NCI Protocol #: Not applicable

DF/HCC Protocol #: 19-224

TITLE: A phase II trial of all-trans retinoic acid (ATRA) in advanced adenoid cystic carcinoma

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Agent: All-trans retinoic acid (ATRA); generic name: *Tretinoin*; trade name: *Vesanoid*®

IND Holder: Glenn J. Hanna, M.D.

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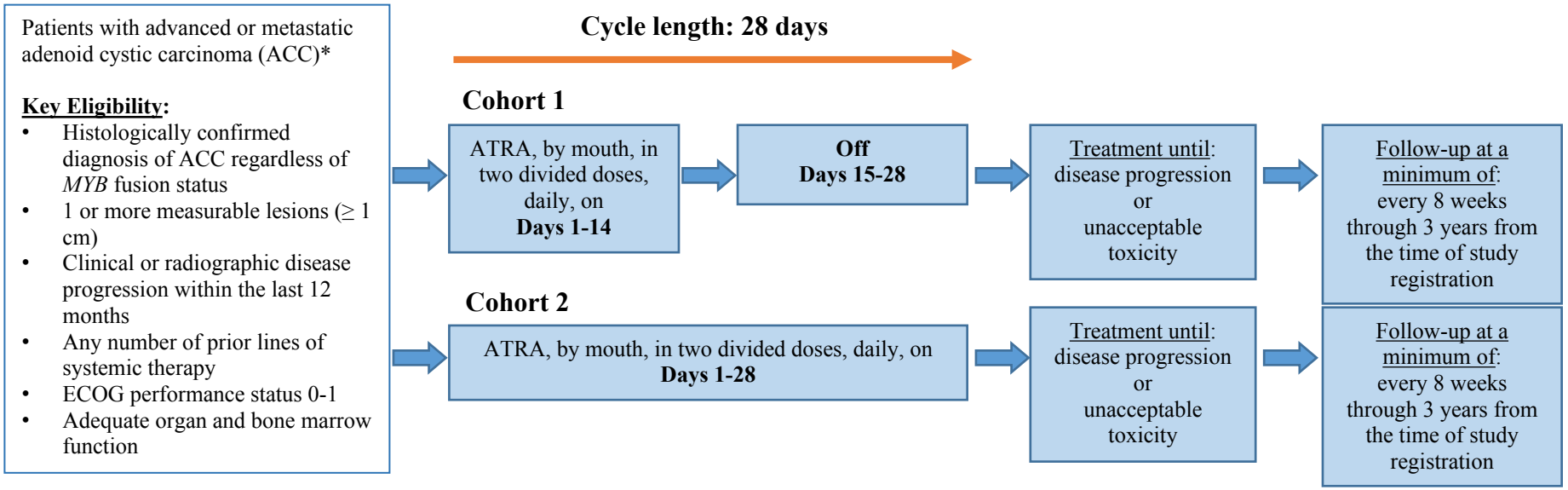
SCHEMA



** pre- and on-treatment tumor biopsies are required, if safe and feasible (a post-treatment biopsy at the time of disease progression will be offered)*

Primary endpoint: objective response rate (ORR) by RECIST v1.1
Secondary endpoints: progression-free survival (PFS), overall survival (OS), changes in *MYB* expression among tumor cells and circulating tumor (ct)DNA

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1. OBJECTIVES

1.1 Study Design

This is an open-label, single arm, phase II study of tretinoin (all trans retinoic acid, ATRA) in patients with advanced, unresectable, or recurrent/metastatic adenoid cystic carcinoma (ACC). All participants will have evidence of disease progression, or lack of benefit from standard therapies. All participants will have measurable disease amenable to disease monitoring. Participants in Cohort 1 will receive tretinoin 45 mg/m^2 orally divided over two daily doses for 14 days of a 28-day cycle until disease progression or unacceptable toxicity. Participants in Cohort 2 will receive tretinoin 45 mg/m^2 orally divided over two daily doses continuously during a 28-day cycle until disease progression or unacceptable toxicity. Our aim is to evaluate the anti-tumor activity of tretinoin by inhibition of MYB expression in advanced ACC patients. Correlative studies are planned to evaluate the effect of tretinoin on *MYB* expression in tumor tissue before and after dosing, and to measure circulating tumor (ct)DNA in serum samples while on therapy.

1.2 Primary Objectives

To evaluate the anti-tumor activity of tretinoin by evaluating the best overall response rate using RECIST v1.1 in participants with advanced adenoid cystic carcinoma.

1.3 Secondary Objectives

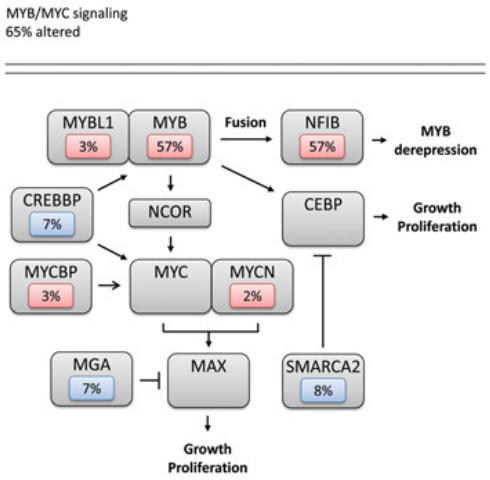
- To estimate progression-free survival (PFS) and overall survival (OS)
- To estimate duration of therapeutic response
- To evaluate the inhibitory effect of tretinoin on *MYB* expression in ACC tumors
- To evaluate ctDNA as a biomarker of disease activity in ACC based on response to therapy
- To develop novel patient-derived tumor xenograft (PDX) models from *MYBL1*-rearranged ACC tumors to understand mechanisms of resistance to tretinoin

2. BACKGROUND

2.1 Study Disease(s)

Adenoid cystic carcinoma (ACC) is a common malignancy arising often in salivary gland tissue, often affecting patients with no identifiable risk factors. Localized disease is generally managed with oncologic resection followed by adjuvant radiotherapy in most cases, given the potential for perineural invasion (PNI) linked with this malignancy [1, 2]. Concurrent adjuvant chemoradiotherapy is sometimes offered to mitigate the risk of locoregional recurrence, but no prospective trials have proved that this strategy is beneficial in terms of disease control rates and survival [3]. Despite initial multimodality therapy in the upfront setting, nearly half of ACC patients develop distant, metastatic spread of disease; but the natural history of such cancers is often reflective of slower growth velocity despite recurrences that involve the lungs, liver and bones [4].

There remain no approved systemic cytotoxic or targeted therapies available for the treatment of salivary gland cancers broadly, and none specifically for ACC. While some agents have demonstrated modest disease activity, there has been no overall or progression-free survival advantage observed with any particular agent(s) – therefore the optimal regimen remains unclear [5]. Cytotoxic chemotherapy combinations using anthracyclines, platinum and fluoropyrimidines, and vinorelbine in some combination may have some benefit in a limited number of ACC patients, but their expected toxicities and lack of durable response make them less than appealing when considering that patients with ACC can have slow growing, metastatic tumor burden but maintain a favorable quality of life over months to years [6, 7]. The **Table** *below* summarizes response rates among ACC patients treated with various cytotoxic regimens [5]. While combination therapies result in greater response rates, there is no clear survival advantage to such an approach and they come with toxicity. Although immune checkpoint blockade has revolutionized cancer immunotherapy with the success of PD-1 inhibitors in a host of solid tumors, ACC does not generally express the ligand of PD-1 (PD-L1) and appears to maintain minimally immunogenic potential [8]. Early studies investigating PD-1 blockade in all salivary cancer patients have been largely disappointing with response rates < 15% in selected PD-L1+ salivary tumors [9].



More recent approaches to treatment have incorporated molecular and genomic profiling data. While no driver mutation is known, these tumors have a low somatic mutation rate when compared with other histologic subtypes of cancer. Multi-targeted tyrosine kinase inhibitors (TKIs) such as sorafenib, and oral therapies targeting c-kit (imatinib, dasatinib) have been investigated with limited objective response rates (< 15%) [10-12]. Next-generation sequencing data has suggested frequent alterations in a diverse array of signaling pathways including: epigenetic modifiers, DNA damage and repair signals, *NOTCH* pathway, and *FGF-IGF-PI3K* signaling [13]; and clinical trials are ongoing to evaluate the anti-tumor effects of drugs aimed at modifying these aberrant signaling cascades in ACC.

Chemotherapy	**# of responses/total patients**	**Response rate (%)**
Cisplatin	2/32	6
Vinorelbine	2/13	15
Mitoxantrone	5/50	10
Epirubicin	2/20	10
Paclitaxel	0/14	0
Cyclophosphamide + doxorubicin + cisplatin	12/43	28
Cisplatin + anthracycline + 5-FU	6/19	32
Cisplatin + vinorelbine	4/9	44
Carboplatin + paclitaxel	2/10	20
Cisplatin + gemcitabine	2/10	20

One molecular aberration that has been identified in sequenced ACC tumors is the *MYB-NFIB* fusion oncogene generated by a t(6;9) translocation which occurs in up to 70-90% of ACC tumors (**Figure** *upper right*) [13, 14]. More than just generating *MYB* gene fusion transcripts, studies have shown that *MYB* mRNA is overexpressed among some of these tumors – pointing to the importance of this molecular event in ACC. Despite the identification of *MYB* has an important target in ACC, no *MYB* inhibitors have been identified. Zon and colleagues recently ran a

chemical screen for bioactive molecules against a *MYB* reporter assay in an animal model and identified retinoic acid compounds, including tretinoin as a potential *MYB*-inhibitory agent [15]. Therefore, evaluating the anti-tumor activity of tretinoin in advanced ACC patients seems a logical next-step in understanding the therapeutic potential of retinoic acids in this disease. Given historical objective response rates of single-agent chemotherapy and multi-targeted TKIs approaching 10% or less, we aim to evaluate if tretinoin may produce similar or superior response rates in advanced ACC.

2.2 IND Agent: Tretinoin (All-trans retinoic acid, ATRA)

2.2.1 Tretinoin

2.2.1.1 Mechanism of action

The retinoids are a pharmacologic class consisting of vitamin A (retinol) and related derivatives. Retinol and its metabolites play a critical role in cell growth, vision, reproduction, and epithelial cell differentiation, along with immune function [16]. Tretinoin is a natural retinol metabolite formed from intestinal oxidation of beta-carotene and from tissue anabolism of retinaldehyde. Normal serum concentrations are 10-20 nanomolar (nM) [17] which is 10 to 100-fold less than the retinoid concentrations achieved with pharmacologic therapy [18].

The *MYB* family of transcription factors is implicated in cell growth and proliferation, having been linked to leukemic transformation as *MYB* blocks differentiation of hematopoietic cells in preclinical models [19, 20]. Moreover, targeting of *MYB* with antisense oligonucleotides blocks hematopoiesis and therefore myeloid leukemic cell proliferation [21]. Expression of *MYB* declines in retinoic acid-treated myeloid leukemic cells, which suggests that the retinoic acid receptor (RAR) partakes in the downregulation of *MYB* expression [22]. A physical interaction appears to exist between *MYBi* and the RAR, while little is actually known about the details of these mechanistic processes [23].

2.2.1.2 Preclinical safety

The HL-60 cell line is derived from an individual with acute promyelocytic leukemia (APML) which proliferates *in vivo* with a dominance of promyelocytes. Early preclinical experiments with tretinoin showed that differentiation (as measured morphologically) was induced at drug concentrations as low as 1 nM, a value $1/500^{th}$ to $1/160{,}000^{th}$ the concentration of butyrate and dimethyl sulfoxide that similarly promote increased differentiation among promyelocytes [24].

2.2.1.3 Clinical pharmacology and safety

Plasma levels of tretinoin after oral administration can be somewhat variable. Following a 45 mg/m^2 oral dose of tretinoin, peak plasma concentrations on the first day of dosing range from 0.1 to 8 μM, with median concentrations of approximately 1 μM. Plasma and urinary concentrations of the parent compound of tretinoin and metabolites was quantified in 13 patients by reverse-phase high-performance liquid chromatography, showing that mean peak plasma levels of tretinoin were around 347 ng/mL and were reached within 1-2 hours after ingestion. Elimination of tretinoin appears rapid with a terminal half-life of around 45 minutes with a monoexponential decay [25, 26]. The only

metabolite detected in plasma or urine was 4-oxo-all-trans RA which accounted for less than 10% of the circulating drug levels in plasma, and less than 1% in urine. No drug was detected in cerebrospinal fluid. Continued oral administration of tretinoin was associated with a decrease in the plasma peak levels and area under the concentration-time curve when measured 2-6 weeks on treatment. It should be noted that this decrease is associated with an increase in urinary excretion of the 4-oxo-all-trans RA metabolite which suggests some degree of drug catabolism over time [18]. While these limitations have resulted in intravenous (IV) formulations being developed, plasma concentrations appear similar to oral dosing in nonhuman primate models, with a similar decline in plasma area under the curve (AUC) as soon as three days into dosing [27].

Further studies have investigated intermittent dosing schedules (7 days on/7 days off) and have shown that plasma AUC declines significantly during the first week of administration from a mean AUC of 145 μM*min on day 1 to a mean of 4 μM*min on day 7. But, plasma tretinoin concentrations at the start of weeks 3 and 11 of this every-other-week schedule were equivalent to those achieved on day 1 of therapy. Thus, intermittent schedules of administration appear to circumvent the low plasma drug exposure that results from sustained upregulation of catabolism that has been observed with daily dosing schedules [28]. This was the rationale for on/off dosing in Cohort 1.

Since tretinoin appears to partially induce its own metabolism, research has focused on inhibitors of tretinoin to combat resistance in APML [29]. Tretinoin is subject to hydroxylation by the cytochrome P450 26A1 isoform (CYP26A1), thus studies have attempted to inhibit tretinoin metabolism by administering ketoconazole or liarozole – despite their known toxicity profiles. This strategy has even been labeled retinoic acid metabolism blocking agents (RAMBAs) to enhance the clinical efficacy of tretinoin.

The toxicity profile of tretinoin is usually minor and includes headache, low grade temperatures, generalized weakness and some component of fatigue – seldom are any of these effects permanent or irreversible. Common toxicity reported in > 10% of treated patients with tretinoin include: peripheral edema (52%), headache (86%), malaise (66%), skin rash or dry red skin (54%), dry mucus membranes or nasal stuffiness, chapped lips (50%), elevated liver function tests, enzymes or bilirubin (50%), ostealgia (77%), or low-grade temperatures (78%) [30, 31]. While uncommon, other significant adverse events have been reported: venous thrombosis and myocardial infarction have been reported in patients without cardiac risk factors, and the risk of thrombosis (arterial or venous) appears increased within the first month of therapy. CNS effects such as dizziness, headaches or malaise have been reported. Hypertriglyceridemia or hypercholesterolemia has been reported in a number of patients which appears reversible following discontinuation of therapy. Pseudotumor cerebri (benign intracranial hypertension) has been reported, especially in children. Tretinoin has a high-risk of teratogenicity [32, 33]. Importantly, the two serious side effects of differentiation syndrome and hyperleukocytosis are **only** observed when tretinoin is used to treat APML.

2.2.1.4 Clinical efficacy

As early as 1996, data was published that established the clinical safety and efficacy of tretinoin in the management of patients with de novo APML. One early protocol utilized a dose of 15-20 mg/m^2/day in 27 patients with newly diagnosed disease and demonstrated a 92% clinical complete

response (CR) rate among 26 evaluable patients within 13-67 days of tretinoin therapy [34]. No cases of retinoic acid syndrome (RAS) or hemorrhagic disseminated intravascular coagulation (DIC) were observed. These data supported the use of low-dose tretinoin in leukemic patients. A further protocol evaluated the efficacy and safety of tretinoin along to induce remission among 53 children with APML. The overall CR rate was 81% with an estimated 5-year disease-free survival (DFS) of 41% and overall survival (OS) of 69% [35]. Moreover, 10-year follow-up of the cohort revealed that OS rates were stable.

Tretinoin has also been successfully and safely combined with chemotherapy. The North American Intergroup regimen is comprised of tretinoin plus daunorubicin (an anthracycline) plus cytarabine (AraC) using the standard 45 mg/m^2/day divided dosing until CR; demonstrating overall CR rates of 90%, and when followed by arsenic trioxide (ATO) consolidation, event-free survival was 80% and OS 86% at 3-years [36]. In the AIDA protocol, tretinoin is combined with idarubicin alone (another anthracycline) but without AraC. Remission rates approached 95% and again utilized the same tretinoin dosing at 45 mg/m^2/day followed by both consolidation and maintenance therapy with an event-free survival at 12 years of 69% [37]. In hopes of avoiding chemotherapy altogether, the Lo-Coco regimen combined tretinoin with ATO at similar dosing and despite shorter follow-up yielded a > 85% remission rate, therefore supporting its role in managing low-to-intermediate risk APML patients, or in those who should not receive anthracycline-based therapy [38]. Combining tretinoin, ATO and gemtuzumab ozogamicin appears to yield response rates of 92%, with an estimated 3-year survival of 85% -- although toxicity has been a concern with an 8% rate of disease-related complications leading to death with this particular regimen [39]. In terms of long-term dosing, as part of consolidation and maintenance therapy for APML, tretinoin is dosed at 45 mg/m^2/day orally days 1-7 during consolidation, and 45 mg/m^2 orally daily on an intermittent schedule of 15 days on every 3-months or 7 days every 2 weeks for up to 1-year during the maintenance phase [36].

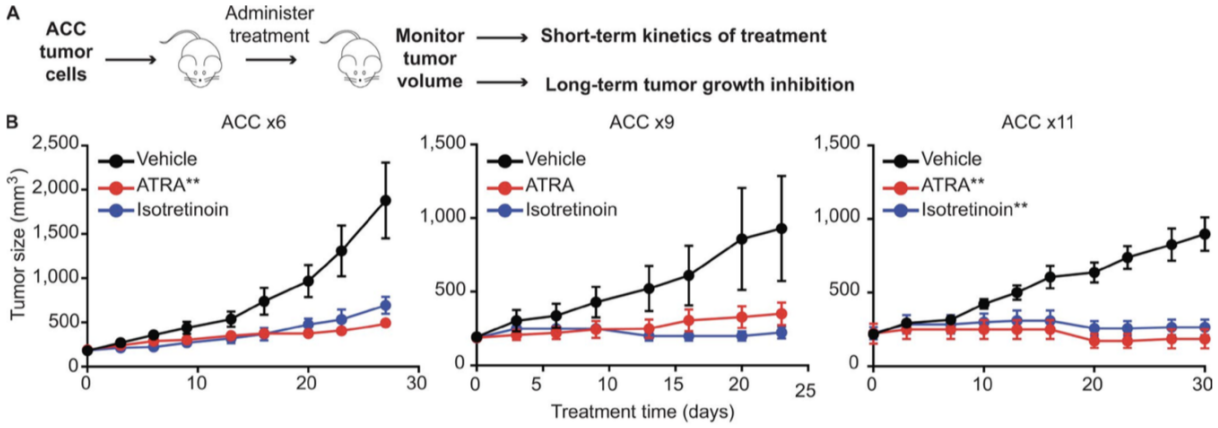
2.3 Rationale

Adenoid cystic carcinoma (ACC) is the second most common salivary gland malignancy, with nearly 1,200 new cases per year in the United States [40]. Over the past decade, genomic characterization of ACC tumors has highlighted recurrent translocations that involve the *MYB* or *MYBL1* genes in upwards of 90% of ACCs [13, 14]. Alterations in *MYB* have been implicated in a variety of cancers, including leukemia, pediatric glioma and colonic adenocarcinomas, as well as in cancers of the colon, breast and prostate; but more recently, ACC-specific *MYB* translocations have been shown to promote transformation in genetically engineered mouse models [41, 42].

To date, no FDA-approved therapies exist for ACC, and no clinical *MYB* inhibitors have been identified. However, given the near universal prevalence of *MYB* or *MYBL1* activation in ACC tumors, targeting of *MYB/L1* remains an attractive therapeutic strategy for ACC patients. Translocations involving *MYB* have been previously described in ACC for bringing strong enhancers into close proximity of the *MYB* locus, and these enhancers are also bound by *MYB* protein, resulting in a positive feedback loop that drives *MYB7* [13, 43]. Mandelbaum and colleagues at our institution recently undertook a chemical screen of bioactive molecules in an *in vivo MYB* reporter assay in zebrafish, and identified the retinoic acid class of compounds, including tretinoin/all-trans retinoic acid (ATRA), as potential *MYB*-inhibitory agents [44]. Specifically, c-myb:GFP-expressing cells from 72-hpf transgenic embryos were treated for 6 hours with 1 μM of retinoic acid agonists. RA

agonists induced a rapid decline in c-myb and GFP transcript expression. Further, while ACC cell lines are not currently available, U937 human cells (a myeloid leukemia line over-expressing c-myb) showed downregulation of c-myb expression within 1 hour of tretinoin treatment in a dose-dependent manner.

In vivo treatment of tretinoin further reduced the growth of human ACC primagraft models in mice. The schematic below [**Figure**, *below*] shows (A) the experimental design for ACC xenograft transplantation, (B) the percentage of tumor growth inhibition among treatment groups with RA



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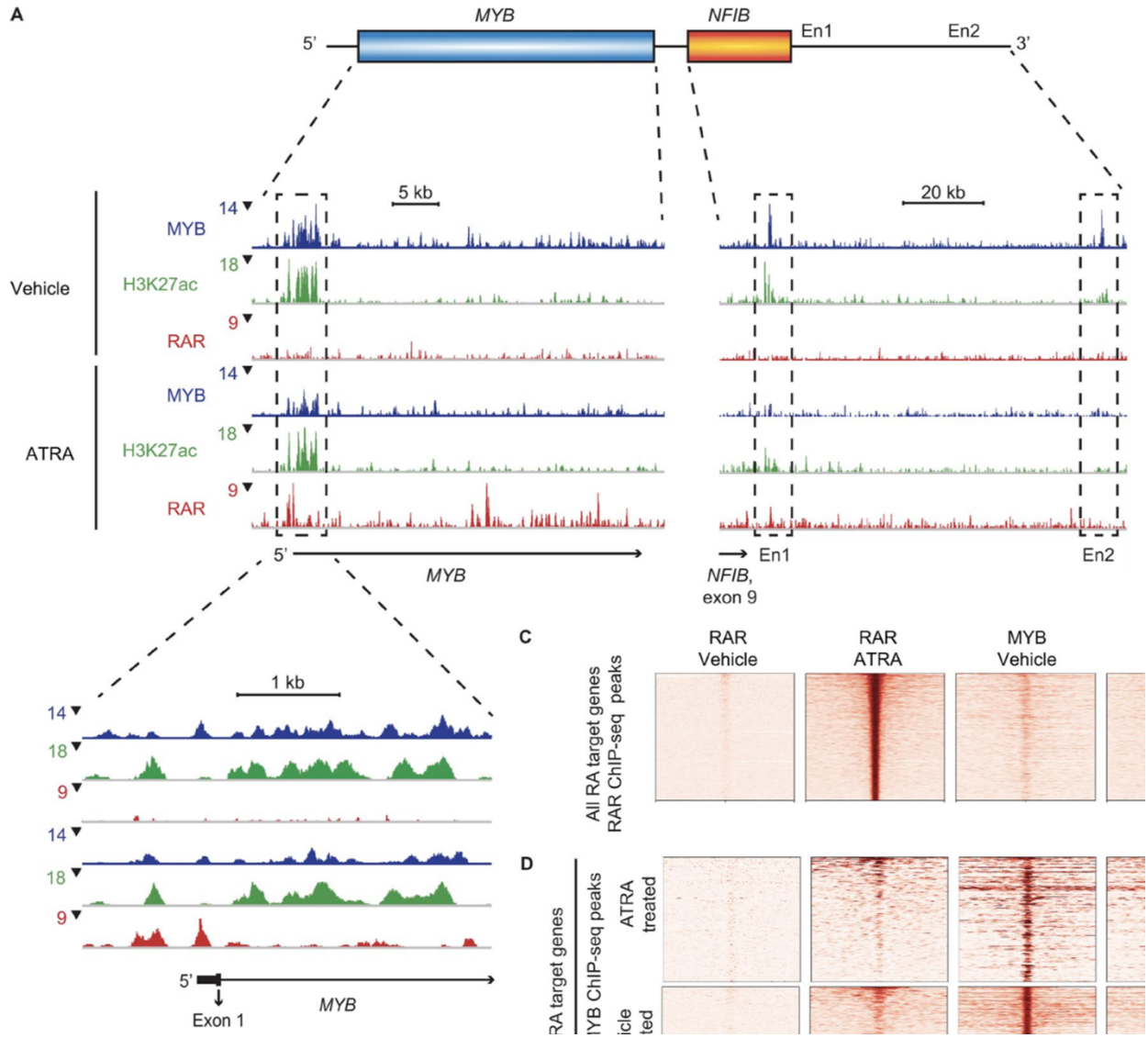
	Tumor Growth Inhibition			
	ACC x6	ACC x9	ACC x11	Average
ATRA	83%	78%	105%	88%
Isotretinoin	70%	95%	94%	86%
Average	77%	87%	100%	87%

compounds, (C) and the final average tumor growth inhibition across three human tumors with RA treatments. The authors were able to show that continuous dosing was required to maintain response, as discontinuation of tretinoin resulted in tumor resurgence in ACC x6, x9.

Tretinoin was mechanistically found to interrupt the autoregulatory enhancer loop that occurs with fusion genes involving *MYB*, thus dramatically lowering the level of the oncogenic fusion protein [**Figure**, *below*]: (A) Chromosomal rearrangements that maintain *MYB* over-expression in ACC are shown with *MYB*, RAR-binding, and H3K27ac profiles shown at the *MYB* locus or downstream of *NFIB* exon 9 in ACC x9 tumors that have *MYB-NFIB* translocations. Previously described translocated *MYB*-bound enhancers are labeled En1 and En2. We observed a strong decrease in *MYB* binding due to tretinoin treatment at two translocated enhancers in the downstream region of *NFIB* that is fused to the *MYB* locus in one of the ACC primagraft models (ACC x9). In addition, there was a concomitant decrease in H3K27ac (marking active enhancers) at these enhancer sites. Upon tretinoin treatment, we found that retinoic acid receptor alpha (RARα) binds physically at the 5' region of the *MYB* gene and at the translocated enhancer, and we confirmed that these sites have RARα binding motifs as determined by the JASPAR database [45]. Tretinoin stimulated increased RAR expression due to positive autoregulation by the bound receptor; these elevated RAR levels cause suppression of *MYB* gene expression or displaces *MYB* binding. Therefore, RARα expression appears to serve as a marker of response to tretinoin.



These findings taken together, plus the existing FDA approval of tretinoin for APML, and its known tempered toxicity profile, together provide a strong rationale for testing whether tretinoin elicits a clinical benefit in ACC patients via *MYB* inhibition. Our goal for this project is to translate the basic and preclinical findings for the use of tretinoin in ACC to the clinic.

2.4 Correlative Studies Background

As part of this clinical study using tretinoin in advanced ACC, we will examine proof of the mechanism of tretinoin in ACC patient-derived tumor specimens, and further aim to identify candidate biomarkers and mechanisms of resistance to this novel therapy. We expect that interrogation of paired tumor specimens and correlation with clinical response will elucidate the inhibitory effects of tretinoin on *MYB* in ACC, therefore revealing possible mechanisms of activity and resistance. We will uncover novel biomarkers of response or resistance to *MYB*-directed therapy in ACC patients, by interrogating a repository of tumor tissue and peripheral blood obtained from

tretinoin-treated ACC patients. Establishing the first *MYBL1*-rearranged and tretinoin-resistant PDX models will yield insights into possible mechanisms of tretinoin activity and resistance.

We intend to interrogate circulating tumor (ct)DNA as a biomarker of ACC activity and of response to tretinoin therapy. Sensitive techniques have been developed recently for analyzing such "liquid biopsies", to monitor ctDNA and circulating tumor cells (CTCs) as surrogate markers for tumor burden [46]. These techniques, in combination with imaging studies, offer a valuable approach for assessing tumor response. Tracking tumor-associated genetic aberrations in the blood can also be employed to assess residual disease, or to detect the emergence of cancer cells that are resistant to the therapy; and monitoring genetic alterations found in the blood plasma can be utilized to detect local recurrence or distant metastasis, as early as 5-10 months before they are detectable by conventional imaging [47, 48]. In the proposed study we aim to establish the utility of ctDNA as a biomarker in ACC. This is the first application of ctDNA as a marker of response to *MYB*-directed therapy in ACC, and will pave the way for future studies to validate ctDNA as a predictor of disease burden and clinical outcomes.

3. PARTICIPANT SELECTION

3.1 Eligibility Criteria

Participants must meet the following criteria on screening examination to be eligible to participate in the study:

1. Participants must have histologically confirmed adenoid cystic carcinoma with evidence of recurrent, metastatic or advanced, unresectable disease that is not amenable to curative surgery with or without radiotherapy.
2. Participants must have at least one RECIST v1.1 measurable non-CNS based lesion, as defined as at least one lesion that can be accurately measured in at least one dimension (longest diameter to be recorded for non-nodal lesions and short axis for nodal lesions) ≥ 1 cm with CT scans or MR imaging.
3. Participants must be willing to undergo fresh tissue core needle biopsy prior to study registration and repeat tumor biopsy while on study for correlatives. Willingness to provide blood samples for research throughout the study is also required.
4. Prior systemic therapy: At least 2 weeks must have elapsed since the end of prior chemotherapy, biological agents (4 weeks for anti-cancer monoclonal antibody containing regimens) or any investigational drug product, with adequate recovery of treatment-related toxicity to NCI CTCAE Version 5.0 grade ≤ 1 (or tolerable grade 2) or back to baseline (except for alopecia or neuropathy). Any number of prior therapies for recurrent/metastatic ACC are permitted.
5. Be ≥ 18 years of age on day of signing informed consent.
6. Have a performance status of 0 or 1 on the ECOG Performance Scale (see *Appendix A*).
7. Participants must have documentation of a new or progressive lesion on a radiologic imaging study performed within 12 months prior to study registration (progression of disease over any

interval is allowed) and/or new or worsening disease-related symptoms within 12 months prior to study registration. This assessment is performed by the treating investigator. Evidence of progression by RECIST v1.1 criteria is not required.

8. Participants must have normal organ and marrow function as defined below (within 14 days prior to study registration):

– leukocytes	≥ 3,000/mcL
– absolute neutrophil count	≥ 1,500/mcL
– hemoglobin	≥ 9 g/dL without transfusion within 7 days of treatment
– platelets	≥ 100,000/mcL
– total bilirubin	≤ 2x upper limit of normal (ULN)
– AST(SGOT)/ALT(SGPT)	≤ 2.5x institutional ULN or ≤ 5x ULN for those with liver metastases
– serum creatinine	≤ 1.5x ULN OR
– creatinine clearance	≥ 60 mL/min/1.73 m^2 for participants with creatinine levels above 1.5x ULN
– coagulation profile	INR ≤ 1.5x ULN unless the participant is receiving an anticoagulant
– triglyceride level	< 500 mg/dL or < 5.7 mmol/L
– cholesterol level	< 400 mg/dL or < 10.34 mmol/L

9. Baseline tumor measurements must be documented from imaging within 28 days prior to study registration. Other non-laboratory tests must be performed within 28 days prior to study registration.
10. Female subjects of childbearing potential should have a negative urine or serum pregnancy test within 14 days of study registration. Female subjects of childbearing potential should have a negative urine or serum pregnancy test repeated within 72 hours prior to receiving the first dose of study medication. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.
11. Female and male subjects of childbearing potential must agree to use an adequate method of contraception (hormonal or barrier method of birth control; abstinence) prior to study entry, for the duration of study participation, and 4 months after completion of tretinoin administration. Contraception is required before starting the first dose of study medication through 120 days after the last dose of study medication. Note: Abstinence is acceptable if this is the usual lifestyle and preferred contraception for the subject. **There is a significant risk of fetal malformation if pregnancy occurs while on tretinoin at any dose level, even if for short exposure periods.**
12. Be willing and able to provide written informed consent for the trial.

3.2 Exclusion Criteria

Participants who exhibit any of the following conditions at screening will not be eligible for admission into the study.

1. Metastatic disease impinging on the spinal cord or threatening spinal cord compression. Patients that have had previous treatment of disease with impinging on the cord with either surgery or radiotherapy with clinical or radiographic evidence of response or stability are eligible.
2. Participant has known active central nervous system (CNS) metastases and/or carcinomatous meningitis. Subjects with previously treated brain metastases may participate provided they are stable (without evidence of progression by imaging for at least four weeks prior to the first dose of trial treatment), and have no evidence of new or enlarging brain metastases. This exception does not include carcinomatous meningitis which is excluded regardless of clinical stability, because of their poor prognosis and because they often develop progressive neurologic dysfunction that would confound the evaluation of neurologic and other adverse events.
3. Concurrent administration of other cancer specific therapy or investigational agents during the course of this study is not allowed.
4. Uncontrolled intercurrent illness including but not limited to ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, or cardiac arrhythmia.
5. Has a known additional malignancy that is progressing or requires active treatment. Exceptions include basal cell carcinoma of the skin or squamous cell carcinoma of the skin that has undergone potentially curative therapy or in situ cervical cancer.
6. Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the subject's participation for the full duration of the trial, or is not in the best interest of the subject to participate, in the opinion of the treating investigator.
7. Subjects who are pregnant, or breastfeeding, or expecting to conceive or father children within the projected duration of the trial, starting with the pre-screening or screening visit through 120 days after the last dose of trial treatment. **Pregnant women are excluded from this study because tretinoin has the potential for teratogenic or abortifacient effects.** Breastfeeding should be discontinued if the mother is treated on this protocol. Women who could potentially become pregnant while undergoing treatment on this protocol must be willing to use 2 methods of contraception.

3.3 Inclusion of Women and Minorities

Both men and women of all races and ethnic groups are eligible for this trial. Women, minorities and other underrepresented populations are all at risk to develop adenoid cystic carcinoma.

4. REGISTRATION PROCEDURES

4.1 General Guidelines for DF/HCC Institutions

Institutions will register eligible participants in the Clinical Trials Management System (CTMS) OnCore. Registrations must occur prior to the initiation of protocol therapy. Any participant not registered to the protocol before protocol therapy begins will be considered ineligible and registration will be denied.

An investigator will confirm eligibility criteria and a member of the study team will complete the protocol-specific eligibility checklist.

Following registration, participants may begin protocol therapy. Issues that would cause treatment delays should be discussed with the Overall Principal Investigator (PI). If a participant does not receive protocol therapy following registration, the participant's registration on the study must be canceled. Registration cancellations must be made in OnCore as soon as possible.

4.2 Registration Process for DF/HCC Institutions

DF/HCC Standard Operating Procedure for Human Subject Research Titled *Subject Protocol Registration* (SOP #: REGIST-101) must be followed.

4.3 General Guidelines for Other Investigative Sites

Not applicable.

4.4 Registration Process for Other Investigative Sites

Not applicable.

5. TREATMENT PLAN

5.1 Treatment Regimen

Treatment will be administered on an outpatient basis. Reported adverse events and potential risks are described in *Section 7*. Appropriate dose modifications are described in *Section 6.* No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the participant's malignancy during the course of treatment.

Participants in **Cohort 1** will receive tretinoin 45 mg/m^2 orally divided over two daily doses for days 1 through 14 of a 28-day cycle (tretinoin will be held on days 15 through 28 of every cycle) until disease progression or unacceptable toxicity. Participants in **Cohort 2** will receive tretinoin 45 mg/m^2 orally divided over two daily doses continuously during a 28-day cycle until disease progression or unacceptable toxicity. Treatment duration is planned for 24 months or 2 years total. Following the first cycle of therapy, tretinoin cycles continue every 28 days or 4-weeks. The pills should be taken with food (preferably with a fat-containing meal).

The first day of each cycle requires a lipid panel (fasting is not required). After completing the study assessment, participants will take the dose with food.

Note: Tretinoin capsules are available in a 10 mg size interval only. Doses ending in ≤ 5 mg should be rounded down to the nearest 10 mg and doses > 5 mg should be rounded up to the nearest 10 mg [e.g. 172 would round *down* to 170 mg; 176 would round *up* to 180 mg]. The higher dose of an uneven dose will be the AM or morning dose.

Tretinoin compliance will be monitored as part of the study. Each participant will be required to maintain a medication diary of each dose of medication. The medication diary will be returned to the clinic study staff at the end of each 28-day cycle.

5.2 Pre-Treatment Criteria

5.2.1 Cycle 1, Day 1

Eligibility and exclusion criteria are provided in *Section 3*. These criteria will be assessed within 28 days of the first day of study treatment to establish eligibility and baseline values.

Informed consent will be obtained after the study has been fully explained to the subject and before the conduct of any screening procedures or assessments. If screening assessments occur within 3 days before start of study treatment, then they may serve as the baseline Cycle 1 Day 1 visit, and Cycle 1 Day 1 labs do not need to be performed.

Demographic information and baseline characteristics will be collected at the Screening Visit. Standard demographic parameters include age, sex, and race/ethnicity (recorded in accordance with prevailing regulations). Baseline characteristics will include ECOG performance status (*Appendix A*), disease status, medical histories, and prior and concomitant medications.

Additional testing required, as per *Section 10*, includes: hematology panel (see **Table 1**), chemistry panel, fasting lipid panel, coagulation panel, urine or serum HCG (in women of childbearing potential; see *Section 3* for when serum HCG testing is required), and an ECG.

Table 1. Clinical Laboratory Testing

Category	Test
Hematology panel	Hematocrit, hemoglobin, platelet count, white cell count with differential (bands, basophils, eosinophils, lymphocytes, monocytes, neutrophils), absolute neutrophil count
Chemistry panel	Chloride, potassium, sodium, BUN, serum creatinine, phosphorus, calcium, albumin, total protein, alkaline phosphatase, ALT, AST, total bilirubin (Note: the frequency of checking magnesium levels is at the discretion of the treating provider)
Lipid panel	(fasting is not required) to include a triglyceride, LDL, HDL, and total cholesterol values
Abbreviations: ALT = alanine aminotransferase; AST = aspartate aminotransferase; BUN = blood urea nitrogen	

A fresh tumor sample should be collected prior to the start of tretinoin therapy in all patients, utilizing a core needle technique. Archival tissue is not permitted. A blood specimen for correlative studies is also needed prior to the start of tretinoin therapy.

Further details about collection and handling of tumor biopsy and blood specimens can be found in *Section 9* and appendices.

5.2.2 Subsequent Cycles

Reasonable effort should be made to conduct study visits on the day scheduled (± 3 days).

Any changes from screening clinical evaluation findings that meet the definition of an adverse event (AE) will be recorded on the AE page of the eCRF.

The participants ECOG performance status, weight (in kilograms), vital signs, interval history since the last visit, and physical examination are performed at each study visit. Concomitant medications should also be reviewed at each study visit. In addition, the adverse event (AE) grid should be completed at each study visit. All laboratory values should be reviewed before proceeding with an additional cycle of treatment. Participants will undergo every-28 day or every 4-week assessments while on study (which coincides with the start of each cycle of therapy). The drug diary for the prior cycle should be reviewed at each study visit by the clinical staff.

5.2.3 Additional On-treatment Assessments

Tumor Assessments:

Tumor assessments will be performed according to RECIST v1.1 (see *Section 11*), at baseline and with response evaluations performed every 8 weeks (every 2 cycles), or as clinically indicated. In the case of response, confirmatory scans will be performed at least 4 weeks after initial response every 8 weeks after the scan that documented the initial response. In the case of progressive disease, confirmatory scans are recommended between 4-8 weeks following the date of initial progression. Continued treatment after initial progression is permitted, provided the patient is thought to be deriving clinical benefit and is counseled regarding the risks and benefits of continued treatment. If progression is confirmed, the date of progression is dated as the time of the original scans for study monitoring purposes.

Research Blood Sample Collections:

Specific instructions for research blood sample collections and draw handling are described in *Sections 9* and *10*, and Appendices.

Tumor Biopsy Collections:

Details about collection and handling of tumor biopsy specimen can be found in *Section 9* and *10,* and Appendices.

5.2.4 End-of-treatment Procedures

End-of-Treatment Visit:

All subjects will be asked to return to the site for a final, end-of-treatment visit, if possible. This visit must be performed within 30 days of final administration of study treatment. End-of-treatment assessments will not have to be repeated if the same assessments were performed within 7 days of this planned visit. The subject will be followed for 30 days after the last study intervention for adverse events.

The reason for the study participant coming off therapy should be clearly documented. For any reason other than for progression of disease, tumor assessments should be performed every 8-12 weeks if a patient comes off the study drug within 1 year of enrollment.

The participants ECOG performance status, weight (in kilograms), vital signs, interval history since the last visit, and physical examination are performed at the end-of-treatment visit. Concomitant medications should also be reviewed at this visit. In addition, the adverse event (AE) grid should be completed at this visit.

5.3 Agent Administration

Tretinoin administration:

All-trans retinoic acid (tretinoin) will be administered by trained medical personnel at the investigational site. Treatment compliance will be monitored through documentation of study treatment administration in the subject's medical record.

Doses of tretinoin at initial and subsequent dosing should use actual (not ideal or adjusted) body weight for calculation of dose.

Tretinoin will be administered orally as an outpatient in two divided daily doses (no in clinic dosing is required). The drug should be taken with food (ideally fat-containing to promote absorption). The dose each day of therapy should be given roughly 12 hours apart ± 30 minutes (suggested timing: 8:00 AM and then again at 8:00 PM daily). Participants should try to avoid shortened or prolonged dosing intervals when possible. If a dose of tretinoin is missed by ≥ 6 hours from the last oral dose, then the next dose should be retimed for the morning of the following day. Any dose of tretinoin that was missed less than 6 hours from the last administration should be taken, and the following dose retimed for the morning of the following day. If tretinoin is not tolerated, or vomiting results in loss of the ingested dose, the dose should be skipped, and the next planned dose attempted when possible. The study drug may be crushed, chewed, or dissolved if necessary.

At each study or on-treatment visit (occurring every 28-days or every cycle), the dose of tretinoin should be recalculated again if there is > 5% change in body weight or body surface area (BSA) per institutional standard practice. Additional intravenous hydration is not required at study visits. An observation period is not required since dosing occurs outside the clinic setting.

5.4 General Concomitant Medication and Supportive Care Guidelines

5.4.1 Concomitant Medication Guidelines

Acceptable Concomitant Medications:

All treatments that the investigator considers necessary for a subject's welfare may be administered at the discretion of the investigator in keeping with the community standards of medical care. Palliative radiation therapy can be prescribed and considered beyond Cycle 3 or in subsequent cycles at the discretion of the overall study PI, if clinically indicated. Pertinent concomitant medications will be recorded on the case report form (CRF). If changes occur during the trial period, documentation of drug dosage, frequency, route, and date may also be included on the CRF.

Prohibited Concomitant Medications:

Azole antifungal medications may increase tretinoin toxicity due to inhibition of cytochrome P450 metabolism of tretinoin, and thus toxicities (including pseudotumor cerebri and renal dysfunction) should be monitored closely during its concomitant use. Investigational agents other than tretinoin. There are no prohibited therapies during the post-treatment follow-up phase.

5.4.2 Supportive Care Guidelines

The following treatments are permitted throughout the duration of the study treatment phase and during follow-up:

- Standard therapies for pre-existing medical conditions unless listed as prohibited therapy below. Any medication intended solely for supportive care (e.g., analgesics, anti-diarrheal, anti-depressants) may be used at the investigator's discretion. At the discretion of the investigator, prophylactic antiemetic and anti-diarrheal medication(s) may be used as per standard clinical practice before subsequent doses of study drugs.
- Anticoagulants: anticoagulation with heparin, heparin derivatives, and/or warfarin may be given at the discretion of the treating physician. Coagulation parameters should be checked at each cycle, or more frequently at the discretion of the treating physician.
- Pain medications administered per standard clinical practice are acceptable while the patient is enrolled in the study.

Patients who experience toxicities should be treated symptomatically as clinically indicated. Medications that are considered necessary for the subject's welfare and that are not expected to interfere with the evaluation of study treatment or be restricted may be given at the discretion of the investigator. Ancillary treatments will be given as medically indicated.

5.5 Criteria for Taking a Participant Off Protocol Therapy

Duration of therapy will depend on individual response, evidence of disease progression and tolerance. In the absence of treatment delays due to adverse event(s), treatment may continue until one of the following criteria applies:

- Disease progression
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse event(s), *see Section 6.0*
- Participant demonstrates an inability or unwillingness to comply with the oral medication regimen and/or documentation requirements
- Participant decides to withdraw from the protocol therapy
- General or specific changes in the participant's condition render the participant unacceptable for further treatment in the judgment of the treating investigator
- Pregnancy

Participants will be removed from the protocol therapy when any of these criteria apply. The reason for removal from protocol therapy, and the date the participant was removed, must be documented in the case report form (CRF). Alternative care options will be discussed with the participant.

When a participant is removed from protocol therapy and/or is off of the study, the relevant Off-Treatment/Off-Study information will be updated in OnCore.

5.6 Duration of Follow-Up

Participants will be followed for best overall response and development and documentation of first disease progression and for survival throughout the course of the trial for 3 years from the time of study registration. Participants removed from protocol therapy for unacceptable adverse event and if they have not developed first disease progression at time of discontinuation of protocol therapy, will continue to be followed until first disease progression and survival until death or 3 years from study registration (whichever occurs first). Participants removed from protocol therapy for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event.

5.7 Criteria for Taking a Participant Off Study

Participants will also be removed from study when any of the following criteria apply:

- Lost to follow-up
- Withdrawal of consent for data submission
- Death

The reason for taking a participant off study, and the date the participant was removed, must be documented in the case report form (CRF). Alternative care options will be discussed with the participant. In addition, the study team will ensure Off Treatment/Off Study information is updated in OnCore in accordance with DF/HCC policy REGIST-101.

In the event of unusual or life-threatening complications, participating investigators must immediately notify the Principal Investigator, Glenn J. Hanna, MD, at Partners pager 46231.

6. DOSING DELAYS/DOSE MODIFICATIONS

Dose delays and modifications will be made as indicated below. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for dose delays and dose modifications. A copy of the CTCAE version 5.0 can be downloaded from the CTEP website:

http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

If possible, symptoms should be managed symptomatically. In the case of toxicity, appropriate medical treatment should be used (including anti-emetics, anti-diarrheals, etc.).

All adverse events experienced by participants will be collected from the time of the first dose of study treatment, through the study and within 30 days of the last study intervention. Participants continuing to experience toxicity at the last scheduled study visit may be kept on the study until the toxicity has resolved, or until the toxicity is deemed irreversible.

Dosing interruptions are permitted in the case of medical/surgical events or logistical reasons not related to study therapy (e.g. elective surgery, unrelated medical events). Subjects should be placed back on study therapy within 1 week or 7 days of any dosing interruption (dosing does not need to be held prior to planned surgery or procedures), unless otherwise discussed with the Principal Investigator. The reason for interruption should be documented in the patient's study record.

Commonly observed toxicities with tretinoin include hepatotoxicity (increased AST and ALT), neurologic changes including headache or pseudotumor cerebri (*see below*), dermatology/skin changes and others including cheilitis, epistaxis, fatigue, musculoskeletal pain, and conjunctivitis.

If a patient experiences a Grade ≥ 3 toxicity that is felt to be related to tretinoin, then tretinoin will be held, until the toxicity has resolved to the Grade 1 level. At that time restart tretinoin at 25% dose reduction (or 33.75 mg/m^2/day divided twice daily) or Dose level -1 (see **Table 2**). Dosing of tretinoin should be as close as possible to 33.75 mg/m^2/day divided twice daily, i.e., rounded to the nearest 10 mg. Doses ending in ≤ 5 mg should be rounded down to the nearest 10 mg and doses > 5 mg should be rounded up to the nearest 10 mg [e.g. 172 would round *down* to 170 mg; 176 would round *up* to 180 mg]. If necessary, the AM or morning dose may differ from the PM or afternoon dose to deliver a total daily dose as close as possible to 33.75 mg/m^2/day divided twice daily.

Recurrent episodes of Grade ≥ 3 toxicity will result in discontinuation of the tretinoin until the toxicity resolves to Grade ≤ 1; tretinoin can then be resumed with an additional 25% reduction (50% total dose reduction, Dose level -2) in the tretinoin dose (22.5 mg/m^2/day divided twice daily). Dosing of tretinoin should be as close as possible to 22.5 mg/m^2/day divided twice daily; rounded to the nearest 10 mg. Doses ending in ≤ 5 mg should be rounded down to the nearest 10 mg and doses > 5 mg should be rounded up to the nearest 10 mg [e.g. 172 would round *down* to 170 mg; 176 would round *up* to 180 mg]. The higher dose of an uneven dose will be the AM or morning dose.

Patients experiencing Grade ≥ 3 toxicity attributable to tretinoin while on 22.5 mg/m^2/day of tretinoin should have tretinoin held until the toxicity resolves to Grade ≤ 1. Tretinoin will then be resumed at the same 50% dose reduction (22.5 mg/m^2/day divided twice daily) while continuing to monitor for toxicity and holding doses as needed for Grade ≥ 3 toxicity. Please notify the study overall PI about patients experiencing Grade ≥ 3 toxicity while on 50% dose reduction. Patients with 3 episodes of Grade 3+ toxicity should be considered for therapy discontinuation in discussion with the overall PI.

If patients whose doses are reduced by 25% (33.75 mg/m^2/day divided twice daily) or 50% (22.5 mg/m^2/day divided twice daily) tolerate the lower dose for 7 days without recurrence of the toxicity that led to dose adjustment, a dose escalation may be attempted. If the patient had been decreased to 50% dosing (22.5 mg/m^2/day divided twice daily) and tolerates an escalation to 75% dosing (33.75 mg/m^2/day divided twice daily) for an additional 7 days, another escalation back to 100% dosing (45 mg/m^2/day divided twice daily) may be attempted.

Tretinoin can be held on study up to 28 days or the length of one full cycle for toxicity reasons, at which point the patient should be removed considered for therapy discontinuation in discussion with the overall PI.

Pseudotumor cerebri

Pseudotumor cerebri (PTC) must be confirmed by proper history, exam and other clinically relevant studies. Symptoms and/or signs of PTC include headache, papilledema, visual field defects (ocular or visual), and absence of focal neurological symptoms, except for occasional cranial nerve (CN) VI palsy. Cerebrospinal fluid should be evaluated and should be negative for signs of infection. However, lumbar puncture should be avoided while active coagulopathy is present. An opening pressure should be obtained; a pressure > 200 mm water helps to establish the diagnosis of PTC. A CT or MRI of the brain (to evaluate for meningitis, intracranial bleed, intracranial thrombosis, etc.) should be performed and should be compatible with PTC. Neurological and ophthalmologic consultations should be obtained in order to assist in making the proper diagnosis. Carefully follow the visual field status of the patient. One should also strongly consider obtaining an MRI/MRV to exclude the possibility of a sagittal sinus thrombosis. Consider acetazolamide (Diamox) therapy and periodic lumbar puncture with therapeutic removal of cerebrospinal fluid as appropriate for age/size.

If PTC develops:

- Hold tretinoin until this toxicity improves to Grade 0 or 1 (“mild” headache)
- Restart tretinoin at 75% of the original dose (33.75 mg/m^2/day divided twice daily). If PTC reoccurs, hold tretinoin as above and then resume at 50% of original dose (22.5 mg/m^2/day divided twice daily
- If the patient tolerates reduced dosing for more than 7 days, dose re-escalation can be attempted. Dose escalation from 50% (22.5 mg/m^2/day divided twice daily) to 75% (33.75 mg/m^2/day divided twice daily) to 100% (45 mg/m^2/day divided twice daily) at 7 day intervals may be attempted if clinically appropriate.

Table 2. Dose De-escalation Schema:

Dose Level	Tretinoin Dose
-2	22.5 mg/m^2/day, two divided doses
-1	33.75 mg/m^2/day, two divided doses
0	45 mg/m^2/day, two divided doses

Note: All treatment modifications must be expressed as a specific dose or amount rather than as a percentage of the starting or previous dose.

Tretinoin Dosing Should be *Delayed* if any of the following are present:

- Grade 3 drug-related creatinine, AST, ALT and/or Total Bilirubin abnormalities
- Grade 3 skin, drug-related adverse event
- Grade 3 drug-related laboratory abnormality, with the following exceptions:
  - Grade 3 lymphopenia
- Any adverse event, laboratory abnormality, or intercurrent illness which, in the judgment of the investigator, warrants delaying the dose of study medication.

Unacceptable Toxicity Warranting Drug *Discontinuation*:

- Any grade 4 adverse event will require *permanent discontinuation* with the following exceptions:
  - Grade 4 electrolyte abnormalities that are < 72 hours in duration
  - Grade 4 neutropenia or lymphopenia which are < 5 days in duration
  - Grade 4 elevation in cholesterol or triglycerides

The consideration to re-initiate study therapy under these exceptions will be made on a case by case basis after considering the overall benefit/risk profile and in consultation with the overall PI.

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of reported and/or potential AEs (Section 7.1) and the characteristics of an observed AE (Section 7.2) will determine whether the event requires expedited reporting **in addition** to routine reporting.

7.1 Expected Toxicities

Virtually all patients treated with tretinoin experience some drug related toxicity, especially headache, fever, weakness, and fatigue. These adverse effects are seldom permanent or irreversible nor do they usually require interruption of therapy. Please see the Package Insert at the bottom of the protocol document for details.

7.2 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a

copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

- **For expedited reporting purposes only:**
  - AEs for the agent(s) that are listed above should be reported only if the adverse event varies in nature, intensity or frequency from the expected toxicity information which is provided.

- **Attribution** of the AE:
  - Definite – The AE *is clearly related* to the study treatment.
  - Probable – The AE *is likely related* to the study treatment.
  - Possible – The AE *may be related* to the study treatment.
  - Unlikely – The AE *is doubtfully related* to the study treatment.
  - Unrelated – The AE *is clearly NOT related* to the study treatment.

7.3 Adverse Event Reporting

7.3.1 In the event of an unanticipated problem or life-threatening complications treating investigators must immediately notify the PI.

7.3.2 PI **must** report any adverse event (AE) that occurs after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment on the local institutional SAE form.

7.3.3 Serious Adverse Events

7.3.3.1 Definition

A **Serious Adverse Event (SAE)** is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening (defined as an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- Requires inpatient hospitalization or causes prolongation of existing hospitalization (see **NOTE** below)
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Is an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the subject or may require intervention [eg, medical, surgical] to prevent one of the other serious outcomes listed in the definition above.) Examples of such events include, but are not limited to, intensive treatment in an emergency room or at

home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization.)

- Suspected transmission of an infectious agent (eg, pathogenic or nonpathogenic) via the study drug is an SAE.

Although pregnancy, overdose, potential drug-induced liver injury (DILI), and cancer are not always serious by regulatory definition, these events must be handled as SAEs.

Any component of a study endpoint that is considered related to study therapy should be reported as an SAE (eg, death is an endpoint, if death occurred due to anaphylaxis, anaphylaxis must be reported).

7.3.3.2 Reporting SAEs

Following the subject's written consent to participate in the study, all SAEs, whether related or not related to study drug, must be collected, including those thought to be associated with protocol-specified procedures. All SAEs must be collected that occur within 100 days of discontinuation of dosing. All SAEs should be followed to resolution or stabilization.

7.4 DF/HCC Adverse Event Reporting Guidelines

Investigative sites within DF/HCC will report AEs directly to the DFCI Office for Human Research Studies (OHRS) per the DFCI IRB reporting policy.

Other investigative sites will report AEs to their respective IRB according to the local IRB's policies and procedures in reporting adverse events. A copy of the submitted institutional AE form should be forwarded to the Overall PI within the timeframes detailed in the table below:

Attribution	DF/HCC Reportable Adverse Events(AEs)				
	Gr. 2 & 3 AE Expected	**Gr. 2 & 3 AE Unexpected**	**Gr. 4 AE Expected**	**Gr. 4 AE Unexpected**	**Gr. 5 AE Expected or Unexpected**
Unrelated Unlikely	Not required	Not required	5 calendar days#	5 calendar days	24 hours*
Possible Probable Definite	Not required	5 calendar days	5 calendar days#	5 calendar days	24 hours*
# If listed in protocol as expected and not requiring expedited reporting, event does not need to be reported.					
* For participants enrolled and actively participating in the study ***or*** for AEs occurring within 30 days of the last intervention, the AE should be reported within 1 business day of learning of the event.					

The Overall PI will submit AE reports from outside institutions to the DFCI OHRS according to DFCI IRB policies and procedures in reporting adverse events.

7.5 Reporting to the Food and Drug Administration (FDA)

The PI, as study sponsor, will be responsible for all communications with the FDA. The PI will report to the FDA, regardless of the site of occurrence, any serious adverse event that meets the FDA's criteria for expedited reporting following the reporting requirements and timelines set by the FDA.

7.6 Reporting to Hospital Risk Management

PI will report to local Risk Management office any participant safety reports, sentinel events or unanticipated problems that require reporting per institutional policy.

7.7 Routine Adverse Event Reporting

All Adverse Events **must** be reported in routine study data submissions to the PI on the toxicity case report forms. **AEs reported through expedited processes (e.g., reported to the IRB, FDA, etc.) must <u>also</u> be reported in routine study data submissions.**

8. PHARMACEUTICAL INFORMATION

Information in *Section 8* is based on the Teva Pharmaceuticals USA, Inc. package insert.

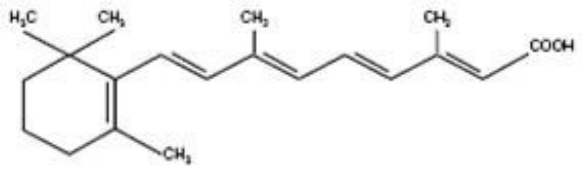
8.1 Study Agent: Tretinoin

All-trans retinoic acid (ATRA); generic name: *Tretinoin*; trade name: *Vesanoid*®

8.1.1 Description

Tretinoin, USP is a retinoid that induces maturation of acute promyelocytic leukemia (APL) cells in culture. It is available in a 10 mg gelatin capsule for oral administration. Each capsule contains the following inactive ingredients: butylated hydroxyanisole, edetate disodium, gelatin, hydrogenated vegetable oil, polysorbate 80, soybean oil, vitamin E, and white wax (beeswax). The ingredients in the capsule shell include black iron oxide, red iron oxide, titanium dioxide and yellow iron oxide. The ingredients in the edible imprinting ink include D&C yellow no. 10 aluminum lake, FD&C blue no. 1 aluminum lake, FD&C blue no. 2 aluminum lake, FD&C red no. 40 aluminum lake, iron oxide black, propylene glycol and shellac glaze.

Chemically, tretinoin, USP is all-*trans* retinoic acid and is related to retinol (Vitamin A) and has the following chemical name: 3,7-Dimethyl-9-(2,6,6-trimethyl-1- cyclohexen-1-yl)-2,4,6,8-nonatetraenoic acid. It is a yellow to light orange crystalline powder, and has the following structural formula:



$C_{20}H_{28}O_2$ Molecular Weight: 300.44

8.1.2 **Form**

Tretinoin Capsules are available as:

10 mg: Two-piece hard gelatin capsule with brown opaque cap and dark yellow opaque body, filled with yellow viscous oily suspension. Imprinted in black ink with stylized barr 808. Available in bottles of 100 capsules (NDC 0555-0808-02).

8.1.3 **Storage and Stability**

Store at 20° to 25°C (68° to 77°F) [See USP Controlled Room Temperature].
Dispense in a tight, light-resistant container as defined in the USP, with a child-resistant closure (as required). It is permissible for DF/HCC site to dispense ordered quantity of tretinoin in standard amber prescription bottle.

KEEP THIS AND ALL MEDICATIONS OUT OF THE REACH OF CHILDREN.
PROTECT FROM LIGHT.

8.1.4 **Availability**

Tretinoin is commercially available.

8.1.5 **Administration**

Tretinoin will be administered by trained medical personnel at the investigational site. Treatment compliance will be monitored through documentation of study treatment administration in the subject's medical record.

Doses of tretinoin at initial and subsequent dosing should use actual (not ideal or adjusted) body weight for calculation of dose. The higher dose of an uneven dose will be the AM or morning dose.

Tretinoin will be administered orally as an outpatient in two divided daily doses (no in clinic dosing is required). The drug should be taken with food (ideally fat-containing to promote absorption). The dose each day of therapy should be given roughly 12 hours apart ± 30 minutes (suggested timing: 8:00 AM and then again at 8:00 PM daily). Participants should try to avoid shortened or prolonged dosing intervals when possible. If a dose of tretinoin is missed by ≥ 6 hours from the last oral dose,

then the next dose should be retimed for the morning of the following day. Any dose of tretinoin that was missed less than 6 hours from the last administration should be taken, and the following dose retimed for the morning of the following day. If tretinoin is not tolerated, or vomiting results in loss of the ingested dose, the dose should be skipped, and the next planned dose attempted when possible. The study drug may be crushed, chewed, or dissolved if necessary.

At each study or on-treatment visit (occurring every 28-days or every cycle), the dose of tretinoin should be recalculated again if there is > 5% change in body weight or body surface area (BSA) per institutional standard practice. Additional intravenous hydration is not required at study visits. An observation period is not required since dosing occurs outside the clinic setting.

8.1.6 **Ordering**

Tretinoin, 10 mg capsules will be sourced locally by DF/HCC pharmacy site, and DF/HCC pharmacy site will be reimbursed via study fund.

8.1.7 **Accountability**

The investigator, or a responsible party designated by the investigator, should maintain a careful record of the inventory and disposition of the tretinoin, using the NCI Drug Accountability Record Form (DARF) or another comparable drug accountability form (See the NCI Investigator's Handbook for Procedures for Drug Accountability and Storage).

8.1.8 **Destruction and Return**

Unused and expired supplies of tretinoin will be destroyed on site per institutional SOP.

9. **BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES**

Background information on the pre-clinical and clinical rationale for these investigations is discussed in *Section 2.0* above.

Research blood sample collections should occur at the following time points: (1) at baseline, within 14 days prior to Cycle 1 Day 1 of protocol therapy (ideally as close to administration of Cycle 1 Day 1 therapy as possible), (2) at the beginning of every new Cycle, following C1 (expected to occur every 28-days) for the first 12 cycles or 1 year on treatment.

If tumor is accessible by surgical or percutaneous approach, a tumor biopsy should be performed at the following time points: (1) at baseline, within 28 days of Cycle 1 Day 1 of protocol therapy (ideally as close to administration of Cycle 1 Day 1 therapy as possible) but *before* the administration of the first dose of study treatment. (2) After cycle 2 (8 weeks on treatment), within 14 days of the start of Cycle 3 Day 1 of protocol therapy – providing the procedure is considered safe, feasible, and accessible at the discretion of the treating investigator.

In all patients in whom a tumor deposit is safely accessible, a baseline tumor biopsy is required prior to the start of tretinoin treatment. In addition, an on-treatment biopsy will be required at after Cycle

2 (or 8 weeks into treatment), within 14 days of the start of Cycle 3 Day 1 of protocol therapy – providing the procedure is considered safe, feasible, and accessible at the discretion of the treating investigator. Patients may also be offered an end-of-treatment biopsy if the reason for discontinuation was progressive disease – again if safe and feasible.

9.1 Evaluating Changes in Tumor MYB Expression in Response to Tretinoin

9.1.1 Collection and Handling of Specimens

Research biopsy kits will be provided by the Zon laboratory.

Biopsies should not be performed on Friday afternoons, as there may not be time for processing of the fresh tissue. If a biopsy must be performed on Friday morning, the lab of Dr. Leonard Zon must be notified ahead of time to ensure that there will be adequate time for processing fresh tissue, since fresh tissue cannot be stored over the weekend. The specimens in formalin may be stored over the weekend and delivered on Monday to the Zon lab. Specimens in formalin should be stored at room temperature until processing.

Ideally **two** core biopsies will be obtained at each biopsy timepoint:
• Two cores should be placed in 10% neutral buffered formalin tube supplied by the study team

After being obtained, processing of the cores is as follows:
• All samples should be de-identified and labeled with the participant initials, participant study ID number and date of procedure.

All leftover tissue will be banked in the laboratory of Dr. Leonard Zon, such that it can be used for additional or optional future analyses as needed:

Dr. Leonard Zon
Karp Research Building, 7th Floor
300 Longwood Ave. Boston, MA 02115
Telephone: 617-919-2069, Fax: 617-730-0222

9.1.2 Specimen Processing and Analysis

Paired tumor biopsies of each participant will also be analyzed by ChIP- and ATAC-seq, to identify changes in *MYB* and RARα binding to chromatin and evaluate chromatin accessibility at relevant regions; the goal is to study molecular changes that can be correlated with response to treatment. We have observed that RARα protein levels are enhanced in the xenograft tumors that are treated with tretinoin for 28 days; as such, this enhancement would serve as an excellent marker of the action of the treatment on the tumor. Most patients in this study will also have had their tumor genomically characterized by a targeted next-generation sequencing platform (DFCI Oncopanel, formerly protocol#11-104, now #17-000) at the time of enrollment. Changes in *MYB* expression and *MYB* gene activation (translocation status) will be correlated with clinical response and patient outcomes. To identify mechanisms of resistance, we will use whole-exome sequencing (WES) to evaluate the tumors of patients who achieve clinical benefit, and who have undergone an optional biopsy at the

time of disease progression, in order to determine the acquisition of novel genomic alterations from activation of other signaling pathways and/or from epigenomic changes. We expect to generate at least one *MYBL1*-rearranged PDX model, because about 10% of ACCs harbor *MYBL1* fusions and take rates are about 50-60%. A second PDX model will also be generated from ACC tumors with primary or secondary resistance to tretinoin.

9.2 Evaluating Circulating Tumor (ct)DNA in Response to Tretinoin

9.2.1 Collection and Handling of Specimens

Research blood specimens should not be performed on Friday afternoons, as there may not be time for processing of the sample. If a collection must be performed on Friday morning, the lab of Dr. Jens Lohr must be notified ahead of time to ensure that there will be adequate time for processing, since these samples should not be stored over the weekend.

Laboratory of Jens G. Lohr, M.D., Ph.D., Dana-Farber Cancer Institute, Dana Building, Room 542, 450 Brookline Avenue, Boston, MA 02215, Phone: (617) 632-2069, Pager: 42255

Ideally **three** blood specimen tubes will be obtained at each collection timepoint:
• Two 10 mL purple-top (EDTA) tubes and one 10 mL Streck tube for circulating tumor (ct)DNA and CTC studies

After being obtained, processing of the blood samples should follow the instructions provided in *Appendix E*

9.2.2 Specimen Processing and Analysis

We will first determine the cancer fraction of ACC in circulating tumor (ct) or cell-free (cf)DNA across disease stages. We will isolate cfDNA from the blood of ACC patients treated with tretinoin, before treatment, and at the time specified in the study calendar. The serum from all of these samples will be isolated, sequencing libraries prepared, and next-generation sequencing performed. Data will be segmented, normalized and bias-corrected, using a custom pipeline. The ACC fraction and the total amount of ACC-derived DNA in cfDNA will be determined at the above time points. The ACC-derived cfDNA fraction, and the total circulating ACC DNA concentration, will be correlated with established clinical parameters of ACC disease burden. In selected patients, we will determine ACC DNA fraction and concentration at very early time points to quantify rapid increases of cfDNA as a consequence of the effective killing of ACC cells, as well as later time points. We will establish whether cfDNA can be used as a quantitative tumor marker of treatment response, identify the copy-number variations in cfDNA, and quantify how many subclones have a distinct copy-number profile. The TITAN algorithm [49] or other computational analysis tools will be applied to determine the clonal composition of ACC-derived cfDNA, and the outgrowth over time of drug-resistant subclones will be quantified. These data should reveal the clonal evolution of ACC following tretinoin treatment over time and will define copy-number events that are associated with developing drug resistance.

We will use deep sequencing of cfDNA or CTCs to determine the changes of clonal composition in

ACC during tretinoin treatment. We plan to determine the somatic resistance mutations or other genetic aberrations in cfDNA and CTCs that occur following tretinoin treatment. To this end, we will perform next generation sequencing of DNA or RNA from CTCs or of cfDNA, and also of CD45+ cells from peripheral blood as matched controls. Somatic mutations can be determined using the Mutect algorithm [50], copy-number variations can be determined using Recapseq, and subclonality and cancer cell fraction of all mutations can be evaluated by the Absolute algorithm [51]. The concordance between cfDNA or CTCs and tumor tissue biopsies of ACC will be assessed for the time points detailed in the study calendar. Computational down sampling of these data will define the sequencing depth at which differences can be detected between cfDNA and tissue biopsy of ACC cells.

10. STUDY CALENDAR

Baseline evaluations are to be conducted within **28 days** prior to study registration (except for pregnancy test and baseline tumor biopsy, as detailed below). If these screening assessments occur 3 days before start of study treatment, then they may serve as the baseline Cycle 1 Day 1 values. Scans must be done within **28 days** prior to study registration.

As detailed in the *Study Calendar*, a negative pregnancy test in women of child-bearing potential must be documented within 72 hours before the first dose of study medication.

A baseline tumor biopsy, obtained within **28 days** before starting protocol therapy, is also required if tumor tissue is safely accessible. A second, on-treatment tumor biopsy sample will be obtained at the end of Cycle 2, within 14 days of Cycle 3 Day 1. A third (optional) biopsy will be performed if deemed safe or feasible at the time of confirmed disease progression (if that is the reason for coming off treatment).

Baseline laboratory evaluations must be completed **within 14 days** prior to study registration. In the event that the participant's condition is deteriorating, laboratory evaluations should be repeated within 48 hours of treatment initiation of the next cycle of therapy.

Assessments must be performed prior to administration of any study agent. Study assessments and agents should be administered within ± 3 days of the protocol-specified date, unless otherwise noted.

	Screening[A]	C1, D1	C2, D1	C2, D16-28	C3 and subsequent cycles	End of protocol Treatment Visit[B]	Post end of protocol treatment
Tretinoin[C]		X	X		X		
Informed consent	X						
Demographics	X						
Medical history	X						
Concurrent meds	X	X----------X					
Physical exam	X	X	X		X	X	
Vital signs	X	X	X		X	X	
Height	X						
Weight[D]	X	X	X		X	X	
Performance status[E]	X	X	X		X	X	
Hematology labs, coagulation profile[F]	X	X	X		X	X	
Serum chemistries and lipid panel[G]	X	X	X		X	X	X
ECG	X						
Tumor biopsy[H]	X			X[H]		X[H]	
Research blood collection[I]	X		X		X[I]	X	
Adverse event evaluation[J]		X----------X					X
Tumor measurements[K]	X	Tumor measurements are repeated **every 8 weeks**. Documentation (radiologic) must be provided for participants removed from study treatment for progressive disease.					X[K]
B-HCG[L]	X[L]						

A. Baseline study evaluations are conducted within 28 days prior to study registration. If screening assessments are 3 days before the start of the start of study treatment, then they may serve as Cycle 1 Day 1 values. Baseline laboratory testing must be completed prior to 14 days of study registration.
B. The reason for protocol treatment removal and the date the participant was removed from protocol treatment must be documented in the study-specific case report form (CRF). Off protocol treatment visit is to occur within 30 days of final administration of study treatment. End of treatment assessments do not have to be repeated if the same assessments were performed within 7 days prior to the visit.
C. Tretinoin is dosed at 45 mg/m^2/day divided into two daily doses for days 1 through 14 of a 28-day cycle (tretinoin will be held on days 15 through 28 of every cycle) until disease progression or unacceptable toxicity ensues in **Cohort 1**; tretinoin is dosed at 45 mg/m^2/day divided into two daily doses continuously during a 28-day cycle until disease progression or unacceptable toxicity in **Cohort 2**. Following the first cycle of therapy, tretinoin cycles continue every 28 days or every 4-weeks. The pills should be taken with food (preferably with a fat-containing meal). Patients are required to complete a study drug diary and return the diary at the start of each Cycle (beginning with Cycle 2), and at the end of treatment.
D. Recorded in kilograms (kg) as the patient's actual (not ideal or adjusted) body weight.
E. Using ECOG performance status scale of 0-5 (*see Appendix A*).
F. Hematology laboratory studies should include: CBC with differential and coagulation profile (PT/INR and aPTT).
G. Serum chemistries should include: a comprehensive metabolic panel with phosphorus and magnesium levels (as appropriate), and a non-fasting lipid panel to include a triglyceride, LDL, HDL, and total cholesterol values.
H. If tumor is accessible by surgical or percutaneous approach, a tumor biopsy should be performed at the following time points: (1) at baseline, within 28 days of Cycle 1 Day 1 of protocol therapy (ideally as close to administration of Cycle 1 Day 1 therapy as possible) but before the administration of the first dose of study treatment. (2) After cycle 2 (8 weeks on treatment), within 14

days of the start of Cycle 3 Day 1 of protocol therapy – providing the procedure is considered safe, feasible, and accessible at the discretion of the treating investigator. (3) Patients may also be offered an optional end-of-treatment biopsy if the reason for discontinuation was progressive disease – again if safe and feasible.

I. Research blood sample collections should occur at the following time points: (1) at baseline, within 14 days prior to Cycle 1 Day 1 of protocol therapy (ideally as close to administration of Cycle 1 Day 1 therapy as possible), (2) at the beginning of every new Cycle, following C1 (expected to occur every 28-days) for the first 12 cycles or 1 year on treatment.
J. Adverse event monitoring should utilize CTCAE version 5.0.
K. Radiographic evaluations should include contrast-enhanced CT imaging of the chest, abdomen, and pelvis; Contrast-enhanced MR imaging of the abdomen may replace CT abdomen and pelvis if further characterization of liver metastases is preferred (at the discretion of the treating physician). A neck CT with contrast should be obtained if appropriate: such as individuals with a history of ACC that arose in the head and neck, or with residual locoregional disease in the head and neck region. CT head or brain imaging is not required. PET-CT imaging can be considered in lieu of CT imaging at any given radiologic assessment, but both studies are often not required. Assessment will be made at baseline and participants will be followed for best overall response and development and documentation of first disease progression and for survival every 8 weeks throughout the course of the trial for 3 years from the time of study registration. Post end of protocol treatment, if first disease progression has not been documented, tumor assessments are to continue every 8 week until first disease progression. After first disease progression, patients will be followed every 8 weeks for survival (by phone) until death or 3 years from study registration (whichever occurs first).
L. Female subjects of childbearing potential should have a negative urine or serum pregnancy test within 14 days of study registration. Female subjects of childbearing potential should have a negative urine or serum pregnancy test repeated **within 72 hours** prior to receiving the first dose of study medication. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required. Women who could potentially become pregnant while undergoing treatment on this protocol must be willing to use 2 methods of contraception.

11. MEASUREMENT OF EFFECT

11.1 Antitumor Effect – Solid Tumors

For the purposes of this study, participants should be re-evaluated for response every 8 weeks. In addition to a baseline scan, confirmatory scans should also be obtained 8 (not less than 4) weeks following initial documentation of objective response.

Response and progression will be evaluated in this study using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [*Eur J Ca* 45:228-247, 2009]. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

11.1.1 Definitions

Evaluable for Target Disease response. Only those participants who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for target disease response. These participants will have their response classified according to the definitions stated below. (Note: Participants who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

Evaluable Non-Target Disease Response. Participants who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

11.1.2 Disease Parameters

Measurable disease. Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥ 20 mm by chest x-ray or ≥10 mm with CT scan, MRI, or calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Note: Tumor lesions that are situated in a previously irradiated area might or might not be considered measurable. If the investigator thinks it appropriate to include them, the conditions under which such lesions should be considered must be defined in the protocol.

Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, abdominal masses (not followed by CT or MRI), and cystic lesions are all considered non-measurable.

Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same participant, these are preferred for selection as target lesions.

Target lesions. All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target lesions. All other lesions (or sites of disease) including any measurable lesions

over and above the 5 target lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow up.

11.1.3 Methods for Evaluation of Disease

All measurements should be taken and recorded in metric notation using a ruler, calipers, or a digital measurement tool. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions. Clinical lesions will only be considered measurable when they are superficial (*e.g.*, skin nodules and palpable lymph nodes) and ≥10 mm in diameter as assessed using calipers (*e.g.*, skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Conventional CT and MRI. This guideline has defined measurability of lesions on CT scan based on the assumption that CT thickness is 5mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size of a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (*e.g.* for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

PET-CT. At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time. Note, however, that the

PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.

Cytology, Histology. These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (*e.g.*, residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

11.1.4 Response Criteria

11.1.4.1 Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters.

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

11.1.4.2 Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis).

Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD): Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not

a single lesion increase.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

11.1.4.3 Evaluation of New Lesions

The finding of a new lesion should be unequivocal (i.e. not due to difference in scanning technique, imaging modality, or findings thought to represent something other than tumor (for example, some 'new' bone lesions may be simply healing or flare of pre-existing lesions). However, a lesion identified on a follow-up scan in an anatomical location that was not scanned at baseline is considered new and will indicate PD. If a new lesion is equivocal (because of small size etc.), follow-up evaluation will clarify if it truly represents new disease and if PD is confirmed, progression should be declared using the date of the initial scan on which the lesion was discovered.

11.1.4.4 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

For Participants with Measurable Disease (*i.e.*, Target Disease)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
CR	CR	No	CR	≥4 wks Confirmation**
CR	Non-CR/Non-PD	No	PR	≥4 wks Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	Documented at least once ≥4 wks from baseline**
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.
** Only for non-randomized trials with response as primary endpoint.
*** In exceptional circumstances, unequivocal progression in non-target lesions may be

accepted as disease progression.

Note: Participants with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration."* Every effort should be made to document the objective progression even after discontinuation of treatment.

For Participants with Non-Measurable Disease (*i.e.*, Non-Target Disease)

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD

* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised

11.1.5 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started, or death due to any cause. Participants without events reported are censored at the last disease evaluation).

Duration of overall complete response: The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented, or death due to any cause. Participants without events reported are censored at the last disease evaluation.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

11.1.6 Survival and Clinical Parameters

Overall Survival: Overall Survival (OS) is defined as the time from randomization (or registration) to death due to any cause, or censored at date last known alive.

Progression-Free Survival: Progression-Free Survival (PFS) is defined as the time from randomization (or registration) to the earlier of progression or death due to any cause. Participants alive without disease progression are censored at date of last disease evaluation.

Time to Progression: Time to Progression (TTP) is defined as the time from randomization (or registration) to progression, or censored at date of last disease evaluation for those without progression reported.

Clinical benefit rate: defined as CR, PR and stable disease (SD) ≥ 24 weeks.

11.1.7 Response Review

For trials where the response rate is the primary endpoint, it is strongly recommended that all responses be reviewed by an expert(s) independent of the study at the study's completion. Simultaneous review of the participants' files and radiological images is the best approach.

12. DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7.0 (Adverse Events: List and Reporting Requirements).

12.1 Data Reporting

12.1.1 Method

The Office of Data Quality (ODQ) will collect, manage, and perform quality checks on the data for this study.

12.1.2 Responsibility for Data Submission

Investigative sites within DF/HCC or DF/PCC are responsible for submitting data and/or data forms to the Office of Data Quality (ODQ) in accordance with DF/HCC policies.

12.2 Data Safety Monitoring

The DF/HCC Data and Safety Monitoring Committee (DSMC) will review and monitor toxicity and accrual data from this study. The committee is composed of medical oncologists, research nurses, pharmacists and biostatisticians with direct experience in cancer clinical research. Information that raises any questions about participant safety will be addressed with the Overall PI and study team.

The DSMC will review each protocol up to four times a year with the frequency determined by the outcome of previous reviews. Information to be provided to the committee may include: up-to-date participant accrual; current dose level information; DLT information; all grade 2 or higher unexpected adverse events that have been reported; summary of all deaths occurring within 30 days of intervention for Phase I or II protocols; for gene therapy protocols, summary of all deaths while being treated and during active follow-up; any response information; audit results, and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

12.3 Multi-Center Guidelines

Not applicable.

12.4 Collaborative Agreements Language

Not applicable.

13. STATISTICAL CONSIDERATIONS

Original Design When Trial Opened.
The trial was originally designed as a two-stage one arm phase II trial with primary endpoint of best overall response with n=14 patients who are eligible and begin protocol treatment to be entered into the first stage. Details of the original design as follows:

The primary endpoint of this study is best overall response rate (CR+PR). A Simon two-stage design will be used to minimize the number of patients exposed to this regimen and the specific sample size and operating characteristics were chosen to be able to show that the response rate is greater than 10% (that found in prior studies) and closer to 28%. Fourteen eligible patients who start protocol treatment are to be accrued in the first stage. If there are ≤ 1 of 14 patients with disease in response, accrual to this trial will be closed with the expectation that there is little evidence that the response rate will reach 28%. The probability that the trial will close early is 58% if the true response rate is 10%. If there are >1 patients with disease in response, accrual will continue until a total of 25 eligible patients who start protocol treatment are entered. If > 4 of 25 patients who are eligible and begin protocol treatment have disease in response, then this regimen will be considered worth further study. The probability of concluding that the regimen is worth further study is 85% if the true response rate is 28%. The probability of concluding that the regimen is worth further study is 9% if the true response rate is 10%. Allowing 2 patients to be entered and then declared ineligible and/or to not start protocol treatment, the overall accrual goal is 27.

Amendment 3: Details, Rational and Design:

Fourteen patients (eligible and began protocol streatment) have been entered into stage 1 for the original design when the trial opened and now referred to as ‘Cohort 1’. Due to preliminary results of low efficacy in Cohort 1, accrual to Cohort 1 is suspended and n=6 additional patients (‘Cohort 2’) are to be entered to assess safety and tolerability of continuous daily dosing. The choice of n=6 is due to lack of concerning SAEs in Cohort 1 as well as to keep within the constraints of original budget and its corresponding drug availability for the testing of continuous dosing. For Cohort 2, AEs plan to be continuously monitored and best overall response assessed. Cohort 2 is a secondary endpoint to assess safety and tolerability of continuous daily dosing in an additional 6 subjects. With n=6 eligible patients who being protocol treatment, the lower limit of one-sided 90% exact binomial confidence interval will be greater than 10% (the null hypothesis in the original design) if at least 3 patients have disease in response (CR or PR). All aspects of the AE profile and outcome of these 6 patients are to be assessed with decisions made regarding amending the trial for any further design changes and continuation or closure of accrual. The table below gives the lower limit of one-sided 90% exact binomial confidence interval based on various

scenarios.

Sample size Cohort 2	# of patients with disease in response	Lower limit of one-sided 90% CI
6	2	9.2%
6	3	20.1%

Analysis and Accrual Estimates
The primary efficacy population includes all eligible patients who begin protocol treatment. Best overall response will be summarized as a proportion with a corresponding exact 95% confidence interval (CI).

For secondary objectives:

- Adverse events will be classified and graded according to the CTCAE v.5.0. Frequencies of adverse events will be summarized among patients who begin protocol therapy.

- The distributions of time-to-event endpoints will be estimated using the Kaplan-Meier method with corresponding 95% confidence intervals for the median or time-specific event time.

With an estimated monthly accrual of 2 patients, accrual to the first stage (Cohort 1) is estimated to take approximately 7-8 months to accrue n = 15 (of which n = 14 who are eligible and who begin protocol treatment). Estimated monthly accrual to Cohort 2 is estimated to be similar (1-2 patients per month). Due to possible delays in initiation of approval and/or in initiation of accrual itself, accrual could take longer. As is customary with this type of design, accrual will be suspended after the first stage (Cohort 1, n = 14 eligible patients who begin protocol therapy) in order to assess outcome; however, this suspension is also dependent on the actual observed accrual rate and the number of patients with confirmation of disease response status while the first stage of the trial is accruing. Accrual to Cohort 2 would be suspended when n=6 eligible patients who begin protocol therapy are entered.

14. **PUBLICATION PLAN**

The results should be made public within 24 months of reaching the end of the study. The end of the study is the time point at which the last data items are to be reported, or after the outcome data are sufficiently mature for analysis, as defined in the section on Sample Size, Accrual Rate and Study Duration (*Section 13.0*). If a report is planned to be published in a peer-reviewed journal, then that initial release may be an abstract that meets the requirements of the International Committee of Medical Journal Editors. A full report of the outcomes should be made public no later than three (3) years after the end of the study. The overall study PI will be responsible for reporting and publishing the data.

15. REFERENCES

1. Bobbio A, C.C., Ampollini L, Bianchi B, Carbognani P, Bettati S, Sesenna E, Rusca M, *Lung metastasis resection of adenoid cystic carcinoma of salivary glands.* Eur J Cardiothorac Surg, 2008. **33**(5): p. 790-3.
2. Spiro RH, H.A., Strong EW., *Adenoid cystic carcinoma of salivary origin. A clinicopathologic study of 242 cases.* Am J Surg, 1974. **128**(4): p. 512-20.
3. Amini A, W.T., Brower JV, Jones BL, McDermott JD, Raben D, Ghosh D, Bowles DW, Karam SD, *Association of Adjuvant Chemoradiotherapy vs Radiotherapy Alone With Survival in Patients With Resected Major Salivary Gland Carcinoma: Data From the National Cancer Data Base.* JAMA Otolaryngol Head Neck Surg, 2016. **142**(11): p. 1100-10.
4. Gao M, H.Y., Huang MX, Ma DQ, Luo HY, Gao Y, Peng X, Yu GY *Clinicopathological study of distant metastases of salivary adenoid cystic carcinoma.* Int J Oral Maxillofac Surg, 2013. **42**(8): p. 923-8.
5. Laurie SA, H.A., Fury MG, Sherman E, Pfister DG, *Systemic therapy in the management of metastatic or locally recurrent adenoid cystic carcinoma of the salivary glands: a systematic review.* Lancet Oncol, 2011. **12**(8): p. 815-24.
6. Airoldi M, P.F., Succo G, Gabriele AM, Ragona R, Marchionatti S, Bumma C, *Phase II randomized trial comparing vinorelbine versus vinorelbine plus cisplatin in patients with recurrent salivary gland malignancies.* Cancer, 2001. **91**(3): p. 541-7.
7. Licitra L, C.R., Grandi C, Palma SD, Guzzo M, Demicheli R, Molinari R, *Cisplatin, doxorubicin and cyclophosphamide in advanced salivary gland carcinoma. A phase II trial of 22 patients.* Ann Oncol, 1996. **7**(6): p. 640-2.
8. Sridharan V, G.E., Liao X, Chau NG, Haddad RI, Severgnini M, Hammerman P, El-Naggar A, Freeman GJ, Hodi FS, Rodig SJ, Dranoff G, Schoenfeld JD, *Immune Profiling of Adenoid Cystic Carcinoma: PD-L2 Expression and Associations with Tumor-Infiltrating Lymphocytes.* Cancer Immunol Res, 2016. **4**(8): p. 679-87.
9. Cohen RB, D.J., Doi T, Piha-Paul SA, Liu SV, Gilbert J, Algazi AP, Damian S, Hong RL, Le Tourneau C, Day D, Varga A, Elez E, Wallmark J, Saraf S, Thanigaimani P, Cheng J, Keam B, *Pembrolizumab for the Treatment of Advanced Salivary Gland Carcinoma: Findings of the Phase 1b KEYNOTE-028 Study.* Am J Clin Oncol, 2018. Feb 21. doi: 10.1097/COC.0000000000000429. [Epub ahead of print].
10. Hotte SJ, W.E., Lamont E, MacKenzie M, Vokes E, Chen EX, Brown S, Pond GR, Murgo A, Siu LL, *Imatinib mesylate in patients with adenoid cystic cancers of the salivary glands expressing c-kit: a Princess Margaret Hospital phase II consortium study.* J Clin Oncol, 2005. **23**(3): p. 585-90.
11. Thomson DJ, S.P., Denton K, Bonington S, Mak SK, Swindell R, Homer J, Sykes AJ, Lee LW, Yap BK, Slevin NJ, *Phase II trial of sorafenib in advanced salivary adenoid cystic carcinoma of the head and neck.* Head Neck, 2015. **37**(2): p. 182-7.
12. Wong SJ, K.T., Hayes DN, Kies MS, Cullen KJ, Tanvetyanon T, Argiris A, Takebe N, Lim D, Saba NF, Worden FP, Gilbert J, Lenz HJ, Razak AR, Roberts JD, Vokes EE, Cohen EE, *Phase II trial of dasatinib for recurrent or metastatic c-KIT expressing adenoid cystic carcinoma and for nonadenoid cystic malignant salivary tumors.* Ann Oncol, 2016. **27**(2): p. 318-23.
13. Ho AS, K.K., Roy DM, Morris LG, Ganly I, Katabi N, Ramaswami D, Walsh LA, Eng S,

Huse JT, Zhang J, Dolgalev I, Huberman K, Heguy A, Viale A, Drobnjak M, Leversha MA, Rice CE, Singh B, Iyer NG, Leemans CR, Bloemena E, Ferris RL, Seethala RR, Gross BE, Liang Y, Sinha R, Peng L, Raphael BJ, Turcan S, Gong Y, Schultz N, Kim S, Chiosea S, Shah JP, Sander C, Lee W, Chan TA, *The mutational landscape of adenoid cystic carcinoma.* Nat Genet, 2013. **45**(7): p. 791-8.

14. Persson M, A.Y., Moskaluk CA, Frierson HF Jr, Cooke SL, Futreal PA, Kling T, Nelander S, Nordkvist A, Persson F, Stenman G, *Clinically significant copy number alterations and complex rearrangements of MYB and NFIB in head and neck adenoid cystic carcinoma.* Genes Chromosomes Cancer, 2012. **51**(8): p. 805-17.

APPENDIX A PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
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).	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.
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.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

APPENDIX B INFORMATION ON POSSIBLE DRUG INTERACTIONS TRETINOIN

Drugs that may interact with tretinoin
• Anti-seizure medications such as carbamazepine, phenobarbital, phenytoin, primidone • Arthritis medications such as leflunomide, tofacitinib • Some oral contraceptives • Some antibiotics, like doxycycline, tetracycline, and tigecycline • Other medications such as aminolevulinic acid, amiodarone, clopidogrel, deferasirox, gemfibrozil, irbesartan, losartan, mifepristone, natalizumab, pioglitazone, rabeprazole, rifampin, ritonavir, rosiglitazone, tranexamic acid
Food and supplements that may interact with tretinoin**
• Echinacea • St. John's Wort • Vitamin A supplements or multivitamins that contain vitamin A

*** Supplements may come in many forms, such as teas, drinks, juices, liquids, drops, capsules, pills, or dried herbs. All forms should be avoided.*

APPENDIX C TRETINOIN DOSING TABLE

Example Tretinoin Dosing Table	
BSA (m^2)	**Dose**
≤ 0.78	20 mg in the AM, 10 mg in the PM
0.79 – 0.99	20 mg twice daily
1 – 1.22	30 mg in the AM, 20 mg in the PM
1.23 – 1.44	30 mg twice daily
1.45 – 1.66	40 mg in the AM, 30 mg in the PM
1.67 – 1.88	40 mg twice daily
1.89 – 2.11	50 mg in the AM, 40 mg in the PM
2.12 – 2.33	50 mg twice daily
2.34 – 2.55	60 mg in the AM, 50 mg in the PM
2.56 – 2.77	60 mg twice daily
2.78 – 2.99	70 mg in the AM, 60 mg in the PM
3 – 3.22	70 mg twice daily
3.23 – 3.44	80 mg in the AM, 70 mg in the PM
≥ 3.45	80 mg twice daily

Note: The higher dose of an uneven dose will be the AM or morning dose.

For additional administration guidelines for administering tretinoin to patients unable to swallow whole capsules either due to young age or level of consciousness (including intubated patients) please see **Appendix D**. Further details about tretinoin can also be found at: https://www.accessdata.fda.gov/drugsatfda_docs/label/2004/20438s004lbl.pdf

APPENDIX D INFORMATION FOR PARENTS/CAREGIVERS FOR GIVING TRETINOIN

Tretinoin (ATRA) gel capsules should be swallowed whole when possible. There is minimal data on the absorption of tretinoin when it is given by other methods. In addition, tretinoin is sensitive to heat, light and air, so avoid exposure to those as much as possible. Alternative methods of delivery should only be used when it is not possible to swallow the gel capsules (i.e., intubated patients, neurologically impaired patients, very young patients).

Note: *Gloves must be worn when handling tretinoin capsules unless the capsules are intact.*

For patients unable to swallow the capsules whole, the following options may be used:

1. Capsules can be chewed then swallowed if the patient is able. A small hole can be poked in the capsules before the patient chews them to make this easier.
2. Capsules can be softened in water and the softened capsule can be chewed/swallowed and/or mixed with pudding or a fatty food and swallowed.
3. Contents of the capsules can be squeezed out and mixed with a fatty food. If at all possible, have the patient suck on the empty capsule in hopes of getting more of the intended dose.
4. If the contents are withdrawn from the capsules and mixed with food, give the dose as soon as possible (*within ONE hour*).

APPENDIX E REQUIRED SPECIMENS, PROCESSING AND SHIPMENT INSTRUCTIONS FOR BIOMARKER, CORRELATIVE AND SPECIAL STUDIES

Peripheral Blood Samples

Specimens will be shipped (via overnight air FEDEX traceable carrier) to and subsequently processed and analyzed at Dana-Farber Cancer Institute. Participating sites are responsible for maintaining a log of all biospecimen and for shipment of bone marrow specimens. An inventory sheet and shipping manifest must accompany each shipment and copies provided to the Coordinating Center.

Participating sites are responsible for shipment of peripheral blood specimens. An initial shipment of collection tubes will be shipped to all DF/HCC and non-DF/HCC participating sites at the time of site initiation. Refill supplies may be requested by contacting the Coordinating Center.

Specimens required per participant per time point:

- 2 purple top tubes (EDTA), 10 mL each
- 1 Streck Cell-Free DNA BCT tube, 10 ml

Specimens must be collected on Mondays, Tuesdays, Wednesdays or Thursdays for same-day shipment (FEDEX). All tubes (purple to and green top) should be shipped with a fridge pack Fed-Ex priority overnight.

Blood samples will be collected at the time points outlined in the study calendar:

Documenting Instructions: Sites must maintain a detailed log of all samples obtained, including:

- Subject Initials
- Subject study ID
- Type of sample
- Visit at which sample was drawn (Screening, Cycle number and day, End of study treatment or Follow-Up)
- Date sample drawn (mm/dd/yyyy)
- Time sample drawn (24 hour clock)
- Date Shipped

Processing Information: There is no required processing for blood samples at each participating site prior to shipment.

Shipping Instructions: Shipments must be sent on the day of collection and cannot be batched.

- Label all specimens with the following:
  - Protocol Number
  - Subject Initials
  - Subject study number
  - Visit at which sample was drawn (Screening, Cycle number and day, End of study treatment or Follow-Up)

  - Date sample drawn (mm/dd/yyyy)
  - Time sample drawn (24 hour clock)
- An inventory sheet including a complete list of samples shipped (patient number, time point, study #) must accompany each shipment. Please sign and date the form, and retain a copy for site record maintenance. Please see form in **Appendix**.
- An electronic copy of the sample list (**Appendix**) and a copy of the shipping manifest must also be sent via email or fax to the Coordinating Center. The listing must also include a **contact name, address and phone number** of the person who is responsible for the shipment. Please email the Coordinating Center to notify of an incoming shipment.
- Please ship Monday, Tuesday, Wednesday or Thursday as shipments cannot be received on weekends and/or on holidays.
- Once drawn, samples should be shipped with a fridge pack Fed-Ex priority overnight to Dana-Farber Cancer Institute attention:

Jens G. Lohr, M.D., Ph.D.
Dana-Farber Cancer Institute
Dana-Building 542
450 Brookline Avenue
Boston, MA 02215
Email: JLOHR@partners.org
Phone: 617-632-2069 or 415-816-8405

APPENDIX F STUDY PARTICIPANT SELF-ADMINISTRATION INSTRUCTIONS

Study Participant Self-Administration Instructions

The study staff will explain how to take the study drug **TRETINOIN** but these are points to remember:

- Tretinoin capsules should be swallowed whole
- Tretinoin is sensitive to heat, light, and air so avoid exposure as much as possible
- Take tretinoin with food
- Please call your doctor or research nurse before taking any new prescription or over-the-counter medications/supplements other than the study drugs.
- For patients unable to swallow the capsules whole, the following options may be used:
    - Capsules can be chewed then swallowed if the patient is able. A small hole can be poked in the capsules before the patient chews them to make this easier.
    - Capsules can be softened in water and the softened capsule can be chewed/swallowed and/or mixed with pudding or a fatty food and swallowed.
    - Contents of the capsules can be squeezed out and mixed with a fatty food. If at all possible, have the patient suck on the empty capsule in hopes of getting more of the intended dose.

NOTE: If the contents are withdrawn from the capsules and mixed with food, give the dose as soon as possible (within ONE hour).

SUMMARY OF CHANGES

Date	Change
12/31/2019 Amendment 2	Addition of cohort 2 throughout the protocol. Fasting is no longer required for the lipid panel.

Study Participant Self-Administration Study Drug Diary

Please record how many capsules you take of TRETINOIN, the time you take them and any comments here below and bring the completed diary as well as your study drug supply, including empty bottles, to every study visit. This will help us keep track of your study drug and how well you are tolerating it.

Participant Identifier: Cycle Number:
Protocol #: Tretinoin (ATRA) Assigned Dose: mg
Doctor: Phone:
Research Nurse: Phone:

You will take the following number of capsules each time (per dose) as listed in the table below:

Study Drug Name	# of capsules to take per time/dose	# of times/doses each day	Approximate time to take drug
Tretinoin (ATRA)			__:__ ☐ a.m. ☐ p.m. __:__ ☐ a.m. ☐ p.m.

Day	Date	Number of Tretinoin Capsules	Time of Dose	Number of Tretinoin Capsules	Time of Dose
1 / 15			_____:_____ □ a.m. / □ p.m. □ Dose Not Taken Why:_____		_____:_____ □ a.m. / □ p.m. □ Dose Not Taken Why:_____
2 / 16			_____:_____ □ a.m. / □ p.m. □ Dose Not Taken Why:_____		_____:_____ □ a.m. / □ p.m. □ Dose Not Taken Why:_____
3 / 17			_____:_____ □ a.m. / □ p.m. □ Dose Not Taken Why:_____		_____:_____ □ a.m. / □ p.m. □ Dose Not Taken Why:_____
4 / 18			_____:_____ □ a.m. / □ p.m. □ Dose Not Taken Why:_____		_____:_____ □ a.m. / □ p.m. □ Dose Not Taken Why:_____
5 / 19			_____:_____ □ a.m. / □ p.m. □ Dose Not Taken Why:_____		_____:_____ □ a.m. / □ p.m. □ Dose Not Taken Why:_____
6 / 20			_____:_____ □ a.m. / □ p.m. □ Dose Not Taken Why:_____		_____:_____ □ a.m. / □ p.m. □ Dose Not Taken Why:_____
7 / 21			_____:_____ □ a.m. / □ p.m. □ Dose Not Taken Why:_____		_____:_____ □ a.m. / □ p.m. □ Dose Not Taken Why:_____
8 / 22			_____:_____ □ a.m. / □ p.m. □ Dose Not Taken Why:_____		_____:_____ □ a.m. / □ p.m. □ Dose Not Taken Why:_____
9 / 23			_____:_____ □ a.m. / □ p.m. □ Dose Not Taken Why:_____		_____:_____ □ a.m. / □ p.m. □ Dose Not Taken Why:_____
10 / 24			_____:_____ □ a.m. / □ p.m. □ Dose Not Taken Why:_____		_____:_____ □ a.m. / □ p.m. □ Dose Not Taken Why:_____
11 / 25			_____:_____ □ a.m. / □ p.m. □ Dose Not Taken Why:_____		_____:_____ □ a.m. / □ p.m. □ Dose Not Taken Why:_____
12 / 26			_____:_____ □ a.m. / □ p.m. □ Dose Not Taken Why:_____		_____:_____ □ a.m. / □ p.m. □ Dose Not Taken Why:_____
13 / 27			_____:_____ □ a.m. / □ p.m. □ Dose Not Taken Why:_____		_____:_____ □ a.m. / □ p.m. □ Dose Not Taken Why:_____
14 / 28			_____:_____ □ a.m. / □ p.m. □ Dose Not Taken Why:_____		_____:_____ □ a.m. / □ p.m. □ Dose Not Taken Why:_____

Participant/Caregiver Signature: _____ Date: _____

Tretinoin (ATRA, Vesanoid®)

("treh-tih-NO-in")

How this drug is given: By mouth

Purpose: Slows the growth of cancer cells in leukemia and other cancers

How to take the drug by mouth

- Take with a meal.
- Swallow this capsule whole; do not crush or chew them. If you have trouble swallowing the capsule, the pharmacist will give you specific instructions.
- If you miss a dose, take it as soon as possible. However, if it is almost time for your next dose, skip the missed dose and go back to your regular dosing schedule. **Do not double dose**.
- Wash hands after taking the medication. Avoid handling crushed or broken capsules.

Storage

- Store this medicine at room temperature, away from heat and moisture. Keep this medicine in its original container, out of the reach of children and pets.

Things that may occur during treatment

- Flu-like symptoms such as fever, chills, headache, muscle and/or joint aches are common. If these occur, your doctor may suggest taking acetaminophen (Tylenol®) to help control the symptoms. Please let your doctor or nurse know if acetaminophen (Tylenol®) does not help, since other medication may be prescribed.
- Some patients may have mild nausea. You may be given medicine to help with this.
- Mild constipation may occur after treatment begins. Please increase your fluid intake and increase fiber in your diet by eating fresh fruits and vegetables. A daily stool softener, such as docusate (Colace®) and/or laxatives such as senna (Senakot®) may be helpful. If these do not help within 48 hours, tell your doctor or nurse. Do not use bulk forming laxatives such as Metamucil® without talking with your doctor or nurse.

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- Loose stools or diarrhea may occur within a few days after the drug is started. You may take loperamide (Imodium A-D®) to help control diarrhea. You may buy this at most drug stores. It is also important to drink more fluids (water, juice, sports drinks). If these do not help, tell your doctor or nurse.
- Your body may not be able to get rid of extra fluid. This is called edema. You may notice some swelling in your arms or legs.
- Skin changes such as dryness or a rash on your body may occur. Ask your doctor or nurse what lotions or creams you may use.
- Eyes may get itchy and watery and bright light may bother you. Natural tears or saline eye drops may help with these symptoms.
- You may have a fast or unusual heartbeat. If you feel any strange changes in your heartbeat, tell your doctor or nurse **right away.**
- Some patients may feel very tired, also known as fatigue. You may need to rest or take naps more often. Mild to moderate exercise can also be helpful in maintaining your energy.
- Restlessness, sedation, depression or confusion can occur. You may notice a change in your mood. Notify your doctor or nurse if you experience any of these symptoms after starting the drug.
- In rare instances of leukemia (APL), an increase in your white blood cells may occur causing high fever, respiratory distress, lung infection, and heart changes.

Things that may occur after treatment ends (even months to years later)

- Fertility and Related Precautions
  - It is very important to use birth control if you are having sex, because this drug could hurt an unborn baby.
  - Women should not breast-feed while receiving this drug.
  - If you are concerned about any of this, please talk with your doctor or nurse.

The above information includes some, but not necessarily all, of the possible side effects of this medication. The side effects in this teaching sheet may not be the same ones you experience. Your side effects may be different, depending on how often you receive treatment (your schedule) and how much you receive each time (your dosage). Side effects may also vary if you take other medications. Please talk with your doctor or nurse if you have questions about possible side effects you may experience. This document should not take the place of conversations with members of your health care team.

If you experience any significant change in your health during or after treatment, contact a member of your health care team right away.

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Managing your oral chemotherapy schedule at home can be challenging. To help you with this, consider using tools to help you keep track of your schedule and any side effects you may have. You can find these tools online at **www.dana-farber.org/OralChemoDiary.**

THIS SPACE RESERVED FOR WRITTEN COMMENTS OR NOTES FOR THE PATIENT AND FAMILY:

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