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Phase Ib 9cUAB30 in Early Stage Breast Cancer to Evaluate Biologic Effect

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Protocol Revision or Amendment # Version 20

SCHEMA

Pilot Study of 9cUAB30 in Early Stage Breast Cancer to Evaluate Biologic Effect

Up to 41 participants with early stage breast cancer – diagnosed by core biopsy

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Invasive breast cancer between 0.5 cm and 5 cm in size
Estrogen receptor positive (ER+) or estrogen receptor negative (ER–),
Her2neu positive or Her2neu negative
OR
Ductal carcinoma in-situ (DCIS) at least 1.0 cm in size,
ER+ DCIS that is Grade 3 or ER- DCIS of any grade

*Beginning November 2021 only estrogen receptor negative (ER-) participants (12 participants) will be enrolled.

↓

Administration of 9cUAB30 240 mg once daily in the evening on an empty stomach (take agent at least 2 hours after last eating and wait at least 1 hour before eating again) for 14-28 days before surgery
(First 5 participants enrolled N=5)
First dose taken in clinic with 5 minute and 2hr. PK blood draws

*After interim analysis**

- Administration of 9cUAB30 240 mg once daily in the evening on an empty stomach (take agent at least 2 hours after last eating and wait at least 1 hour before eating again) for 14-28 days before surgery (N=31)

OR

- Administration of 9cUAB30 160 mg once daily in the evening on an empty stomach (take agent at least 2 hours after last eating and wait at least 1 hour before eating again) for 14-28 days before surgery (N=36)

↓

Home BP Check Day 7

↓

Telephone Contact and Home BP Check Day 8 (+/-1 day)

↓

Home BP Check Day before surgery

↓

Definitive Breast Surgery Day 15-29

↓

Telephone Contact 7 days (+/-1 day) after surgery and 4-5wks after surgery

Slides from core biopsy and surgical specimens will be obtained for each enrolled participant along with historical matched control tissues.
Study Endpoints:
Primary: Change in proliferation (Ki-67)
Secondary:

- Change in apoptosis (Cleaved Caspase 3)
- Differences in gene expression from pre and post treatment samples using a custom gene panel from Nanostring Technologies
- Examine Safety and PK drug levels in first 5 participants for interim analysis
- PK drug levels
- Increase gene expression of type I immune cells in the tumor immune environment
- Examine safety in comparison to known Retinoid toxicity

Exploratory: Increase activated type I dendritic cells in peripheral blood (participants 6-25)

**If the interim analysis for dose 240mg does not establish Cmax >957 ng/mL or has ≥ 2 subjects experience grade 2 hypertriglyceridemia of any attribution then the study dose will be changed to 160mg.*

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1. OBJECTIVES

The overall goal is to determine if 9cUAB30 can produce a potentially beneficial biologic effect on human breast cancer at a non-toxic dose. We hypothesize that comparative molecular analysis of pre- and post-treatment tissue samples of breast cancers of patients treated with 14-28 days of oral RXR-selective 9cUAB30 will demonstrate significantly reduced proliferation, increased apoptosis and changes consistent with ligand-RXR binding, as reflected in downstream markers, evaluated at the mRNA level by Nanostring, with selective confirmation at the protein level by IHC.

1.1 Primary Objective

We hypothesize that comparative molecular analysis of pre- and post-treatment tissue samples of breast cancers of participants treated with 14-28 days of oral RXR-selective 9cUAB30 will demonstrate significantly reduced proliferation.

1.2 Secondary Objectives

1.2.1 Determine if 14-28 days of oral RXR-selective 9cUAB30 treatment increases apoptotic index, as measured by cleaved caspase 3 assay.

1.2.2 Examine the differences in gene expression from baseline to post-exposure breast cancer samples using a custom gene panel from Nanostring Technologies. Examined genes will be selected as described below and will include up to 48 genes. Prospective biomarkers on the panel will include but are not limited to (A) biomarkers associated with cell proliferation or apoptosis: PCNA, p21, p27, TUNEL assay, caspase 3; (B) potential biomarkers of rexinoid activity reported in published studies: ABCA1, ABCG1, RARβ, SREBP-1c, cyclin D1, IGFBP6; (C) potential biomarkers of response to 9cUAB30 as defined in our preliminary studies: RET, MEN1, mTOR, AKT1, AKT2, 4EBP1, eIF4G1, S6K, IGFBP5, TGFβR1, PPARα, PPARγ (D) housekeeping genes for normalization: GAPDH, TUBB, PGK1, GUSB, and CLTC. Additionally, as possible, we will perform IHC for a subset of 5 of the most differentially regulated genes found on Nanostring to verify that gene expression data also results in protein differences.

1.2.3 To examine if the Cmax and safety of 9cUAB30 in the first 5 participants is affected by reducing the number of capsules at the 240mg dose level. That is using 3 capsules of 80mg each instead of 12 capsules of 20mg each.

1.2.4 To examine the Cmax of all participants at baseline and on the day of surgery

1.2.6 Determine if treatment with 2-4 weeks of 9cUAB30 prior to surgery will increase gene expression of type I immune cells in the tumor immune environment of all participants except the first 5

1.2.7 Assess the overall safety of 9cUAB30 in comparison with known retinoid toxicity.

1.3 Exploratory Objective

1.3.1 Determine if treatment with 2-4 weeks of 9cUAB30 prior to surgery will increase activated type I dendritic cells in peripheral blood.

2. BACKGROUND

2.1 Study Disease

In 2019, an estimated 268,600 new cases of invasive breast cancer and 48,100 new cases of DCIS were diagnosed in women in the United States and approximately 41,760 women were expected to die from breast cancer.[1] Prevention of breast cancer not only is key to reducing breast cancer mortality but also to improving quality of life by reducing the number of patients who have to undergo surgery, chemotherapy and radiation therapy to treat their cancers.

Women at increased risk for breast cancer are candidates for hormonal prevention strategies. Two selective estrogen modulators (SERMs), Tamoxifen or raloxifene, are highly effective (≈ 50%) in reducing risk of ER+ breast cancers. Aromatase inhibitors (AIs) prevent the recurrence of invasive ER+ cancers as well as or better than SERMs. Currently, Exemestane, an AI, is included in management guidelines as an alternative to tamoxifen or raloxifene to reduce risk of invasive ER+ breast cancer in postmenopausal women at increased risk for breast cancer. However, because of the real and perceived risks of chemoprevention, patient uptake of current chemoprevention strategies remains low.[2] [3]Studies show that only 12% of women at high risk for breast cancer opt for Tamoxifen as a preventive agent. The main reason women are declining Tamoxifen is due to concerns about toxicity.[4] Moreover, a significant population of high-risk women will develop cancers that are not prevented by currently approved prevention strategies. For example, women with mutant BRCA-1 usually develop tumors that are ER-negative/PR-negative/Her2neu negative. Evidence is sparse that these patients will respond to traditional hormonal prevention.[5] There is a need for an oral preventive agent with an attractive side effect profile that will prevent both ER-positive and more aggressive ER-negative breast cancers.

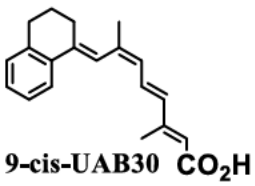
2.2 Study Agent

Retinoids, which target the retinoic acid receptors (RARs), and rexinoids, which target the retinoid X receptors (RXRs), represent two classes of potential drugs with potent capacity to prevent breast cancers and have been studied extensively. 9-cis-Retinoic acid (9cRA) is a potent agonist for RXRs,[6-10]which activates RXR homodimer-mediated transcription or heterodimer-mediated transcription with other receptors of the steroid/thyroid superfamily (e.g. RARs, VDR, PPAR, TR and orphan receptors). These two pathways lead to activation of gene transcription that is responsible for the induction of differentiation and apoptosis. Thus, RXRs are the master regulators of gene expression of the RAR- and RXR-signaling pathways, and both pathways can be controlled by 9cRA.[8-11] 9-cis-retinoic acid has been shown to be effective in preventing mammary gland tumors in N-*methyl*-N-*nitrosurea* (MNU) animal models, unfortunately, the effective dose is near its maximally tolerated dose.[12]

Targretin and other RXR-selective ligands, or rexinoids, have been developed in an effort to identify a rexinoid that will be safe for chronic human use. Targretin is the best studied rexinoid and FDA approved to treat cutaneous T-cell lymphoma. Targretin shares little structural similarity to 9cRA, and human clinical trials revealed it was better tolerated than 9cRA. Targretin did not produce many of the classic retinoic acid associated toxicities of 9cRA (bone fractures; skin lesions and redness; elevated serum calcium) but does produce dose-limiting hyperlipidemia (both elevated cholesterol and triglycerides) and hypothyroidism.[13,14] The lower toxicity of Targretin relative to 9cRA did not reduce potency of this rexinoid in cancer prevention. Several studies demonstrated Targretin was as effective as 9cRA in reducing the number and tumor burden of ER+ mammary cancers.[12,15] Other groups established that the effects of combining Targretin or 9cRA with a SERM were additive and the combination regimen significantly decreased tumor

number/burden.[16-18] More recently, Brown demonstrated Targretin prevents ER– cancers in rodents in a dose-dependent manner.[19,20] These studies and other reveal the potential of rexinoids as cancer preventive agents.

2.2.1 9cUAB30 – a novel rexinoid for chemoprevention

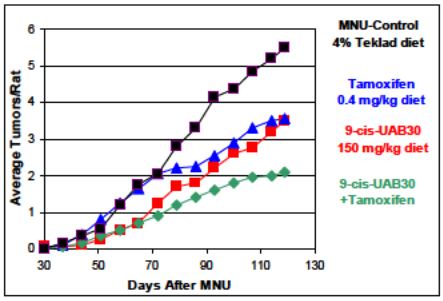


The aim of medicinal chemists at University of Alabama-Birmingham (UAB) was to design a rexinoid that was selective and potent for epithelial cells but was not an agonist in tissues that promote lipid biosynthesis (e.g., liver). The goal was to make rexinoids attractive cancer preventive agents by minimizing side effects. In 1998, we reported on the design and synthesis of 9cUAB30 and showed it to be a highly selective agonist for each RXR subtype.[21] 9cUAB30 is a novel RXR-selective retinoid, which is a conformationally constrained analog of 9 cis UAB30. In animal models we have shown that it prevents both ER-positive and ER-negative mammary cancers with similar potency to Targretin. Additionally, modulation of proliferation and apoptosis was seen after only 7 days of treatment.[22] Importantly we used gene expression array profiling to demonstrate that 9cUAB30 is not an agonist in liver, which is the desired hallmark of a tissue-selective rexinoid.[23]

2.2.2 Animal Model Studies

2.2.2.1 MNU – ER+ mammary cancer prevention rodent model

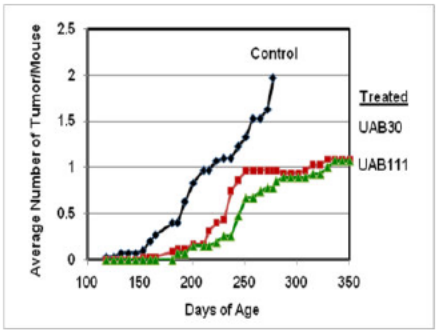
We first evaluated 9cUAB30 in an ER-positive mammary cancer prevention rodent model (MNU).[24,25] Using this model we showed that 9cUAB30 was as effective as either Targretin or 9cRA in preventing ER-positive cancers. 9cUAB30 reduced the number of tumors by approximately 30% (65-70% effective) which was similar to the reduction in number of tumors by tamoxifen.[26] Low-dose 9cUAB30 in combination with Tamoxifen resulted in additive tumor prevention (see inset). [11]We also demonstrated the combination of low-dose 9cUAB30 and the AI, vorozole, was additive [67% decrease from combination versus 37% for low-dose 9cUAB30 and 42% decrease for vorozole].



Efficacy of 9cUAB30 and tamoxifen, either alone or in combination, on the time of appearance of MNU-induced mammary cancers [11]

2.2.2.2 MMTV-erbB2 transgenic model – ER– mammary cancers

In addition, it appears that 9cUAB30 may prevent ER-negative mammary cancers. RAR agonists and RXR agonists control cell proliferation and induce apoptosis, and they may prevent cancers regardless of estrogen status.[27] Two different transgenic models [MMTV-erbB2 and the C3(1)SV40 T/t-antigen transgenic models] have been developed to evaluate if agents prevent ER-negative mammary cancers.[18,20] Each preclinical animal model responds to either Targretin or 9cRA in a dose-dependent manner.[18,20] We evaluated 9cUAB30 in the MMTV-erbB2 transgenic model at 200 mg/kg diet. As shown in the inset, 9cUAB30 is very effective in preventing the number (and tumor burden) of ER-negative MMTV-erbB2 mammary cancers by approximately 50%. The 200 mg/kg diet (20 mg/kg BW mouse) is equivalent to a 120 mg/day dose for a 60 kg human.



Efficacy of 9cUAB30on the time of appearance of MMTV-erbB2 transgenic model (Data not published)

2.2.3 Preclinical toxicity studies

In a series of preclinical studies conducted via the NCI DCP RAPID program, 9cUAB30 was found to have no lipid toxicity in two species (rat and dog), [28] and it had fewer side effects than Targretin. 9cUAB30 displayed favorable pharmacology and was not mutagenic.[29,30]

2.2.4 Human Studies

After IND approval in 2009 (IND#101,604), DCP funded the first Phase 1 trial. Between November 2008 and May 2009, 14 healthy volunteers at the University of Wisconsin (UW) were treated with single doses of 9cUAB30, 4 subjects at 5mg, 6 subjects at 10 mg, and 4 subjects at 20 mg.[31] Subjects were 50% male and 50% female and all were white, non-Hispanic. Subjects were enrolled in groups of 4 sequentially into the progressively higher dose groups; a single dose of 5, 10, or 20 mg of 9cUAB30 was given orally in the morning on an empty stomach. No subjects at dose level 5 mg or 20 mg experienced ≥grade 2 toxicity. There were few observed ≤ grade 1 laboratory abnormalities, including 2 subjects with grade 1 hypertriglyceridemia After Tmax (2-3 hours after dose), 9cUAB30 levels declined steadily. Plasma half-life increased in a dose dependent manner, averaging 2.79 hours in the 5-mg group, 5.76 hours in the 10-mg group, and 7.21 hours for the 20-mg group. In regression analysis, AUC increased linearly across the examined dose range of 5 to 20 mg; a linear relationship between AUC and dose was found to be significant (β=38.7, $p < 0.001$). Cmax, increased with dose, but not in a linear fashion; it was found to be proportional to the log of dose (β= 4.43, $p < 0.001$). The average plasma clearance ranged from 25 to 39 L/h compared to the average renal clearance which ranged from 0.018 to 0.103 L/h.

We initiated a double-blind, placebo-controlled single and multi-dose study of 9cUAB30 in healthy volunteers at 3 participating organizations (UW, UAB, and University of Iowa). Over the course of two different protocols (UWI09-8-02, UWI10-16-01R), 44 participants were enrolled into the study in cohorts of 10 (8 9cUAB30:2 placebo). The starting dose level was 20 mg followed by dose cohorts at 40 mg, 80 mg, 160 mg, and 240mg daily. Subjects received a single, initial dose of study drug (fasting) on day 1 with full pharmacokinetic (PK) and safety monitoring for 24 hours and continued observation off study drug until returning the morning of day 8 for PK sampling and initiation of continuous daily dosing (day 8 -35) when they would return for full PK monitoring for 24 hours after their last dose. Summary PK parameters are presented in Table 1; we observed a linear to near linear increase in peak concentrations and AUC from 20 to 160 mg per day of 9cUAB30. There was no further increase in 9cUAB30 concentrations (Cmax or AUC) from 160 to 240 mg dosing, in fact there was even some evidence to suggest a possible decline in drug levels raising the possibility of absorption issues/interference due to the large number of gelatin capsules at 240 mg (12 capsules). Comparison of baseline and day 36 pharmacokinetics parameters did not suggest drug accumulation or altered metabolism (whether an increase or decrease in absorption or clearance) with repeated dosing.

Table 1: AUC by Dose Level Cohorts 20mg-240mg Multi-day Study (UWI09-8-02, UWI10-16-01R)

Parameter, Time	0 mg (n=10)		20 mg (n=8)		40 mg (n=8)		80 mg (n=8)		160 mg (n=8)		240 mg (n=8)	
Cmax* (ng/ml)												
Day 1	0	(0-0)	128	(41.0-252)	176	(19.8-430)	623	(353-2620)	975	(348-1590)	812	(246-1830)
Day 36	0	(0-0)	122	(54.8-143)	201	(54.5-247)	1060	(220-1690)	1420	(486-5120)	889	(477-1370)

Absolute Change	0	(0-0)	-10.7	(-111-53.7)	-47.8	(-183-185)	492	(-930-751)	950	(-1100-3990)	186	(-1290-623)
AUC*												
Day 1	0	(0-0)	1200	(304-3640)	1570	(181-1900)	5710	(1850-12500)	13000	(2190-62000)	8560	(2000-23400)
Day 36	0	(0-0)	1030	(529-2420)	2280	(865-16000)	8290	(3270-13500)	14400	(10700-40700)	12300	(4990-15400)
Absolute Change	0	(0-0)	-86.3	(-2660-1280)	831	(348-14400)	1730	(-3170-6510)	5820	(-2250-27600)	2710	(-863-7310)

*Data are presented as median (range)

Subjects were followed for 30 days after their last study dose and escalation to the next dose cohort occurred after all 10 subjects at a dose level had completed the course with ≤1 subject experiencing unacceptable, attributable toxicity. Characteristics of the 44 subjects were; mean age - 40.9 (SD 13.5); ECOG performance status – all zero; 27 male; 17 female; 39 subjects - white, 5 - African American; all identified as not Hispanic or Latino. Early evaluation of observed toxicity did not reveal any consistency or pattern of toxicity normally seen with rexinoids. Reports of nausea or headache were rare and did not persist despite continued administration of study drug. Skin changes and fatigue were rarely reported. Serum lipid abnormalities were noted but rarely consistently elevated from baseline.

Table 2: Percentage of subjects with worst grade of adverse event for CTCAE v4.0 category

	Grade		0 mg (n=12)		20 mg (n=8)		40 mg (n=9)		80 mg (n=8)		160 mg (n=9)		240 mg (n=8)	
CTCAE Category Overall	0	None	2	(17)	2	(25)	0	(0)	2	(25)	1	(11)	0	(0)
	1	Mild	5	(42)	2	(25)	5	(56)	2	(25)	3	(33)	3	(38)
	2	Moderate	5	(42)	2	(25)	4	(44)	4	(50)	4	(44)	2	(25)
	3	Severe	0	(0)	2	(25)	0	(0)	0	(0)	1	(11)	3*	(38)

*All grade 3 events were considered "Unlikely" to be related to treatment. To be a dose-limiting toxicity, they would have had to be at least "Possibly" related.

2.3 Rationale

The goal of this Phase 1b window trial is to ensure that 9cUAB30 produces a biological effect in humans and to identify candidate RXR signaling pathways that may play a role for cancer prevention. We believe the low toxicity of the rexinoid 9cUAB30 will be well-received for prevention of breast cancers in the high risk population, and potentially even better received for women who have had cancer and wish to prevent the occurrence of a second tumor. The results of this study will help to determine the suitability of continued study with 9cUAB30 as a chemoprevention drug in trials proposed by the NCI DCP in the Clinical Development Plan of 9cUAB30.

Treatment with 9cUAB30 in animal models reduced proliferation and number of tumors in both ER+ and ER- breast cancer animal models, suggesting a chemopreventive effect of the drug. To evaluate the anti-proliferative effect in humans, we chose the pre-surgical window trial design. The model of short-term administration of a drug between the window of diagnosis to operative intervention, allows for evaluation of biomarker response from baseline (core biopsy) to post-treatment (surgery). Several pre-surgical trials

have shown the ability of SERMs to reduce proliferation as measured by Ki67 after short-term administration. In these trials administration of tamoxifen or other SERM results in decrease of Ki67 by approximately 40% when surgical resection samples are compared with pretreatment core biopsy samples.[32-35] We expect that 9cUAB30 will reduce proliferation and increase apoptosis.

Because breast cancer prevention/therapy preclinical mouse model data with Targretin, 9cRA and 9cUAB30 suggest a linear relationship between increasing dose/concentration and anti-cancer effects, it is important to explore the highest 9cUAB30 dose that pharmacodynamics suggest. The new formulation of 9cUAB30 will significantly lower the gelatin: active ingredient ratio (12 capsules vs 3 capsules) thus raising the potential of higher tissue exposure at 240 mg as compared to 160 mg. Therefore, we plan to "pilot" the 240 mg dose in initial participants with planned assessment of C_{2hr} (Tmax usually at 2 hours) at 2 hours post initial dose. If real time assessment of 9cUAB30 concentrations observe a significant increase over 957 ng/ml levels we will continue at 240 mg/day. If initial concentrations are not appreciably >957 ng/ml with 240 mg/day, participants will be treated at 160 mg/day.

Further, we plan to evaluate the potential effect of 9cUAB30 on various breast cancer pathways, shedding light into the mechanism of action to help design future trials.

Increasing evidence suggests that tobacco and alcohol use are risk factors in the development of intraepithelial neoplasia and cancer. In addition, tobacco and alcohol use may adversely affect agent intervention, for example by altering the safety profile or metabolism of a drug. Standardized assessments of tobacco and alcohol use during clinical trials will aid in understanding the potential relationship between the use of these products and clinical endpoints or cancer prevention biomarkers. Therefore, NCI, DCP is including assessment of tobacco and alcohol use at baseline and at surgery, to determine the potential impact of tobacco and alcohol use on 1) treatment toxicity and symptom burden, and 2) the efficacy of treatment intervention.

The rexinoic acid receptor bexarotene decreases Th2 tumor immune environment and increases CD8+ T-cells in breast tumors of TgMMTV-neu transgenic mouse mammary tumor model.

The rexinoic acid receptor (RXR) agonists bexarotene and 9cUAB30 have been shown to prevent breast cancer through inhibiting proliferation.[43,44,45] RXR has also been shown to have immune roles, although the effects vary depending on the immune environment, including preventing T-cell apoptosis and inhibiting the viral response to infection.[46 47,48] The breast cancer tumor immune microenvironment can determine both whether a tumor will be responsive to therapy and predict a patient's risk of relapse.[49,50] A type I immune environment is essential both to recruit $CD8^+$ T-cells to the tumor and to ensure that these $CD8^+$ T-cells are active to destroy the tumor.[51,52] On the other hand, Th2 regulatory T-cells induce a type II immune response which suppresses $CD8^+$ T-cell activity.[53] The majority of breast tumors develop an immunosuppressive immune environment which begins to develop in DCIS and increases as the tumor progresses to IBC.[54] This immunosuppressive environment prevents the level of anti-tumor immune infiltration in the majority of breast tumors that is necessary for improved immune tumor destruction and better prognosis.[55]

We have demonstrated that bexarotene can decrease both hyperplasia and invasive breast cancer while increasing $CD8^+$ T-cells in the tumor in TgMMTV-neu mice. Furthermore, bexarotene was able to enhance the efficacy of a multi-antigen vaccine to prevent breast cancer in these mice suggesting an anti-tumor immune role for bexarotene. When mice were vaccinated with the HER2-IGFBP2-IGF1R vaccine and concurrent bexarotene, the percent of mice that did not develop tumors increased from 65% to 87% (HR 18.1 95% CI 6.4 to 63.2, $p<0.0001$). Bexarotene alone only inhibited tumor development in 15% of the mice that was not significantly more than in the adjuvant controls ($p=0.307$).[56] Similar to human breast

cancer, TgMMTV-neu mice develop a type II tumor immune environment.[7] When treating TgMMTV-neu mice that had existing 100 mm^3 tumors with bexarotene, we found the tumor immune environment became more type I with increased $CD8^+$ T-cells in the tumor and decreasing $GATA3^+$ cells (a marker of Th2 T-cells) in the tumor (Fig. 3). Because of bexarotene's role in modulating the immune environment, we wanted to determine if the rexinoic acid receptor in human immune cells.

In evaluating RXR expression in human peripheral blood mononuclear cells (PBMC), we found that RXR had the highest expression in antigen presenting cells (APC). RXR is expressed in almost 30% of APC, 24.9±13% of macrophages, 38.6±14% of plasmacytic dendritic cells (pDC), 33.1±16% of monocytic dendritic cells (mDC) (Fig. 4). Further, when PBMC were treated with increasing doses of bexarotene for 48 hours, there was increased co-stimulatory CD40 expression on mDC (indicating mDC activation, p=0.03 between 0 and 20 uM bexarotene, Fig 5A) and pDC (indicating pDC activation, p=0.05 between 0 and 20 uM bexarotene, Fig 5B). There was also increased type I cytokine release including IL-1β (p=0.04 between 0 and 20 uM bexarotene Fig 6A) and TNFα (p=0.03 between 0 and 20 uM bexarotene Fig 6B) but not type II cytokine release of IL-10 (p=0.99) and IL-4 (p=0.97) (Fig 6C and D). Infiltration of activated dendritic cells has been associated with improved prognosis in multiple solid tumors.[58,59] These data suggest that bexarotene can activate and bias DCs to develop into type I DCs and therefore may have an immune component of its anti-tumor activity.

Activation of monocytic dendritic cells is conserved between RXR agonists bexarotene and 9cUAB30.

While bexarotene may have both an anti-proliferative and immune anti-tumor role, it has been associated with significant toxicities including pancreatitis, hypertriglyceridemia, headache, and thyroid abnormalities.[60] A second novel retinoid 9cUAB30 is much better tolerated, with only grade 1 and 2 toxicities in an initial 14-person healthy volunteer dose escalation study.[31] 9cUAB30 has been shown to be effective with tamoxifen in hormone-induced mammary cancers in rats reducing tumor growth by 57%.[61] From preliminary experiments comparing the activation of dendritic cells by 9cUAB30 and bexarotene in parallel, 9cUAB30 can also increase CD40 expression on monocytic dendritic cells (p=0.05 at 20 uM as compared to 0 uM 9cUAB30, Fig 7D) and plasmacytic dendritic cells (p=0.02 at 20 uM as compared to 0 uM 9Cuab30, fig 7E) suggesting that this activity is conserved across RXR agonists. We therefore aim to evaluate whether increasing doses of 9cUAB30 can activate type 1 dendritic cells and enhance the type I immune environment in women with early stage breast cancer. We hypothesize that the activation of type I dendritic cells by the RXR agonist 9cUAB30 can contribute to its anti-cancer activity.

3. SUMMARY OF STUDY PLAN

Eligible participants will be ≥18 years of age and newly diagnosed pre- or post-menopausal patients with core biopsy proven invasive breast cancer between 0.5 cm and 5 cm in size or DCIS that is ≥ 1.0 cm in size and is either Grade 3 ER+ DCIS or ER- DCIS of any grade

Patients agreeing to participate will take 9cUAB30 240 mg (160 mg contingent on interim analysis) by mouth daily for 14 – 28 days until the time of surgery. Tissue from the core biopsy will be used for the pre-treatment biomarker analysis. The tissue obtained from the surgical excision will be used for post-treatment biomarker analysis. We plan to enroll ER+ participants and ER- participants for a total of up to 41 participants undergoing intervention. We expect the study to be complete within 18-24 months. Beginning in November 2021, participants will be limited only to estrogen receptor negative status.

At baseline all participants will have a complete medical history, physical examination and review of concomitant medications. Participants will undergo laboratory testing to determine eligibility within 30 days prior to day 1 of the study. Baseline laboratory tests will include WBC, differential, hemoglobin,

hematocrit, platelets, INR, PTT, AST, ALT, total bilirubin, alkaline phosphatase, BUN, creatinine, calcium, glucose, albumin, electrolytes (Na, K, Co2, Cl), HDL, LDL, total cholesterol, triglycerides, thyroid stimulating hormone, FT3, and FT4 (may omit any if collected as part of initial cancer workup documented in the medical record within 30 days of study Day 1). A plasma and urine sample for pharmacokinetic (PK) analysis will also be obtained. An additional 30 ml blood sample will be obtained to evaluate and measure the immune response of 9cUAB30 in participants 6-25.

For the first 5 participants, two extra plasma PK samples will be collected after administering the first dose of the study agent at 5 minutes and 2 hours post the drug administration to evaluate the Cmax. After the first 5 participants complete study participation, their PK samples will be evaluated to determine the Cmax and adverse events will be evaluated to determine safety of the 240mg dose. Based on this evaluation, it will be determined to continue study dose at 240mg or to reduce to 160mg (refer to section 13.9).

For all study participants, 1 H&E stained slide, 5 unstained slides of 5µm thickness and 5 unstained slides of 10µm thickness will be requested from the diagnostic core biopsy formalin fixed paraffin embedded (FFPE) tissue block containing tumor using the existing diagnostic core biopsy material already collected.

As part of the assessment of participant evaluability, a pathologist will examine the deepest level cut from blocks of the core biopsy and surgical resection specimens containing tumor. If the estimated residual tumor in the block is considered sufficient to enable cutting of at least 5 unstained slides at 5µm thickness, needed to support the future performance of Ki-67 and caspase 3 assays, the participant can be considered evaluable.

Once a surgery date is scheduled, participants meeting eligibility requirements will begin oral 9cUAB30 for daily treatment for at least 14 (but not longer than 28 days) days at the 240 mg/day dose level (160 mg contingent on interim analysis) prior to surgery. The day the participant begins taking 9cUAB30 is considered Day 1 of the study. Each participant will be provided with a blood pressure (BP) monitor to track the BP values at home. During the visit, participants will be instructed on how to use the monitor. The instructions are noted on the BP diary (Appendix C) for reference at home. Participants will be asked to measure and document the blood pressure values in the BP diary on Day 7, Day 8 (approximately 24 hours later) and the day prior to surgery. All BP readings should be taken in the morning. They will be instructed to notify the study team if the value of the reading is Systolic ≥160 and Diastolic ≥100. The study coordinator will contact the participant on Day 8 by telephone to assess adverse events and other symptoms, review medications and review the BP readings. Study team will also contact participants on the day before surgery to remind them to take last dose of medication, bring the pill diary and blood pressure monitors and bring all the pill bottles given to them. Participants will be encouraged to contact the investigator or study coordinator with any new symptoms or concerns at any time during the trial.

Surgery must occur between Days 15 and 29 of the study. On the day of surgery or the day before surgery laboratory tests will be obtained and include WBC, differential, hemoglobin, hematocrit, platelets, INR, PTT, AST, ALT, total bilirubin, alkaline phosphatase, BUN, creatinine, calcium, glucose, albumin, electrolytes (Na, K, Co2, Cl), HDL, LDL, total cholesterol, triglycerides, thyroid stimulating hormone, FT3, FT4, as well as a plasma and urine sample for PK. The history and physical examination will be obtained and/or updated within 24 hours of the procedure. An additional 30 ml blood sample will be obtained during this visit to evaluate and measure the immune response of 9cUAB30 by comparing it to the sample obtained at baseline for participants 6-25.

At the time of surgery, tissue samples will be obtained of remnant tumor and processed as is the usual standard at each institution. One H&E stained slide, 5 unstained slides of 5µm thickness and 5 unstained

slides of 10µm thickness will be requested from the formalin fixed paraffin embedded (FFPE) tissue blocks containing tumor as described in section 10.2.1

The plasma and urine samples for PK will be sent to University of Wisconsin - CP lab for evaluation. The immunogenicity samples will be sent to University of Washington – Tumor Vaccine Group. All other baseline and pre-surgical laboratory studies will be performed at each participating institution.

All participants will receive $30 for completing the screening visit, $70 for completing the surgery visit and returning the blood pressure machine as compensation towards the extra efforts taken such as travel expenses, parking, time missed from work or other expenses associated with the study visits. The first 5 participants will be given additional $50 for the additional time required for the study visit.

The study participants will be matched with historical nonrandomized controls from each site based on sex (female), pathologic stage, receptor status, grade, and menopausal status from each site. These samples will be obtained from blocks no more than 5 years old. The pre-surgical and post-surgical tissue samples (from the participants and matched controls) will be batched and analyzed concurrently utilizing immunohistochemistry and Nanostring to measure any effects of 9cUAB30 on tumor cell proliferation (Ki67) and activation of RXR downstream pathways (gene expression). Slides will be analyzed via immunohistochemistry at Wisconsin and via Nanostring at UAB per existing protocols.

Seven days after surgery and 4-5 weeks after surgery a follow-up phone call will be performed for safety assessment.

4. PARTICIPANT SELECTION

Potential participants will be screened and recruited from the University of Alabama at Birmingham, University of Wisconsin, University of Minnesota, University of Iowa and Northwestern University. Beginning in November of 2021, study sites will be limited to University of Alabama at Birmingham, University of Wisconsin, and Northwestern University.

4.1 Inclusion Criteria

1. Female only. The sample size of males affected by breast cancer is limited, hence we will not be able to collect significant data for analysis of the effect of study drug on breast cancer in males
2. Age $\geq$ 18 years. Because no dosing or adverse event data is currently available on the use of 9cUAB30 in participants <18 years of age.
3. ECOG performance status $\leq$ 1 or Karnofsky $\geq$ 70% (convert the Karnofsky score into the ECOG scale based on Appendix A).
4. Invasive breast cancer diagnosed by needle core biopsy, between 0.5 cm and 5 cm in size based on imaging, that is ER+ or ER-, Her2neu positive or negative

   OR

   DCIS of the breast diagnosed by core needle biopsy and at least 1.0 cm in size based on imaging. Grade 3 ER+ DCIS will be allowed as well as ER- DCIS of any grade. For DCIS-only lesions, the imaging abnormality corresponding to the cancer must be at least 1.0 cm in size (i.e. calcifications, distortion or mass on mammogram, or mass or nonmass enhancement on MRI).
5. Participants must have normal organ and marrow function as defined below:
   - WBC $\geq$3000/mm^3;
   - Platelets $\geq$ 100,000/mm^3,

- Hemoglobin >10 g/dL;
- Bilirubin ≤ upper limit of institutional normal;
- AST ≤ upper limit of institutional normal
- Creatinine ≤ upper limit of institutional normal
- Triglycerides ≤1.5xULN
- Cholesterol ≤1.5xULN

6. Participants must agree to discontinue all supplements containing vitamin A while taking study medication and for thirty days after the last dose of study medication.
7. Have not been treated with chemotherapy, or biological therapy in the last 5 years. We do not know if the previous treatment will have an effect on the tissues to be examined.
8. Have not used tamoxifen, raloxifene, or other antiestrogen compounds within 6 months of study entry. If used within 5 years of study entry, total duration of use must be less than 6 months.
9. Have not used exogenous hormone replacement therapy or hormonal contraception in the year prior to diagnosis. The use of non-systemic estrogen (such as vaginal estrogen use) is allowed.
10. The effects of 9cUAB30 on the developing human fetus are unknown. Since retinoids are known to be teratogenic, to avoid any complications due to unintentional pregnancies only postmenopausal women and some premenopausal women (as outlined below) will be eligible. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her study physician immediately.
    - Women will be considered postmenopausal if one of the following is met:
        i. Prior bilateral oophorectomy
        ii. 60 years of age or older
        iii. Age less than 60 years; amenorrheic for 12 or more months; and follicle-stimulating hormone (FSH) in the postmenopausal range or had prior hysterectomy.
    - Premenopausal women without childbearing potential are eligible to participate if one of the following criteria is met:
        i. Prior hysterectomy
        ii. Prior fallopian tubal ligation (cut, tied, or sealed)
        iii. Prior placement of permanent intratubal contraceptive devices (e.g. Essure)
        iv. Partner with prior vasectomy and willing to use barrier method (e.g. condoms)
11. Participants must have the ability to understand, and the willingness to sign, a written informed consent document.

4.2 Exclusion Criteria

1. Participant taking medications that might interact with 9cUAB30; See section 5.4 for a detailed list of potentially interacting medications
2. Participant who has started or increased dosage of lipid-lowering agents in the last 30 days of enrollment.
3. Participant receiving any other investigational agents within 30-days of enrollment nor during study participation with the exception of ^{18}F-FFNP investigational imaging agent. See section 5.4 for a detailed description of ^{18}F-FFNP trial co-enrollment conditions.
4. Participant with a history of allergic reactions attributed to compounds of similar chemical or biologic composition of retinoids.

5. Participant with an uncontrolled intercurrent illness including, but not limited to;
    a. ongoing or active infection,
    b. symptomatic congestive heart failure,
    c. unstable angina pectoris,
    d. cardiac arrhythmia,
    e. a persistent grade 3 hypertension***
    f. psychiatric illness/social situations that will limit compliance with study requirements.
6. Participant who is breastfeeding or planning to breastfeed for a month post last dose of study agent
7. Participant known to be HIV-positive, as we do not know the effects of study drug on suppression of the immune system.
8. Participant with a history of a second cancer diagnosis or reoccurrence <2 years from study entry with the exception of a history of squamous or basal cell carcinoma of the skin <2 years from study entry will not be excluded from this study. This is to eliminate the residual effects of any previous treatments for those cancers.
9. Participant with history of ipsilateral breast radiation.
10. Participant's core biopsy slides suggest that later re-sectioning will not contain sufficient tumor to allow for an adequate evaluation of Ki67 and caspase 3 assays, at a minimum.

***To assess hypertension during eligibility verification, see below instructions:
For grade 1, grade 2, and non-persistent grade 3 hypertension, repeat blood pressure reading after 5 minutes. If the average reading of the two measurements is grade 3 (systolic BP ≥160 mm Hg or diastolic BP ≥100 mm Hg) the patient is not eligible. If the average reading of the two measurements is less than or equal to grade 2, then the participant is eligible. If the average of the 2 readings is grade 1 or grade 2 hypertension, document the appropriate level hypertension on the baseline symptom form.

4.3 Inclusion of Women and Minorities

Women of all races and ethnic groups are eligible for this trial.

4.4 Recruitment and Retention Plan

Potential participants will be screened and recruited from the University of Alabama at Birmingham, University of Wisconsin, University of Minnesota, University of Iowa and Northwestern University. Breast cancer surgeon investigators at each site will be instrumental in achieving accrual targets. The duration of the accrual is expected to take 18- 24 months. Beginning in November of 2021, study sites will be limited to University of Alabama at Birmingham, University of Wisconsin, and Northwestern University.

Each site will review the surgeon's clinic patient list to identify potential participants. Through review of the medical record, the study team will determine if a patient is eligible and contact them for interest in the study. Review of medical records prior to consenting to the research study is very minimum risk of harm to the potential study participants and involves no study related procedures for which written consent is normally required outside of the research context.

The potential participant may be initially approached by their physician and the study coordinator will then speak with them more in depth about the study.

We plan to have staggered enrollment for the study. We will enroll 5 participants at the 240mg dose level

and after analysis of the PK data collected from the first 5 participants, it will be determined whether to continue with 240mg dose level or to modify the dose to 160mg for rest of the study. If the study continues at 240mg dose level, the remaining 31 participants will be accrued. If the dose is determined to need to be reduced to 160mg, 36 participants will be accrued at 160mg. We expect a participant dropout rate of less than 5%.

5. AGENT ADMINISTRATION

Intervention will be administered on an outpatient basis. Reported AEs and potential risks are described in Section 6.2.

5.1 Dose Regimen and Dose Groups

The study drug 9cUAB30 240 mg (3 capsules of 80 mg) or *160 mg (2 capsules of 80 mg) will be taken daily for a period of at least 14 days but not to exceed 28 days.

*Depending on if interim dose modification rules are met.

5.2 9cUAB30 Administration

All study drug will be dispensed by research pharmacies at the participating organizations. Three bottles (30 capsules per bottle) will be distributed to study participants at the 240mg dose level. If the interim dose modification rules are met and the study is modified to 160mg daily, two bottles (30 capsules per bottle) will be distributed to the remaining participants. Individually labeled participant-specific bottles of 9cUAB30 will be distributed to the investigational pharmacy at each site. The daily dose will be self-administered at approximately the same time every evening on empty stomach (2 hours after eating and wait for 1 hour before eating again). The last dose will be the day before surgery.

5.3 Run-in Procedures

Not applicable

5.4 Contraindications

Due to the effects demonstrated in other Rexinoids, the following medications are contraindicated:

- bile acid sequestrants (cholestyramine, colesevelam, colestipol)
- ezetimibe
- fibric acids (fenofibrate, gemfibrozil)
- nicotinic acid (niacin)

Because of potential effects on drug metabolism other contraindicated agents are:

- St John's Wort
- oral ketoconazole
- tetracycline
- oral corticosteroids
- CYP2B6 Substrates (Sensitive): Bupropion, cyclophosphamide (Activated to acrolein by CYP2B6), efavirenz, irinotecan, ketamine, promethazine, propofol, selegiline,

All medications listed above should be avoided while participants are taking study agent, they may

resume use on the day of surgery.

Because of potential confounding effects on pharmacokinetics, participants may not take other topical or oral retinoids. These agents are:

- retinol
- Retinal
- tretinoin (Retin-A)
- isotretinoin
- alitretinoin
- etretinate
- acitretin
- Tazarotene
- bexarotene
- Adapalene

For participants who choose to co-enroll in an ^{18}F-FFNP PET/MRI trial, Day 1 of 9cUAB30 study drug administration must be at least 1 day after the FFNP administration to ensure the AE reporting period for the FFNP trial has concluded.

5.5 Concomitant Medications

All medications (prescription and over-the-counter), vitamin and mineral supplements, and/or herbs taken by the participant will be documented on the concomitant medication CRF and will include: 1) start and stop date, dose and route of administration, and indication. Medications taken for a procedure (*e.g.*, biopsy) should also be included. Medications given in the hospital as part of the standard surgical protocol will not be documented as concomitant medications. However, any medications given to treat an adverse or unexpected event following surgery will be documented.

5.6 Dose Modification

No dose modifications will occur for an individual participant. The study dose may be reduced from 240 mg/day to 160 mg/day if interim dose modification rules are met (see Section 13.9). No other dose modifications will be allowed.

5.7 Adherence/Compliance

Subjects that maintain 90% compliance for the time period beginning day 1 of the trial and ending on the day before surgical intervention will be considered compliant. Compliance will be measured through pill counts conducted by the study coordinator or institutional pharmacy and recorded in the CRF.

6. PHARMACEUTICAL INFORMATION

6.1 9cUAB30 (IND 101,064, NCI, DCP)

This Phase Ib clinical study investigating the biologic effect of a novel retinoid 9- cis-UAB30 (9cUAB30) will be conducted under an IND sponsored by NCI, DCP. 9cUAB30 [8- (3',4'-dihydro-1-(2'H)-naphthalen-1'-ylidene)-3,7-dimethyl-2,4,6-octatrienoic acid], has been synthesized at the University of Alabama (D. Muccio) and is undergoing development through the NCI, DCP RAPID program. This new RXR-selective retinoid is a conformationally- constrained analogue of 9-cis-retinoic acid (9-cis-RA) (Muccio et al. 1998).

Evidence continues to accumulate from in vitro and in vivo studies, as well as from clinical trials, that retinoids have therapeutic effects in several neoplastic diseases. Several RXR agonists (rexinoids) have undergone clinical evaluation as potential chemopreventive compounds. 9cUAB30 is a yellow powder which will be formulated into capsules as subsequently described. Active capsules are size 1, Swedish orange, hard-gelatin capsules containing 80 mg 9cUAB30 plus pregelatinized starch, colloidal silicon dioxide, crospovidone and magnesium stearate. All capsules are manufactured under contract to NCI, DCP, and are packaged in 60 cc white HDPE bottles, 30 capsules per bottle, with 33/400 polypropylene caps.

6.2 Reported Adverse Events and Potential Risks

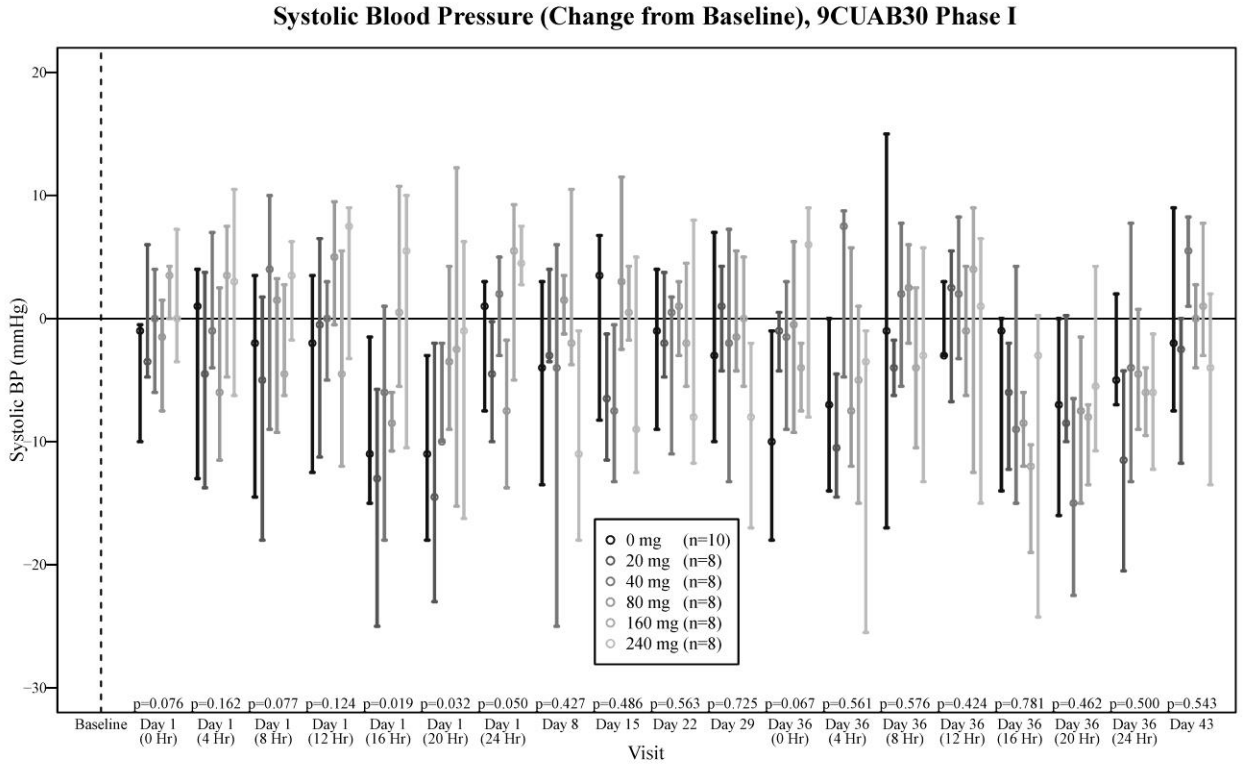
Nonclinical PK evaluations show that 9cUAB30 is orally available in mice, rats, and dogs. In mice, plasma 9cUAB30 levels were similar after one and seven days of dosing, indicating no drug accumulation. Increases in the area under the drug concentration curve (AUC) were less than proportional to dose possibly due to an increased rate of clearance (Cl) and decreased half-life ($t_{½}$) seen at the same time. In rats, drug levels increased with dose. In dogs, the time to maximal drug concentration (t_{max}) was 2–3.5 hours and $t_{½}$ was 2.7–4 hours. *In vitro* metabolic studies suggest that human metabolism may be similar to canine metabolism and differ significantly from murine (rat) metabolism. In isolated human liver microsomes, the major cytochrome P 450 (CYP) isozymes responsible for 9cUAB30 metabolism were CYP2C8, CYP2C9 and CYP2C19; at 10 μM, 9cUAB30 also induced CYP2B6. A 9cUAB30 glucuronide conjugate can be formed by uridine diphosphate (UDP) glucuronosyltransferase 1A9 (UGT1A9).

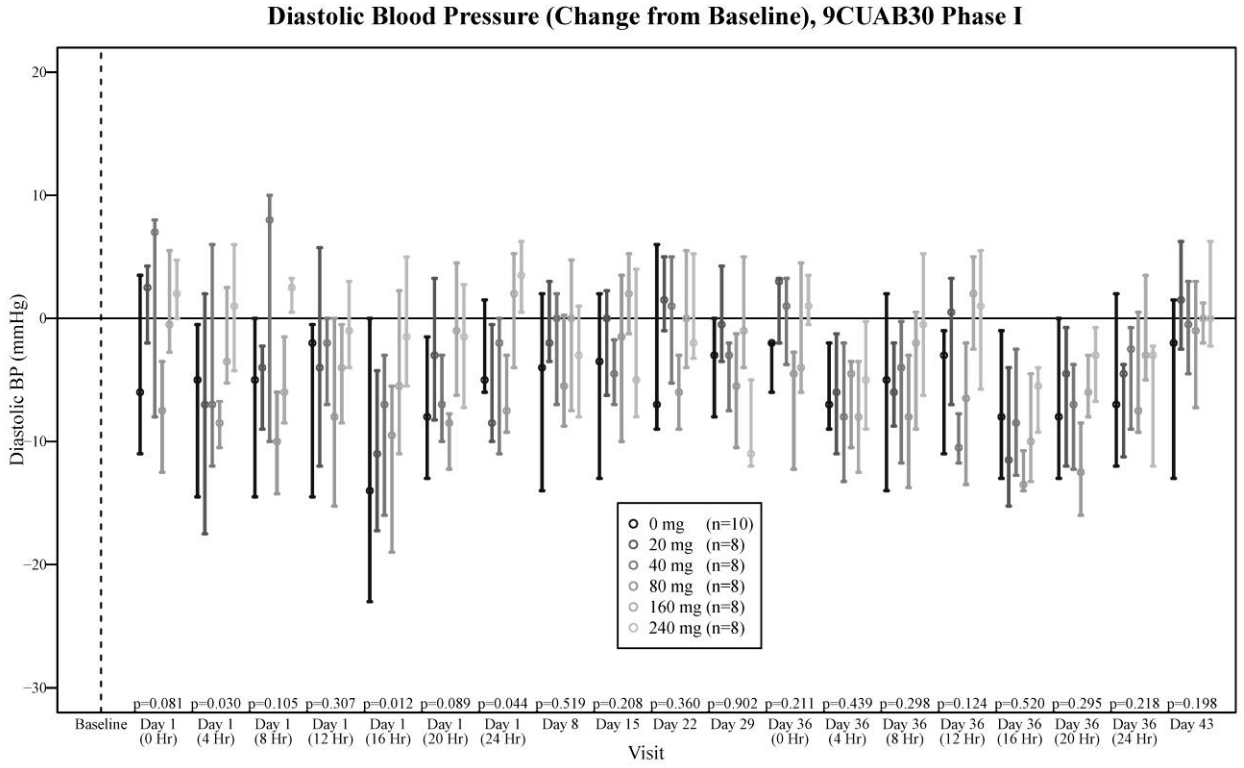
9cUAB30 has been evaluated in multiple nonclinical toxicity and safety pharmacology studies sponsored by NCI, DCP. In a 28-day oral toxicity study of 9cUAB30 in rats, target organs were the liver and hematologic system. Dose-dependent increases in liver weight as a percent of brain weight, vacuolation in liver tissue consistent with glycogen deposition, and increased serum aspartate aminotransferase (AST) and alkaline phosphatase (ALP) levels were observed. Hematologic changes included dose-dependent decreases in prothrombin time (PT), increases in fibrinogen, and, at the highest dose (100 mg/kg-bw-day), eosinopenia and neutrophilia. The no observed adverse effect level (NOAEL) was considered to be 3 mg/kg-bw/day. In a 28-day oral toxicity study of 9cUAB30 in female dogs, no treatment-related toxicities were observed even at the highest dose (100 mg/kg-bw/day), which was considered the no observed adverse effect level (NOAEL). In safety pharmacology studies, no cardiac effects were seen in female dogs treated with single doses of 9cUAB30 up to 300 mg/kg-bw/day. Additionally, in a functional observational battery (FOB) study in rats, no effects on central nervous system (CNS) or behavior were seen with single doses of 9cUAB30 up to 100 mg/kg-bw/day. In genotoxicity studies, 9cUAB30 was negative in the *in vivo* mouse bone marrow micronucleus assay and the bacterial reverse mutation test (Ames). In the Chinese hamster ovary (CHO) cell assay, 9cUAB30 did not increase chromosome aberrations or polyploidy after three-hour exposure to low levels of 9cUAB30 in the absence of metabolic activation. However, longer duration (24 hours) in the presence or absence of metabolic activation, or higher doses of 9cUAB30 with metabolic activation, increased chromosome aberrations and polyploidy. Because of variable outcomes and lack of a dose response in the CHO cell assay, 28-day *in vivo* mutagenicity and six-month oncogenicity studies were undertaken in transgenic mice. In the Big Blue *lacI/cII* transgenic mouse mutagenicity study, no dose-related increases in mutation frequency were found in the liver, kidney, or spleen. In addition, 9cUAB30 was not oncogenic in $p53^{+/-}$ transgenic mice, and there was no evidence of vitamin A-like toxicity. These results, combined with the negative oncogenicity and mutagenicity data, suggest an acceptable toxicity profile for 9cUAB30.

9cUAB30 has been tested in normal volunteers in a phase 1, first-in-human, open-label, single ascending dose study (UWI06-8-03) and in a 28-day double-blind, placebo-controlled, randomized, multiple ascending dose (MAD) study (under two protocols UWI09-8-02, UWI10-16-01R). In the first study, single doses of 5, 10, and 20 mg 9cUAB30 were well tolerated and orally available, with a t_{max} of 2.2–3.1 hours.

Most adverse events (AEs) were grade 1 in severity; Grade 3 AE's were rare and likely unrelated to study agent. There were no grade 4, or serious AEs (SAEs), and no dropouts. The most common treatment-emergent AE (TEAE) was headache with the first dose and little to no occurrence thereafter raising the possibility of caffeine withdrawal due to fasting as a potential cause. Another common TEAE was grade 1 (1 grade 3 event on day 1 prior to initial dose) hypertension which was more frequently observed with higher doses (see Table 3). Because of this observation, we more closely examined Blood Pressure results throughout the study (see figure 2). Based on absolute BP readings there is no evidence of increased BP during the first 24 hours after their initial dose (data not shown) and no evidence of an increase from baseline through day 36 (figure 2). The increase in numbers of TEAE's implies participants entered the study with borderline hypertension and resultant repeated measurements led to sporadic BP elevations meeting CTCAE requirements across participants and sustained elevations within participants where not seen.

Figure 2:





*Median and first and third quartiles are displayed

Other common TEAEs were increased blood chloride, decreased hemoglobin (anemia), and hypercholesterolemia. Rare events such as rash, dry skin, sweating, burping after meals, shaking, temperature of body feeling warmer, were also observed. Interestingly, commonly associated retinoid/rexinoid toxicities were not observed with any frequency or significant grade (skin changes, headache, hyperlipidemia, leukopenia, etc.), supporting the hypothesis that the conformational changes to 9cUAB30 structure as compared to 9cRA result in less toxicity. Our data supports that 9cUAB30 daily dosing of ≤240 mg/day for up to 28 days continuously is safe.

Table 3: TEAEs by treatment Reported by ≥Three Subjects in UWI09-8-02, UWI10 16 01R

System Organ Class Preferred Term	**0 mg (n=12) n(%)**	**20 mg (n=8) n(%)**	**40 mg (n=9) n(%)**	**80 mg (n=8) n(%)**	**160 mg (n=9) n(%)**	**240 mg (n=8) n(%)**
Any TEAE	10 (83)	6 (75)	9 (100)	6 (75)	8 (89)	8 (100)
Gastrointestinal Disorders	1 (8)	3 (38)	2 (22)	2 (25)	2 (22)	0 (0)
Nausea	1 (8)	1 (13)	1 (11)	0 (0)	2 (22)	0 (0)
Vomiting	1 (8)	1 (13)	1 (11)	0 (0)	0 (0)	0 (0)
General Disorders and Administrative Site Conditions	1 (8)	3 (38)	0 (0)	1 (13)	2 (22)	0 (0)
Fatigue	0 (0)	2 (25)	0 (0)	0 (0)	2 (22)	0 (0)
Infections and Infestations	5 (42)	0 (0)	0 (0)	2 (25)	2 (22)	2 (25)
Sinusitis	2 (17)	0 (0)	0 (0)	0 (0)	1 (11)	2 (25)
Investigations	4 (33)	1 (13)	5 (56)	2 (25)	2 (22)	5 (63)
Blood Chloride Increased	1 (8)	0 (0)	1 (11)	2 (25)	1 (11)	0 (0)
Cholesterol High	1 (8)	1 (13)	2 (22)	1 (13)	1 (11)	1 (13)
Increased LDL Cholesterol	1 (8)	1(13)	0 (0)	1 (13)	0 (0)	0 (0)
Metabolism and Nutrition Disorders	3 (25)	2 (25)	2 (22)	3 (38)	1 (11)	0 (0)
Hypertriglyceridemia	2 (17)	2 (25)	2 (22)	2 (25)	1 (11)	0 (0)
Musculoskeletal And Connective Tissue Disorders	1 (8)	1 (13)	0 (0)	1 (13)	1 (11)	4 (50)
Back pain	0 (0)	1 (13)	0 (0)	0 (0)	1 (11)	1 (13)
Nervous System Disorders	4 (33)	2 (25)	3 (33)	3 (38)	3 (33)	4 (50)
Headache	3 (25)	2 (25)	3 (33)	3 (38)	2 (22)	4 (50)
Respiratory, Thoracic, and Mediastinal Disorders	0 (0)	1 (13)	0 (0)	2 (25)	1 (11)	2 (25)
Nasal Congestion	0 (0)	1 (13)	0 (0)	2 (25)	0 (0)	1 (13)
Skin and Subcutaneous Tissue Disorders	1 (8)	1 (13)	0 (0)	3 (38)	4 (44)	4 (50)
Dry Skin	0 (0)	1 (13)	0 (0)	2 (25)	0 (0)	1 (13)
Pruritus	0 (0)	0 (0)	0 (0)	0 (0)	1 (11)	3 (38)

System Organ Class Preferred Term	**0 mg (n=12) n(%)**	**20 mg (n=8) n(%)**	**40 mg (n=9) n(%)**	**80 mg (n=8) n(%)**	**160 mg (n=9) n(%)**	**240 mg (n=8) n(%)**
Rash Acneiform	1 (8)	0 (0)	0 (0)	1 (13)	1 (11)	2 (25)
Vascular Disorders	5 (42)	2 (25)	7 (78)	4 (50)	7 (78)	8 (100)
Hot Flashes	2 (17)	0 (0)	0 (0)	0 (0)	1 (11)	0 (0)
Hypertension	5 (42)	2 (25)	7 (78)	4 (50)	7 (78)	8 (100)

6.3 Availability

9cUAB30 is an investigational agent supplied to investigators by the Division of Cancer Prevention (DCP), NCI. The agent is formulated into 80mg capsules.

6.4 Agent Distribution

Agents will only be released by NCI, DCP after documentation of IRB approval of the DCP-approved protocol and consent is provided to DCP and the collection of all Essential Documents is complete (see DCP website for description of Essential Documents).

DCP guidelines require that the agent be shipped directly to the institution or site where the agent will be prepared and administered. NCI, DCP-supplied agents are requested by Consortium Lead Organization (CLO), University of Wisconsin to be dispatched to Participating Organization (PO) by completing the DCP Clinical Drug Request form (NIH-986) (to include complete shipping contact information) and faxing or mailing the form to the DCP agent repository contractor:

John Cookinham
MRIGlobal
DCP Repository
1222 Ozark Street
North Kansas City, MO 64116
Phone: (816) 360-3805
FAX: (816) 753-5359
Emergency Telephone: (816) 360-3800

DCP does not permit the transfer of agents between institutions (unless prior approval from DCP is obtained). DCP does not automatically ship agents; the site must make a request.

6.5 Agent Accountability

The Investigator, or a responsible party designated by the Investigator, must maintain a careful record of the inventory and disposition of all agents received from DCP using the NCI Drug Accountability Record Form (DARF). The Investigator is required to maintain adequate records of receipt, dispensing and final disposition of study agent. Include on receipt record from whom the agent was received and to whom study agent was shipped, date, quantity and batch or lot number. On dispensing record, note quantities and dates study agent was dispensed to and returned by each participant.
DCP requirements for agent accountability and the required forms are available on the DCP website.

6.6 Packaging and Labeling

9cUAB30 capsules will be packaged by NCI, DCP. Each bottle will contain 30 capsules of 80mg 9cUAB30. Two-part labels will be placed on each bottle. One part stays on bottle, and one part goes into the participant chart or other designated research record as a source document. Sites will be responsible for recording the Participant ID (PID) number on the bottle once they are dispensed. The label remaining on the bottle will identify study specific information, such as Study title, DCP protocol number, dosing instructions, recommended storage conditions, the name and address of the distributor, and a caution statement indicating that the agent is limited by United States law to investigational use only and the agent should be kept out of reach of children.

6.7 Storage

The agent should be stored at a temperature of between 2°and 8° C. Participants transporting study medication from clinic to home will be provided with a cold, but unfrozen, ice pack. Participants will be instructed to store study medication in the refrigerator.

6.8 Registration/Randomization

CLO will be informed about the expected date and time of the scheduled screening visit as soon as it has been scheduled.

PID will be assigned sequentially to all participants who sign consent form at the PO and will designate the recruitment site, the study and the participant. Once a participant has signed a consent form and has satisfied all eligibility criteria:

- Fax (608-467-5900) or email (multisite_research@cancer.wisc.edu) the participant's eligibility checklist signed by Investigator and copy of the signed consent form to CLO during normal business hours (M-F 8:00 am – 4:00 pm Central Time).
- CLO will verify eligibility documents are complete and assign bottle numbers to dispense the study medication.
- CLO will email a confirmation of accrual to the study coordinator at the participating institution including the bottle numbers to be assigned to the participant. If a confirmation is not received within two hours, the coordinator should contact the CLO to confirm that eligibility documents were received.
- CLO will be responsible for tracking number of ER+ and ER- participants to ensure correct tracking of accrual. A notification of accrual will be sent to each PO after a participant has been assigned to treatment.
- After receiving the confirmation from CLO, this information should be conveyed to the local site investigational pharmacist to process the study drug request.

6.9 Blinding and Unblinding Methods

Not applicable

6.10 Agent Destruction/Disposal

DCP-supplied agents: at the completion of investigation, all unused study agent will be returned to NCI, DCP Repository according to the DCP "Guidelines for AGENT RETURNS" and using the DCP form "Return Drug List".

7. CLINICAL EVALUATIONS AND PROCEDURES

7.1 Schedule of Events

Evaluation/ Procedure	Screening Visit	Day 1	Day 7	Day 8 (± 1 Day)	Day before surgery (-2 Days)	Surgery Day 15-29	7 days after surgery (± 1 Day)	4-5 weeks after surgery
Informed consent	X							
Assess eligibility	X							
Medical history	X							
Physical exam	X					X		
Vital signs/ height and weight	X[1]		X[2]	X[2]	X[2]	X[10]		
Laboratory tests[3] - CBC with differential - PTT, INR - CMP - TSH/FT3/FT4 - HDL/LDL/Chol/TG - FSH[5]	X[5]					X[4]		
Urine for PK	X					X		
Plasma for PK	X	X[6]				X		
Immunogenicity sample	X[7]					X[7]		
Concomitant medications	X			X	X	X	X	X
Dispense study agent	X							
Study medication start		X						
Collect study agent						X		
Providing drug diary and instructions	X							
Provide Blood Pressure monitors	X							
Review agent diary/record						X		
Adverse events	X			X	X	X	X	X
Telephone contact				X	X		X	X
Tobacco and Alcohol assessments	X[8]					X[9]		

1. Participant will be given a BP monitor, instructed on its use, and given instructions on recording and reporting BP readings. See section 11.1.1 Reportable AEs, for how the blood pressure should be obtained.
2. Home BP check (morning) only. For Day 7 and Day 8, the readings should be approximately 24 hours apart. If the participant has missed taking their BP reading on Day 7 and Day 8 they should be encouraged to note two readings on the following days at least 24 hours apart from each other.
3. CBC with differential will include WBC, differential, platelets, hemoglobin and hematocrit. CMP will include electrolytes (Na, K, Cl, CO_2) glucose, BUN, creatinine, calcium, albumin, total bilirubin, AST, ALT, alkaline phosphatase.
4. Participant must be fasting for 10 hours for the surgery Day 15-29 lab collection.
5. FSH will be collected for confirmation of post-menopausal status at screening for women under 60 who are amenorrheic for 12 or more months.
6. For first 5 participants only, plasma PK samples will be collected 5 minutes and 2 hours after the first dose has been administered.
7. For participants 6-25.
8. Appendix D – administer the baseline version of tobacco and alcohol assessments

9. Appendix D – administer the follow-up version of tobacco and alcohol assessments
10. Blood pressure on Surgery Day 15-29 will only be obtained if the Day before Surgery average blood pressure is above Systolic ≥ 120 or Diastolic ≥ 80.

7.2 Baseline Testing/Prestudy Evaluation

7.2.1 Screening Visit (within 30 days of Day 1)

Newly diagnosed early stage breast cancer patients will be screened as potential participants from the University of Alabama at Birmingham, University of Wisconsin, University of Minnesota, University of Iowa, and Northwestern University. A history and physical examination will be performed as part of standard of care by providers at each institution.

The procedures to be performed to evaluate eligibility are:

- Invasive breast cancer or DCIS confirmed on pathology report; eligible participants must have invasive breast cancer between 0.5 cm and 5.0 cm in size based on imaging or for DCIS-only lesions, the imaging abnormality corresponding to the cancer must be at least 1.0 cm in size (i.e. calcifications, distortion or mass on mammogram, or mass or nonmass enhancement on MRI).
- Estrogen receptor status must be confirmed on pathology report. Estrogen receptor positivity defined as per 2010 ASCO-CAP Guidelines[36] – with at least 1% positive tumor nuclei in the sample on testing in the presence of expected reactivity of internal (normal epithelial elements) and external controls.
    - Eligible participants must have invasive breast cancer that is ER+ or ER-,
      OR
    - Eligible participants must have DCIS that is ER+ and grade 3, or that is ER- and any grade.

  *Beginning in November 2021, participants will be limited to estrogen receptor negative status.

- Her2neu status must be confirmed on pathology report for those with invasive cancer.[37] Her2neu status is defined as positive and negative based on the 2013 ASCO-CAP guideline. As recommended by the guideline, Her2neu equivocal results must have a reflex or new test to determine Her2neu status.
- Review of the participant's medical history.
- Measurement of height and weight.
- Evaluation of vital signs including temperature, pulse, blood pressure, and respiration rate.
    - For grade 1, grade 2, and non-persistent grade 3 hypertension, repeat blood pressure reading after 5 minutes.
    - If the average reading of the two measurements is grade 3 (systolic BP ≥160 mm Hg or diastolic BP ≥100 mm Hg) the patient is not eligible.
    - If the average reading of the two measurements is less than or equal to grade 2, then the participant is eligible.
    - If the average of the 2 readings is grade 1 or grade 2 hypertension, document the appropriate level hypertension on the baseline symptom form.
- A physical examination. A gynecologic examination may be waived. The physical examination may be performed by any healthcare professional (i.e. physician, nurse practitioner, physician assistant) who is allowed by local policy to perform physical examinations.
- Assessment of Performance Status, using the ECOG method (convert a Karnofsky score into an ECOG score using the scale in Appendix A).
- Review of concomitant medications including over the counter medications and nutrition supplements
- Review and discussion of study including review of informed consent document.

- Patients agreeing to participate in the study will sign the informed consent document.

After obtaining written informed consent, a participant is registered with CLO and assigned a unique PID number (see section 6.8) and eligibility evaluations may occur. Eligibility evaluations determine if the participant is a fit candidate for study participation. All eligibility evaluations (including labs) must be completed within 30 days of Day 1. The eligibility checklist is provided to each site to help determine if the participant is eligible for the trial.

- Administer the baseline version of tobacco and alcohol assessment questionnaire – Appendix D
- Perform the laboratory tests as outlined in section 7.1. Results from laboratory tests or physical examinations performed outside the context of the research study may be used to confirm eligibility, provided these procedures were conducted within 30 days of Day 1.
- A fasting or non-fasting lipid panel may be obtained, if drawn within 30 days of Day 1. If non-fasting lipid levels are outside of the range for eligibility, a fasting lipid panel can be drawn. For the fasting lipid panel, participants should be fasting for at least 10 hours prior to this draw.
- Collect urine and blood sample for baseline PK studies.
- Collect 30 mL blood sample for immune studies for participants 6-25.

If deemed eligible, follow the below mentioned procedures:

For the first 5 participants:

a. Since the participants will be fasting for the laboratory tests, they can have a meal after blood draw for laboratory tests while they wait to get their laboratory results.
b. Once the lab results are obtained to determine eligibility, dispense the study agent in 3 bottles of 30 capsules of 80mg each and administer the first dose of 240mg (3 capsules) at the clinic visit after minimum of 2 hours post their last meal. The participant will be able to eat again after both PK blood draws are performed. This will be considered as Day 1.
c. Document the date and time in the pill diary and provide the diary to participant with instructions on how to complete.
d. First post-dose PK sample of 3mL blood will be collected within 5 minutes of drug administration.
e. Second post-dose PK sample of 3 mL blood will be collected 2 hours from the time the drug was taken. Participants should not eat between the PK blood draws.
f. The study dose has to be taken in the evening on empty stomach (2 hours after last eating and wait for 1 hour before eating again), every day until the day before surgery.
g. They will be provided with a BP monitor to measure BP at home. While they are in clinic, they will be instructed on how to use the monitor. The instructions are noted on the BP diary for reference at home (Appendix C). Participants will be asked to measure and document the blood pressure values in the BP diary on Day 7, Day 8 (approximately 24 hours later) and day prior to surgery. All BP measurements should be taken in the morning. They will be instructed to notify the study team if the value of the reading is Systolic ≥160 or Diastolic ≥100 (grade 3). The study coordinator will contact the participants on Day 8 by telephone to assess adverse events and other symptoms, review medications and review the BP readings.

For participants 6 onwards:

a. Review guidelines for agent administration with the participant and provide the diary to participant with instructions on how to complete.
b. Dispense study agent – 9cUAB30 based on the dose of the study determined by interim analysis. Participant will be given enough study drug for the duration of participation in the treatment phase of the study.
    - Dose 240mg (3 capsules) – dispense 3 bottles of 30 capsules each or

- Dose 160mg (2 capsules) – dispense 2 bottles of 30 capsules each.

c. Participants may start taking the medication in the evening on the date of dispense, in the evening on empty stomach (2 hours after last eating and wait for 1 hour before eating again) until the day before surgery.
d. They will be provided with a BP monitor to measure BP at home. While they are in clinic, they will be instructed on how to use the monitor. The instructions are noted on the BP diary for reference at home (Appendix C). Participants will be asked to measure and document the blood pressure values in the BP diary on Day 7, Day 8 (approximately 24 hours later) and day prior to surgery. All BP measurements should be taken in the morning. They will be instructed to notify the study team if the value of the reading is Systolic ≥160 or Diastolic ≥100 (grade 3). The study coordinator will contact the participants on Day 8 by telephone to assess adverse events and other symptoms, review medications and review the BP readings.

7.3 Evaluation During Study Intervention

7.3.1 Day 1

Participant starts study medication. This day will be determined based on when the surgery will be scheduled for the participant. The study drug should be started at minimum 14 days before scheduled surgery and not more than 28 days before surgery. In order to avoid any further delay of surgery due to participation in the study, it will be recommended that the study medication be started as soon as the participant is deemed eligible. For the first 5 participants this will be in clinic on the day of screening visit.

7.3.2 Day 7

Home BP monitoring in the morning. They will note the BP value and time reading taken in the BP diary and call the study team if they see a value of systolic ≥160 or diastolic ≥100. Participants will be instructed to repeat the reading after 5 minutes if the value of the reading is Systolic ≥ 120 or Diastolic ≥ 80. The average reading of the two measurements will be noted in the database and used for adverse event determination. See section 11.1.1 Reportable AEs, for how the blood pressure should be obtained.

7.3.3 Day 8 (telephone contact)

- Home BP monitoring in the morning. They will note the BP value and time reading taken in the BP diary and call the study team if they see a value of systolic ≥160 or diastolic ≥100. Participants will be instructed to repeat the reading after 5 minutes if the value of the reading is Systolic ≥ 120 or Diastolic ≥ 80. The average reading of the two measurements will be noted in the database and used for adverse event determination. See section 11.1.1 Reportable AEs, for how the blood pressure should be obtained.
- Day 8 (+/- 1Day) is a telephone contact: the study coordinator will attempt to make contact with the participant at least two times.
- If the coordinator is unable to reach the participant between Day 7 and 9 a protocol deviation is filed.
- The coordinator will continue his/her attempts to reach the participant until contact is made;
- Concomitant medication review
- Review adverse events/symptom assessment
- The coordinator will also review the blood pressure readings documented on the blood pressure diary and if the participant has missed taking their BP reading on both Day 7 and Day 8 then they should be encouraged to collect the missed readings on the following two consecutive days approximately 24 hours apart from each other. If the participant has missed taking their BP reading on only Day 8 then they should be encouraged to collect the missed reading on the following morning.

- During this telephone contact if any adverse events are reported that require treatment or further evaluations, the participants will be asked to visit site for further evaluations of the symptoms.

7.3.4 Day before surgery (telephone contact)

- Home BP monitoring in the morning. They will note the BP value and time reading taken in the BP diary and call the study team if they see a value of systolic ≥160 or diastolic ≥100. This has to be taken by participant day before surgery and not on the day the study coordinator calls participant. For grade 1, grade 2, and grade 3 hypertension blood pressure readings, repeat blood pressure reading after 5 minutes. The average reading of the two measurements will be noted in the database and used for adverse event determination. See section 11.1.1 Reportable AEs, for how the blood pressure should be obtained.
- Day before surgery telephone contact (- 2 Days): the study coordinator will attempt to make contact with the participant at least two times. If the coordinator is unable to reach the participant before surgery, a protocol deviation is filed.
- Concomitant medication review
- Review adverse events/symptom assessment
- Remind of the last dose of study medication.
- Remind that a study team member will meet them the day of their surgery before surgery for study related procedures.
- Remind to bring study medication bottles, blood pressure monitor and diary to the clinic visit on the day of surgery.

7.4 Evaluation at Completion of Study Intervention

7.4.1 Day 15-29 (Pre-surgery – assessments must occur before surgery)

- Pre-surgery assessments may be completed up to 1 day prior to scheduled surgery (if surgery is scheduled for Monday, then pre-surgery assessments may be completed on Friday of the prior week). If pre-surgery assessments are completed prior to the day of surgery, the participant should be reminded that their last dose of medication should be the day before surgery.
    - If visit occurs on the day prior to surgery, pill bottles and diaries should be collected on the Day of Surgery to allow participant to take the last dose and document it.
- Evaluation of vital signs including temperature, pulse, and respiration rate.
    - Blood pressure on Surgery Day 15-29 will only be obtained if the average Day before Surgery blood pressure is above Systolic ≥ 120 or Diastolic ≥ 80. An assessment of blood pressure should be collected using the study-supplied wrist monitor.
        - For grade 1, grade 2, grade 3 hypertension blood pressure readings, repeat blood pressure after 5 minutes. The average reading of the two measurements will be noted in the database and used for adverse event determination.
- A physical examination. A gynecologic examination may be waived. The physical examination may be performed by any healthcare professional (i.e. physician, nurse practitioner, physician assistant) who is allowed by local policy to perform physical examinations.
- Assessment of Performance Status, using the ECOG method (convert a Karnofsky score into an ECOG score using the scale in Appendix A).
- Administer the follow-up version of tobacco and alcohol assessment questionnaire – Appendix D
- Review of concomitant medications including over the counter medications and nutrition supplements.

- Perform the laboratory tests as outlined in section 7.1. The lipid panel (cholesterol, HDL cholesterol, LDL cholesterol, triglycerides) must be drawn after the participant has fasted for 10 hours (water is OK).
- Collect 10 mL urine and 3 mL blood for pharmacokinetic studies (prior to study medication dosing if the visit is 1 day prior to surgery)
- Collect 30 ml blood sample for immune studies for participants 6-25.
- Safety assessment and review of adverse events
- Pill accountability by the coordinator
- Blood pressure monitor will be collected from participant and all the documented readings will be checked for grades and documented in the adverse event log accordingly.

7.5 Post-intervention Follow-up Period

7.5.1 7 days after surgery (+/- 1 Day)

- Follow-up telephone contact: the study coordinator will attempt to make contact with the participant at least two times.
- If the coordinator is unable to reach the participant within this time frame a protocol deviation is filed.
- The coordinator will continue his/her attempts to reach the participant until contact is made;
- Concomitant medication review;
- Review adverse events/symptom assessment: if any AEs not related to surgery are concerning to either the participant or the study coordinator/nurse the participant will be scheduled for a clinic visit and evaluation.

7.5.2 4-5 Weeks after Surgery

- Follow-up telephone contact: the study coordinator will attempt to make contact with the participant at least two times.
- If the coordinator is unable to reach the participant within this time frame a protocol deviation is filed.
- The coordinator will continue his/her attempts to reach the participant until contact is made;
- Concomitant medication review;
- Review adverse events/symptom assessment: if any AEs not related to surgery are concerning to either the participant or the study coordinator/nurse the participant will be scheduled for a clinic visit and evaluation.

All participants will receive $30 for completing the screening visit and $70 for completing the surgery and returning the blood pressure machine visit as compensation towards the extra efforts taken such as travel expenses, parking, time missed from work or other expenses associated with the study visits. The first 5 participants will be given additional $50 for the additional time required for the study visit.

7.6 Methods for Clinical Procedures

- Participants will be fasting for 10 hours prior to serum lipid evaluation for the Day 15-29 Visit.

8. CRITERIA FOR EVALUATION AND ENDPOINT DEFINITION

8.1 Primary Endpoint

Our primary endpoint is that treatment with 9cUAB30 for 14-28 days will result in reduced proliferation as measured by Ki-67 expression in breast cancer tissue obtained at surgical resection compared with tissue obtained at diagnostic core biopsy/baseline.

8.2 Secondary Endpoints

Our secondary endpoint is that treatment with 9cUAB30 for 14-28 days will result in increased apoptosis in breast cancer tissue obtained at surgical resection compared with tissue obtained at diagnostic core biopsy/baseline. Additionally, we plan to explore the effect of 9cUAB30 on a molecular level using Nanostring technology providing further insight into its mechanism of action. Examined genes will include up to 48 genes. Prospective biomarkers on the panel will include but are not limited to (A) biomarkers associated with cell proliferation or apoptosis: PCNA, p21, p27, TUNEL assay, caspase 3; (B) potential biomarkers of rexinoid activity reported in published studies: ABCA1, ABCG1, RARβ, SREBP-1c, cyclin D1, IGFBP6; (C) potential biomarkers of response to 9cUAB30 as defined in our preliminary studies: RET, MEN1, mTOR, AKT1, AKT2, 4EBP1, eIF4G1, S6K, IGFBP5, TGFβR1, PPARα, PPARγ (D) housekeeping genes for normalization: GAPDH, TUBB, PGK1, GUSB, and CLTC. Additionally, as possible, we will perform IHC for a subset of 5 of the most differentially regulated genes found on Nanostring to verify that gene expression data also results in protein differences.

One of our secondary endpoint will be to examine if Cmax at Day 1 in the first 5 participants is increased from 957ng/ml by reducing the number of capsules for the 240mg dose level. Dose of each capsule has been changed from 20mg per capsule to 80 mg per capsule. We will examine if there is any difference in absorption of the study agent with this formulation. Additionally, we will examine adverse events in these participants for safety due to the change in formulation.

In all of the participants we will examine the urine and plasma drug concentration levels of 9cUAB30 at baseline and on the day of surgery.

Determine if treatment with 9cUAB30 modifies the tumor immune environment to a type I immune environment. Tumor biopsy specimens collected prior to 9cUAB30 therapy and at the time of surgery will be tested as well as external comparator tumors before and after surgery untreated with 9cUAB30 and matched by site. These tissues are being prepared for Nanostring to evaluate for expression of RXR genes. We propose to use these same RNA preparations to evaluate the Nanostring pan-cancer immune profiling panel on the tumor tissue. We predict that there will be a shift to type I gene expression (including CD8, TBET, IFNG, and IL-1B included in the panel) and decrease in GATA3 and type II gene expression similar to what we have seen with bexarotene therapy in the TgMMTV-neu mouse (Fig 3).

Figure 3: Tumor immune environment with increased CD8+ T-cells and decreased immune suppressive GATA3 cells with bexarotene therapy. TgMMTV-neu mice (n=4) were treated with four weeks of either PBS and sesame oil (control) or 50 mg/m2 bexarotene for four weeks and their tumors were evaluated at sacrifice. For each mouse the average of three independent high-powered fields (20X) were averaged. (A) Percent of DAPI+ CD8+ cells (B) Percent of DAPI+GATA3+ cells

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Figure 4. The rexinoic acid receptor is highly expressed on antigen presenting cells. 2X106 peripheral blood mononuclear cells from women without breast cancer were evaluated for expression of the rexinoic acid receptor (RXR) by flow cytometry. CD4: RXR+CD3+ CD4+, CD8: RXR+ CD3+, CD8+, NK T-cells: RXR+ CD56+, B-cells: RXR+ CD20+, Macrophages: RXR+ HLA DR+ CD14+, Plasmacytic dendritic cells: RXR+ LIN- HLA DR+ CD123+ (pDendritic), Monocytic dendritic cells: RXR+ LIN- HLA DR+ CD11c+ (mDendritic), and myeloid derived suppressor cells (MDSC) Monocytic MDSC (mMDSC) RXR+HLA DR- CD14+CD11b+ and plasmacytic MDSC (pMDSC) RXR+ HLA DR- LIN- CD33+CD11b+

Figure 5. Rexinoic acid activation activates antigen presenting cells. 1.5X106 peripheral blood mononuclear cells from women without breast cancer (n=8) were stimulated by increasing concentrations of bexarotene for 48 hours. Monocytic dendritic cells (CD11c+HLA DR+ LIN-) (A) and plasmacytic dendritic cells (CD123+ HLA DR+ LIN-) (B) have increased CD40+ expression suggesting increased

Figure 6. Increasing concentration of bexarotene induces a type I immune environment. 1.5X106 peripheral blood mononuclear cells from women without breast cancer (n=8) were stimulated by increasing concentrations of bexarotene for 48 hours. PBMC released increased type I IL-1B (A) and TNF-a (B) but not type II IL-10 (C) or IL-4 (D) by Luminex assay. * $p<0.05$ ** $p<0.01$.

8.3 Exploratory Endpoint

For participants 6-25, we will determine if 9cUAB30 increases activated dendritic cells in the peripheral blood. Three 10 mL vials of whole blood drawn in sodium heparin tubes will be obtained and shipped fresh to the University of Washington prior to starting the 9cUAB30 therapy and at surgery. The University of Washington Tumor Vaccine Group Immune Monitoring Laboratory has developed a flow cytometry panel that includes CD11c-AF700, CD14-V450, CD123-PE, and HLA-DR BV605 to evaluate for antigen presenting cells. As immature dendritic cells activate and mature, they increase expression of co-stimulatory molecules CD40 FITC and CD86 PECy7 as well as percent of MHC class II receptors on their cell surface.[62,63] We will measure the expression of CD40, CD86, and MHC II on CD11c and CD123 dendritic cells by FACS analysis. We expect that there will be at least a doubling in the percent of CD11c dendritic cells that are expressing CD40 and CD86 activation markers as that has been what we have seen *in vitro* with increasing doses of 9cUAB30 (Fig 7).

Figure 7. Both bexarotene and 9cUAB30 can activate monocytic dendritic cells. 1.5X106 PBMC cells from women without breast cancer (n=5) were stimulated by increasing concentrations of (A-C) bexarotene and (D-F) 9cUAB30 for 48 hours. Monocytic dendritic cells (CD11c+HLA DR+ LIN-) (A and D) and plasmacytic dendritic cells (CD123+ HLA DR+ LIN-) (B but not D) have increased CD40+ expression suggesting increased activation. Macrophages do not show activation with either bexarotene or 9cUAB30

It has been found that use of some Retinoids have resulted in various toxicities such as, skin toxicity, angular chelitis, headache, hyperlipidemia, vision changes and elevations in hepatic transaminases. During this study all safety information will be obtained for participants who have taken 9cUAB30 and we will compare this safety profile to that seen with other Retinoids.

8.4 Off-Agent Criteria

Participants may stop taking study agent for the following reasons: completed the protocol-prescribed intervention, adverse event or serious adverse event that will compromise the surgery, inadequate agent supply, noncompliance, concomitant medications, lost to follow-up, pregnancy or medical contraindication.

Participants will continue to be followed, if possible, for safety reasons and in order to collect endpoint data according to the schedule of events.

8.5 Off-Study Criteria

Participants may go 'off-study' for the following reasons: the protocol intervention and any protocol-required follow-up period is completed, adverse event/ serious adverse event, lost to follow-up, non-compliance, concomitant medication, medical contraindication, withdraw consent, death, determination of ineligibility (including screen failure), pregnancy, or subject wishes.

8.6 Study Termination

NCI, DCP as the study sponsor has the right to discontinue the study at any time. If not stopped early, the study will be considered terminated when the final report is written.

9. CORRELATIVE/SPECIAL STUDIES

9.1 Rationale for Methodology Selection

The primary goal of the study is to ensure that 9cUAB30 produces a biological effect in humans to justify pursuing more large-scale studies of 9cUAB30. To assess response to 9cUAB30, expression levels of biomarkers will be assessed pre and post treatment with immunohistochemistry (IHC) and Nanostring technology. We have incorporated a nonrandomized matched "control" cohort to better understand baseline variability in biomarker analysis between diagnostic core biopsy and surgical resections.

If the treatment agent 9cUAB30 is effective against breast cancer, it should reduce proliferation and/or kill breast cancer cells. In paraffin blocks, proliferation is assessed by immunohistochemistry via measuring changes in Ki67 and cell death is measured by the caspase 3 assay and routine histology. Another measure of proliferation is PCNA. However, all cells of breast cancer frequently stain with PCNA so it is not very useful. Paraffin embedded biopsy tissue will be compared with paraffin embedded tissue after definitive surgery following treatment with 9cUAB30. These assays will be performed on 5 µm tissue sections from paraffin embedded tissues. Prior to analyzing treated cases, there will be a study of the variability of Ki67 and caspase 3 between biopsies and definitive surgery (i.e., excision of any remaining tumor either in a lumpectomy or in a mastectomy). This study will be performed on retrospective samples which have not been treated between the biopsy and definitive surgery from each institution. Other than this, there will be no existing data with which to compare results of treatment with 9cUAB30 in this format of analysis.

Specifically, at each institution, pretreatment core biopsy FFPE blocks containing breast cancer will be used to obtain 5 unstained slides of 5µm thickness for immunohistochemistry analysis to be performed at Wisconsin and 1 H&E stained slide, 5 unstained slides of 10µm thickness for Nanostring analysis to be performed at UAB. Post treatment, 1 H&E stained slide, 5 unstained slides of 5µm thickness and 5 unstained slides of 10µm thickness will be prepared from diagnostic FFPE blocks from the definitive surgical resection for analysis also to be performed at Wisconsin and UAB.

Additionally, each institution will provide matched samples (at the direction of UAB Pathology Core lab) from archival diagnostic core biopsy FFPE blocks containing breast cancer and archival FFPE blocks from the corresponding definitive surgical resection. Each matched case will be used to obtain 1 H&E stained slide, 5 unstained slides of 5µm thickness and 5 unstained slides of 10µm thickness from the archived core biopsy and 1 H&E stained slide, 5 unstained slides of 5µm thickness and 5 unstained slides of 10µm

thickness from the archived surgical specimen. These will be prepared and sent to Wisconsin and UAB as directed for analysis.

9.2 Comparable Methods

Immunohistochemistry

In paraffin sections, immunohistochemistry is the best assay for proliferation and activated caspases are the best assay for cell death by apoptosis. The antibody for Ki67 will be a rabbit monoclonal (SP6). We have experience with both SP6 and the mouse monoclonal MIB-1 antibodies and prefer SP6. The most important variable in measuring proliferation and apoptosis is probably the time between the end of treatment with 9cUAB30 surgery and definitive surgery. This should be as constant as practicable.

Similarly, the best time to measure changes in proliferation is a function of the time after therapy with 9cUAB30 ends and the length of therapy with 9cUAB30. An answer to this will also require a pilot study.

Nanostring

Gene expression analysis will be performed using the Nanostring nCounter platform. The Nanostring nCounter Dx Analysis System delivers direct, multiplexed measurements of gene expression through the digital readout of the relative abundance of mRNA transcripts isolated from formalin fixed paraffin embedded (FFPE) tissue. Multiple validation studies of this technology compared q-PCR, microarray, and Nanostring techniques.

Results showed that Nanostring technology achieved superior gene expression quantification results compared to arrays and/or IHC in archived FFPE samples.[37] [38-40] Furthermore, robust data could be obtained from RNA extracted from 30 year old archived FFPE tumors.[41] These reports support the utility of the Nanostring nCounter platform to yield highly robust and reproducible data.

Flow Cytometry (Exploratory only):

Whole blood will be processed from baseline and prior to surgery and frozen for batch evaluation. Dendritic cell activation will be evaluated by a dendritic cell antibody panel. For evaluation of immune components in whole blood, 12 color flow cytometry will be performed using an LSR2 Flow Cytometer (BD Biosciences). Batched flow cytometry using validated methods from the Cancer Immunotherapy Trials Network will allow for samples from both baseline and after treatment with 9cUAB30 to be run with the same controls and to allow direct comparison. Flow cytometry is the best method to measure activation markers in the peripheral blood of individual dendritic cell populations (Autissier Cytometry 77A:410 2010).

10. SPECIMEN MANAGEMENT

10.1 Laboratories

The University of Wisconsin will perform the immunohistochemistry analysis on the tissue provided from each of the sites, as well as pharmacokinetic urine and blood testing. The University of Alabama at Birmingham will perform the Nanostring analysis.

<u>Immunohistochemistry</u>

Each institution will provide unstained slides from archival formalin-fixed paraffin embedded blocks containing malignant tissue that will support provision of ~ 100 mg, from both the pre-surgical core biopsy as well as the definitive surgical resection as detailed in section 10.2.1. A total of 5 unstained slides of 5µm thickness (for the UWCCC TRIP lab) and 1 H&E stained slide, 5 unstained slides of 10µm thickness (for the Yang lab) will be required. If the core biopsy will not support the preparation of the requested slides, alternatively, 1 H&E stained slide, and 5 unstained slides at 5 µm will be accepted. The preparation of these slides will be coordinated with the UAB Tissue Biorepository Laboratory and UWCCC TRIP to assure the slides are prepared and submitted shortly before the analysis will occur.

Nanostring analysis

Using the Nanostring platform, the gene expression of the following genes will be assessed:

- Potential biomarkers of rexinoid activity: ABCA1, ABCG1, RARβ, SREBP1c, cyclinD1, IGFBP6
- Potential biomarkers of response to 9cUAB30: RET, MEN1, mTOR, AKT1, AKT2, 4EBP1, eIF4G1, S6K, IGFBP5, TGFbR1, PPARα, PPARγ
  Additionally, 5 housekeeping genes (GAPDH, TUBB, PGK1, GUSB, and CLTC) will be used as internal controls.
- Pan-cancer immune profiling panel

Slides will be processed with the same protocol designed by Nanostring for their FDA cleared Prosigna Breast Cancer Prognostic Gene Signature Assay. Briefly, H&E stained slides will be examined by Dr. Al Diffalha, who will determine the tumor surface area and delineate the tumor. Tumor surface area will be used to estimate the amount of tissue required. Sections will then be macrodissected and RNA is extracted using a commercially available FFPE kit from Roche (also used exclusively by Nanostring) and quantified. As little as 50ng of high quality RNA can be used to hybridize overnight with gene-specific probe pairs consisting of a Reporter and Capture probes. The fluorescent Reporter probe consists of a 35-50 base probe sequence that is complementary to the mRNA target and a DNA sequence that hybridizes to a "color code" unique to each target. After hybridization, the samples are purified through a series of automated magnetic bead capture steps and washes on the nCounter Prep Station, bound to the surface of a nCounter Cartridge (via the Capture probe) and subjected to a high voltage to align and immobilize the Reporter probes to be read in the nCounter Digital Analyzer.

Flow Cytometry Analysis:

The blood for flow cytometry analysis of dendritic cell activation will be performed from SOPs form the University of Washington Tumor Vaccine Group. In brief, 30 mL of blood will be collected at baseline and after 9cUAB30 therapy prior to surgery and sent directly to the University of Washington Immune Monitoring Laboratory where it will be frozen and stored in liquid nitrogen. Samples will be thawed and evaluated in batches. The samples will be stained with a cell surface marker dendritic cell panel including CD11c AF700, CD123 PE-Cy5, HLA-DR BV605, and activation markers CD40 FITC and CD86 BV650 and fixed with 2% paraformaldehyde prior to evaluation. The dendritic cell panel has been previously validated for use in clinical studies but the activation markers CD40 and CD86 will require further validation. Flow cytometry will be performed on a BD Biosciences LSRII cytometer.

10.2 Collection and Handling Procedures

The University of Wisconsin Comprehensive Cancer Center's (UWCCC) CP Laboratory (Cancer Pharmacology Lab) will serve as the central repository for blood and urine specimens. The University of Alabama at Birmingham (UAB) Tissue Biorepository Laboratory and UWCCC TRIP will serve as the central repositories for tissue specimens. The UWCCC CP Laboratory will prepare sample kits for each

subject enrolled on the study – for both the slides being sent to UAB and UWCCC, and the blood and urine being sent to UWCCC. Each site will receive two kits as they activate the study. The CP lab will send additional kits to the sites as needed based on the rate of accrual.

Each kit will be sent in a box to each site. Each box will contain:

- Styrofoam cooler for blood and urine samples and airbill – which go to UWCCC
- Slide mailers, shipping box and airbill for slides – which go to UWCCC and UAB

The University of Washington will ship 2 kits for the 30 mL peripheral blood to each of the sites prior to start of the trial and then will ship further kits to the sites as needed based on recruitment. The University of Washington will train the sites on packing and shipping the blood. All blood processing and storage during the trial will be performed at the University of Washington and after analysis any remaining specimens will be returned to the Division of Cancer Prevention repository.

10.2.1 Methods for preparing tissue specimens of participants and historical matched controls.

NOTE:

- Unstained sections are to be mounted on charged slides
- Historical matched samples should be prepared at the same time as the participant tissue sample.

From both the participant's and historical matched sample of pre-surgical core biopsy and the definitive surgical resection:

- A pathologist should identify, from each procedure, a FFPE archival block that contains an adequate amount of malignant tumor tissue to support the preparation of 11 slides (1 H&E stained slide, 5 unstained slides of 5μm thickness and 5 unstained slides of 10μm). If the core biopsy will not support the preparation of 11 slides, 1 H&E stained slide, and 5 unstained slides at 5μm will be acceptable.
- Samples that are considered adequate and eligible for analysis:
    - From the core biopsy – contain >20% malignant nuclei
    - From the surgical tumor sample – contain >10% malignant nuclei
- After notification to the respective laboratory, the slides are to be labeled with the study number – UWI 2015-05-01, the PID # assigned to the participant, followed by a P (for pre-surgical core) or S (for surgical) to denote the time-point, followed by a 10 or a 5 (to denote the thickness of the cut), followed by the # of the cut (to denote the order in which the slides were cut from the block) as follows:
    - Labeling of Pre-Surgical Core Biopsy Slides
        - 1 for H&E staining: PID-P
        - 5 unstained to be cut at 5 microns: PID-P5-1, PID-P5-2, … PID-P5-5
        - 5 unstained to be cut at 10 microns: PID-P10-6, PID-P10-7, … PID-P10-10
    - Labeling of Surgical Slides:
        - 1 for H&E staining: PID-S
        - 5 unstained to be cut at 5 microns: PID-S5-1, PID-S5-2, … PID-S5-5
        - 5 unstained to be cut at 10 microns: PID-S10-6, PID-S10-7, …PID-S10-10

  For historical matched control samples – contact CLO to get the Sample ID (SID)

    - Labeling of Historical Matched Pre-Surgical Core Biopsy Slides
        - 1 for H&E staining: SID-HP
        - 5 unstained to be cut at 5 microns: SID-HP5-1, SID-HP5-2, … SID-HP5-5
        - 5 unstained to be cut at 10 microns: SID-HP10-6, SID-HP10-7, … SID-HP10-10
    - Labeling of Historical Matched Surgical Slides

    - 1 for H&E staining: SID-HS
    - 5 unstained to be cut at 5 microns: SID-HS5-1, SID-HS5-2, … SID-HS5-5
    - 5 unstained to be cut at 10 microns: SID-HS10-6, SID-HS10-7, … SID-HS10-10
- Unstained sections are to be prepared as follows for participant's samples as well as historical matched samples, cut and prepared in the order listed, placed on the labeled charged slides, and are NOT to be melted:
    - Core Biopsy
        - 1 H&E stained slide
        - 5 unstained at 5µm thickness (mounted on charged slides)
        - 5 unstained at 10µm thickness (mounted on charged slides)
        - If core will not support the requested # of slides, prepare only the 5 unstained at 5µm thickness and do not prepare the 5 unstained at 10µm thickness
    - Surgical Resection
        - 1 H&E stained slide
        - 5 unstained at 5µm thickness (mounted on charged slides)
        - 5 unstained at 10µm thickness (mounted on charged slides)
        - If core will not support the requested # of slides, prepare only the 5 unstained at 5µm thickness and do not prepare the 5 unstained at 10µm thickness

- Complete the Slide Submission Form located in the lab manual.

10.2.2 Methods for drawing and processing the pharmacokinetics of blood

At each pharmacokinetic time point (baseline, 5 minutes post day 1 dose, 2 hours post day 1 dose and Day 15-29 prior to surgery), draw one 3ml green top heparinized tube. Centrifuge the tube for 10 minutes to separate plasma. Divide the plasma into 2 cryovial tubes. Label the 2 cryovial tubes with the labels provided in the kit. Make sure to add the participant number, initials, and date of processing to the labels. Freeze the cryovial tubes at -70/ -80°C, and save for transport to the UWCCC CP Laboratory.

10.2.3 Methods for processing the pharmacokinetics of urine

Request the participant to void and record date and time. Store samples immediately at 4°C. Aliquot approximately 10ml of urine into a falcon tube for urine pharmacokinetics. Freeze urine aliquot within 1-4 hours at -70 / -80°C. Discard any remaining urine.

10.2.4 Methods for processing for whole blood.

30 mL of blood will be collected from participants 6-25 at baseline and on day of surgery (Day 15-29) prior to surgery in green top sodium heparin tubes and shipped directly to the University of Washington. Whole blood will cryopreserved in freezing media (10% dimethylsulfoxide and 90% media which includes 12.5% human serum albumin and RPMI-1640 with L-glutamine and 25 mM HEPES) and stored in liquid nitrogen until use in the assay.10.3 Shipping Instructions

All samples will be shipped in compliance with the International Air Transport Association (IATA) Dangerous Goods Regulations.

10.3.1 Methods for shipping of tissue slides:

Maintain at room temperature until the slides are packaged and shipped to UAB and Wisconsin within 24

hours of preparation. Slides should be prepared and shipped Monday – Wednesday. They should not be shipped on a Thursday, Friday, on a holiday, or on the day prior to a holiday.

Unstained slides are to be packaged securely in provided slide holders, and packaged carefully (they should not vibrate in the container) to avoid breakage of the slides.

Although slides from more than one participant are encouraged to be prepared and shipped together, it is critical that slides from different participants be packaged in separate slide holders/mailers to avoid confusion.

Slides are to be shipped via overnight delivery, at room temperature, with the Slide Submission Form(s) (Appendix B), and de-identified copies of the pathology reports (labeled with the PID only) using the provided pre-printed airbill to:

1 H&E stained slide and 5 unstained slides cut at 10 microns:

Deborah Della Manna
1700 6th Ave South
176F HSROC Room 2220B
Birmingham, AL 35249-6832

5 unstained slides cut at 5 microns:

CP Lab K4/559 CSC
600 Highland Avenue Madison
WI 53792-5669

Call the CP Lab to inform them of a shipment (608) 263-5369 or email the CP lab mailing list 3plab@lists.medicine.wisc.edu.

10.3.2. Methods for shipping the plasma and urine pharmacokinetics samples:

Once the participant has completed the study, ship the carton containing the pharmacokinetic samples for one participant to the address below. If multiple participants are completing the study, samples from multiple participants can be combined in one shipment.

CP Lab K4/559 CSC
600 Highland Avenue Madison
WI 53792-5669

Call the CP Lab to inform them of a shipment (608) 263-5369 or email the CP lab mailing list 3plab@lists.medicine.wisc.edu

Use the express courier service indicated by the shipping label contained in the kit. Closely follow all special shipping instructions outlined in the kit instruction packet.

Ship the PK samples only on Monday, Tuesday or Wednesday to ensure safe arrival during the week. Do

not ship the day before a holiday.

Fill the styrofoam cooler with dry ice and put all of the blood and urine samples into the cooler. Place the cooler into the shipping box in which you received the kit.

10.3.3 Method for shipping blood samples for immunology

For the 30 mL of peripheral blood for immune evaluation, blood will be packaged per protocol in the shipping box received in the kit and shipped FED-EX priority overnight for next day delivery. The University of Washington Immune Monitoring Laboratory can receive blood shipped Monday through Thursday except the day prior to a national holiday. Prior to shipment, the Immune Monitoring Laboratory must be contacted (contact uwiml@uw.edu to inform that samples are shipped). Any remaining samples after evaluation for the trial will be returned to the Division of Cancer Prevention repository.

UW IML CITN CENTRAL LAB
Attention: Yi Yang
850 Republican street
Brotman 219
Seattle WA 98109

10.3.4 Sample Shipment Schedule:

Sample Type	Time Point	Lab to send (see address above)	Shipment
Tissue Slides	➢Baseline ➢Surgery	UAB Tissue Biorepository Laboratory – Alabama UWCCC CP Lab - Wisconsin	Batch Shipment (Pre-surgical and surgical samples)
Historical Tissue Slides	➢ Baseline ➢ Surgery	UAB Tissue Biorepository Laboratory – Alabama UWCCC CP Lab - Wisconsin	To be sent along with the participant that it matches. Batch shipment
Plasma for PK	➢Baseline ➢Surgery	UWCCC CP lab - Wisconsin	Batch Shipment
Urine for PK	➢Baseline ➢Surgery	UWCCC CP lab - Wisconsin	Batch Shipment
Blood for immunology	➢Baseline ➢Surgery	UW IML CITN CENTRAL LAB - Washington	Same day shipment (ambient)

10.4 BioBanking

Biologic specimens collected during the conduct of each clinical trial that are not used during the course of the study will be considered deliverables under the contract and thus the property of the NCI. At study completion, NCI reserves the option to either retain or relinquish ownership of the unused biologic specimens. If NCI retains ownership of specimens, the Contractor shall collect, verify and transfer the requested biologic specimens from the site to a NCI-specified repository or laboratory at NCI's expense.

11. REPORTING ADVERSE EVENTS

DEFINITION: AE means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. An AE can therefore be any unfavorable and unintended sign), symptom, or disease temporally associated with participation in a study, whether or not related to that participation. This includes all deaths that occur while a participant is on a study.

Please note that all abnormal clinical laboratory values that are determined to be of clinical significance based on a physician's assessment are to be reported as AEs. Those labs determined to be of no clinical significance or of unknown clinical significance (per the physician's assessment) should not be reported as AEs. Any lab value of unknown clinical significance should continue to be investigated/followed-up further for a final determination, if possible.

A list of AEs that have occurred or might occur can be found in §6.2 Reported Adverse Events and Potential Risks, as well as the Investigator Brochure or package insert.

11.1 Adverse Events

11.1.1 Reportable AEs

11.1.1.1 All AEs that occur after the informed consent is signed and baseline assessments are completed (including run-in) must be recorded on the AE CRF (paper and/or electronic) whether or not related to study agent.

How to obtain blood pressure for the study (for study team and participants)

- Use the **wrist blood pressure monitors** provided for the study to record all readings for the study.
- When the blood pressure is being obtained, the participant should sit for at least 5 minutes before taking the blood pressure.
- Place the cuff on participant's wrist as instructed in the wrist blood pressure monitor's manual.
- The arm used to measure blood pressure should be supported and ensure the arm is at heart level.
- The participant should be instructed not to talk during this time, keep their back rested and legs uncrossed.

In the case of an elevated blood pressure, in which it was repeated, the average of the values should be used for Adverse Event determination.

11.1.1.2 AEs not related to surgery that occur from the time of surgery through the 4-5 week post surgery follow-up visit must be recorded on the AE CRF (paper and/or electronic) whether or not related to study agent.

11.1.1.3 All SAEs, including all hospitalizations, during the study period will be reported as per DCP SAE reporting procedures, with the following exception:

Hospitalization for planned surgery will not be reported. However, if this hospitalization event lasts longer than the usual period at the institution (as determined by the Site Principal Investigator or Sponsor), it will be reportable as an SAE. Adverse events (AEs) relevant to the prolongation of this hospitalization will also be collected and reported on AE CRFs.

11.1.2 AE Data Elements:
The following data elements are required for AE reporting.

- AE verbatim term
- NCI Common Terminology Criteria for Adverse Events version 4.0 (CTCAE v4.0) AE term (MedDRA lowest level term)
- CTCAE (MedDRA) System Organ Class (SOC)
- Event onset date and event ended date
- Treatment assignment code (TAC) at time of AE onset
- Severity grade
- Attribution to study agent (relatedness)
- Whether or not the event was reported as a SAE
- Whether or not the subject dropped due to the event
- Outcome of the event

11.1.3 Severity of AEs

11.1.3.1 Identify the AE using the CTCAE version 4.0. The CTCAE provides descriptive terminology (MedDRA lowest level term) and a grading scale for each AE listed. A copy of the CTCAE can be found at http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

AEs will be assessed according to the grade associated with the CTCAE term. AEs that do not have a corresponding CTCAE term will be assessed according to the general guidelines for grading used in the CTCAE v4.0. as stated below.

CTCAE v4.0 general severity guidelines:

Grade	Severity	Description
1	Mild	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
2	Moderate	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)*.
3	Severe	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**.
4	Life-threatening	Life-threatening consequences; urgent intervention indicated.
5	Fatal	Death related to AE.

ADL

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

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

11.1.4 Assessment of relationship of AE to treatment

The possibility that the AE is related to study agent will be classified as one of the following: not related, unlikely, possible, probable, definite.

11.1.5 Follow-up of AEs

All AEs, including lab abnormalities that in the opinion of the investigator are clinically significant, will be followed according to good medical practices and documented as such. AEs with a "possibly", "probably" or "definitely" attribution to study agent that have occurred prior to surgery will be monitored up to 4-5 weeks after surgery via phone calls.

Participants with AEs "possibly", "probably" or "definitely" related to study agent that are not resolved at the 4 – 5 weeks post-surgery phone contact, should be referred to their primary care provider for follow-up.

11.2 Serious Adverse Events

11.2.1 DEFINITION: Regulations at 21 CFR §312.32 (revised April 1, 2014) defines an SAE as any untoward medical occurrence that at any dose has one or more of the following outcomes:

- Death
- A life-threatening AE
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to perform normal life functions
- A congenital anomaly or birth defect
- Important medical events that may not be immediately life-threatening or result in death or hospitalization should also be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require intervention to prevent one of the other outcomes.

11.2.2 Reporting SAEs to DCP

11.2.2.1 The Lead Organization and all Participating Organizations will report SAEs on the DCP SAE Report Form found at http://prevention.cancer.gov/clinical-trials/clinical-trials-management/protocol-information-office/pio-instructions-and-tools/2012-consortia.

11.2.2.2 Contact the DCP Medical Monitor by phone or email (email preferred) within 24 hours of knowledge of the event.

Edward Sauter, MD, PhD
Breast and Gynecologic Cancer Research Group
Division of Cancer Prevention
9609 Medical Center Dr., Rm 5E326
Rockville, MD 200850
Office Tel (240)276-7657
Cell: (240)944-3279
Email: edward.sauter@nih.gov

Include the following information when calling the Medical Monitor:

- Date and time of the SAE
- Date and time of the SAE report
- Name of reporter
- Call back phone number

- Affiliation/Institution conducting the study
- DCP protocol number
- Title of protocol
- Description of the SAE, including attribution to drug

11.2.2.3 The Lead Organization and all Participating Organizations will email (do not FAX) written SAE reports to DCP's Regulatory Contractor CCS Associates, Inc. (CCSA; phone: 650-691-4400) at safety@ccsainc.com within 48 hours of learning of the event using the fillable PDF SAE Report Form.

11.2.2.4 The DCP Medical Monitor and CCSA regulatory & safety staff will determine which SAEs require FDA submission as IND safety reports.

11.2.2.5 The Lead Organization and all Participating Organizations will comply with applicable regulatory requirements related to reporting SAEs to the IRB/IEC.

11.2.3 Follow-up of SAE
Site staff should send follow-up reports as requested when additional information is available. Additional information should be entered on the DCP SAE Report Form in the appropriate format. Follow-up information should be sent to DCP as soon as available SAE's will be followed for 30 days after completion of treatment. Any ongoing SAE's will be referred to the primary care physician for follow-up.

12. STUDY MONITORING

12.1 Data Management
All of the procedures outlined in the University of Wisconsin Chemoprevention Consortium standardized Data Management Plan (approved 09/23/2019) will be followed in this protocol. Please refer to this document for additional details on data management procedures.
This study will report clinical data using the OnCore web-based application clinical trials/database. OnCore will be the database of record for the protocol and is subject to NCI and FDA audit. All OnCore data entry will be performed at the CLO where staff is trained in OnCore per our DMP and applicable regulatory requirements such as 21 CFR; Part 11.

12.2 Case Report Forms
All data entry will be performed by the Consortium lead Organization (or CLO, the University of Wisconsin) staff. Participant data will be entered into protocol-specific electronic case report forms (CRF's), developed from the standard set of DCP Chemoprevention CRF Templates and utilizing NCI-approved Common Data Elements (CDE). PO's will be required to submit data for quality assurance review and entry into the OnCore database within 10 business days of each study encounter. All data entry will be completed by the CLO within 15 business days of the scheduled visit, as defined in the approved master Data Management Plan. Amended e-CRFs will be submitted to the DCP Protocol Information Office for review and approval.

12.3 Source Documents
The CLO will provide participating sites with standardized, pre-printed source document forms that serve a dual role as source documents and as CRF's, which may be used to ensure that all data items are collected. These pre-printed source document forms must be signed and dated by the person completing the form. If the policy at the site mandates the use of an approved local institutional form or source documentation, the local institutional form may be submitted for the forms supplied by the CLO, as long as all required data points are included on the local institutional form. All de-identified source documents will be sent

electronically or by mail to the CLO monitor. All data is due at the CLO within 10 business days of each study visit.

12.4 Data and Safety Monitoring Plan

The standard University of Wisconsin Chemoprevention Consortium DSMP was approved by the DCP on 03/20/2018. In addition to the standard procedures outlined in the consortium DSMP, we will be modifying the DMP and will perform additional monitoring of data for this trial.

12.5 Sponsor or FDA Monitoring

The NCI, DCP (or their designee), pharmaceutical collaborator (or their designee), or FDA may monitor/audit various aspects of the study. These monitors will be given access to facilities, databases, supplies and records to review and verify data pertinent to the study.

12.6 Record Retention

Clinical records for all participants, including CRFs, all source documentation (containing evidence to study eligibility, history and physical findings, laboratory data, results of consultations, *etc*.), as well as IRB records and other regulatory documentation will be retained by the Investigator in a secure storage facility in compliance with Health Insurance Portability and Accountability Act (HIPAA), Office of Human Research Protections (OHRP), Food and Drug Administration (FDA) regulations and guidances, and NCI/DCP requirements, unless the standard at the site is more stringent. The records for all studies performed under an IND will be maintained, at a minimum, for two years after the approval of a New Drug Application (NDA). For NCI/DCP, records will be retained for at least three years after the completion of the research. NCI will be notified prior to the planned destruction of any materials. The records should be accessible for inspection and copying by authorized persons of the Food and Drug Administration. If the study is done outside of the United States, applicable regulatory requirements for the specific country participating in the study also apply.

12.7 Cooperative Research and Development Agreement (CRADA)/Clinical Trials Agreement (CTA)

Not Applicable

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Description

We aim to enroll 24 ER+ participants and 12 ER- participants for a total of 36 participants. We plan to "pilot" the 240 mg dose in 5 initial participants with planned assessment of Cmax (Tmax usually at 2 hours) at 2 hours post initial dose (see section 2.3 for further information). In the original 9cUAB30 Phase I study, we saw a flattening of the otherwise linear PK concentration curve; there was a mean Cmax of 957 ng/ml (SD 611) at the 240 mg dose level, similar to the mean Cmax of 983 (SD 434) for the 160 mg dose level. If Cmax had continued to be linear, we will have expected to see a Cmax of 1668 at the 240 mg dose level (calculated using linear regression with a forced intercept of 0, omitting the 240 mg level). We will test the hypothesis H_0: Cmax=957 (concentration curve is flat) vs the hypothesis H_A:Cmax=1668 (concentration curve is linear) with a one-tailed one-sample Student t-test. Assuming a standard deviation of 611, we will have 0.69 power to reject H_0: Cmax=957 given that the true mean Cmax is 1668 using a one-tailed one-sample t-test of level 0.05. Based on the results of this analysis, if we do not reject H_0 and the dose gets changed to 160 mg after enrolling 5 participants, 36 additional participants will be enrolled to meet total of 36 participants for data analysis on 160 mg dose and 41 participants total in the study. If we reject H_0 we

will continue at 240 mg/day and enroll 31 more subjects.

This is a single arm open label pre-surgical "window trial" of the novel rexinoid 9cUAB30. The primary objective is to evaluate whether therapy with 9cUAB30 substantially reduces proliferation in breast cancer cells. To test the hypothesis that proliferation decreases in 9cUAB30 treated cases, we plan to examine Ki67 from newly diagnosed breast cancer patients. For each enrolled participant, we will obtain baseline core biopsy tissue samples and post-treatment definitive surgical tissue samples. To account for potential confounding variability associated within the tumor as a result of core biopsy, we plan to include a matched historical nonrandomized "control" group which will have an initial sample as well as post-surgical sample. This matched group will come from archived tissue samples of core biopsy and definitive surgical tissue samples available at each participating Institution. The primary endpoint will be the absolute change in Ki67 expression in each treated participant and matched "control" pair.

The primary analysis will compare the difference in absolute change in Ki67 between treatment and matched "control" group using a one-tailed paired t-test or Wilcoxon signed-rank test, as appropriate, at a significance level of 0.05. We chose the one-tailed test as we are only interested in detecting the reduction in Ki67 expression level with 9cUAB30 as compared to controls.

13.2 Randomization/Stratification

We aim to enroll 24 ER+ participants and 12 ER- participants for a total of 36 participants. As this is a non-randomized trial, stratification for randomization is not applicable. One of the analyses to be performed will be stratified by estrogen receptor status.

13.3 Accrual and Feasibility

According to Dowsett et al., there was a mean decrease in Ki67 of 59.5% and an estimated standard deviation (SD) of 57.5% with tamoxifen, the least effective among tamoxifen alone, anastrozole alone, and tamoxifen/anastrozole combination.[34]As we do not know if 9cUAB30 will have as much of a decrease as tamoxifen, we will use a range of more conservative estimates, from 50-75% of 59.5%, or 29.8-44.6%. According to Chen et al., there was a mean increase in Ki67 of 1.7% with a SD of 7.6% based on three untreated observations with the time interval comparable to the proposed study.[43] However, three observations for estimation of SD is not reliable. Instead we used the widest interval-based SD estimate of 14.8% (from the 2-4 day interval), to be conservative. We also conservatively used an estimate of 0% for change in the control group instead of 1.7%, again, because of the sample size consideration.

Since we are using matched pairs, we needed to estimate the mean and SD of the difference between the matched pair. With 0% as our control estimate, the estimate of the range of the mean difference is thus 29.8-44.6%. The SD is estimated using the equation $\sqrt{\sigma_T{}^2 + \sigma_C{}^2 - 2\rho\sigma_T\sigma_C}$ where σ_T is the SD of the treatment group, σ_C is the SD of the control group, and ρ is the correlation between the matched pair. The value of this equation increases as ρ decreases in value, so an estimate using a smaller correlation coefficient is more conservative. This correlation is unlikely to be negative. Also, if the effect on Ki67 is not dependent on the factors that will be used to choose the matched controls, the correlation is likely to be close to 0. For these reasons, we use 0 as the estimate of ρ; this gives an estimate of 59.4% for the SD of the difference between the matched pair.

We will enroll 36 participants, and obtain 36 matched pairs. Stratified analyses will be conducted if we reach a minimum of 12 participants (and matched pairs) with ER-. Assuming 20% of the participants cannot be evaluated at the end of study due to dropout or unevaluable/unavailable specimen, we will have approximately 28 participants, with 9 participants with ER- and 19 with ER+. The range of mean difference

in the change of 29.8-44.6% and the estimated SD of 59.4% result in an effect size ranging from 0.502-0.751. The table below shows the attainable power of a one-tailed level 0.05 paired test under various effect sizes. With 32 participants, the study will have 0.82-0.98 power to detect the range of effect size of 0.502-0.751. With the 9 participants and matched controls in the ER- group, there will be a power of 0.39-0.65 to detect an effect size range of 0.502 -0.751 between the treatment group and the matched controls. With the 19 participants and matched controls in the ER+ group there will be a power of 0.67-0.93 to detect the same effect size range. Because of the nature of the study being exploratory, we are not making adjustment for multiplicity of testing in subgroups defined by ER status.

	Subgroup		
Effect Size	All (n=28)	ER+ (n=19)	ER- (n=9)
0.751	0.98	0.93	0.65
0.626	0.94	0.83	0.52
0.502	0.82	0.67	0.39

Power for Different Estimated Effect Sizes

13.4 Primary Objective, Endpoint(s), Analysis Plan

The primary objective will be to evaluate if there is a significant decrease in proliferation (Ki67) expression in breast cancer cells from baseline to post-exposure in participants treated with 9cUAB30 compared with matched controls. The primary endpoint is the absolute change from baseline to post-exposure in 9cUAB30 treated and matched controls in Ki-67 in breast epithelial cells obtained by needle core biopsy. The baseline, post-exposure, absolute change in Ki-67, and difference in absolute change between the treated and matched controls will all be summarized with descriptive statistics. The primary analysis will compare the absolute change in Ki-67 between treatment and matched "control" group using a one-tailed paired t-test or Wilcoxon signed-rank test, as appropriate, at a significance level of 0.05.

13.5 Secondary Objectives, Endpoints, Analysis Plans

The first of secondary objectives is to evaluate if there is a difference in the change in apoptosis (caspase 3) from baseline to post-exposure in breast cancer epithelial cells in participants treated with 9cUAB30 compared with matched controls. In the second of secondary objectives, we will examine the differences in gene expression from baseline to post-exposure breast cancer samples using a custom gene panel from Nanostring Technologies. Examined genes will be selected as described below and will include 23 genes. Prospective biomarkers on the panel will include (A) biomarkers associated with cell proliferation or apoptosis: PCNA, p21, p27, caspase 3; (B) potential biomarkers of rexinoid activity reported in published studies: ABCA1, ABCG1, RARβ, SREBP-1c, cyclin D1, IGFBP6; (C) potential biomarkers of response to 9cUAB30 as defined in our preliminary studies: RET, MEN1, mTOR, AKT1, AKT2, 4EBP1, eIF4G1, S6K, IGFBP5, TGFβR1, PPARα, PPARγ; and (D) housekeeping genes for normalization: GAPDH, TUBB, PGK1, GUSB, and CLTC. Additionally, as possible, we will perform IHC for a subset of 5 of the most differentially regulated genes found on Nanostring to verify that gene expression data also results in protein differences.

One of the secondary objectives will be to examine if the Cmax at day 1 is increased from 957 ng/ml by reducing the number of capsules for the 240 mg dose level. The endpoint for this objective is Cmax measured at day 1. This will be tested using a one-tailed one-sample Student t-test.

Observed toxicities seen in this study will be compared with those known to occur from retinoid use. This comparison will be qualitative by necessity and descriptive in nature. As such these will be described in descriptive statistics.

13.6 Reporting and Exclusions

All participants who receive any 9cUAB30 will be analyzed for adverse events. Subjects that maintain 90% compliance for the time period beginning day 1 and ending on the day before surgical intervention will be considered compliant. Compliance will be measured though pill counts conducted by the study coordinator and recorded in the CRF. For biomarker modulation, all participants that have evaluable pre- and post-treatment samples will be included in the analysis. Compliance and availability of evaluable samples for biomarker studies will be included in descriptive statistics.

13.7 Evaluation of Toxicity

All participants will be evaluable for toxicity from the time of their first dose of 9cUAB30. The toxicity will be tabulated by severity with descriptive statistics.

13.8 Evaluation of Response

All participants included in the study will be assessed for response to intervention with 9cUAB30, specifically decrease in proliferation and increase in apoptosis. All of the participants who met the eligibility criteria will be included in the main analysis. All conclusions regarding efficacy will be based on all eligible participants.

13.9 Interim Analysis

There is no planned interim analysis of the primary endpoint of the study. Safety data will be monitored by the consortium's data monitoring committee.

There will be an interim analysis after first 5 participants complete the study at dose 240 mg. This will be to analyze the Cmax and evaluate toxicity data collected for the first 5 participants.

The dose of the study will be reduced to 160 mg if one of the following criteria is met:

1. If the mean Cmax is not >957 ng/mL
2. If ≥ 2 subjects experience grade 2 hypertriglyceridemia of any attribution

If the study dose is reduced to 160mg due to the toxicity criteria at 240mg, then another cohort of 5participants will be evaluated only for toxicity.

If any subjects experience grade 4 or higher toxicity, the study will be suspended for accrual. The study chair will consult with the DSMC for study termination or other course of action. The PI will work with the NCI DCP Investigators to make a decision in consideration of the DSMC's recommendation.

13.10 Ancillary Studies

Not applicable

14. ETHICAL AND REGULATORY CONSIDERATIONS

14.1 Form FDA 1572

Prior to initiating this study, the Protocol Lead Investigator at the Lead or Participating Organization(s) will provide a signed Form FDA 1572 stating that the study will be conducted in compliance with regulations for clinical investigations and listing the investigators, at each site that will participate in the protocol. All personnel directly involved in the performance of procedures required by the protocol and the collection of data should be listed on Form FDA 1572.

14.2 Other Required Documents

14.2.1 Current (within two years) CV or biosketch for all study personnel listed on the Form FDA 1572 and Delegation of Tasks form for the Lead Organization and all Participating Organizations.

14.2.2 Current medical licenses (where applicable) for all study personnel listed on Form FDA 1572 and Delegation of Tasks form for the Lead Organization and all Participating Organizations.

14.2.3 Lab certification (*e.g*., CLIA, CAP) and lab normal ranges for all labs listed on Form FDA 1572 for the Lead Organization and all Participating Organizations.

14.2.4 Documentation of training in "Protection of Human Research Subjects" for all study personnel listed on the FDA Form 1572 and Delegation of Tasks form for the Lead Organization and all Participating Organizations.

14.2.5 Documentation of Federalwide Assurance (FWA) number for the Lead Organization and all Participating Organizations.

14.2.6 Signed Investigator's Brochure/Package Insert acknowledgement form

14.2.7 Delegation of Tasks form for the Lead Organization and all Participating Organizations signed by the Principal Investigator for each site and initialed by all study personnel listed on the form

14.2.8 Signed and dated NCI, DCP Financial Disclosure Form for all study personnel listed on Form FDA 1572 for the Lead Organization and all Participating Organizations

14.3 Institutional Review Board Approval

Prior to initiating the study and receiving agent, the Investigators at the Lead Organization and the Participating Organization(s) must obtain written approval to conduct the study from the appropriate IRB. Should changes to the study become necessary, protocol amendments will be submitted to the DCP PIO according to DCP Amendment Guidelines. The DCP-approved amended protocol must be approved by the IRB prior to implementation

14.4 Informed Consent

All potential study participants will be given a copy of the IRB-approved Informed Consent to review. The investigator or designee will explain all aspects of the study in lay language and answer all questions regarding the study. If the participant decides to participate in the study, he/she will be asked to sign and date the Informed Consent document. The study agent(s) will not be released to a participant who has not signed the Informed Consent document. Subjects who refuse to participate or who withdraw from the study will be treated without prejudice.

Participants must be provided the option to allow the use of blood samples, other body fluids, and tissues obtained during testing, operative procedures, or other standard medical practices for further research purposes. If applicable, statement of this option may be included within the informed consent document or may be provided as an addendum to the consent. A Model Consent Form for Use of Tissue for Research is available through a link in the DCP website.

Prior to study initiation, the informed consent document must be reviewed and approved by NCI, DCP, the Consortium Lead Organization, and the IRB at each Organization at which the protocol will be implemented. Any subsequent changes to the informed consent must be approved by NCI, DCP, the Consortium Lead Organization's IRB, and then submitted to each organization's IRB for approval prior to initiation.

14.5 Submission of Regulatory Documents

All regulatory documents are collected by the Consortia Lead Organization and reviewed for completeness and accuracy. Once the Consortia Lead Organization has received complete and accurate documents from a participating organization, the Consortium Lead Organization will forward the regulatory documents to DCP's Regulatory Contractor:

Paper Document/CD-ROM Submissions:
Regulatory Affairs Department
CCS Associates, Inc.
2001 Gateway Place, Suite 350 West
San Jose, CA 95110
Phone : 650-691-4400
Fax : 650-691-4410

E-mail Submissions :
regulatory@ccsainc.com

Regulatory documents that do not require an original signature may be sent electronically to the Consortium Lead Organization for review, which will then be electronically forwarded to DCP's Regulatory Contractor.

14.6 Other

This trial will be conducted in compliance with the protocol, Good Clinical Practice (GCP), and the applicable regulatory requirements.

15. FINANCING, EXPENSES, AND/OR INSURANCE

All laboratory tests and clinic visits required for study participation will be done at no cost to the participant or his/her insurance company. Although it is not expected, taking part in this study may lead to added costs to participants.
All participants will receive $30 for completing the screening visit and $70 for completing the surgery visit and returning the blood pressure machine as compensation towards the extra efforts taken such as travel expenses, parking, time missed from work or other expenses associated with the study visits. The first 5 participants will be given additional $50 for the additional time required for the study visit.

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APPENDIX A
Performance Status Criteria
ECOG Performance Status Scale

Grade	Descriptions
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (*e.g.*, light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

Karnofsky Performance Scale

Percent	Description
100	Normal, no complaints, no evidence of disease.
90	Able to carry on normal activity; minor signs or symptoms of disease.
80	Normal activity with effort; some signs or symptoms of disease.
70	Cares for self, unable to carry on normal activity or to do active work.
60	Requires occasional assistance, but is able to care for most of his/her needs.
50	Requires considerable assistance and frequent medical care.
40	Disabled, requires special care and assistance.
30	Severely disabled, hospitalization indicated. Death not imminent.
20	Very sick, hospitalization indicated. Death not imminent.
10	Moribund, fatal processes progressing rapidly.
0	Dead.

APPENDIX B

UWI2015-05-01: Pilot Study of 9cUAB30 in Early Stage Breast Cancer to Evaluate Biologic Effect	PID#: __ __ __ __ __ __ __ Date: __ __/__ __/__ __ __ __

PILL DIARY

INSTRUCTIONS

Please read the instructions noted below before you start taking the study medication.

You have been given ___ pill bottles containing the study agent. (30 capsules of 80mg in each bottle) **Please keep your study agent in the refrigerator.**

- Take ___ capsules (_____mg dose) by mouth in the evening on an empty stomach (at least two hours after last meal). After taking your study medication, you must wait for 1 hour before eating or drinking anything.
- The number of doses you take depends on the number of days until your surgery. Therefore, you may or may not have leftover study medication in the bottles.
- Do **NOT** take study medications on the **day of surgery**.
- Please complete this Pill Diary daily using black or blue pen only.
- Record the time, number of capsules, any symptoms or side effects that you notice and initial the line.
- If you miss a dose or lose any capsule, please note this in the comments section of the table.
- Return this Pill Diary at your next visit.
- Return the pill bottles and any unused capsules at your next visit.
- **Return the pill bottles even if it is empty.**

In case you have any questions or concerns, please feel free to call the study team at_____

Initial and Date (person explaining the instructions):

Dose	Date	Time	# of capsules taken	Comments (symptoms or side effects noticed, reason for missed dose, etc.)	Initials
Day 1.					
Day 2.					
Day 3.					

Dose	Date	Time	# of capsules taken	Comments (symptoms or side effects noticed, reason for missed dose, etc.)	Initials
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.					

Dose	Date	Time	# of capsules taken	Comments (symptoms or side effects noticed, reason for missed dose, etc.)	Initials
Day 24.					
Day 25.					
Day 26.					
Day 27.					
Day 28.					

Participant Signature: _____ Date: _____

Reviewer Signature: _____ Date: _____

Blood Pressure Diary

Instructions for taking blood pressure reading using the Monitor provided:

- Put on cuff and place that arm comfortably on top of the table.
- Sit in a sturdy chair with both feet on ground and apart from each other; do not cross your legs while sitting.
- Wait in this position for 5 minutes and relax while taking normal breaths. After 5 minutes press the start button on the monitor.
- The monitor reads and records the blood pressure and pulse. Please note the values for blood pressure on the pill diary along with the time.
- Enter the value noted beside SYS under the column Systolic and the value noted beside DIA under diastolic column in the table below.
- The reading should to be taken in the morning on **Day 7, Day 8** and on the **day before surgery**.
- If the value of the reading is Systolic ≥ 120 or Diastolic ≥ 80 the blood pressure should be repeated after 5 minutes
- Please notify the study team if the value of the reading is Systolic ≥160 or Diastolic ≥100.

In case you have any questions or concerns, please feel free to call the study team at _____.

Initial and Date (person explaining the instructions): _____

Dose Day	Date	Time	Systolic	Diastolic	Initials
Day 7					
Day 7 (after 5 mins)					
Day 8					
Day 8 (after 5 mins)					
Day before Surgery					
Day before Surgery (after 5 mins)					

Participant Signature: _____ Date: _____

Reviewer Signature: _____ Date: _____

APPENDIX C

UWI2015-05-01: Pilot Study of 9cUAB30 in Early Stage Breast Cancer to Evaluate Biologic Effect	PID#: __ __ __ __ __ __ __ Date: __ __/__ __/__ __ __ __

TOBACCO ASSESSMENT – BASELINE

Instructions:
When a number is requested in the response, please enter a whole number (i.e. "4") and not a range or fraction of a number.

Section A. Basic Cigarette Use Information

1. Have you smoked at least 100 cigarettes (5 packs = 100 cigarettes) in your entire life?

   ☐ Yes
   ☐ No → **Skip to Section B**
   ☐ Don't know/Not sure → **Skip to Section B**

2. How old were you when you first smoked a cigarette (even one or two puffs)?
   _____ Years old

3. How old were you when you first began smoking cigarettes regularly?

   _____ Years old
   ☐ Check here if you have never smoked cigarettes regularly.

4. How many total years have you smoked (or did you smoke) cigarettes? Do not count any time you may have stayed off cigarettes.

   _____ Years (If you smoked less than one year, write "1.")

5. On average when you have smoked, about how many cigarettes do you (or did you) smoke a day? (A pack usually has 20 cigarettes in it).
   _____ Number of cigarettes per day

6. Do you NOW smoke cigarettes?

   ☐ Everyday
   ☐ Some days
   ☐ Not at all → **Skip to question 8**

7. How soon after you wake up do you smoke your first cigarette?

   ☐ Within 30 minutes
   ☐ After 30 minutes

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8. How long has it been since you last smoked a cigarette (even one or two puffs)?

First check which one of the following choices applies to you. Then, if applicable, write a number on the line for how many days, weeks, months, or years it has been since your last cigarette.

☐ I smoked a cigarette today (at least one puff)
☐ 1-7 days → Number of days since last cigarette _____
☐ Less than 1 month → Number of weeks since last cigarette _____
☐ Less than 1 year → Number of months since last cigarette _____
☐ More than 1 year → Number of years since last cigarette _____
☐ Don't know/Don't remember

Section B. Use of Other Forms of Tobacco

9. Have you ever used other forms of tobacco, not including cigarettes?

☐ Yes
☐ No → **Skip to Section C**

10. How often do you/did you use other forms of tobacco?

☐ Every day → Number of times per day _____
☐ Some days → Number of days _____ per ☐ Week ☐ Month ☐ Year

11. Which of the following products have you ever used regularly?
Check all that apply

☐ Cigarettes
☐ E-cigarettes or other electronic nicotine delivery system
☐ Traditional cigars, cigarillos or filtered cigars
☐ Pipes
☐ Waterpipe
☐ Hookah
☐ Clove cigarettes or kreteks
☐ Bidis
☐ Smokeless tobacco, like dip, chew, or snuff
☐ Snus
☐ Paan with tobacco, gutka, zarda, khaini
☐ Other, Please specify:_____

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12. If you do not currently use other forms of tobacco, but did in the past, how long has it been since you last used other forms of tobacco regularly?

- ☐ Within the past month (0 to 1 month ago)
- ☐ Between 1 and 3 months (1 to 3 months ago)
- ☐ Between 3 and 6 months (3 to 6 months ago)
- ☐ Between 6 and 12 months (6 to 12 months ago)
- ☐ Between 1 and 5 years (1 to 5 years ago)
- ☐ Between 5 and 15 years (5 to 15 years ago)
- ☐ More than 15 years ago
- ☐ Don't know/Not sure
- ☐ Never used other forms of tobacco regularly

Section C. Second-Hand Smoke Exposure

13. Are you currently living with a smoker?

- ☐ Yes
- ☐ No

14. In the past 30 days, have you lived in a place where other people smoked cigarettes indoors?

- ☐ Yes
- ☐ No

15. In the past 30 days, have you worked in a place where other people smoked cigarettes indoors?

- ☐ Yes
- ☐ No

16. Thinking of all your childhood and adult years, have you ever lived in a place where other people smoked cigarettes indoors?

- ☐ Yes → In total, for about how many years? _____ If less than 1, write "1."
- ☐ No

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17. Thinking of all the years you have worked, have you ever worked in a place where other people smoked cigarettes indoors?

☐ Yes → In total, for about how many years? _____ If less than 1, write "1."
☐ No

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(Signature of person completing) *(MM/DD/YYYY)*

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TOBACCO ASSESSMENT - FOLLOW-UP

Instructions:
When a number is requested in the response, please enter a whole number (i.e. "4") and not a range or fraction of a number.

1. Do you NOW smoke cigarettes?

☐ Everyday
☐ Some days
☐ Not at all → **Skip to Question 3.**
☐ Never smoked → **Skip to Question 4**

2. On average, when you smoked, about how many cigarettes do you (or did you) smoke a day? (A pack usually has 20 cigarettes in it).

_____ Number of cigarettes per day

3. How long has it been since you last smoked a cigarette (even one or two puffs)?

First check which one of the following choices applies to you. Then, if applicable, write a number on the line for how many days, weeks, months, or years it has been since your last cigarette.

☐ I smoked a cigarette today (at least one puff)
☐ 1-7 days → Number of days since last cigarette _____
☐ Less than 1 month → Number of weeks since last cigarette _____
☐ Less than 1 year → Number of months since last cigarette _____
☐ More than 1 year → Number of years since last cigarette _____
☐ Don't know/Don't remember

4. Since your last visit, have you used other forms of tobacco, not including cigarettes?

☐ Yes
☐ No (**End)**

5. How often do you/did you use other forms of tobacco?

☐ Every day → Number of times per day _____
☐ Some days → Number of days _____ per ☐ Week ☐ Month ☐ Year

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6. Since your last visit, which of the following products have you used? ***Check all that apply***

- ☐ Cigarettes
- ☐ E-cigarettes or other electronic nicotine delivery system
- ☐ Traditional cigars, cigarillos or filtered cigars
- ☐ Pipes
- ☐ Waterpipe
- ☐ Hookah
- ☐ Clove cigarettes or kreteks
- ☐ Bidis
- ☐ Smokeless tobacco, like dip, chew, or snuff
- ☐ Snus
- ☐ Paan with tobacco, gutka, zarda, khaini
- ☐ Other, Specify _____

7. If you do not currently use other forms of tobacco, but did in the past, how long has it been since you last used other forms of tobacco regularly?

- ☐ Within the past month (0 to 1 month ago)
- ☐ Between 1 and 3 months (1 to 3 months ago)
- ☐ Between 3 and 6 months (3 to 6 months ago)
- ☐ Between 6 and 12 months (6 to 12 months ago)
- ☐ Between 1 and 5 years (1 to 5 years ago)
- ☐ Between 5 and 15 years (5 to 15 years ago)
- ☐ More than 15 years ago
- ☐ Don't know/Not sure
- ☐ Never used other forms of tobacco regularly

The following instructions pertain to questions 8 - 10. During each of the following time frames, please indicate whether you smoked cigarettes every day, some days, or not at all.

8. During study treatment

- ☐ Smoked every day
- ☐ Smoked some days
- ☐ Did not smoke at all
- ☐ Don't know/not sure
- ☐ Not applicable

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9. After the end of study treatment

- ☐ Smoked every day
- ☐ Smoked some days
- ☐ Did not smoke at all
- ☐ Don't know/not sure
- ☐ Not applicable (I have not completed the study treatment)

10. Since your last visit to this clinic

- ☐ Smoked every day
- ☐ Smoked some days
- ☐ Did not smoke at all
- ☐ Don't know/not sure

This assessment was completed by: ☐ Study Team Member ☐ Participant

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(Signature of person completing) *(MM/DD/YYYY)*

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ALCOHOL ASSESSMENT – BASELINE

Instructions:

For the following questions about drinking alcoholic beverages, a drink means a 12 oz. beer, a 5 oz. glass of wine, or one and a half ounces of liquor.

When a number is requested in the response, please enter a whole number (i.e. "4") and not a range or fraction of a number.

1. In your entire life, have you had at least 12 drinks of any kind of alcoholic beverage?

   ☐ Yes
   ☐ No **(End)**
   ☐ Refused **(End)**
   ☐ Don't know/Not sure

2. In the past 12 months, on average, how often did you drink any type of alcoholic beverage?

   _____ (Enter the number of days you drank based on the timeframe checked below. Enter 0 if you never drank and skip to Question 6.)

   ☐ Week
   ☐ Month
   ☐ Year
   ☐ Refused
   ☐ Don't know/Not sure

3. In the past 12 months, on those days that you drank alcoholic beverages, on average, how many drinks did you have per day?

   _____ (Enter the average number of drinks per day)

   ☐ Refused
   ☐ Don't know/Not sure

4. In the past 12 months, on how many days did you have 5 or more drinks of any alcoholic beverage?

   _____ (Enter the number of days you had 5 or more drinks, or enter 0 if none.)

   ☐ Refused
   ☐ Don't know/Not sure

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5. Was there ever a time or times in your life when you drank 5 or more drinks of any kind of alcoholic beverage almost every day?

   ☐ Yes
   ☐ No
   ☐ Refused
   ☐ Don't know/Not sure

6. If you do not currently drink alcoholic beverages, but did in the past, how long has it been since you last drank regularly?

   ☐ Within the past month (0 to 1 month ago)
   ☐ Between 1 and 3 months (1 to 3 months ago)
   ☐ Between 3 and 6 months (3 to 6 months ago)
   ☐ Between 6 and 12 months (6 to 12 months ago)
   ☐ Between 1 and 5 years (1 to 5 years ago)
   ☐ Between 5 and 15 years (5 to 15 years ago)
   ☐ More than 15 years ago
   ☐ Don't know/Not sure
   ☐ Never drank regularly

7. At the heaviest point, either now or in the past, on the days when you drank, about how many drinks did you drink a day on the average?

   _____ (Enter the number of drinks a day)

   ☐ Refused
   ☐ Don't know/Not sure

8. How many years have you been drinking (or did drink) regularly?

   _____ years

   ☐ Refused
   ☐ Don't know/Not sure

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9. At what age did you begin drinking regularly?

_____ years of age

☐ Refused
☐ Don't know/Not sure

10. What type(s) of alcohol do you drink? (Mark ALL that apply)

☐ Wine
☐ Liquor
☐ Beer
☐ Wine cooler

This assessment was completed by: ☐ Study Team Member ☐ Participant

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ALCOHOL ASSESSMENT - FOLLOW-UP

Instructions:For the following questions about drinking alcoholic beverages, a drink means a 12 oz. beer, a 5 oz. glass of wine, or one and a half ounces of liquor.

1. During the past 30 days, did you drink any alcoholic beverages?

☐ Yes
☐ No **(End)**
☐ Refused **(End)**
☐ Don't know/Not sure

2. During the past 30 days, how many days per week or per month did you drink any alcoholic beverages, on the average?
_____ (Enter number of days you drank based on the timeframe checked below. Enter 0 if you did not drink.)

☐ Week
☐ Month
☐ Refused
☐ Don't know/Not sure

3. On the days when you drank, on average, about how many drinks did you have?
_____ (Enter the average number of drinks you had per day.)

☐ Refused
☐ Don't know/Not sure

4. In the past 30 days, on how many days did you have 5 or more drinks per day?
_____ (Enter the number of days you had 5 or more drinks, or enter 0 if none.)

☐ Refused
☐ Do not know/Not sure

This assessment was completed by: ☐ Study Team Member ☐ Participant

Completed By: _____ Date __ __ / __ __ / __ __ __ __
(Signature of person completing) *(MM/DD/YYYY)*

Completed By: _____
(Printed name of person completing)