supplies of blinded drug at 3, 6, and 9 months after registration. Sites may dispense a 6 month supply. However, sites that dispense a 6 month supply will be responsible for reconciliation of all medication returns. Institutional staff will contact the Registration Office for a study medication code number. Drug orders for these additional supplies should be submitted at least one week prior to each of these visits using the Alliance A211102 Drug Order Form.

Open-label metformin: All patients will be unblinded at 12 months (see Section 6.9). All patients, except those who were randomized to placebo and who opt not to cross over to open-label metformin will receive open-label metformin. After re-registering the patient per Section 6.9, site staff will order patient-specific supplies of open-label metformin using the A211102 Drug Order Form. Orders should be placed at least 1 week prior to the patients' 12-, 15-, 18-, and 21-month visits.

Open-label metformin will be provided as 100 count bottles. Sites are responsible to label the bottles according to their respective state pharmacy laws. A sample label is below:



The Alliance A211102 Drug Order Form is available at the Alliance and CTSU Web sites.

Outdated or remaining drug/product should be destroyed on-site per procedures in place at each institution.

15.1.9 Nursing Guidelines:

- Metformin can have numerous drug to drug interactions that can cause either hyper or hypoglycemia. Assess patient's concomitant medications (including OTC) and instruct patients not to start any new medications while on the study without checking with the study team.
- Warn patients of gastrointestinal side effects when initially starting agent, including diarrhea, nausea, abdominal discomfort, flatulence, indigestion and vomiting. Agent should be taken with food to minimize these side effects.
- There is a rare but serious risk of lactic acidosis. Instruct patients to report nausea or weakness. Patient with these symptoms should have their electrolytes checked and/or corrected under the direction of the physician.
- Drug may cause hypoglycemia. Instruct patients in the signs and symptoms of hypoglycemia and to seek medical attention if they experience these.
- Instruct patients that if they need to have any procedure (i.e. CT scan with contrast) with iodinated contrast they must discontinue metformin per institutional practice prior to the procedure and must not resume it until their creatinine level is satisfactory (per institutional practices).

15.2 Placebo

A placebo tablet will be provided which will be similar in appearance to the metformin HCl 850mg tablet.

Formulation: Pharm Ops, Inc., Inc. will formulate and produce the placebo tablets. The tablets will contain microcrystalline cellulose and other inactive ingredients: croscarmellose sodium, colloidal silicon dioxide and magnesium stearate.

16.0 STATISTICAL CONSIDERATIONS AND METHODOLOGY

16.1 Study Design

16.1.1 Study Populations

A total of 177 premenopausal women will be pre-registered, among which 96 patients with atypia (Masood Score 14-17) on RPFNA at baseline are expected to be randomized between

Metformin arm and placebo control arm. It is expected that the accrual rate will be 1 patient per month and that accrual will be completed about 8 years after study activation. The randomization will be stratified for known BRCA mutation (BRCA1 or BRCA2 mutation vs. no mutation), prior excisional biopsy (atypical epithelial hyperplasia, atypical lobular hyperplasia, flat epithelial hyperplasia vs. DCIS vs. LCIS), and baseline fasting insulin > 2x ULN vs. ≤ 2x ULN.

At 12 months the women in the placebo arm will be allowed to take metformin. Based on experience from 300 women undergoing serial RPFNA at Duke, it is anticipated that approximately 40% of women who are randomized to the metformin arm will have disappearance of atypia at 12 months and 60% will have persistent atypia. The estimated drop-out rate is 5% at 6 months and 10% at 12 months. Over 98% of women with atypia will have atypia on repeat RPFNA on the non-metformin arm.

We propose to test RPFNA on both right and left breasts at, 12 and 24 months after registration/randomization for the presence or absence (Masood score ≤13) of atypia by Masood Cytology Index Score. The RPFNA at 24 months is optional for women who continue on the placebo control arm and will not receive metformin. The outcomes collected at 24 months will be exploratory. In analysis, we will consider taking a transformation, such as logarithm, of positive variables in order to improve the normality and variance stabilization. Among the 96 randomized patients, we expect that about 10% of patients will drop out at month 12. So, the power analyses are based on N= 88 patients (44 per arm).

16.2 Statistical Analysis and Power Calculation

16.2.1 Primary Objective:

Test for the presence or absence of atypia in RPFNA after 12 months (24 month optional for placebo group for patients who remain on placebo arm and will not receive metformin); Metformin versus placebo control:

16.2.1.1 Primary Analysis

Multiple Masood scores from each breast will be averaged for each time point. The primary endpoint of the study is the binary outcome of atypia (Masood score ≥ 14). In this analysis, the sampling units will be women, so that, if a woman has atypia in one breast and no atypia in the other breast, this woman will be called atypia. Presence of atypia at month 12 will be compared between two arms using the chi-square test in univariate analysis, and regressed on group indicator, age, race, stratification factors, and baseline Masood score using logistic regression in multivariate analysis. As an exploratory analysis, we will also conduct a regression analysis including the timing of baseline RPFNA (intraoperative vs. in clinic) and the number of breasts with atypia at baseline in addition to these covariates. With 44 women per arm, the primary analysis has 90% of power to detect a difference of 90% vs. 60% of atypia between placebo and metformin arms at month 12 by a chi-square test with alpha=5%.

In order to address the worst score rule, we will repeat above analyses using the maximum, instead of average, Masood score from each breast.

16.2.1.2 Exploratory Analysis

An exploratory analysis will be conducted using the breasts as sampling units, not women, so that all eligible breasts of eligible patients will be included in data analysis. The variance of the test statistic for comparing the presence of atypia at month 12 will be estimated using the generalized estimating equation (GEE) approach[53] to account for the possible dependency among the binary atypia observations from each patient.

In another exploratory analysis, we will compare the continuous Masood score between two arms. The sampling unit in this analysis will be breast. The change rate (ratio) in Masood score at month 12 from baseline will be compared between metformin group and placebo control group using two-sample t-test for univariate analysis with 2-sided alpha=5%. We will also conduct multivariate analysis to regress the month 12 Masood score on group indicator, age, race, prior excisional biopsy, and baseline Masood score.

Since the sampling unit in this analysis is breast, the Masood score observations from two breasts may be potentially correlated. In order to account for possible dependency between the data points from two breasts of each woman, the variances of t-test (in univariate analysis) and regression estimates (in multivariate analysis) will be estimated using the GEE.

It is expected that about 25% of women (n = 11) will contribute one breast and the remaining 75% of women (n = 33) will contribute samples from both breasts, so that there will be about77 (= 11x1 + 33x2) data points (breasts) per arm in this analysis. Assuming that the intracluster correlation coefficient between two breasts is 0.3, this analysis will have 84% power to detect a difference of 0.4 times the standard deviation (SD).

Similar analyses will be conducted for 24 months data.

The women or breasts with unsatisfactory repeat RPFNAs will be considered missing at random in the analysis.

16.2.2 Secondary Objectives:

16.2.2.1 Test for Masood score and the presence of atypia or disappearance of atypia in RPFNA after 12 (for both arms) and 24 months for Metformin arm:

We will investigate the trend of Masood score and presence of atypia measured at month 0 and 12 for metformin arm and placebo arm. Breasts will be the sampling units in the analysis of Masood score, while either women (in the primary analysis) or breasts (in the secondary analysis) will be regarded as sampling units in the analysis of atypia. At first, we will perform descriptive analyses for summary statistics of Masood score and atypia at each measurement time. We will also generate a line plot connecting the Masood scores of each breast over time to investigate the time trend.

We will compare the mean Masood scores between 0 vs. month 12 using paired t-tests. In this analysis, we will use the breasts as sampling units. The possible dependency between data points from two breasts of each woman will be adjusted using the GEE method using the working independent correlation model[55]. Assuming that about 25% of women (n=11) will contribute one breast and the remaining 75% of women (n=33) will contribute samples from both breasts for each arm, we will have 90% of statistical power to detect a difference of 0.3 times SD if the intracluster correlation coefficients between breasts and two time points are 0.3 for each woman. We will conduct McNemar test to compare the proportion of atypia between month 0 and month 12. These analyses will be conducted for each arm, and the results will be descriptively compared between two arms. We will summarize the 24-month data for the metformin arm.

16.2.2.2 Compare Masood Cytology Score value at 0, 12, and 24 months in right and left breast from the same individual in the metformin and non-metformin group:

Women with DCIS will not have two breasts for comparison. We anticipate that this will constitute approximately 25% of the women who participate in this study. Based on our published CALGB study[21] we expect that we will not observe concordance between right and left breast Masood Cytology Score. Our analysis here will further investigate these preliminary observations. A scatter plot (for right vs. left) will be generated to demonstrate dependency between right and left breast Masood cytology scores at each measurement time. Also from each scatter plot, the correlation coefficient and its p-value will be estimated. Only 75% of women (N = 33 per arm) contributing two breasts will be included in this analysis. In order to investigate the time trend in dependency between right and left breast Masood cytology scores, we will compare the correlation coefficients over different time points. We will also make a plot of differences (right-left) or ratios (right/left) over different time-points, and test if there exists any time trend in difference or ratio. The p-value will be calculated accounting for the dependency of the longitudinal data using GEE method[55].

16.2.2.3 Test the reproducibility of RPPM in duplicate RPPM determination from single bilateral RPFNA specimen:

At each time of month 0 and 12, we will generate duplicated RPPM data on the 50 endpoints. The reproducibility of RPPM at each time point will be tested using (i) unsupervised hierarchical clustering analysis to check if the duplicated data points are clustered together, and (ii) scatter plots between paired RPPM and estimated correlation coefficients. We will use an ANOVA method to test if the intracluster correlation coefficient is 0 or not.

16.2.2.4 Correlate baseline RPPM values with presence of atypia at month 12 and month 24:

We will combine data set of metformin and no-metformin groups (N = 88) and use the breasts as the sampling units in this analysis. The possible dependency in observations between the left and right breasts for each woman will be adjusted using the GEE approach[55].

The baseline RPPM value of each protein will be correlated with Masood cytology score at month 12 using a simple linear regression method. In a multivariate analysis, Masood score at month 12 will be regressed on the baseline RPPM value of each protein, group indicator, age, race, prior excisional biopsy, and baseline Masood scores. Similar analysis will be conducted using the change rate of Masood score between baseline and month 12 in place of Masood score at month 12.

The baseline RPPM value of each protein will be correlated with presence and absence of atypia at month 12. A total of 50 proteins will be tested, so that the significance of each protein will be adjusted for multiple testing by controlling the family-wise error rate (FWER) using the permutation method. We expect that about 60% of the women will have persistent atypia at 12 months. The 50 proteins were selected from a previous study so that they are expected to have reasonable effect sizes. Assuming that each woman contributes samples from only one breast and the proteins have correlation coefficient of 0.1, the multiple testing procedure controlling the FWER at 10% level by two-sided tests will have about 90% probability to detect 15 or more proteins if 25 of the 50 proteins have an effect size of 0.8 times SD [Jung and Young, 2011][56]. Since about 75% of women are expected to contribute samples from both breasts to the study, the real power for this analysis will be higher.

In a multivariate analysis, presence of atypia at month 12 will be regressed on the baseline RPPM value of each protein, group indicator, age, race, prior excisional biopsy, and baseline Masood scores.

A similar analysis will be conducted to correlate baseline RPPM value of each protein with Masood score and presence/absence of atypia at month 24.

16.2.2.5 Record and analyze patient-reported knowledge of the arm to which they were randomized:

In March, 2019 the study team was notified of an unexpected unblinding incident. An enrolled patient informed her physician that she was able to google the code (serial number) on the pills to uncover whether she was taking metformin or placebo. Notably, all metformin tablets have a serial number printed on them while the placebo pills do not have any such information; in other words, the placebo tablets were not designed to look exactly like the metformin tablets and the risk of potential unblinding due to the lack of similarity was known prior to study activation, and this risk was deemed acceptable. The Division of Cancer Prevention at NCI and the Alliance Data and Safety Monitoring Board were notified of this single unblinding incident.

Because the primary outcome is an objective measurement, the trial results are not at significant risk of bias due to the single known incident of unblinding. Furthermore, the remaining secondary, tertiary, and correlative endpoints are not subjective in nature. Coupled with the fact that this study is intended to serve as proof of principle for the use of RPFNA to test for the presence or absence of atypia before, during, and after the administration of a prevention agent, this unblinding incident is unlikely to affect the validity of the study.

At the time of this amendment, ≤ 10% of the patients had withdrawn from the study and the study discontinuation rate was balanced across the randomized arms; therefore, any premature unblinding of the study agents was likely to be limited in scope. However, it is important to understand the true extent and impact of the unblinding issue. Therefore, at the end of study intervention (i.e. at 12 months) but before the patient is unblinded and before the patient crosses over to metformin, patients will be asked the following question: "Did you know with certainty which agent you were randomized to receive during the first 12 months of the study?" This single data field will be captured in Rave, our database of record, and will allow us to report on the magnitude of the premature unblinding as a patient-reported outcome. This information will also allow us to conduct sensitivity analyses on the primary and secondary endpoints by excluding patients who were unblinded to assess the impact (if any) of the premature unblinding on the study results. For patients who have completed the study, discontinued early, or who are on study but beyond the 1-year intervention at the time of this amendment, the site coordinators will still contact the patient either by phone or during a protocol scheduled visit to ask them this question. Although it is conceivable that some patients may decline to provide a response, we anticipate that the success rate for obtaining a response to this single question will be high and, therefore, this information will be useful to contextualize the study findings.

16.3 Study Monitoring

16.3.1 This study will be monitored by the Alliance Data Safety Monitoring Board (DSMB), an NCI-approved functioning body. Reports containing efficacy, adverse event, and administrative information will be provided to the DSMB as per NCI guidelines.

16.3.2 Results Reporting on [redacted]: At study activation, this study will have been registered within the [redacted] web site. The Primary and Secondary Endpoints (i.e., "Outcome Measures") along with other required information for this study will be reported on [redacted]

16.4 Inclusion of women and minorities

Breast cancer occurs primarily in women. Men and children are generally not subject to breast cancer and they will be excluded from this study. Efforts will be made to enroll individuals of all races and ethnic backgrounds.

Racial Categories	DOMESTIC PLANNED ENROLLMENT REPORT				
	Ethnic Categories				
	Not Hispanic or Latino		Hispanic or Latino		Total
	Female	Male	Female	Male	
American Indian/Alaska Native	1	0	0	0	1
Asian	3	0	0	0	3
Native Hawaiian or Other Pacific Islander	1	0	0	0	1
Black or African American	15	0	1	0	16
White	60	0	15	0	75
More Than One Race	0	0	0	0	0
Total	80	0	16	0	96

17.0 PATHOLOGY CONSIDERATIONS/TISSUE BIOSPECIMENS

None

18.0 BUDGET

See the A211102 Study Funding Sheet, available on the A211102 page of the Alliance and CTSU web sites.

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APPENDIX I: MODEL CONSENT FORM

NCI Informed Consent Template for Cancer Treatment Trials
(English Language)

***NOTES FOR LOCAL INVESTIGATORS:**

The goal of the informed consent process is to provide people with sufficient information for making informed choices. The informed consent form provides a summary of the clinical study and the individual's rights as a research participant. It serves as a starting point for the necessary exchange of information between the investigator and potential research participant. This template for the informed consent form is only one part of the larger process of informed consent. For more information about informed consent, review the "Recommendations for the Development of Informed Consent Documents for Cancer Clinical Trials" prepared by the Comprehensive Working Group on Informed Consent in Cancer Clinical Trials for the National Cancer Institute. The Web site address for this document is [redacted]

- A blank line, _____, indicates that the local investigator should provide the appropriate information before the document is reviewed with the prospective research participant.
- Suggestion for Local Investigators: An NCI pamphlet explaining clinical trials is available for your patients. The pamphlet is entitled: "Taking Part in Cancer Treatment Research Studies". This pamphlet may be ordered on the NCI Web site at [redacted] to request a free copy.
- Optional feature for Local Investigators: Reference and attach drug sheets, pharmaceutical information for the public, or other material on risks. Check with your local IRB regarding review of additional materials.

**These notes for {authors and} investigators are instructional and should not be included in the informed consent form given to the prospective research participant.*

A211102, Testing for Atypia in Random Periareolar Fine Needle Aspiration (RPFNA) Cytology After 12 Months Metformin (1,1-Dimethylbiguanide Hydrochloride) Chemoprevention Versus Placebo Control in Premenopausal Women

This is an important form. Please read it carefully. It tells you what you need to know about this research study. If you agree to take part in this study, you need to sign this form. Your signature means that you have been told about the study and what the risks are. Your signature on this form also means that you want to take part in this study.

This is a clinical trial, a type of research study. Your study doctor will explain the clinical trial to you. Clinical trials include only people who choose to take part. Please take your time to make your decision about taking part. You may discuss your decision with your friends and family. You can also discuss it with your health care team. If you have any questions, you can ask your study doctor for more explanation.

This study is conducted by the Alliance for Clinical Trials in Oncology, a national clinical research group supported by the National Cancer Institute. The Alliance is made up of cancer doctors, health professionals, and laboratory researchers, whose goal is to develop better treatments for cancer, to prevent cancer, to reduce side effects from cancer, and to improve the quality of life of cancer patients.

You are being asked to take part in this research study because you are considered to be high risk for the development of breast cancer in the future.

Why is this research study being done?

The purpose of this research study is to test whether metformin, a drug commonly used to treat diabetes, is able to get rid of atypia (early cell changes that are thought to be a marker of breast cancer risk) in women at increased risk for breast cancer. We will test for the presence of atypia in the breast after metformin is given to see if it can get rid of atypia. We would like to compare the effects, good and/or bad, of metformin or placebo on atypia to find out which is better.

Note: The standard drug used for the “breast cancer prevention” is tamoxifen. If you are eligible to take tamoxifen, you must be offered tamoxifen prevention as part of your clinical care and you must have refused tamoxifen treatment to be on this study. Metformin and tamoxifen are not similar and function differently. This study is not investigating the use of tamoxifen. Metformin is not approved for preventing breast cancer, but is currently being tested to determine if it can prevent breast cancer.

How many people will take part in the research study?

About 177 women will take part in this study.

What will happen if I take part in this research study?

In order to find out whether you are eligible to participate in this study, you will have a procedure called "Random Periareolar Fine Needle Aspiration" (RPFNA) of your breasts to test for atypia (marker of breast cancer risk). This procedure, which will be repeated if you are eligible to participate in the study, is described below. **If the pathologist does not identify atypia in the samples you will not be able to participate.**

Before you begin the study …

You will need to have the following exams, tests or procedures to find out if you can be in the study. If you have had some of them recently, they may not need to be repeated. This will be up to your study doctor.

- Medical history (questions about your health and any medications you are taking)
- General physical examination including your weight, height, and BMI
- Waist/hip ratio
- A breast exam
- A standard digital mammogram
- Routine blood tests (Blood counts, fasting blood sugar, insulin levels, kidney function)
- Before start of treatment, you will have an evaluation to see if any of the possible side effects are already present before treatment.

- **Random Periareolar Fine Needle Aspiration (RPFNA)**. RPFNA is a method for collecting cells from the breasts. Your doctor will use a small needle to remove and collect cells from both of your breasts. Your doctor will do this by inserting 4 needles into two locations on each of your breasts (8 needles per breast) and removing cells. Usually, this procedure will be done on both breasts. However, if you have been diagnosed with DCIS in one breast, you will have this procedure done only on the other breast. Or, if you are having surgery to remove atypia from one breast, your surgeon may decide to perform the RPFNA procedure on the other breast only during the surgery.

  Analysis of RPFNA samples will be done at laboratories associated with the Alliance. The RPFNA will be reviewed by a pathologist looking at any changes in the cells under the microscope. In the event that the pathologist identifies cells in your RPFNA sample that are suspicious for cancer, your doctor will discuss these results with you and your doctor will determine what to do next.

During the study…

If the exams, tests and procedures show that you can be in the study, and you choose to take part, you will be registered and "randomized" into one of the study groups described below. Randomization means that you are put into a group by chance (as in the flip of a coin). A computer program will place you in one of the study groups. Neither you nor your doctor can choose the group you will be in. You will have an equal chance of being placed in either group.

Group 1
Patients randomized to Group 1 will receive metformin tablets.

Group 2
Patients randomized to Group 2 will receive placebo tablets, which are not active (like a sugar pill).

The placebo is given in Group 2 so that participants in both groups receive similar-looking pills. This way, neither you nor your doctor can tell which group you are in, and that makes the study more objective. After 12 months of treatment, you will be told by your clinic staff whether you were receiving metformin or the placebo. If you were randomized to receive placebo, you will be asked if you wish to switch to metformin pills or stop taking study drug (see below). There is no plan to tell you whether you are receiving metformin or placebo during the first 12 months of the study except in the case of an emergency.

When you start the study treatment, you will take 1 metformin/placebo pill every day for the first 4 weeks (called the "ramp-up period"). After this, you will take one pill by mouth two times a day – one pill in the morning and one in the evening. The pills should be taken with food. If you miss a dose, you should skip that dose and not try to make it up. The drug dosage may be reduced, or may be stopped temporarily or permanently if you suffer side effects that are unacceptable. You are encouraged to maintain a healthy weight and moderate degree of exercise. You are also encouraged to consult your doctor or study nurse if you decide to stop study drug permanently.

Month	**Number of metformin/placebo pills**
1	1 pill each day by mouth (4 week ramp up period)
2-12	2 pills each day by mouth

After 12 months of treatment, you will be told by your clinic staff whether you were receiving metformin or the placebo.

If you were receiving metformin, you will continue taking the drug for 12 more months.

Month	Number of metformin pills
13-24	2 pills each day by mouth

If you were receiving the placebo, you will have the option to either:

1) Begin taking metformin for one year (also called "crossover,") OR
2) Stop taking study drug

If you choose to cross over to treatment with metformin, you will start with one pill for 4 weeks and then increase to two pills per day.

Month(s)	Number of metformin pills
13	1 pill each day by mouth (4 week ramp up period)
14-24	2 pills each day by mouth

If you choose to stop taking the study drug, you will be asked to continue with the tests and procedures as scheduled below. You will have the option to skip the RPFNA procedure that is scheduled at 24 months.

Tests and procedures
During the study you will have the following tests and procedures, which will be done around 6, 12, and 24 months after you start the study.

- Medical history (questions about your health and any medications you are taking)
- General physical examination including your weight and BMI
- Waist/hip ratio
- A breast exam
- Routine blood tests (Blood counts, fasting blood sugar, insulin levels, kidney function)
- Evaluation of any side-effects you are having.
- You will be given a Patient Medication Diary to complete during the ramp-up period. Before the end of the ramp-up period, you will receive a phone contact to remind you to increase your treatment to twice daily after 28 days and to ask about any side-effects you may be having.

RPFNA: In addition to the RPFNA procedure that you will have before you start the study, you will be asked to undergo this procedure after you have received metformin or placebo for 12 months. After you have received treatment for 24 months, you will asked to undergo the RPFNA a third time, unless you were randomized to group 2 and have chosen not to continue treatment after 12 months.

Blood samples: You will be asked to provide blood samples for research that are being done to see how the study is affecting your body. About 4 tablespoons of blood will be collected at each time point. These samples will be collected before you start study treatment, and at around 6, 12, and 24 months after you start the study. These samples will be used to learn about proteins and genes (also called biomarkers) that might be related to atypia. These samples will be tested at laboratories associated with the Alliance as part of this study. It is hoped that this will help investigators better prevent and understand breast cancer. The results of these tests are not currently part of standard clinical care and are only for research testing. Therefore, neither you nor your doctor will receive any specific results and the results will not be used in planning your care. These tests are for research purposes only and you will not have to pay for them.

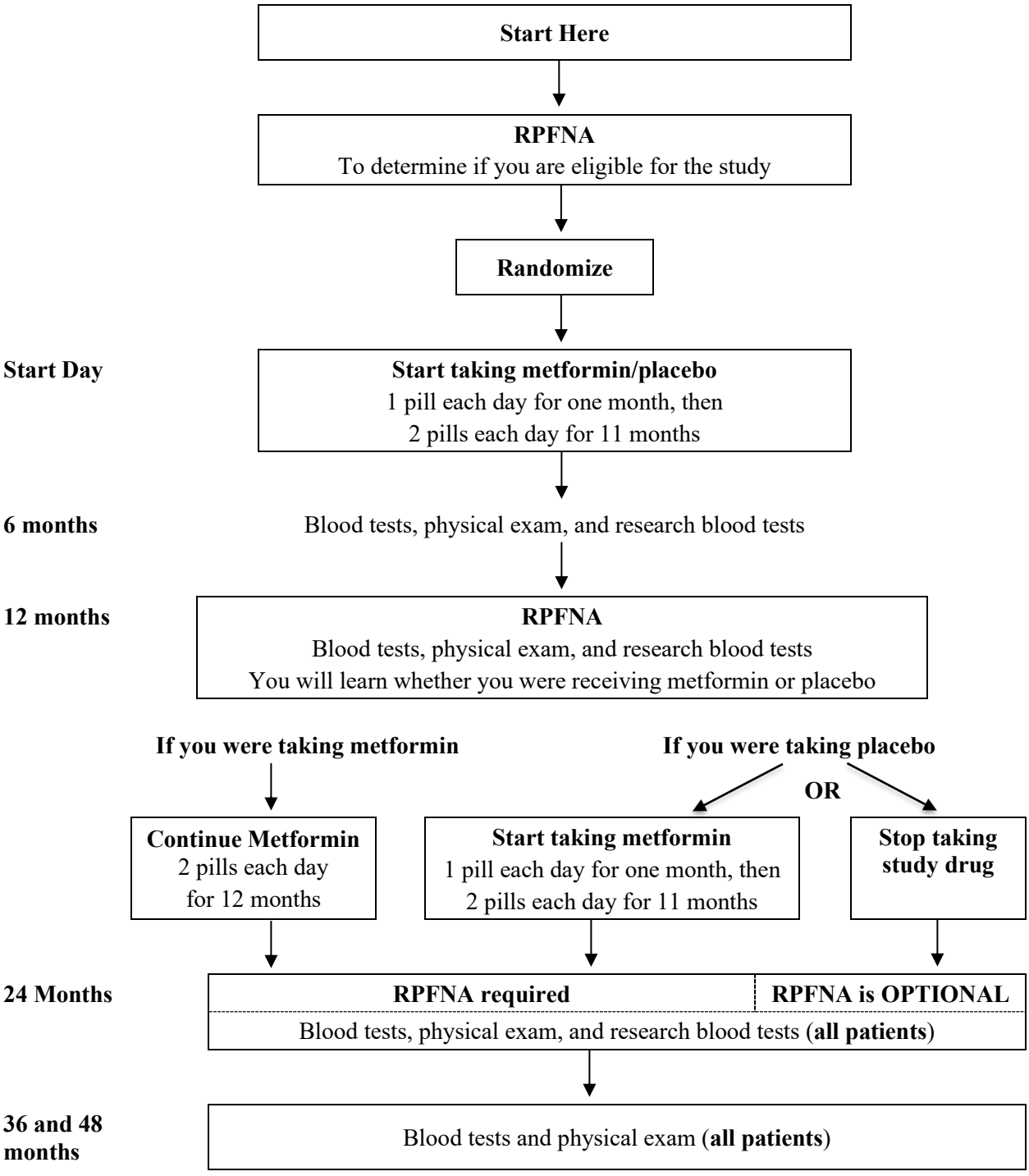
Please see the "Additional Studies" sections at the end of this consent form for additional information regarding your RPFNA tissue and blood samples.

When I am finished taking the study drug…

You will be asked to return to the clinic 36 and 48 months after you started the study, with the following tests being done at each time point:

- Medical history (questions about your health and any medications you are taking)
- General physical examination including your weight and BMI
- Waist/hip ratio
- A breast exam
- Routine blood tests (Blood counts, fasting blood sugar, insulin levels, kidney function)
- Evaluation of any side-effects you are having.

Study Plan:
Another way to find out what will happen to you during the study is to read the chart below. Start reading at the top and read down the list, following the lines and arrows.



How long will I be in the research study?

The duration of the study is 48 months.

Can I stop being in the research study?

Yes. You can decide to stop at any time. Tell the study doctor if you are thinking about stopping or decide to stop. He or she will tell you how to stop safely.

It is important to tell the study doctor if you are thinking about stopping so any risks from the treatment can be evaluated by your doctor. Another reason to tell your doctor that you are thinking about stopping is to discuss what follow up care and testing could be most helpful for you.

The study doctor may stop you from taking part in this study at any time if he/she believes it is in your best interest; if you do not follow the study rules; or if the study is stopped.

What side effects or risks can I expect from being in the research study?

You may have side effects while on the study. Everyone taking part in the study will be watched carefully for any side effects. However, doctors don't know all the side effects that may happen. Side effects may be mild or very serious. Your health care team may give you medicines to help lessen side effects. Many side effects go away soon after you stop taking metformin. In some cases, side effects can be serious, long lasting, or may never go away. There also is a risk of death.

You should talk to your study doctor about any side effects that you have while taking part in the study.

RPFNA risks: You may experience some risks and side effects associated with RPFNA, however, there are no known lasting health risks from undergoing a RPFNA. There is some minimal discomfort after the RPFNA procedure. The main potential risk is bruising at the site of the procedure, which is common. There is a less than 1% risk of infection after the RPFNA technique. Other potential risks associated with the procedure occur infrequently but include: bleeding, slight pain, and hematoma (large bruise), which are standard risks of any invasive procedure, including procedures like blood sampling.

Most likely risks of metformin (greater than 20%) These symptoms are generally temporary, during the start of treatment, and disappear without stopping the drug.

- Loose stools (Diarrhea)
- Feeling sick to your stomach (Nausea)
- Throwing up (Vomiting)
- Abdominal bloating/discomfort
- Indigestion
- Gas (Flatulence)
- Loss of appetite (Anorexia)

Less likely [Occasional] risks of metformin (5 – 20%)

- Loss of taste or metallic taste (during start of therapy)
- Minor weight loss

Rare risks of metformin (1 to 4%)

- Hypoglycemia (low blood sugar)
- Rash, redness or itchiness
- Decrease in your red blood cell count
- Liver function test abnormalities as seen by blood tests or hepatitis (inflammation of your liver). These effects will disappear when Metformin is stopped.

Rare but Serious risks of metformin (less than 1%)

- Lactic acidosis (a high acid level in the blood) occurs rarely (3 cases per 100,000 years of use) and can cause death. Individuals at risk of this complication include persons with diabetes who have kidney abnormalities, heart muscle abnormality or who are over 80 years of age. Symptoms of lactic acidosis may include tiredness, muscle aches, difficulty breathing, vomiting, or severe abdominal pain. If you develop these symptoms or any serious medical condition while taking Metformin, you should stop the medication and seek medical treatment - your regular physician or your local Emergency Department – the physician you see should be informed that you are taking Metformin.

As with any medication, allergic reactions are a possibility.

Risks for blood draws: The risks of drawing blood include pain, bruising or rarely infection at the needle site.

CT or MRI Scans: There are risks associated with metformin when receiving scans with contrast. If you need to have a CT or MRI scan while taking metformin/placebo, you should inform the radiology staff.

Reproductive risks: You should not become pregnant while on this study because the drugs in this study can affect an unborn baby. Women should not breastfeed a baby while on this study. It is important you understand that you need to use birth control while on this study. Check with your health care provider about what kind of birth control methods to use and how long to use them. Some methods might not be approved for use in this study.

For more information about risks and side effects, ask your study doctor.

Are there benefits to taking part in the research study?

Taking part in this study may or may not make your health better. While doctors hope metformin will be useful in preventing breast cancer, there is no proof of this yet. We do know that the information from this study will help doctors learn more about metformin as a treatment to help prevent breast cancer. This information could help future patients who have a higher risk of getting breast cancer.

What other choices do I have if I do not take part in this research study?

You do not have to be in this study to receive treatment.
Your other choices may include:

- Getting treatment or care to help prevent your cancer without being in a study
- Taking part in another study
- Receiving standard treatment, i.e., tamoxifen
- Getting no treatment

Talk to your doctor about your choices before you decide if you will take part in this study.

Will my medical information be kept private?

We will do our best to make sure that the personal information in your medical record will be kept private. The study doctors have a privacy permit to help protect your records if there is a court case. However, we cannot guarantee total privacy. Your personal information may be given out if required by law. If information from this study is published or presented at scientific meetings, your name and other personal information will not be used.

Organizations that may look at and/or copy your medical records for research, quality assurance, and data analysis include:

- Alliance for Clinical Trials in Oncology (Alliance)
- Institutional Review Boards
- The National Cancer Institute (NCI) and other government agencies, like the Food and Drug Administration (FDA), involved in keeping research safe for people
- The Cancer Trials Support Unit (CTSU), a service sponsored by the NCI to provide greater access to cancer trials

A description of this clinical trial will be made available on [redacted] This Web site will not include information that can identify you. At most, the Web site will include a summary of study results. You can search this Web site at any time.

[Note to Informed Consent Authors: the above paragraph complies with the new FDA regulation found at 21 CFR 50.25(c) and must be included verbatim in all informed consent documents. The text in this paragraph cannot be revised.]

[Note to Local Investigators: The NCI has recommended that HIPAA regulations be addressed by local institutions. The regulations may or may not be included in the informed consent form depending on local institutional policy.]

What are the costs of taking part in this research study?

You and/or your health plan/ insurance company will need to pay for some or all of the costs of procedures being done in this study. Some health plans will not pay these costs for people taking part in studies. Check with your health plan or insurance company to find out what they will pay for. Taking part in this study may or may not cost your insurance company more than the cost of getting regular treatment.

The study agents, metformin and placebo, will be provided free of charge while you are taking part in this study. The cost of getting the metformin and placebo ready and giving it to you is not paid by the study, so you or your insurance company may have to pay for this. You will not be paid for taking part in this study.

You will not need to pay for tests and procedures that are done just for this research study. These tests are:

- Research blood test(s)

For more information on clinical trials and insurance coverage, you can visit the National Cancer Institute's Web site at [redacted]. You can print a copy of the "Clinical Trials and Insurance Coverage" information from this Web site. Another way to get the information is to call [redacted] and ask them to send you a free copy.

What happens if I am injured because I took part in this research study?

It is important that you tell your study doctor, _____ [investigator's name(s)]; if you feel that you have been injured because of taking part in this study. You can tell the doctor in person or call him/her at _____ [telephone number].

You will get medical treatment if you are injured as a result of taking part in this study. You and/or your health plan will be charged for this treatment. The study will not pay for medical treatment.

What are my rights if I take part in this research study?

Taking part in this study is your choice. You may choose either to take part or not to take part in the study. If you decide to take part in this study, you may leave the study at any time. No matter what decision you make, there will be no penalty to you and you will not lose any of your regular benefits. Leaving the study will not affect your medical care. You can still get your medical care from our institution.

We will tell you about new information or changes in the study that may affect your health or your willingness to continue in the study.

In the case of injury resulting from this study, you do not lose any of your legal rights to seek payment by signing this form.

Who can answer my questions about the research study?

You can talk to your study doctor about any questions or concerns you have about this study. Contact your study doctor _____ [name(s)] at _____ [telephone number].

For questions about your rights while taking part in this study, call the _____ [name of center] Institutional Review Board (a group of people who review the research to protect your rights) at _____ [telephone number]. [Note to Local Investigator: Contact information for patient representatives or other individuals in a local institution who are not on the IRB or research team but take calls regarding clinical trial questions can be listed here.]

Where can I get more information?

You may call the National Cancer Institute's Cancer Information Service at:

[redacted]

You may also visit the NCI Web site at [redacted]

- For NCI's clinical trials information, go to: [redacted]
- For NCI's general information about cancer, go to [redacted]
- For NCI's general information about cancer in Spanish, go to [redacted]

You will get a copy of this form. If you want more information about this study, ask your study doctor.

ADDITIONAL STUDIES SECTION (REQUIRED):

Please note: This section of the informed consent form is about additional research studies that are being done with people who are taking part in the main study.

Research on Tissue

Please read this section of the informed consent on related research studies and ask about anything that is not clear to you. This is to inform you of the possible risks, benefits and limits of giving your samples for research.

As described above, you will be required to have RPFNA tissue and blood samples taken to use for protein and gene research studies as part of the main study.

What are samples and where are they stored?

A sample is any material taken from your body such as tissue, blood, urine and other fluids. If you agree, your samples will be stored for research in a Cooperative Group bank supported by the National Cancer Institute. A Cooperative Group bank contains samples and information. Your samples are kept along with those from other people in this bank. Researchers then ask for samples from the bank to study them.

What information will be collected?

Your tissue and some of your blood will be sent along with your initials and the date of collection to a laboratory at City of Hope Hospital in California. Some of your blood samples will be sent to the Alliance Bank. Any personal information sent with the samples to the Alliance bank is not given to researchers. Your privacy will be protected to the fullest extent possible. This will be discussed later in the section "How will information related to my samples be protected?"

Other information that might be stored for future research by the Alliance includes:

- Dates of medical procedures
- Any diagnosis and stage of your disease (if you have cancer)
- Your age and race
- Medical and family history
- Treatments you had
- How you responded to treatments

What will happen to my samples?

Your samples will be stored in the Alliance Bank. The samples will be kept until they are used up or destroyed. The samples are given a code to protect your privacy before they are used. Any related information given to researchers will also be coded. Researchers will receive the code instead of any information that might directly identify you.

You or your doctor will not be given reports or other information about the research that uses your samples. This information will not be put into your health record. Results may be used for future research.

You will not be named or identified by other personal information if any results are published. Most publications contain results from many patients.

Your samples and related information will be used only for research and will not be sold. It is possible that research may help to create new products or treatments. If this should happen, you will not be paid.

Because the information gained from the research studies performed on your samples can be very useful to the research community, several groups including the National Institute of Health (NIH) have requested that some of these data be placed in a central database. Therefore, some of the coded research information may be sent to a central database. The goal is to speed up the process of discovery of new treatments, prevention and diagnosis of disease. The information will continue to be made available for approved research. Your name or contact information will not be put in the database.

What kind of research will be done with my samples?

Many types of research use normal or atypical (marker of cancer risk) or cancerous samples. Researchers can study proteins, RNA and DNA (genes). The study of genes (DNA) is often called genetic research.

Your samples may be looked at:

- To see if a trait is passed down in families from one generation to the next (inherited). This type of research may help to explain why some cancers run in families or why some people have side effects of treatment while others do not. This is often studied through blood cells and DNA (genes).
- To learn about changes in the body that happen after you were born (non-inherited). For example, being in the sun too much can cause changes in cells that lead to skin cancer.

Will it help me to give my sample for research?

Using your samples for research will probably not help you. We do hope the research results will help people in the future. The best way to prevent, find or treat cancer and other diseases is by studying human samples and data.

What are the risks of giving my samples for research?

- There can be mild pain, or some bleeding or bruising when blood is drawn. Rarely, an infection can happen where the needle was placed. Feeling dizzy or fainting can also happen, but may only last a few minutes after blood is drawn.
- There is a risk that your information could be misused. The chance of this happening is very small. We have many protections in place to lower this risk. See the next section, "How will the information related to your samples be protected?" Your privacy will be protected to the fullest extent possible.
- There can be a risk in knowing genetic information. New health information about inherited traits that might affect you or your blood relatives could be found during a research study. Even though your genes are unique, you share some of the same genes with your blood relatives. Very rarely health or genetic information could be misused by employers, insurance companies, and others. For example, life insurance companies may charge a higher rate based on this information.

Some states have laws to protect against genetic discrimination. A new federal law called the Genetic Information Non-Discrimination Act, or GINA is in effect. This law helps to lower the risk of health insurance or employment discrimination. The law does not include other types of misuse by life insurance or long term care insurance. To learn more about the GINA Law, please check the Internet or ask the study staff.

Although we are not able to know all of the risks from taking part in research on inherited traits, we believe that the risks to you and your family are very low, because research results will not be returned to you or your doctor.

How will information related to my samples be protected?

We have many ways to protect the information related to your samples:

- The samples and information sent to the Alliance Bank will receive a unique code. Researchers only receive coded samples and information, and will not be able to link the code to you. Only approved people in the Alliance can match you to the code on your samples and related information.
- Strict security safeguards are in place to reduce the chance of misuse or unplanned release of information. Steps we take include, but are not limited to, restricted access to buildings, rooms and freezers housing patient samples, numeric coding of both patient data and samples, and password protected access to databases housing patient data.
- Before samples are given to researchers, studies are reviewed for the quality of the science and for patient protection. Records from research studies can be reviewed by the Cooperative Group, by the sponsor, and by government agencies. This is to make sure the research follows the rules of the Cooperative Group and state or federal laws.
- In most cases, research results will not be returned to you or your doctor. If research results are required to make a decision regarding your treatment on this study then research results may be shared with you or your doctor. If research results are published, your name and other personal information will not be given.

ADDITIONAL FUTURE STUDIES SECTION (OPTIONAL):

Storage of Your Specimens:

We would like to keep any leftover tissue and blood samples for future research. The choice to allow your left over samples to be stored and used for future research is up to you. This may help researchers learn more about how to prevent, find and treat cancer and other diseases. No matter what you decide, it will not affect your medical care and it will not affect whether you take part in other studies.

The research that may be done with your specimens is not designed specifically to help you. It might help people who have cancer and other diseases in the future. Reports about research done with your specimens will not be given to you or your doctor. These reports will not be put in your health record. The research will not have an effect on your care. The choice to let us keep left over specimens for future research is up to you.

Making your choice

The choice to take part is up to you. You may choose not to let us use and store your leftover samples. If you decide not to let us store and use your samples, you will still receive the same medical care. You may also take part in other research studies.

If you decide that your samples can be kept, you may change your mind at any time. Contact the study staff at your hospital and let them know that you do not want your samples used for research. Then, any sample that remains in the bank will no longer be used. Samples or related information that have already been given to or used by researchers cannot be returned or destroyed.

Thank you for considering whether to allow your samples to be used for the research described above and/or banked for future research.

1. My coded samples and related coded information may be kept for use in future research to learn about, prevent, find or treat cancer. This may also include research on inherited traits (genes passed on in families).

   Yes____ No____

2. My coded samples and related coded information may be kept for use in future research to learn about, prevent, find or treat other health problems (for example: diabetes, Alzheimer's disease, or heart disease). This may also include research on inherited traits (genes passed on in families).

   Yes____ No____

3. Someone from my hospital or the Alliance may contact me in the future to ask to take part in more research.

   Yes____ No____

This is the end of the section about additional studies.

Signature

I have been given a copy of all _____ *[insert total of number of pages]* pages of this form. I have read it or it has been read to me. I understand the information and have had my questions answered. I agree to take part in this study.

Printed Participant Name: _____

Participant Signature: _____

Date: _____

Printed name of person obtaining informed consent:

_____

Signature of person obtaining informed consent:

_____

Date _____

APPENDIX II: STANDARD OPERATING PROCEDURE FOR RPFNA

Collection and Analysis of Breast Cells by Random Periareolar Fine Needle Aspirate (RPFNA) in Women at High Risk for Breast Cancer on Study

Procedure: Participants will undergo RPFNA of both breasts (at the 2 and 10 o'clock position) prior to treatment with metformin or placebo and after treatment with metformin or placebo. RPFNA will be used to collect breast tissue samples. The entire procedure is expected take 30 minutes. Personnel required will include the provider performing the procedure and an assistant.
Note: Only the contralateral breast can be aspirated in women with DCIS and in those undergoing surgery for an atypical lesion.

Prior to Procedure:

- RPFNA kits will be assembled in quart-size Ziploc bags. Each kit should be labeled with the study name and CRA contact information. Each kit must include:

Item	Quantity
12 cc Syringe	17
20 gauge needles (1.0-1.5")	17
25 gauge needles (1.0-1.5")	5
6 cc Syringe	5
1 mL Syringe (with 25 gauge 5/8 inch needle)	5
Cytolyte	2 vials
10% formalin	2
8.4% Injectable Sodium Bicarbonate	approx. 5 mL
Lidocaine 1% (with Epi)	1 (20 mL)
RPFNA Procedure Documentation Form	1

- Provider/CRA will remind the participant not to use aspirin or other NSAIDs for the 7 days prior to the procedure and for 24 hours after the procedure.
- At least one day prior to the procedure, the participant will be given Emla crème. (Participating sites will need to provide this through their clinic or by writing a pharmacy prescription for the participant).

Day of Procedure:

- On the day of the procedure the CRA will be available to help the clinician performing RPFNA collect the sample and bring/ship the Cytolyte containing the cellular material to the laboratory of Victoria Seewaldt.
- On the day of the procedure the clinician will prepare the modified Cytolyte: two 15 mL tubes containing 10 mL modified Cytolyte (Cytolyte 9 mL + 1 mL 10% formalin freshly prepared on the morning of aspiration).
- On the day of the procedure the participant will apply the Emla crème in a "inch area ~1 inch from the areola at 2 and 10 o'clock on each breast 45 minutes to 1 hour before the procedure.
- Prior to the procedure the CRA will label the Cytolyte tube with the study name and number, study participant ID #, and the date of collection.
- The CRA will set up the procedure room prior to the participant's arrival.
- Four 1 mL syringes with 25 gauge 5/8 inch needles should be set up to contain 4 parts lidocaine and 1 part sodium bicarbonate (0.8 mL lidocaine with epinephrine followed by 0.2 mL of sodium bicarbonate).
- Four 6 cc syringes with 25 gauge 1.0-1.5 inch (depending on participant breast size) needle setups should be put together. These syringe/needle setups will include 5 mL total of lidocaine/sodium

bicarbonate with 4 parts lidocaine and 1 part sodium bicarbonate (4 mL lidocaine with epinephrine followed by 1 mL sodium bicarbonate).
- 16 syringe/needle setups (20 gauge 1.0-1.5 inch needles depending on participant breast size) should be put together (therefore one extra syringe/needle setup will be available during the procedure if needed). These should be pre-wetted with RPMI or saline to the hub of the needle. RPMI/saline should be removed just prior to the procedure for each syringe.

Procedure:
- The Emla crème will be removed with a soft cloth before the procedure. The affected areas will be cleaned with an alcohol swab prior to anesthetizing the area.
- 1% lidocaine **with** epinephrine will be used for anesthesia. A small wheel of lidocaine will be made on the skin at each site of the Emla crème with the 1 mL syringe with a 25 gauge needle. This will be followed by the deeper injection of 1% lidocaine at each site (2 and 10 o'clock on each breast) using a 1.0-1.5 inch 25 gauge needle.
- 3 to 5 minutes should be taken to let the lidocaine spread and numb the underlying tissue, as well as to be absorbed. It is recommended that all sites be anesthetized prior to beginning the procedure.
- Sixteen, 1.0-1.5 inch 20 gauge needles attached to 13 cc syringes will be used for RPFNA at each anesthetized site (total 16 needle setups). The breast should be held up in the opposite hand that is performing the cell collection. Just before using the needle, squirt out the RPMI/saline from the syringe. Then, with the needle under the skin, suction will be applied in a steady manner and multiple needle passes per needle/syringe setup will be performed. When the hub of the needle has a visible amount of material present, that needle can be withdrawn and a new needle set used. It is important that when withdrawing the needle some air enter the needle, but not so much that all the cellular material sprays throughout the plastic syringe.
- A used needle setup will be handed to the CRA. The CRA will forcefully plunge the contents of the syringe/needle into the modified Cytolyte container (Cytolyte 9 mL + 1 mL 10 % formalin freshly prepared on the morning of aspiration). The syringe setup should subsequently be washed with modified Cytolyte from this same tube to remove any remaining cellular material (wash/plunge an additional 3-4 times).
- The entire sample from the right breast is pooled into one tube of modified Cytolyte labeled RIGHT - and the entire sample from the left breast is pooled into the second tube of modified Cytolyte labeled LEFT.

After the Procedure:
- Following the procedure each breast should be treated with ice packs over the sites of the procedure for 10 minutes.
- The participant should maintain compression of the breasts for the next 24 hours, even while sleeping. This can be done using by wearing a tight fitting sports bra or the Kling wrap/Breast Binder. The compression will prevent further bleeding and swelling.
- Recommend that the participant take acetaminophen within the hour to alleviate any discomfort as the lidocaine wears off.
- Provider will complete the RPFNA Procedure Documentation Form, to document the procedure.
- CRA or provider will make a follow-up phone call the following day to check in with the participant and review any side effects of the procedure.

For participants at UVM/FAHC: Samples will be taken directly to the cytopathology laboratory where the sample will be spun down and processed into cytology slides using the thin preparation method.

For participants at other sites: The 2 tubes of modified Cytolyte will be appropriately packaged (see Section 14.0) and sent to the laboratory of Victoria Seewaldt (see shipping address), where it will be processed as described in the protocol.

Shipping address:

[redacted]

APPENDIX III: PATIENT MEDICATION DIARY

Start Date: _____

Alliance Patient Number: _____ Patient Initials (LFM) _____

INSTRUCTIONS TO PATIENT:

- Complete this form during the four week ramp-up period.
- Please record the date you start taking pills for this study.
- You should take one pill by mouth once a day.
- After 4 weeks, you will receive a telephone call to discuss this medication diary.

Day of the Week	Day of the Week	Day of the Week	Day of the Week	Day of the Week	Day of the Week	Day of the Week
Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21
Day 22	Day 23	Day 24	Day 25	Day 26	Day 27	Day 28

COMMENTS:

SIGNATURE: _____

DATE: _____ *(dd/mm/yyyy)*

APPENDIX IV: NURSE/CRA PHONE CONTACT GUIDE

Patient Phone No. _____
Best Dates/Times to call_____

FOLLOW UP:

1. Phone call schedule:
   - Call patient at home after one month to document compliance and address problems. If the patient is seen in clinic, this phone call can be omitted.

   - Call patient at home for those women on placebo arm and crossing over after 12 months.

2. **Physician's office will record the following information in the patient record.**
   - Date of phone call
   - Date patient finished ramp-up period _____
   - Total number of pills taken during ramp-up period: _____
   - Date patient started protocol treatment: _____
   - Questions/Comments

     If the patient reports any side effects, please document:
     - Side effects:
       - Abdominal distension - Severity and attribution, if applicable
       - Bloating - Severity and attribution, if applicable
       - Diarrhea - Severity and attribution, if applicable
       - Nausea - Severity and attribution, if applicable
       - Vomiting - Severity and attribution, if applicable
       - Any others - Severity and attribution, if applicable.

3. Remind the patient to increase medication to twice daily after 28 days.

Physician/Nurse/Data Manager's signature _____

Date _____

APPENDIX V: FOLLOW-UP RPFNA PHONE CONTACT

Introduction:

Good morning/afternoon. This is _____speaking and I'm calling from _____. Thank you for participating in our chemoprevention study comparing Metformin to placebo. I would like to ask a few questions about how you are doing following the Random Periareolar Fine Needle Aspiration procedure. Please also understand that your answers to these questions will be kept confidential and when the data from this research study is reported your data will be included in a summary without your name or any other identifying information. Is now a good time?

___Yes (continue) ___No: *When may I call you back?* _____

1. *Did you experience any pain after the RPFNA procedure*? ☐ Yes ☐ No

If no, skip to question #3.

If yes*, how long did it last?* _____

2. *If yes, did you take any medication for the pain?* ☐ Yes ☐ No

If no, skip to question #3.

If yes, *which medication and how many doses?* _____

_____

3. *Do you have any bruising after the RPFNA procedure?* ☐ Yes ☐ No

4. Did you experience any of the following after the RPFNA procedure?

Fever ☐ Yes ☐ No

If yes, when did it start, how bad was it, did you take any medication for it, did it resolve?

Comments: _____

Bleeding ☐ Yes ☐ No

If yes, when did it start, how bad was it, did you take any medication for it, did it resolve?

Comments: _____

Indication of infection, such as redness, warmth, odor, or discharge at the site of the needle insertions? ☐ Yes ☐ No

Comments: _____

_____

Physician/Nurse/Data Manager's signature _____ Date _____