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# A Randomized, Placebo-Controlled, Double-Blind, Dose Escalation, Single Dose, and Steady-State Pharmacokinetic Study of 9cUAB30 in Healthy Volunteers

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## Abstract

9cUAB30 is a synthetic analogue of 9-cis retinoic acid with chemoprevention activity in cell lines and animal models. The purpose of this phase I placebo-controlled, double-blinded, dose escalation study of 9cUAB30 was to evaluate its safety, pharmacokinetics, and determine a dose for future phase II studies. Participants received a single dose of study drug (placebo or 9cUAB30) on day 1 followed by a 6-day drug-free period and then 28 days of continuous daily dosing starting on day 8. Fifty-three healthy volunteers were enrolled into five dose cohorts (20, 40, 80, 160, and 240 mg). Participants were randomized within each dose level to receive either 9cUAB30 ($n = 8$) or placebo ($n = 2$). 9cUAB30 was well tolerated, with no dose limiting toxicities reported and no evidence of persistent elevations in serum triglycerides or cholesterol. Treatment-emergent grade 3 hypertension occurred in 1 of 8 participants at the 20 mg dose level and in 2 of 8 at the 240 mg dose level, all considered unlikely related to study agent; no other grade 3 adverse events were observed. The AUC increased, as expected, between day 1 (single dose) and day 36 (steady state). Pharmacokinetics were linear in dose escalation through 160 mg. 9cUAB30 administered by daily oral dosing has a favorable safety and pharmacokinetic profile. On the basis of the observed safety profile and lack of linearity in pharmacokinetics at doses greater than 160 mg, the recommended phase II dose with the current formulation is 160 mg once daily.

## Introduction

Retinoids, which target the retinoic acid receptors (RAR), and rexinoids, which target the retinoid X receptors (RXR), are effective antiproliferative agents used in various health conditions, including cancer, and have shown promise as cancer chemoprevention agents (1, 2). 9-*cis* retinoic acid (9-cRA), a potent RXR agonist, activates both RAR:RXR heterodimer-mediated transcription as well as 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; 3, 4). These nuclear receptors contain binding domains for both ligand molecules and DNA, acting ultimately as ligand-induced transcription factors that regulate cellular processes, including differentiation and apoptosis (5, 6). Thus, RXRs are the master regulators of gene expression of the RAR and RXR signaling pathways, and both pathways can be controlled by 9-cRA.

Bexarotene and other RXR-selective ligands, have been developed in an effort to identify a rexinoid that will be safe for chronic human use. Bexarotene administration is not associated with many of the classic retinoic acid associated toxicities of 9-cRA (e.g., bone fractures, skin lesions and redness, elevated serum calcium), but does cause dose-limiting hyperlipidemia (both elevated cholesterol and triglycerides) and hypothyroidism,

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requiring administration of both levothyroxine and a lipid-lowering agent in clinical trials (7). Despite these and other toxicities, the proven anticancer effects of rexinoids have led to the pursuit of vitamin A derivatives with lower toxicity.

9cUAB30 is a synthetic analogue of 9-cRA with little or no RAR-binding activity relative to 9-cRA and other RARs (8, 9). 9cUAB30 is a tissue-selective RXR agonist with agonist activity in epithelial tissue but with little or no potency in liver tissues (10, 11). 9cUAB30 has been assessed *in vitro* with human cell cultures (12, 13). Human hepatocytes demonstrated no signs of cytotoxicity with treatment of 9cUAB30 up to 50 μmol/L (14), although when human breast cancer cells were treated with 9cUAB30, they showed a significant inhibition of cell proliferation and apoptotic levels 2.5 to 3.5 times that of untreated cells (12).

The chemopreventive activity and toxicity of 9cUAB30 has been tested in animal models with anticancer potential in both $ER^+$ and $ER^-$ breast cancer models shown (13, 15, 16). At a dose level of 200 mg/kg/day, 9cUAB30 decreased N-methyl-N-nitrosourea (MNU-) induced mammary cancer incidence by 63% compared with controls (13). It was as effective as either bexarotene or 9-cRA in preventing ER-positive cancers. When 9cUAB30 was coadministered with tamoxifen, the combination also produced a significant reduction in the size of established tumors (13). 9cUAB30 was evaluated in the MMTV-erbB2 transgenic model at 200 mg/kg/day. 9cUAB30 reduced the number (and tumor burden) of ER-negative MMTV-erbB2 mammary cancers by approximately 50%. Therefore, RXR agonists, which control cell proliferation and induce apoptosis may prevent cancers regardless of estrogen status. Serum triglycerides levels were measured in rats after 7 days of treatment with the 9-cRA 60 mg/kg/day or 9cUAB30 dosed at 200 or 800 mg/kg/day. Although 9-cRA significantly increased serum triglycerides (5-fold) compared with untreated controls, 9cUAB30 did not affect serum triglyceride levels at either dose (16). Importantly, we used gene expression array profiling to demonstrate that 9cUAB30 is not an agonist in the liver, which is the desired hallmark of a tissue-selective rexinoid (17). In addition, toxicity was not reported even when doses significantly higher than needed for chemoprevention activity were tested (13, 15, 16, 18).

A prior study of 9cUAB30 in healthy volunteers demonstrated oral bioavailability with a $T_{max}$ occurring after approximately 3 hours, with a mean half-life ranging from 2.79 to 7.21 hours. Both $C_{max}$ and AUC increased in a dose proportional manner. The percent excreted unchanged in urine over 24 hours was 4.85% for the 5 mg dose, 2.46% for the 10 mg dose, and 17% for the 20 mg dose, suggesting primarily hepatic metabolism. 9cUAB30 was well tolerated with only one participant experiencing grade 2 toxicity (19).

The purpose of this phase I, placebo-controlled, double-blinded, dose escalation study of 9cUAB30 was to evaluate safety, characterize single-dose and steady-state pharmacokinetics, and determine a dose for future phase II studies.

## Materials and Methods

### Participant eligibility and recruitment

Healthy adults between the ages of 18 and 65 years with an ECOG performance status $\leq 1$ (Karnofsky score $\geq$ 80%) were eligible for this study (Table 1). Participants were required to have WBC $\geq$ 3,000/$mm^3$, platelets $\geq$ 100,000 $mm^3$, hemoglobin >10 g/dL, triglycerides $\leq 1.5\times$ ULN and cholesterol $\leq 1.5\times$ ULN, as well as normal hepatic and renal function and normal electrolyte levels. Participants could not be taking potentially interacting medications, vitamin supplements, lipid-lowering agents, oral corticosteroids, or any other retinoids. Enrolled women and the female partners of enrolled men were required to use two forms of birth control during the trial. Participants were excluded if they were taking other investigational agents, had a history of allergic reactions attributed to compounds similar in composition to 9cUAB30, had an uncontrolled intercurrent illness, cancer diagnosis within last

**Table 1.** Baseline participant characteristics

| Characteristics | All ($n = 53$) | 0 mg ($n = 11$) | 20 mg ($n = 8$) | 40 mg ($n = 9$) | 80 mg ($n = 8$) | 160 mg ($n = 9$) | 240 mg ($n = 8$) |
|---|---|---|---|---|---|---|---|
| Sex | | | | | | | |
| Female | 34 (64) | 7 (64) | 4 (50) | 5 (56) | 4 (50) | 8 (89) | 6 (75) |
| Male | 19 (36) | 4 (36) | 4 (50) | 4 (44) | 4 (50) | 1 (11) | 2 (25) |
| Race | | | | | | | |
| White | 46 (87) | 10 (91) | 7 (88) | 6 (67) | 7 (88) | 9 (100) | 7 (88) |
| Black or African American | 7 (13) | 1 (9) | 1 (12) | 3 (33) | 1 (12) | 0 (0) | 1 (12) |
| Age (y) | 42.8 ± 14.2 | 43.5 ± 14.9 | 35.8 ± 14.3 | 40.1 ± 15.2 | 47.9 ± 10.6 | 38.0 ± 12.5 | 52.0 ± 13.8 |
| Weight (kg) | 83.7 ± 18.1 | 87.1 ± 18.1 | 79.7 ± 17.8 | 84.3 ± 16.1 | 84.3 ± 22.2 | 74.8 ± 13.6 | 91.8 ± 21.1 |
| BMI ($kg/m^2$) | 28.9 ± 6.4 | 30.2 ± 5.5 | 26.6 ± 4.9 | 28.2 ± 4.9 | 27.1 ± 5.1 | 29.3 ± 9.7 | 31.6 ± 7.6 |
| Blood pressure (mm Hg) | | | | | | | |
| Systolic | 121 ± 13.5 | 122 ± 7.1 | 121 ± 17.2 | 121 ± 6.5 | 119 ± 15.0 | 118 ± 11.8 | 126 ± 22.5 |
| Diastolic | 74.7 ± 8.7 | 76.7 ± 7.4 | 72.6 ± 11.2 | 73.3 ± 7.5 | 75.8 ± 10.5 | 71.8 ± 7.3 | 78.0 ± 9.5 |

NOTE: Data are expressed as mean ± SD or number of patients (%).

5 years, HIV, or were pregnant or nursing. The study was conducted in accordance with the U.S. Common Rule, approved by the Institutional Review Board and the investigators obtained informed written consent from all subjects.

### Trial design

The trial was conducted at the University of Wisconsin (Madison, WI), which served as the coordinating site, the University of Alabama-Birmingham (Birmingham, AL) and the University of Iowa (Iowa City, IA). Healthy volunteers were recruited in groups of 10 to escalating dose levels ranging from 20 mg to 240 mg orally daily. This protocol was conducted under two protocol and NCT entries due to the funding mechanism. Study recruitment was from November 2011 to September 2013 for protocol NCT01336387 and October 2014 to July 2016 for NCT01935960. Interruptions in drug supply temporarily stopped the study between August 2013 and June 2014, and October 2015 and July 2016 (NCT01935960). Participants were randomized within each dose level to receive either 9cUAB30 ($n = 8$) or placebo ($n = 2$). This allocation was to ensure an equal sample size of 8 between placebo and each of the four active dose levels, before the fifth dose level of 240 mg was added.

On day 1, participants were admitted for a 24-hour inpatient research unit stay and received the first dose of 9cUAB30. Twenty-four–hour pharmacokinetic sampling and blood samples for biomarkers were obtained, followed by a 6-day drug-free washout period. Study drug was restarted on day 8 and continued through day 36, when participants again had 24-hour pharmacokinetic sampling, blood samples for biomarkers, optional skin biopsies (80 and 160 mg dose levels only) and toxicity evaluation. Additional follow-up for safety evaluations occurred 7 and 30 days after stopping drug per protocol.

Adverse events were graded using the National Cancer Institute-Common Terminology Criteria for Adverse Events (NCI-CTCAE; 20). Grade 3 toxicities (CTCAE version 4.0) that were definitely, probably, or possibly related to 9cUAB30 were considered dose limiting. Any participant experiencing an attributable grade 3 or greater toxicity was discontinued from the protocol. If two participants experienced a grade 3 toxicity at the same dose level, the blinding was to be broken by the UWCCC Chemoprevention Consortium Data and Safety Monitoring Committee (DSMC). If the participant was receiving placebo, or if only one participant was receiving active study agent, enrollment into the cohort would continue as planned. If both participants were receiving active drug, that cohort would be terminated. If no participant experienced a dose-limiting toxicity after completion of enrollment to a dose level, escalation to the next dose level would proceed after all 10 participants, with $\geq$90% compliance, had been observed for toxicity through day 42 of the study. All adverse events were followed until resolution to grade 1 or less.

### Biomarker sample schedule

Plasma samples were collected for analysis of 9cUAB30 plasma concentrations at 0, 0.5, 0.75, 1, 1.5, 2, 4, 8, 12, 20, and 24 hours postdose on days 1 and 36, as well as during the weekly laboratory visits. Samples for analysis of biomarkers were obtained at 0, 2, and 12 hours postdose on study days 1 and 36. All samples were stored between $-80°C$ and $-70°C$.

### Bioanalytic methodology

Our previously described LC/MS-MS method was used to measure plasma 9cUAB30 concentrations (19, 21). Briefly, liquid–liquid extraction was used to prepare the samples and samples were analyzed on an Applied Biosystems/MDS Sciex API 4000 equipped with a Turbo V Atmospheric Pressure Chemical Ionization (APCI) source in positive ion mode. 9-cRA was the internal standard.

An eight-point standard curve ranging from 1.56 to 8,000 ng/mL with a trend line $r^2$ of 0.998 over the range was used for sample quantitation. The lower limit of quantitation was 1.56 ng/mL, and the lower limit of detection was 0.78 ng/mL. Intraday and interday variability was less than 15% for standards. Quality control samples were run at the beginning, middle, and end of all runs and analytic runs were rejected if quality control samples varied by >15% from the expected concentration.

### Pharmacokinetic analyses

Noncompartmental methods with Phoenix WinNonlin version 6.4 (Pharsight Corporation) were used to calculate pharmacokinetic parameters. The trapezoidal rule from time 0 to peak concentration, and the trapezoidal rule from the peak concentration to the last measurable plasma concentration was used to estimate the AUC.

### Gene expression

Whole blood in PAXgene tubes frozen at $-80°C$ was thawed overnight at room temperature, and then RNA was extracted by use of the PAXgene Blood RNA Kit (Qiagen, 762164). RNA was then quantified via spectrophotometer and converted to cDNA by standard methods. Briefly, the quantitative PCR reaction was constituted as follows: 10 µL master 2× SYBR green master mix; 1 µL of 10 µmol/L Primer F; 1 µL of 10 µmol/L Primer R; 8 µL RNA in nuclease-free water (total loading, 100 ng). PCR amplification was performed on a Bio-Rad CFX96 using GAPDH as a loading control, as described above with the exception of 50 ng total RNA loaded and the following primers were

used: DNMT1: 5′-agcaagaagtgaagcccgta-3′ 5′-ccagttacttgggaggctga-3′; CYP2B6: 5′acct-gcaggaaatcaatgct-3′ 5′-tctggtggctgaattcactg-3′; GAPDH 5′- ggcctccaaggagtaagacc-3′ 5′-aggggtctacatggcaactg-3′. Results were normalized to GAPDH expression, and then analyzed according to Bio-Rad CFX96 software.

### Telomerase assay

CPT tubes frozen at −80°C were thawed at room temperature and centrifuged for 10 minutes at 1,100 × *g*. The supernatant was decanted and the cell pellet was washed with 1× PBS. The resuspended cells were spun again at 1,100 × *g* for 10 minutes, and the supernatant was aspirated and discarded. The cell pellet was then lysed in 200 μL of CHAPS lysis buffer (TRAPeze XL Telomerase Detection Kit, EMD Millipore S7707). Cells were left on ice for 30 minutes, and then spun 12,000 × *g* for 20 minutes at 4°C. Protein concentration was determined by spectrophotometer. Once diluted to appropriate concentration in CHAPS (TRAPeze XL Telomerase Detection Kit, EMD Millipore S7707), the sample was frozen on dry ice and stored a −80°C until use.

Samples were thawed at room temperature and then placed on ice. Duplicate samples were run in which one set was heat inactivated at 85°C for 10 minutes beforehand. Control cells were used as an upper bound, while controls with no cells or no Taq polymerase were used as negative controls. Serial dilutions of TSR8 stock were used as standards (TRAPeze XL Telomerase Detection Kit, EMD Millipore S7707) for the assay.

The reactions were transferred to a black optical plate, and analyzed via SpectraMax M3 at the following wavelengths: FL 485 nm excite/535 nm emit, R 585 nm excite/620 nm emit. The log(10) of the ratio ΔFL/ΔR was calculated as total product generated (TRAPeze XL Telomerase Detection Kit, EMD Millipore S7707).

### Statistical analysis

The pharmacokinetic parameters (collected on days 1 and 36) that were calculated were $C_{max}$, AUC, $T_{max}$, $T_{1/2}$, clearance (CL), and volume of distribution ($V_d$). Levels obtained from single plasma samples taken on days 8, 15, 22, and 29 were summarized with descriptive statistics, including means, SDs, medians, and interquartile ranges by dose, visit, and time point, as available. Scatter plots were used to explore possible associations between the dose and the pharmacokinetic measures. Jonckheere–Terpstra trend test was performed to determine the significance of the association between increasing dose level and the pharmacokinetic measures. Regression analysis was performed to explore the relationship between pharmacokinetic measures and covariates such as dose, gender, and body weight.

Participant toxicity throughout the study was summarized and analyzed on the basis of the presence or absence of any toxicities, worst CTCAE grade, and strongest investigator defined relationship examined and characterized by dose.

## Results

### Participants

Fifty-three participants enrolled between late 2011 and 2016. Overall, 11 participants received placebo (one was replaced due to a dosing error), eight received 20 mg daily, nine received 40 mg daily (one was replaced due to a dosing error), eight received 80 mg daily, nine received 160 mg daily (one was replaced due to participant withdrawal secondary to a hemorrhagic ovarian cyst considered unlikely to be related to the study drug), and eight received 240 mg daily.

The baseline participant characteristics are summarized in Table 1. The majority of participants were white (46/53, 87%), female (34/53, 64%) with a mean age of 43 years, and all were non-Hispanic. There were no significant differences in demographic characteristics between any of the dosing cohorts, although the 240 mg cohort was both the oldest (cohort mean, 52.0 years of age) and had the highest body mass index (BMI; cohort mean, 31.6 kg/m$^2$).

**Table 2.** 9cUAB30 treatment emergent adverse events across dose level

| | 0 mg (*n* = 11) | | | | 20 mg (*n* = 8) | | | | 40 mg (*n* = 9) | | | | 80 mg (*n* = 8) | | | | 160 mg (*n* = 9) | | | | 240 mg (*n* = 8) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Toxicity** | **Pts with event (%)** | **1** | **2** | **3** | **Pts with event (%)** | **1** | **2** | **3** | **Pts with event (%)** | **1** | **2** | **3** | **Pts with event (%)** | **1** | **2** | **3** | **Pts with event (%)** | **1** | **2** | **3** | **Pts with event (%)** | **1** | **2** | **3** |
| Hypertension[a] | 5 (45) | 2 | 3 | 0 | 2 (25) | 1 | 0 | 1 | 7 (78) | 5 | 2 | 0 | 4 (50) | 3 | 1 | 0 | 6 (67) | 5 | 2 | 0 | 8 (100) | 4 | 2 | 2 |
| Pruritus[a] | 0 (0) | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 1 (11) | 1 | 0 | 0 | 2 (25) | 2 | 0 | 0 |
| Skin (rash/dry skin) | 1 (9) | 1 | 0 | 0 | 1 (13) | 1 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 2 (25) | 2 | 0 | 0 | 1 (11) | 1 | 0 | 0 | 2 (25) | 2 | 0 | 0 |
| Fatigue | 0 (0) | 0 | 0 | 0 | 2 (25) | 2 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 2 (22) | 2 | 0 | 0 | 1 (13) | 1 | 0 | 0 |
| Headache | 2 (18) | 2 | 0 | 0 | 2 (25) | 1 | 1 | 0 | 2 (22) | 2 | 0 | 0 | 3 (38) | 3 | 0 | 0 | 2 (22) | 2 | 0 | 0 | 4 (50) | 3 | 1 | 0 |
| Increased triglycerides | 2 (18) | 1 | 1 | 0 | 2 (25) | 2 | 0 | 0 | 2 (22) | 1 | 1 | 0 | 2 (25) | 2 | 0 | 0 | 1 (11) | 1 | 0 | 0 | 4 (50) | 3 | 1 | 0 |
| Increased LDL chol. | 2 (18) | 2 | 0 | 0 | 1 (13) | 1 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 1 (13) | 1 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 2 (25) | 2 | 0 | 0 |
| Increased total chol. | 1 (9) | 1 | 0 | 0 | 1 (13) | 1 | 0 | 0 | 2 (22) | 2 | 0 | 0 | 1 (13) | 1 | 0 | 0 | 1 (11) | 1 | 0 | 0 | 1 (13) | 1 | 0 | 0 |
| Nausea | 1 (9) | 1 | 0 | 0 | 1 (13) | 1 | 0 | 0 | 1 (11) | 1 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 2 (22) | 1 | 1 | 0 | 0 (0) | 0 | 0 | 0 |
| Nasal cong./sinusitis | 2 (18) | 2 | 0 | 0 | 1 (13) | 0 | 1 | 0 | 0 (0) | 0 | 0 | 0 | 2 (25) | 2 | 0 | 0 | 1 (11) | 0 | 1 | 0 | 2 (25) | 0 | 2 | 0 |

NOTE: Data are expressed as number of patients (%). Patients are categorized into maximum CTCAE grade v4.0.
[a]$P < 0.05$ for Jonckheere–Terpstra test for trend.

### Safety

Table 2 lists frequency of pertinent treatment-emergent adverse events (TEAE). No dose-limiting toxicities were observed and dose escalation was discontinued at 240 mg. Overall, 9cUAB30 was well tolerated and the incidence of toxicities commonly associated with retinoids (e.g., nausea, hyperlipidemia, headache, or skin changes) were not meaningfully different from placebo. There were no grade 4 or 5 adverse effects, but there were six grade 3 AEs: two in the 20 mg cohort (one participant with grade 3 hypertension and one with grade 3 ophthalmic/cataract), one in the 160 mg cohort (grade 3 abdominal pain), and two in the 240 mg cohort (two participants with grade 3 hypertension). All grade 3 AEs were considered unlikely to be related to 9cUAB30.

The most common adverse event, hypertension, was also seen in 45% of participants receiving placebo. However, the frequency of hypertension was significantly different across dose levels ($P < 0.05$) with a trend for more frequent and higher grade events with increased dose (see Fig. 1 and Table 2). Despite an apparent increase in frequency and severity of gradable hypertension events, examination of individual and cohort blood pressure values recorded frequently during day 1 to 2 and weekly thereafter did not show a demonstrable increase in actual systolic or diastolic values or any consistent change from baseline according to dose or duration receiving study medication (Fig. 1). Baseline blood pressure values (mean ± SD) across treatment groups ranged from 118 ± 11.8 to 126 ± 22.5 mm Hg (systolic) and 71.8 ± 7.3 to 76.6 ± 7.1 mm Hg (diastolic) with mean changes of approximately −20 to +20 mm Hg in systolic or diastolic blood pressure at various time points after 9cUAB administration.

Total serum cholesterol, LDL and HDL cholesterol, and triglyceride levels were measured at baseline and then weekly for 6 weeks. Multiple adverse events including three participants with grade 1 decreases in HDL cholesterol (one on placebo, two at 240 mg), were



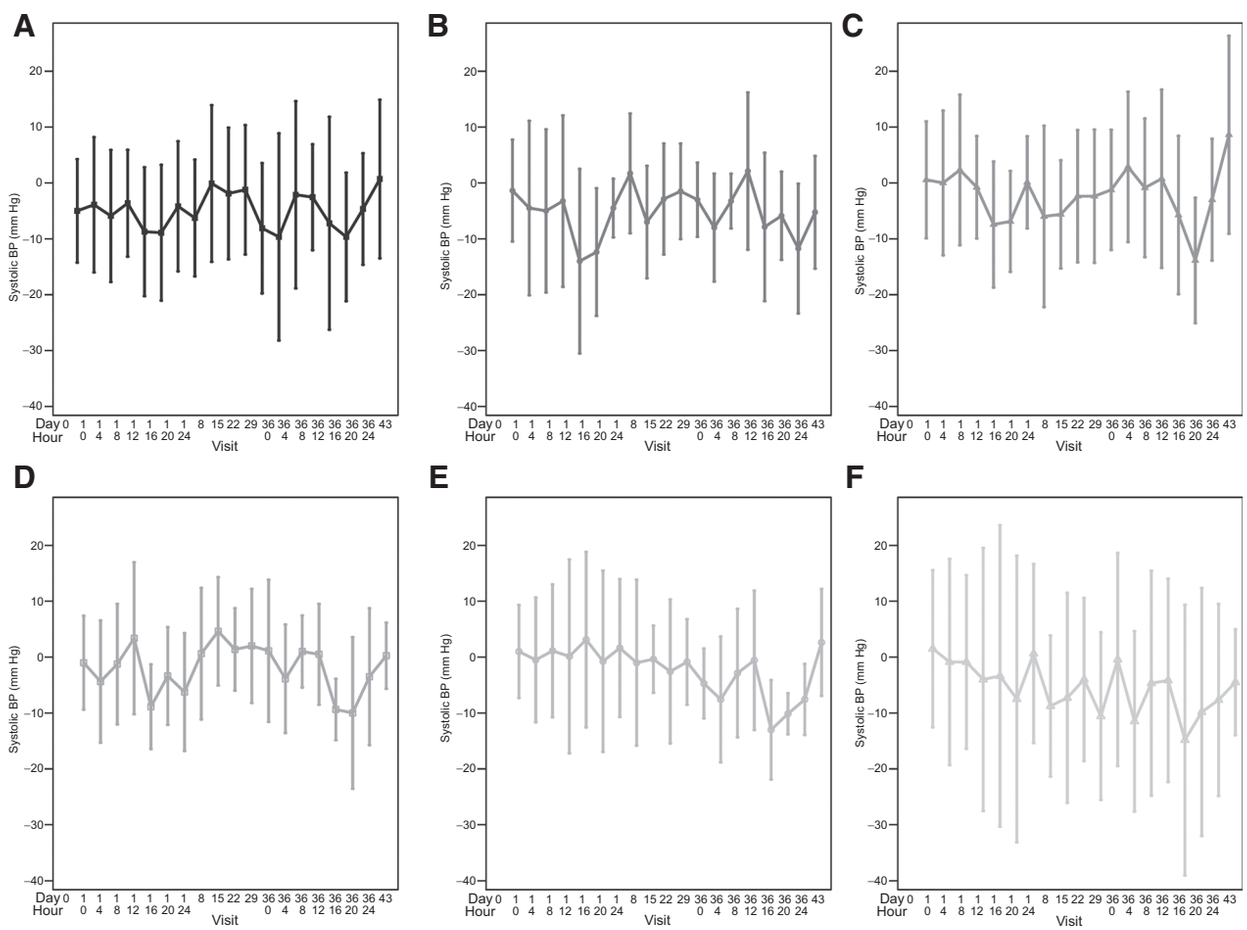


**Figure 1.**

Systolic blood pressure changes from baseline by visit day and hour by dose cohort. Each bar represents the mean ± SD for the dose level. **A,** Placebo ($n = 11$); **B,** 20 mg ($n = 8$); **C,** 40 mg ($n = 9$); **D,** 80 mg ($n = 8$); **E,** 160 mg ($n = 9$); **F,** 240 mg ($n = 8$).

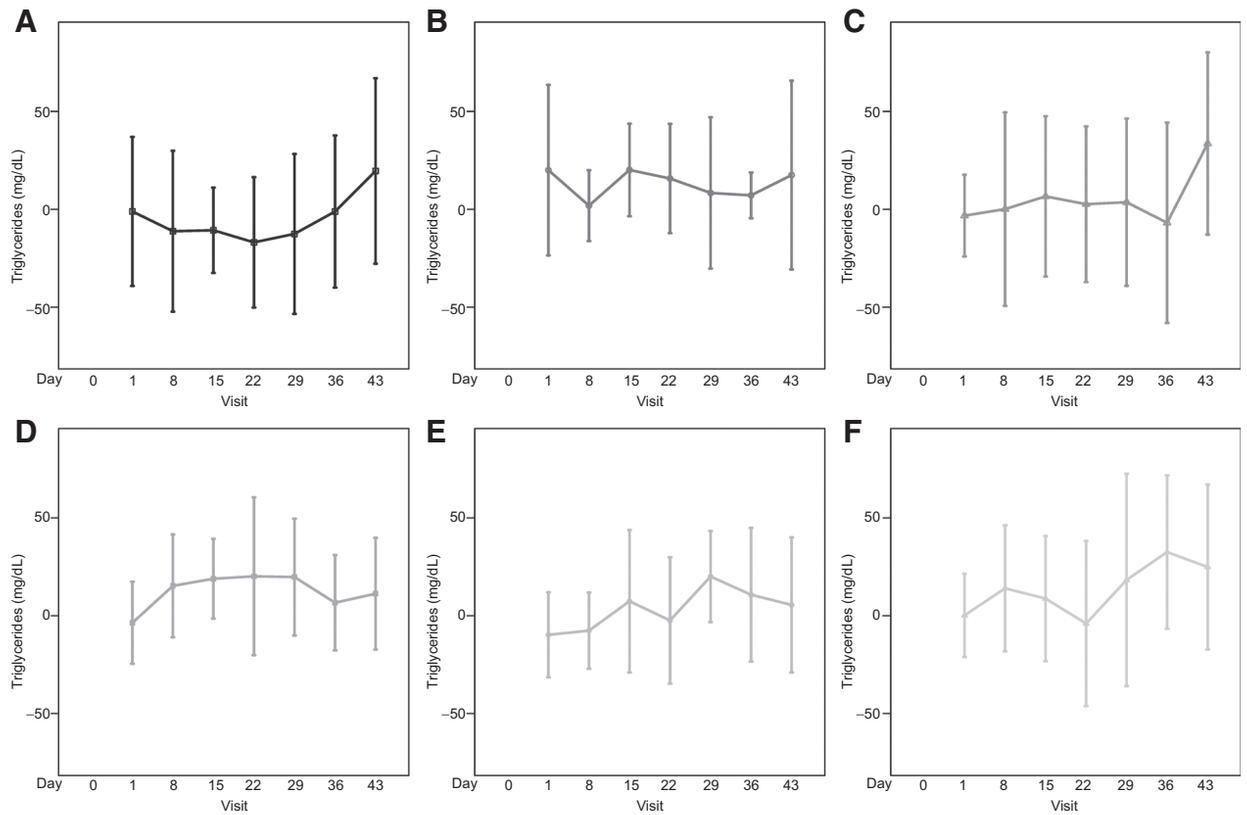


**Figure 2.**

Triglyceride changes from baseline by visit day by dose cohort. Each bar represents the mean ± SD for the dose level. **A,** Placebo ($n = 11$); **B,** 20 mg ($n = 8$); **C,** 40 mg ($n = 9$); **D,** 80 mg ($n = 8$); **E,** 160 mg ($n = 9$); **F,** 240 mg ($n = 8$).

observed, although no patients required administration of lipid-lowering medications to control hyperlipidemia. Alterations in serum triglyceride levels by cohort over the 6 weeks of measurements are shown in Fig. 2. Examination of serum triglyceride and cholesterol levels revealed no consistent trend toward increase or decrease during study participation.

Adverse events related to skin (pruritus, rash, dry skin) are reported in Table 2. Pruritus (grade 1) occurred more frequently in participants receiving higher doses of 9cUAB30 ($P < 0.05$) with no events reported among participants receiving placebo or 20, 40, or 80 mg of drug versus four events at the highest dose levels (one at 160 mg and three at 240 mg). The rest of the adverse event profile, including laboratory assessments of hepatic, renal, and thyroid function, and headache were similar for treated participants and those receiving placebo.

### 9cUAB30 pharmacokinetic parameters

Dose linearity was shown by linear regression, with day 36 AUC increasing linearly across the examined dose range of 20 to 160 mg, while dose linearity was lost at 240 mg (Table 3). Because individuals in the 240 mg cohort had the highest BMI among all cohorts, weight-based dose was also assessed, and a linear relationship between AUC and dose/weight was found to be a better fit and significant ($P < 0.0001$). Lack of linearity at the 240 mg dose cohort was also noted for $C_{max}$ (Table 3). All calculated pharmacokinetic parameters are summarized in Table 3.

Plasma half-life ranged from approximately 5 to 12 hours and appeared independent of dose and day. As expected, both the $C_{max}$ and AUC were higher at day 36 when compared with baseline for dose levels 80, 160, and 240 mg, suggesting accumulation with chronic dosing. These doses achieved mean $C_{max}$ concentrations of 1,090, 1,900, and 923 ng/mL, respectively, on day 36. In addition, AUCs of 8,350, 18,900, and 11,900 ng/h/mL were achieved at these dose levels on day 36. The $C_{max}$ and AUC were similar between day 1 and day 36 for the 20 mg and 40 mg dose level, suggesting lack of accumulation at lower dose levels.

There were no significant differences in gene expression or telomerase after 9cUAB30 administration, likely related to the small sample size and inadequate power to detect small differences.

**Table 3.** Summary of 9cUAB30 plasma pharmacokinetics

| | Parameter, time | $C_{max}$[a] (ng/mL) | AUC[a] (ng-h/mL) | $T_{1/2}$ (h) | $T_{max}$ (h) | $CL_P$ (L/h) | $CL_R$ (L/h) | $V_d$ (L) |
|---|---|---|---|---|---|---|---|---|
| 20 mg[b] ($n = 8$) | $D_1$ | 130 (±64.4) | 1,540 (±1,250) | 25.7 (±35.7) | 3.44 (±2.13) | 23.9 (±22.3) | 0.0501 (±0.0427) | 494 (±411) |
| | $D_{36}$ | 117 (±28.7) | 1,220 (±618) | 15.1 (±11.5) | 2.06 (±0.86) | 21.3 (±10.1) | 0.0548 (±0.0303) | 372 (±222) |
| 40 mg[b,c,d] ($n = 8$) | $D_1$ | 197 (±127) | 1,220 (±713) | 8.59 (±3.47) | 2.00 (±1.34) | 68.6 (±76.9) | 0.0718 (±0.0326) | 894 (±1270) |
| | $D_{36}$ | 172 (±68.7) | 3,840 (±4,970) | 21.3 (±22.5) | 3.00 (±2.31) | 18.6 (±12.1) | 0.0482 (±0.0322) | 354 (±131) |
| 80 mg[c] ($n = 8$) | $D_1$ | 857 (±735) | 6,490 (±4,190) | 7.83 (±4.45) | 2.00 (±0.89) | 17.3 (±13.5) | 0.0220 (±0.0187) | 200 (±181) |
| | $D_{36}$ | 1,090 (±523) | 8,350 (±3070) | 7.68 (±6.35) | 3.22 (±2.35) | 11.6 (±5.88) | 0.0254 (±0.0177) | 127 (±129) |
| 160 mg[c,d] ($n = 8$) | $D_1$ | 983 (±434) | 16,900 (±18,800) | 30.6 (±42.3) | 3.38 (±2.20) | 21.9 (±23.3) | 0.0284 (±0.0212) | 351 (±178) |
| | $D_{36}$ | 1900 (±1570) | 18,900 (±10,900) | 12.2 (±5.26) | 2.71 (±1.22) | 10.8 (±4.43) | 0.0210 (±0.0163) | 196 (±121) |
| 240 mg[c] ($n = 8$) | $D_1$ | 957 (±611) | 9,780 (±7,040) | 17.2 (±17.1) | 2.75 (±1.04) | 44.7 (±40.5) | 0.0484 (±0.0274) | 640 (±462) |
| | $D_{36}$ | 923 (±407) | 11,900 (±3,760) | 11.6 (±3.27) | 3.83 (±4.11) | 23.8 (±12.3) | 0.0411 (±0.0169) | 371 (±136) |

NOTE: Data are expressed as mean ± SD.

[a]$P < 0.05$ for Jonckheere–Terpstra test for trend for day 1 and day 36.

[b]From study UWI09-8-02 (NCT01336387).

[c]From study UWI10-16-01R (NCT01935960).

[d]One subject in the each of the 0, 40, and 160 mg cohorts did not complete the day 36 pharmacokinetic collection and were excluded from day 36 and absolute change analyses.

## Discussion

Rexinoids, including fenretinide, isotretinoin, bexarotene, and 13-cis retinoic acid, have been extensively evaluated or used as cancer therapeutic or chemoprevention agents; however, experience in chronic use, especially in the setting of cancer prevention, has been limited by toxicities and/or intolerance (e.g., hypertriglyceridemia and/or skin toxicity; refs. 10, 22). 9cUAB30, which binds primarily to the RXR receptor, was designed to retain anticancer activity while minimizing adverse effects (8).

In this placebo-controlled, dose escalation study, we demonstrated that 9cUAB30 in doses up to 240 mg a day continuously for up to 28 days was well tolerated, with no participants experiencing dose-limiting toxicities. Observed hypertriglyceridemia in this study was grade 1–2, did not differ between the placebo and active treatment cohorts, and was not related to dose and duration, suggesting it was unrelated to 9cUAB30 administration and consistent with normal intrapatient variability. Potential study drug-related skin adverse events were observed. Pruritus ("itchy or dry skin") was mild (grade 1), short in duration and infrequent, except at the highest doses (160 and 240 mg), raising the possibility that this adverse event is dose dependent. Over the brief time on study (5 weeks), no participants went off study due to skin toxicity.

Grade 1–2 hypertension was observed in 45% of participants receiving placebo compared with 12.5%, 78%, 50%, 78%, and 62.5% of participants receiving 20, 40, 80, 160, and 240 mg, respectively. Grade 3 hypertension was rare (one subject at 20 mg) at dosing cohorts below 240 mg/day. Two possibly related grade 3 TEAEs were observed in the 240 mg dose cohort, including one participant in this cohort who had grade 3 hypertension at baseline that resolved and then reoccurred while on study agent. Notably, participants in this cohort were the oldest, heaviest, and had the highest baseline blood pressures. Blood pressure recordings of participants throughout the study suggested little to no effect of 9cUAB30: both systolic and diastolic blood pressure measurements fluctuated by ±20 mm Hg with no discernable pattern, suggesting that the observed changes in blood pressure most likely represent random variability.

Steady-state plasma concentrations ranged from 172 ng/mL at the 40 mg dose level to 1,900 ng/mL at the 160 mg dose level. Preclinical studies of 9cUAB30 demonstrated transcriptional activation of an RXR-responsive promoter reporter construct at an $EC_{50} = 118$ nmol/L (34 ng/mL), and RXR receptor-binding assays in competition with radiolabeled 9-cRA at an $IC_{50} = 284$ nmol/L (82 ng/mL). Hansen and colleagues demonstrated that 0.1 μmol/L (29 ng/mL) of 9cUAB30 inhibits colony formation (12). In addition, 1 μmol/L concentrations of 9cUAB30

(corresponding to 294 ng/mL) is required to prevent KLF4-mediated tumor initiation (23). Therefore, concentrations achieved by the 80 mg dose and higher doses all exceed the predicted biologically active concentrations.

9cUAB30 exhibits high intersubject pharmacokinetic variability, with observed SDs of the AUC and $C_{max}$ approaching and sometimes exceeding the mean values. This effect appeared most pronounced at the 160 mg dose level, and occurred with both single dose and steady-state administration. Although food effects commonly result in variability in absorption, study participants were advised to self-administer 9cUAB30 on an empty stomach, and on the days of single-dose administration, 9cUAB30 dosing was observed. Therefore, a food effect seems an unlikely explanation for the high pharmacokinetic variability. One potential explanation is the formation of a pharmacobezoar (24). Participants at the 160 mg dose level ingested 8 capsules, which may have physically interacted with each other and altered absorption resulting in increased variability in pharmacokinetic parameters. Although pharmacokinetic variability was not as apparent at the 240 mg level where participants ingested 12 capsules a day, fewer patients may have been able to ingest the full dose, also providing a potential explanation for the nonlinearity with dose observed. Importantly, 9cUAB30 is being reformulated to substantially reduce the pill burden, which may also improve absorption.

Currently, the only FDA-approved rexinoid for cancer treatment is bexarotene, which was approved in 1999 for cutaneous T-cell lymphoma at a dose of 300 mg/m$^2$/day. Use of this agent is limited by adverse effects, including triglyceride elevations requiring lipid-lowering agents in 70% and hypothyroidism requiring supplementation in 50% of treated individuals (7). Although this trial did not compare 9cUAB30 to bexarotene, no subjects receiving 9cUAB30 developed symptomatic elevations in lipids or hypothyroidism, suggesting 9cUAB30 may be better tolerated. Moreover, because the daily dose of bexarotene in an average sized adult is 600 mg and the dose of 9cUAB30 required to achieve plasma concentrations associated with RXR transcription is 80 mg, 9cUAB30 may also be the more potent agent.

9cUAB30 is a promising chemopreventive agent for a variety of malignancies, including breast and skin cancer. On the basis of this agent's capability to prevent MNU-initiated mammary cancers in a rat model, a phase Ib clinical trial (NCT02876640) is being conducted in women with early-stage breast cancer. The primary objective of this study is to evaluate the ability of 9cUAB30 to reduce proliferation comparing pre- and posttreatment breast tissue. 9cUAB30 also prevents the formation of squamous cell carcinoma (SCC) in the Kruppel-like factor 4 (KLF4) transgenic mouse model (23) and inhibits tumor growth and increases survival in a murine neuroblastoma xenograft model (25), suggesting a potential chemopreventive role for this agent in populations highly susceptible to SCC, such as organ transplant recipients (26).

In conclusion, this phase I dose escalation study of the novel retinoid 9cUAB30 determined 160 mg per day to be the recommend phase II dose. This dose was very well-tolerated and provided serum concentrations well over those required for RXR activation and preventive efficacy in preclinical models and tolerability.

## Disclosure of Potential Conflicts of Interest

D.D. Muccio has ownership interest (including patents) in a patent. No potential conflicts of interest were disclosed by the other authors.

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