BARBARA ANN Karmanos

CANCER INSTITUTE

Wayne State University

EISENBERG CLINICAL TRIALS OFFICE
PROTOCOL CLARIFICATION MEMO

Study #:	2009-165 (NCI Protocol #8474)
Protocol Title:	A Phase 2 Study of Suberoylanilide Hydroxamic Acid (SAHA) in Subjects with Locally Advanced, Recurrent or Metastatic Adenoid Cystic Carcinoma (ACC) (IND 71976)
From:	Ulka Vaishampayan MD
Date:	August 16, 2016
Subject:	Clarification of Protocol Version Date for Amendment 17

On the Summary of Changes page, the protocol date is listed incorrectly. It is listed as 5/12/2016; however, the correct version date is 8/16/2016. The protocol version date is listed correctly in the footer and throughout the rest of the protocol.

Signature: _____ Date: 9/6/16

Ulka Vaishampayan MD
Principal Investigator
Karmanos Cancer Institute

SUMMARY OF CHANGES

For Protocol Amendment # 17 to: A Phase 2 Study of Suberoylanilide Hydroxamic Acid (SAHA) in Subjects with Locally Advanced, Recurrent or Metastatic Adenoid Cystic Carcinoma (ACC) (IND 71976)

NCI Protocol #: 8474
Local Protocol #: 2009-165

Protocol Date: 05/12/2016

#	Section	Page(s)	Change
1	All	All	Changed Version Date in footer to 08/16/16
2	SOC	i	Amendment number changed to 17
3	Face page	1	"Resident" removed from Priscila Hermont Goncalves' name
4	Face Page	6	Updated Version date to 8/16/16
5	Appendix I	86	Font changed to Times New Roman
6	Consent Form		There are no consent form revisions.

NCI Protocol #: 8474

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TITLE: A Phase 2 Study of Suberoylanilide Hydroxamic Acid (SAHA) in Subjects with Locally Advanced, Recurrent or Metastatic Adenoid Cystic Carcinoma (ACC) (IND 71976)

Coordinating Center: Barbara Ann Karmanos Cancer Institute/Wayne State University

Principal Investigator: Patricia Mucci LoRusso, DO
Associate Director of Innovative Medicine
Yale Cancer Center
333 Cedar Street, WWW217
P.O. Box 208028
New Haven, CT 06520-8028
Phone: (203) 785-5944
Fax: (203) 785-4116
E-mail: patricia.lorusso@yale.edu

Co-Investigators: Ulka Vaishampayan, M.D.- KCI Site Principal Investigator
Karmanos Cancer Institute, HW04HO
4100 John R Street, Detroit, MI 48201
Phone: (313) 576-8718
Fax: (313) 576-8487
E-mail: vaishamu@karmanos.org

Priscila Hermont Goncalves, MD
National Cancer Institute, National Institutes of Health
10 Center Drive, Building 10, Room 6N110
Bethesda, MD, 20892
Phone: 301-402-1541
Email: priscila.goncalves@nih.gov

Dr. Jeffrey Trent
445 N 5th Street, Suite 600
Phoenix, AZ 85004
jtrent@tgen.org
602-343-8419
602-343-8448 FAX
(Note: Dr. Trent is not participating in patient care for this study)

Dr. Michael Barrett
13208 E. Shea Blvd.
Scottsdale, Arizona 85259

mbarrett@tgen.org
602- 358-8383
602- 358-8360 FAX
(Note: Dr. Barrett is not participating in patient care for this study)

Panayiotis (Panos) Savvides, MD, PhD, MPH (site PI)
Department of Medicine
University Hospitals of Cleveland
Case Western Reserve University
11100 Euclid Avenue
Cleveland, OH 44106
Phone: 216-844-5946
Fax: 216-844-1721
Email: psavvides@case.edu

Joel Saltzman, MD
Lake University – Ireland Cancer Center (LUICC)
9485 Mentor Avenue
Mentor, OH 44060
Phone: 440-205-5755
Fax: 440-205-5792
Email: jns70@yahoo.com

Mark Bergman, MD
Lake University – Ireland Cancer Center (LUICC)
9485 Mentor Avenue
Mentor, OH 44060
Phone: 440-205-5755
Fax: 440-205-5792
Mark.Bergman@uhhospitals.org

Vinay Gudena, MD
Ireland Cancer Center at Firelands Regional Medical Center
701 Tyler Street
Sandusky, OH 44870
Phone: 419-557-7480
Fax: 419-557-7533
Vinay.Gudena@uhhospitals.org

Amy Reese, MD
Ireland Cancer Center at Firelands Regional Medical Center
701 Tyler Street
Sandusky, OH 44870
Phone: 419-557-7480

Fax: 419-557-7533
Amy.Reese@uhospitals.org

Joseph Bokar, MD
Department of Medicine
University Hospitals of Cleveland
VA Hospitals of Cleveland
Case Comprehensive Cancer Center
11100 Euclid Avenue
Cleveland, OH 44106
Email: Joseph.Bokar@uhHospitals.org

Afshin Dowlati, MD
Department of Medicine
University Hospitals of Cleveland
Case Comprehensive Cancer Center
11100 Euclid Avenue
Cleveland, OH 44106
Email: Afshin.Dowlati@uhHospitals.org

Charles Nock, MD
Department of Medicine
University Hospitals of Cleveland
Case Comprehensive Cancer Center
11100 Euclid Avenue
Cleveland, OH 44106
Email: Charles.Nock@uhHospitals.org

Joseph Chao M.D.
City of Hope Comprehensive Cancer Center
Medical Office Building
1500 E. Duarte Road
Duarte, CA 91010
Phone: 626-471-9200
Fax: 626-301-8233
Email: jchao@coh.org

Stephen Shibata, MD
City of Hope National Medical Center
Department of Medical Oncology
Medical Office Building
1500 E. Duarte Road
Duarte, CA 91010
Phone: 626-359-811 X1
Email: sshibata@coh.org

Alice Chen, MD
Medical Oncology Branch
National Cancer Institute
31 Center Drive
Bldg 31 Room 3A44
Bethesda, MD 20892
Phone: 301-496-4291
Fax: 301-496-0826
Email: chenali@mail.nih.gov

Stephen Koehler, MD
City of Hope Medical Group, Inc.,
209 Fair Oaks Avenue
South Pasadena, CA 91030
Phone: 626/396-2900
Fax: 626/799/2770
Email: Skoehler@cohmg.com

Ammar Sukari M.D.
Karmanos Cancer Institute
4100 John R, HW04HO
Detroit, MI 48201
Phone: 313-576-8751
Fax: 313-576-8702
Email: sukaria@karmanos.org

Alan L. Ho M.D., Ph.D.
Memorial Sloan-Kettering Cancer Center
Department of Medicine, Solid Tumor Service
1275 York Avenue, Schwartz 1210B
New York, New York 10065
Phone : 212-639-3311
Fax : 212-717-3278
Email : hoa@mskcc.org

Lillian L Siu, FRCPC, MD
Princess Margaret Hospital/Ontario Cancer Institute (OCI)
610 University Avenue, Suite 5-718
Toronto, Ontario
M5G 2M9
Canada
Tel: 416-946-2911
Fax: 416-946-4467
Email: lillian.siu@uhn.on.ca

Aaron Hansen, MD
Princess Margaret Cancer Centre
610 University Ave
Toronto, Ontario, M5G 2M9
Canada
T: +1 416-946-4501 ext 3426
F: +1 416-946-2890

Les Folio, DO DO, MPH, FAOCR
Col (ret), USAF, MC, SFS
Physician Lead for Computed Tomography,
Radiologist, Body Imaging;
Radiology and Imaging and Sciences
Clinical Center, National Institutes of Health

Clinical Professor, Radiology, George Washington University Hospital

NIH address:
National Institutes of Health
10 Center Drive; Building 10; Room 1C 340
Bethesda, MD, 20892
Voice 301.435 8622
Cell 240.281.8832
Fax 301.480.2229
Email: les.folio@nih.gov
(Note: Dr. Folio is not participating in patient care for this study)

Statisticians:

Lance K. Heilbrun, Ph.D
Assistant Director, Biostatistics Core
Karmanos Cancer Institute
Office: Mid Med Building, 3rd Floor
87 East Canfield
Detroit, MI 48201
E-mail: heilbrun@karmanos.org
phone: 313 / 576-8652
FAX: 313 / 576-8656

Daryn Smith, M.S.
Karmanos Cancer Institute
Office: Mid Med Building, 3rd Floor
87 East Canfield
Detroit, MI 48201

Phone: 313-576-8649
Email: smithda@karmanos.org

Responsible Data Manager: Kelly Schneider, BS, CCRP
Karmanos Cancer Institute, Clinical Trials Office
4100 John R Street, Detroit, MI, 48201
Phone: 313 576-9749
Fax: 313 576-9684
Email: schneidk@karmanos.org

NCI Supplied Agent: Suberoylanilide Hydroxamic Acid (SAHA); NSC 701852, IND 71976

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October 1, 2014 (Amendment 14)
July 23, 2015 (Amendment 15)
May 12, 2016 (Amendment 16)
August 16, 2016 (Amendment 17)

SCHEMA

Treatment Plan:
Pre-treatment Period
Screening assessments will be performed as described below:

Days -21 to-1: (within 21 days prior to start of treatment). Informed consent; medical history; physical exam (PE) with vital signs, including height and weight; ECOG performance status and review of concomitant medications and electrocardiogram (EKG) will be ordered. Tumor tissue from a previous paraffin block will be obtained for correlative studies.

Days -14 to -1: (within 14 days of start of treatment). PE with vital signs and weight; review of any new events in medical history and medication; ECOG performance status will be repeated. Assessment and tumor imaging (CT scans and optional PET/CT scans) will also be obtained.

Day -7 to day 1 prior to study drug start: Hematology, comprehensive metabolic chemistry profile, coagulation profile and urinalysis will be obtained. Women of childbearing potential will have a serum pregnancy test done.

Treatment Period:
Vorinostat will be administered orally once daily continuously at 400 mg. Each treatment cycle will consist of 4 weeks of daily treatment.

Study visits will be conducted on weeks 2 and 4 (±1 day) for the first cycle, then for the second cycle on week 5 and 7, (±1 day); then from week 9 on, every four weeks (±1 day) thereafter for the remainder of the study. Patient medical histories, physical examinations, laboratory tests, assessments of compliance with study medication and recording of adverse events will be conducted during study visits. Further blood may be drawn for research correlatives. For patients that have been on study longer than 2 years, office visits can be done every 12 weeks (±7 days), with medical history, concurrent meds, physical exam, vital signs, weight, performance status, and adverse events evaluation done also at that time. For patients that have been on study longer than 2 years, hematology labs and serum chemistry can be done every 6 weeks (±7 days).

The severity duration and relationship to vorinostat will be determined for each adverse experience (AE) (Section 11).

Blood will be drawn on days 1 prior to first drug intake, and on days 7 (+- 1 day) and 28 (+- 1day), prior to drug intake, to assess for correlative studies.

Patients will have radiological tumor assessment by computed tomography (CT) scan (or magnetic resonance imaging - MRI) on days -14 to -1 (prior to the start of vorinostat) and day 56 (±3 days). They will be followed with CT scans or MRI every 2 cycles / 8 weeks (± 1 week) thereafter. Patients may receive scans every 3 cycles/12 weeks (± 1 week) after completing 6 months of treatment on study. Patients that have been on study longer than 2 years can have tumor measurements (CT scans or MRI) done every 24 weeks (±10 days).

Predicated on obtaining funding, additional scans (optional) will be performed. These will include PET/CT scans prior to treatment, Day 28 (±3 days), and Day 56 (±3 days). An additional CT scan or MRI will also be performed on Day 28 (±3 days) for comparison purposes. The Day 28 CT/MRI and the PET/CT scans will be optional studies- due to financial issues (funding).

Vorinostat will be withheld for any possible or probable drug related grade 3 to 4 adverse event. .Patients will be allowed to have 2 dose reductions (from 300mg/day D1-7 to 300mg/day D1-5) due to grade 3 nausea, emesis or diarrhea (not controlled by appropriate supportive care measures); due to other drug-related grade 3/4 non-hematological toxicity; due to grade 3/4 neutropenia associated with fever and/or grade 4 neutropenia and/or grade 4 thrombocytopenia (section 6). In the event a patient is experiencing clinical benefit from vorinostat and requires further dose reduction, a discussion between CTEP and PI will ensue.

Patients who cannot be retreated within 3 weeks (21 days) of the end of the previous cycle should be removed from study, unless they are deriving benefit. In the later case, a discussion with the PI and/or CTEP senior investigator will also ensue.

Patients who experience either stable disease, complete response, or partial response by RECIST criteria will be allowed to continue on vorinostat as long as they are experiencing benefits without intolerable/life-threatening side effects.

Post-treatment Period:

Patients with progression of disease (PD), unacceptable toxicity, or uncontrolled intercurrent illness will be taken off the trial. Other criteria for discontinuation will include: patient withdrawal of consent, noncompliance with study medication or visits, or any change that would render the patient ineligible for further treatment. In those cases, patients will be entered on the post-treatment period. The end of study visit should occur within 30 days of the last dose of the study drug. Patient medical histories, physical examinations and laboratory tests will be obtained. Tumor imaging if not done in the last 30 days, will be ordered at this time. The investigator or designee will obtain follow-up information on the patient at 180 days after the last dose of the study drug or until death (if before 180 days).

Correlative Studies:

The correlative portion of this trial is described the Laboratory Correlates section (appendix D). In a retrospective pilot study, CT and or MRI scans already obtained from all the patients in the trial will be re-reviewed. RECIST imaging will be compared to volumetric density (viable tumor volume VTV) CT and MR measurements. The objective is to establish whether volumetric density/percent necrosis algorithms such as VTV more accurately assess the extent of the disease and response to therapy than standard RECIST criteria.

Table of Contents

1. OBJECTIVES

1.1. Primary Objectives

- To evaluate the efficacy by means of response rate (based on RECIST 1.1 criteria) of vorinostat in the treatment of patients with locally advanced, recurrent or metastatic adenoid cystic carcinoma (ACC).

1.2 Secondary Objectives

- To characterize the safety and tolerability of vorinostat in this patient population.
- To assess the time to tumor response (TTR).
- To assess the response duration (RD).
- To evaluate progression free survival (PFS).
- To assess overall survival (OS).

1.3 Exploratory Objectives

- To assess the association between a metabolic response by PET/CT after one cycle of chemotherapy and subsequent best tumor response according to standard anatomic response evaluation criteria (RECIST).
- To assess the association between a metabolic response by PET/CT after the first and second chemotherapy cycle and PFS.
- Flow sort diploid, aneuploid, and tetraploid populations of tumor cells from FFPE tissue blocks from patients who benefited from SAHA therapy and from patients who did not demonstrate a durable benefit.
- Profile the genomes of each cell population using oligonucleotide CGH arrays. This will confirm the identification of tumor populations in each sample and provide genomic landmarks for NGS analysis.
- Perform whole exome analysis of the sorted tumor population and matching germ line sample for each of the patients selected
- To assess stable disease duration (SDD)
- To assess the association between response to vorinostat treatment and HR23B on tumor paraffin blocks
- Retrospectively compare volumetric density (viable tumor volume=VTV) with pre-determined RECIST of target lesions in cross sectioning imaging (CT/MR) already obtained
- Correlate VTV, RECIST and treatment response (partial response, stable disease, progressive disease and stable disease over 6 months)

2. BACKGROUND

2.1 Adenoid Cystic Carcinoma

Tumors of the salivary gland comprise 3 to 6% of all head and neck neoplasms in adults; the

incidence is one to three per 100,000 people per year (1) Of the several types of salivary gland cancer, adenoid cystic carcinoma is the second most common type, accounting for 22% of cases in some series (2). ACC is an indolent cancer with 5-year survival rates as high as 50-90% and up to 25% 15-year survival rates. Surgery with wide resection is the main modality of treatment for localized ACC. Adjuvant radiation therapy is reserved for patients with poor prognostic factors, such as close or positive margins, vascular or perineural invasion, lymph node metastasis or high grade tumors. Loco-regional recurrence and distant metastasis especially to the lung are not uncommon. A watch and wait approach is appropriate if a patient has metastasis but has few or no symptoms due to the indolent nature of the tumor.

Response to chemotherapy agents is around 20% with single agents and 50% with combination therapy (3-10). There is no evidence that combination therapy is better than single agents and it is unknown if survival is improved with chemotherapy. Active single agents include cisplatin, 5-FU, doxorubicin, mitoxantrone and vinorelbine. New agents have also been studied, but so far their use remains investigational. In the largest study utilizing imatinib in patients with ACC that overexpressed c-kit, no partial or complete responses were seen, stable disease was achieved in 9 patients, some of them over 12 months (11). In a phase 2 study of trastuzumab in salivary gland tumors that overexpressed Her-2, a partial response was seen in 1 out of 14 patients (12). Another phase 2 trial utilizing lapatinib, a pan-Erb inhibitor (EGFR and HER-2) showed promising activity with 79% of ACC patients with stable disease, 47% of more than 6 months (13). A phase 2 trial of gefitinib showed no responses but stable disease in 68% of the patients (14). The use of cetuximab also showed no response, but stable disease in 50% of the patients with salivary gland tumors (15). A combination trial investigated the use of bortezomib and doxorubicin. Bortezomib was given until progression in patients with metastatic ACC. Doxorubicin was added at time of progression. There were no responses to single agent bortezomib, however, 64% of patients had stable disease (16).

Stable disease duration has been described in several trials (11,13,15,17,18) as so far partial or complete responses have been rarely obtained with the current regimens or experimental therapies. It is unclear if stable disease duration represents a marker of drug activity or only the indolent nature of this tumor. However, most phase II studies report stable disease duration for 6 months or more as an endpoint, therefore, especially in view of tumors that were progressing prior to study entry, this may be a relevant exploratory endpoint.

There is no established standard chemotherapy for the treatment of ACC. Therefore there is a need to perform high quality clinical trials in this setting. Multicenter cooperative trials should be included in order to maximize patient's resources due to the rarity of this cancer.

2.2 Suberoylanilide Hydroxamic Acid (SAHA)

Suberoylanilide hydroxamic acid (SAHA; NSC 701852) is a small molecule inhibitor of histone deacetylase (HDAC) that binds directly in the enzyme's active site in the presence of a zinc ion (19). Because aberrant HDAC activity has been implicated in a variety of cancers, development of HDAC inhibitors is a rational approach to the design of targeted anticancer

therapeutics. Several HDAC inhibitors from multiple chemical classes have been developed and are currently in clinical trials. Trichostatin and butyric acid were among the first HDAC inhibitors to be administered to patients, but these were found to be clinically unsuitable due to potency and formulation issues (20,21). Depsipeptide was originally selected for clinical study based on its antiproliferative effects; subsequently it was discovered to antagonize HDACs (22) and was the first HDAC inhibitor to demonstrate clinical efficacy (23). Of the three classes of HDACs, SAHA targets most human Class 1 (related to the yeast transcriptional regulator Rpd3) and Class 2 (similar to the yeast Hda1) enzymes (24,25); the third class of HDACs (homologues of yeast sir2) requires NAD+ for activity and is not inhibited by SAHA. Among those currently in clinical trials, SAHA is the most potent HDAC inhibitor that can be administered orally with excellent bioavailability.

Mechanism of Action

The HDACs exert their targeted action during post-translational acetylation of core nucleosomal histones, which affects chromatin structure, thereby regulating gene expression. DNA that is wrapped around condensed, non-acetylated histones is transcriptionally inactive, whereas acetylation of N-terminal histone lysine residues exposes DNA to important transcription factors that promote transcriptional activity (26,27)The dynamic equilibrium between histone acetylation and deacetylation is regulated by histone acetyltransferases (HATs) and HDACs. The action of HDACs on nucleosomal histones leads to tight coiling of chromatin and silencing of expression of various genes, including those implicated in the regulation of cell survival, proliferation, differentiation, and apoptosis (28). The effects of HDACs are not limited to histone deacetylation. HDACs also act as members of a protein complex to recruit transcription factors to the promoter region of genes, including those of tumor suppressors, and they affect the acetylation status of specific cell cycle regulatory proteins (27)

Nonclinical Activity

SAHA was identified originally by its ability to induce differentiation of murine erythroleukemia cells at micromolar concentrations (29,30). Subsequently, it was found to induce differentiation or arrest growth of a wide variety of human carcinoma cells. To date, SAHA activity has been reported in transformed hematopoietic cells, such as multiple myeloma (MM) (31-33), acute promyelocytic leukemia (APL) (34), acute lymphocytic leukemia (35), chronic myelogenous leukemia (36,37), Waldenstrom's macroglobulinemia (33), and cutaneous T-cell lymphoma (CTCL) (38). Activity has also been reported in cell lines representing other tumor types including bladder transitional cell carcinoma (39), breast cancer (40-42), prostate cancer (43), head and neck squamous carcinoma (44), and colon carcinoma (45).

The antitumor activity of SAHA was demonstrated in several *in vivo* models of cancer, including a xenograft model of human CWR22 prostate cancer cells (43), a mouse model of APL containing the promyelocytic leukemia zinc-finger-retinoic acid receptor α fusion gene (PLZF-RARα) (46), and an *N*-methylnitrosourea-induced mammary tumor model in rodents (47).

SAHA has also showed activity when administered daily by intraperitoneal (IP) injections in the CWR22 and PLZF-RARα models and by oral (PO) administration in the carcinogen-induced mammary tumor model.

SAHA Combination Studies

Accumulating evidence has demonstrated the effectiveness of HDAC inhibitors in combination with several other agents *in vitro*. The combination of SAHA and DNA hypomethylating agents (5-azacytidine or decitabine) acts synergistically to induce apoptosis, differentiation, and/or cell growth arrest in various cancer cell lines (48,49). When SAHA was combined with the anti-metabolite 5-fluorouracil, a supra-additive to additive antiproliferative effect in wild type and mutant-p53 colorectal cancer cells was observed (50). SAHA with imatinib mesylate (Gleevec®) may be effective in chronic myelogenous leukemia (CML) cells that resist imatinib mesylate through increased Bcr-Abl expression (36,37). Minimally toxic concentrations of the proteasome inhibitor bortezimib combined with SAHA resulted in increased apoptosis in human leukemia cells (37). In cells from RARα-PLZF/RARα-PLZF transgenic mice and in cells harboring t(15;17) (RARα-PML fusion genes), SAHA induced significant apoptosis and growth inhibition, effects that were increased by adding all-*trans* retinoic acid (46). Pre-treating four human cancer cell lines (including a brain tumor line) with SAHA increased the killing efficiency of etoposide, ellipticine, doxorubicin, or cisplatin, but not of the topoisomerase I inhibitor camptothecin (51). However, treating cells in the reverse order (anticancer drug followed by SAHA) was no more cytotoxic than the anticancer drug alone. Finally, overexpression of Bcl-2 or Bcl-XL in leukemia and MM cell lines abolished SAHA-induced apoptosis but did not affect its differentiation or cell cycle regulatory effects (45,52,53). This suggests that agents blocking Bcl-2 expression or function could be effectively combined with SAHA. Studies done with other HDACs have suggested that a consequence of HDAC inhibitor-induced heat shock protein 90 (Hsp 90) acetylation is depletion of the Hsp 90 client protein Her-2 and increased apoptosis of breast cancer cell lines induced by taxotere, trastuzumab, epothilone B, and gemcitabine (54,55). In addition to these potentially additive or synergistic interactions between SAHA or other HDACs and various anticancer drugs, SAHA has shown synergistic activity when combined with radiotherapy in prostate cancer cell spheroids (56).

Phase 1 Clinical Experience

A phase 1 study evaluated vorinostat administered intravenously (IV) to patients with advanced solid tumors and hematologic malignancies (57). Vorinostat was administered using two schedules: 2-hour IV infusion daily x 3 q21d, or daily x 5 for 1 to 3 weeks. No dose-limiting toxicities (DLTs) were observed in eight patients administered vorinostat daily x 3 q21d at doses of 75, 150, 300, 600, and 900 mg/m²/day. One solid tumor patient given 900 mg/m² on the daily x 5 schedule for 3 weeks developed acute respiratory distress and grade 3 hypotension. Among five other patients at this dose, no additional DLTs were observed. Among 12 hematologic and 17 solid tumor patients enrolled on the daily x 5 schedule (300, 600, and 900 mg/m²/day), therapy was delayed for 1 week for grade 3/4 leukopenia and/or thrombocytopenia in two of five patients with hematologic malignancies given 600 mg/m² for 3 weeks. The

maximum tolerated dose (MTD) for hematologic malignancy patients was 300 mg/m² daily x 5 for 3 weeks. An MTD for solid tumors was not determined because the study was terminated when an oral formulation became available. The mean terminal half-life ($t_{1/2}$) ranged from 21 - 58 minutes, and the dose was linearly proportional to the area under the concentration versus time curve (AUC). Acetylated histones were detected in peripheral blood mononuclear cells (PBMCs) up to 4 hours post-infusion at higher dose levels. This observation was confirmed by immunohistochemical analyses of post-therapy tumor biopsies. Four patients, including two with lymphoma and two with bladder cancer, experienced objective tumor regression with clinical improvement in tumor-related symptoms.

As of 29-May-2009 the clinical safety of vorinostat is supported by data from 854 patients (858 patient-exposures) who have been treated in Merck & Co., Inc sponsored Phase I, II, and III studies (Investigator's Brochure, 2009) conducted in patients with both hematologic malignancies and solid tumors. An additional 290 patients were enrolled in a randomized, blinded study where patients are randomized in a 1:1 fashion to vorinostat or placebo. Merck & Co., Inc., including Banyu (Japan), has sponsored 30 vorinostat studies that are completed (11),closed (3), or ongoing (14). The NCI is working in collaboration with Merck & Co., Inc. to further evaluate vorinostat. The NCI-sponsored studies of vorinostat are in progress or are planned in a variety of different indications both in hematologic malignancies as well as solid tumors. To date, 1,230 patients have received vorinostat as either monotherapy or combination chemotherapy in 43 studies sponsored by the NCI.

In addition, vorinostat is also being evaluated in studies initiated and sponsored by independent Investigators, both as monotherapy and as combination chemotherapy. As of 29-May-2009, 44 of these Investigator initiated studies have enrolled 326 patients. These active studies are enrolling and additional studies are planned.

Therefore, over 2,574 patients have received at least one dose of vorinostat in studies sponsored by either Merck and Co., Inc., the NCI or independent Investigators.

The total daily doses studied ranges from 200 mg to 900 mg. The tolerability of oral vorinostat appears to be determined by total daily dose and the length of consecutive days of dosing. The maximum tolerated dose (MTD) for continuous daily dosing without a rest period is 400 mg daily or 200 mg BID. The MTD for intermittent dosing is 300 mg BID x 3 consecutive days per week, or 250 mg TID x 14 consecutive days followed by a 7-day rest. Dose-limiting toxicities (DLTs) of single agent vorinostat were mainly non-hematologic (anorexia, dehydration, diarrhoea, and fatigue); hematologic toxicities are primarily anaemia and thrombocytopenia, most of which were mild to moderate. The majority of these DLTs occurred within the first month on oral vorinostat. The DLTs were manageable because these toxicities resolved quickly after drug administration
was interrupted. The optimal dose, dose frequency, and dose duration remains under active investigation.

The pharmacokinetics and toxicokinetics of vorinostat have been evaluated in

mice, rats, dogs and humans. Rapid oral absorption has been noted in all species studied. Vorinostat (0.5 to 50 μg/mL) exhibited moderate reversible binding to plasma proteins. In human plasma, vorinostat appears to bind primarily to human serum albumin; however, some binding of vorinostat was also observed in solutions of human α1-acid glycoprotein.

Vorinostat is excreted exclusively as metabolites, the majority of metabolites are derived from glucuronidation and hydrolysis with β-oxidation pathway metabolites accounting for the rest. As vorinostat is not eliminated via cytochrome P450 pathways, it is anticipated that vorinostat will not be subject to drug-drug interactions when co-administered with drugs that are known to be CYP inhibitors. Although vorinostat was not a potent reversible inhibitor of the cytochrome P450 isozymes (IC50 >75 μM), gene expression studies detected some potential for suppression of CYP2C9 and CYP3A4 activities at ≥10 μM vorinostat however, these changes were observed at concentrations higher than the pharmacologically relevant serum concentration of 2 μM (C_{max}).

Pharmacokinetic analysis of 26 patients has indicated that the bioavailability of oral vorinostat is approximately 46%. Vorinostat doses in the range of 200 - 600 mg produced maximum concentration (C_{max}) and drug exposure (AUC) curves that were linearly proportional. The $t_{1/2}$ of oral vorinostat ranges from 92 to 150 minutes, and preliminary data suggest that administration of vorinostat with food does not appear to substantially alter the rate or extent of absorption. Inhibition of HDAC activity was achieved in PBMCs at the 200 mg dose level. At dose levels of 400 and 600 mg, the duration of HDAC inhibition lasted $\geq$10 hours.

Significant antitumor activity has been observed among members of the above patient trial population. In a phase 1 study of oral vorinostat, seven patients with heavily pre-treated diffuse large B-cell lymphoma were entered. Among these patients, one complete response (CR) and one partial response (PR) were observed. In addition, one patient has had a significant PET scan response. The PR lasted 5 months, the PET scan response lasted 6 months, and the CR was ongoing with a duration >12 months at last report. Decrease in tumor mass, pleural effusion, and improvement of tumor-related pain or shortness of breath have also been observed in patients with mesothelioma. In an ongoing phase 2 study of vorinostat in patients with heavily pretreated CTCL or peripheral T-cell lymphoma, objective responses have been observed (including PRs in 5 of 13 patients), and 8 of 10 patients reported decreased pruritus.

Zolinza® (vorinostat) was approved by the U.S. Food and Drug Administration (FDA) on 06-Oct-2006 for the treatment of cutaneous manifestations in patients with cutaneous T-cell lymphoma (CTCL) who have progressive, persistent or recurrent disease on or following two systemic therapies. Phase I and II clinical studies of Vorinostat sponsored by Merck & Co., Inc., Banyu (Japan), and the NCI have emonstrated confirmed anti-tumor activity in patients with acute myeloid leukemia (AML), advanced multiple myeloma, B-cell non-Hodgkin's lymphoma, squamous cell laryngeal carcinoma, thyroid carcinoma, breast carcinoma, non-small cell lung carcinoma, glioblastoma multiforme, and myelodysplastic syndrome.

Potential for Drug Interactions

Vorinostat has a low propensity to cause or be affected by drug-drug interactions. In vivo animal models and in vitro human systems have demonstrated that the major pathways of metabolism of vorinostat involve glucuronidation, and hydrolysis followed by β-oxidation (Investigator's Brochure, 2009). As vorinostat is not eliminated via cytochrome P450 (CYP) pathways, it is anticipated that vorinostat will not be subject to drug-drug interactions when co-administered with drugs that are known to be CYP inhibitors or inducers. Since vorinostat was neither a potent inhibitor nor an inducer of CYP enzymes, drug-drug interactions caused by vorinostat are not anticipated. Additionally, vorinostat is not recovered intact in urine to any appreciable extent. Therefore, compounds known to affect renal elimination are not expected to affect the pharmacokinetics of vorinostat.

Prolongation of prothrombin time (PT) and International Normalized Ratio (INR) were observed in patients receiving vorinostat concomitantly with coumarin-derivative anticoagulants. Physicians should carefully monitor PT and INR in patients concurrently administered vorinostat and coumarin derivatives.

Vorinostat should not be administered concomitantly with other HDAC inhibitors (e.g., valproic acid) as class-specific adverse reactions may be additive. Severe (Grade 4) thrombocytopenia with associated gastrointestinal bleeding and anaemia has been reported with the concomitant use of vorinostat and valproic acid.

Patients who have received HDAC inhibitors or compounds with HDAC inhibitor like activity, such as valproic acid, for anti-neoplastic therapy should not enroll in vorinostat oncology trials. Patients who have received such agents for other indications, e.g epilepsy, may enroll on vorinostat trials after a 30-day wash-out period.

2.3 FDG PET/CT

Assessment of tumor response to therapy plays a central role in drug development as well as clinical patient management. Currently response is mainly evaluated by measuring tumor size in CT and classifying tumor shrinkage according to standard criteria, such as those of the World Health Organization (WHO) or Response Evaluation Criteria for Solid Tumors (RECIST) 1.1 criteria (58-60). However, response rates as assessed by these criteria are not well correlated with patient survival. Furthermore, response is evaluated not earlier than 2-3 months after start of therapy. This represents a significant clinical problem, since many treatment regimens are only active in the minority of the patients, meaning that the majority of patients undergo prolonged therapy without benefits. Several studies have suggested that positron emission tomography (PET) with the glucose analog fluorodeoxyglucose (FDG) may be used to monitor tumor response very early in the course of therapy. Quantitative changes in tumor FDG uptake 2-3 weeks after start of therapy have been shown to correlate well with subsequent tumor shrinkage and patient survival in some tumor types (table 1). Conversely, the absence of a measurable decrease in tumor FDG uptake after the first chemotherapy cycle has been found to predict lack

of tumor shrinkage and poor patient survival.

Table 1: Prognostic relevance of quantitative changes in tumor FDG uptake during chemo- or chemoradiotherapy

Tumor	Author	Year	N	criteria	median survival		p-value
					responder	non-responder	
Esophagus	Weber(61)	2001	37	-35%	>48	20	0.04
	Wieder(62)	2004	22**	-30%	>38	18	0.011
Gastric	Ott(63)	2002	35	-35%	>48	17	0.001
Head and Neck	Brun(64)	2002	47**	median*	>120	40	0.004
Ovarian	Avril (65)	2005	33	-20%	38	23	0.008
Lung	Weber(65)	2003	57	-20%	9	5	0.005
	Hoekstra (66)	2005	56	-35%	43	18	0.04

* Median metabolic rate at the time of the follow-up study.
** Chemoradiotherapy, otherwise chemotherapy.

Thus, FDG-PET has the potential to improve patient management by signaling the need for early therapeutic changes in non-responders, thereby avoiding the side effects and costs of ineffective treatment. Furthermore, as an early indicator of clinical benefit, FDG-PET may also facilitate oncologic drug development by shortening Phase II trials and detecting clinical benefit earlier in Phase III investigations.

An exploratory objective of this study (pending funding) will be to assess the association between a metabolic response after one and two cycles of chemotherapy and subsequent best tumor response according to standard anatomic response evaluation criteria (RECIST 1.1). The PET studies will be optional.

Rationale for Studying the Time Course of Tumor FDG Uptake During Chemotherapy

To the best of our knowledge, there is very few data published on the use of PET scans to monitor response in ACC (67,68). Non small cell lung cancer (NSCLC) was chosen as a model because the data on this tumor type is more mature and abundant.

A previous study has indicated that, in patients with advanced NSCLC, changes in tumor FDG uptake after one cycle of chemotherapy are well correlated with patient survival (65). In addition, a study evaluating neoadjuvant chemotherapy has also shown that metabolic activity after one chemotherapy cycle is highly predictive of patient survival (66). A second study in patients undergoing neoadjuvant chemoradiotherapy retrospectively analyzed PET scans of patients with locally advanced NSCLC imaged after three cycles of induction chemotherapy and after completion of chemoradiotherapy (45 Gy) (69). Changes in tumor FDG uptake at both time points were predictive of patient survival. However, most of the patients underwent only two and not three PET studies. There are some data in locally advanced breast and esophageal cancer that PET imaging after one cycle of chemotherapy may be a slightly better predictor of patient survival than PET after 2-6 cycles (62,70,71).

Both patients treated in the NCI protocol 8057 (section 2.4) experienced subjective clinical and/or laboratorial improvement around 2-3 weeks after starting treatment with vorinostat. Therefore, this trial will measure changes in tumor FDG uptake after one and two cycles of chemotherapy.

Definition of a Metabolic Response in FDG-PET

Based on the reproducibility of the FDG-signal in untreated tumors, relative changes of approximately 20% are very unlikely to be due to measurement errors or spontaneous fluctuations of tumor metabolic activity (72,73). These data establish the minimal effect of treatment on tumor metabolic activity that can be assessed by FDG-PET. However, a measurable change in metabolic activity does not necessarily imply that treatment has a beneficial effect for the patient. A recent study evaluated the prognostic implications of a measurable change in tumor glucose utilization in patients with advanced NSCLC, who were treated with palliative platinum-based chemotherapy. A "metabolic response" in PET was prospectively defined as decrease of the SUV of the primary tumor by at least 20%. A total of 57 patients were included in the study and 28 tumors showed a metabolic response after the first chemotherapy cycle. Median progression-free survival of metabolic nonresponders was only 1.8 months versus 5.9 months for metabolic responders. Median overall survival of metabolic responders was 8.4 months and only 5.0 months for metabolic nonresponders (65). The sensitivity and specificity of a metabolic response for prediction of best response according to RECIST criteria were 95% and 74%, respectively.

These data indicate that a measurable change in tumor FDG uptake after the first cycle of chemotherapy is associated with a palliative effect of therapy. These data and the data on the test-retest reproducibility of FDG-PET suggest that a 20% decrease in tumor FDG uptake may be

used to define a metabolic response in patients with NSCLC. However, we will use slightly stricter criteria for a metabolic response in the present study and define a metabolic response as a decrease of tumor FDG uptake by 25%. The rationale for this definition is that the test/retest reproducibility of FDG-PET in a multicenter trial may be lower than previously reported in single-center studies. Therefore, a 20% threshold may not be robust enough for a multicenter trial. Defining a metabolic response as a 25% decrease of tumor FDG uptake makes this study also consistent with previous recommendations by the EORTC (74).

Although data in the literature support the use of a 25% decrease in tumor FDG uptake as a criterion for a metabolic response, we cannot exclude that other threshold values may allow a better prediction of patient outcome. Therefore, receiver operating characteristic (ROC) curves will be calculated for prediction of best response by quantitative changes in FDG uptake after one and two cycles of chemotherapy. Predictive accuracy will be evaluated through 95% confidence intervals for the area under the ROC curve (75)

All PET/CT data will follow the guidelines specified in appendices B and C (76) .

2.4 Rationale

Case Reports

Two patients with ACC were treated in the NCI protocol 8057 (Phase I and pharmacokinetic study of vorinostat for solid tumors and lymphomas in patients with varying degrees of hepatic dysfunction) at the Karmanos Cancer Institute (KCI), Detroit, MI. Both patients displayed significant subjective clinical and/or laboratorial improvement. One patient also experienced partial response on the liver metastasis. Based on those cases, we propose to investigate the activity of vorinostat in patients with ACC. A brief summary of those cases is described below.

Case 1

40 year old Caucasian female with a diagnosis of ACC stage III of the right parotid in 1997. Patient underwent right neck dissection and total parotidectomy on 11/03/97. Adjuvant radiation therapy to the left maxilla and mandible at 6200cGY was delivered. Metastatic disease to the bone, lung and liver were diagnosed in August of 2006. Palliative radiation therapy to the bone was delivered from T9 to L1 (30Gy) and from L3 to S2 (30Gy). Patient opted for a watch and wait approach until January of 2008.

Previous treatments included a phase 1 trial utilizing a hedgehog inhibitor from 01/22/08 to 03/24/08. Treatment was discontinued due to progression of disease. Patient subsequently enrolled on another phase 1 trial utilizing a telomerase inhibitor from 05/15/08 to 06/16/08, discontinued due to progression of disease in the liver.

Patient enrolled on the liver dysfunction NCI protocol 8057 on 07/24/08. A flat dose of 400mg of vorinostat was given on day -6 (07/24/08), followed by a 200mg daily dose starting on 07/31/08. At the time of enrollment, patient's liver enzymes and bilirrubin levels placed her on

the moderate liver dysfunction cohort (see table below). On 08/21/08, a decrease in bilirrubin levels was observed as well as a minor decrease in alanine aminotransferase (ALT). Decrease in the liver span and firmness on physical examination were noticed. Patient reported improvement in symptoms, such as less bloating since the start of treatment. CT scans done at the end of 8 weeks of treatment showed stable disease, but on cycle 3 day 1 (09/11/08), ALT level was normal, AST was only mildly elevated (grade 1) and bilirrubin was normal. On 10/02/08, due to complete normalization of liver enzymes, vorinostat dose was increased from 200mg to 400mg (normal dose cohort). Patient's CT scans showed partial response in the liver after 5 months into the trial, with stable disease in the lung. Liver enzymes (AST and ALT) and bilirrubin return to normal limits (table 2). Patient was taken off trial on April 30, 2009 due to interstitial pneumonitis (possibly drug related).

Adverse events observed included pulmonary embolism (probably drug related), grade 1 elevation of hemoglobin and alkaline phosphatase. Patient also experienced grade 2 thrombocytopenia, completely resolved. Patient also experienced adrenal insufficiency, needing steroids replacement and had 3 episodes of hospitalization due to aspiration pneumonia.

Table 2 – Laboratory values according to vorinostat cycle

	07/24/08 (D-6)	07/31/08 (C1D1)	08/21/08 (C2D1)	09/11/08 (C3D1)	10/02/08 (C4D1)	02/10/09 (C10D13)	03/12/09
AST	219	160	200	64	27	27	23
ALT	173	137	120	45	33	25	20
Bilirrubin	4.0	1.9	1.4	0.8	0.5	0.3	0.3
Alkaline phosphatase	1421	1305	1418	1103	774	232	208

Case 2

40 year old Caucasian male with a diagnosis of ACC stage IVB of the left nasopharynx in January 2003. Patient was treated with a LeFort I approach resection through the sphenoid sinus and pterigopalatine fossa on the same month. Tumor was not totally resected, due to extension to carotid artery. He subsequently received adjuvant radiation therapy by intensity-modulated external beam radiation therapy (IMRT) to the nasal cavity and sinus (at a dose of 50.4Gy) as well as radiosurgery boost to the residual sinus lesion (10Gy). Radiation therapy was completed in May of 2003. Patient developed metastatic lung disease in May of 2006. Lung biopsy confirmed metastatic ACC. At the same time, a renal mass was found in his left kidney. Biopsy was performed and it was positive for chromophobe renal cell carcinoma. It was surgically resected by left radical nephrectomy in August 29, 2006 with no evidence of recurrence from this cancer until the present moment.

Patient was treated with sorafenib from May 2007 to June 2007. Treatment was discontinued upon patient's request; no evaluation for response was performed. Re-irradiation to the recurrent disease on the left pterygopalatine fossa, left trigeminal nerve and cavernous sinus

at a dose of 34Gy was performed from August 08, 2007 to August 20, 2007.

Previous treatments included a phase 1 trial utilizing a hedgehog inhibitor from 11/19/07 to 02/14/08. Treatment was discontinued due to progression of disease and worsening of symptoms. Patient subsequently enrolled on another phase 1 trial utilizing a telomerase inhibitor from 03/24/08 to 05/2708, discontinued due to progression of disease. He underwent another phase 1 trial with a vascular disrupting agent from 07/07/08 to 08/18/08. Treatment was discontinued due to intolerable side effects.

Treatment on the NCI protocol 8057 with vorinostat at 400mg orally daily (normal dose cohort) was started on 10/09/08. Clinical benefit was noted within the first month of treatment with vorinostat, as demonstrated by decrease in facial pain, increase in energy levels and weight gain. CT thorax after 8 weeks of treatment showed decrease in the size of the lung metastasis in the range of 10-20%. Significant adverse events included grade 2 asymptomatic elevation of creatinine, which after a 5-day discontinuation of the drug returned to the patient's baseline. Grade 1 decrease in hemoglobin and platelets were also observed. Patient remained on the study until 01/12/09, off study due to progression of disease.

3. PATIENT SELECTION

3.1 Eligibility Criteria

3.1.1 Patients must have histologically or cytologically confirmed locally advanced, recurrent or metastatic adenoid cystic carcinoma.

3.1.2 Patients must have measurable disease, defined as at least one lesion that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥20 mm by chest x-ray, as ≥10 mm with CT scan, or ≥10 mm with calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters). See section 10.1 for the evaluation of measurable disease.

3.1.3 Patients must have locally advanced and/or recurrent and/or metastatic disease not amenable to potentially curative surgery or radiotherapy. Any prior number of chemotherapy regimens is allowed. A minimum of at least 4 weeks since prior chemotherapy or radiation therapy should have elapsed, 6 weeks if the last regimen included BCNU or mitomycin C.

3.1.4 Age ≥18 years. Because no dosing or adverse event data are currently available on the use of vorinostat in patients <18 years of age, children are excluded from this study but will be eligible for future pediatric single-agent trials, if applicable.

3.1.5 Life expectancy of greater than 12 weeks.

3.1.6 ECOG performance status 0–2 (Karnofsky ≥60%; see Appendix A).

3.1.7 Patients must have normal organ and marrow function as defined below:

• leukocytes	≥3,000/mcL
• absolute neutrophil count	≥1,500/mcL
• platelets	≥100,000/mcL
• total bilirubin	within normal institutional limits(WNL)
• AST(SGOT)/ALT(SGPT)	≤2.5 X institutional upper limit of Normal (ULN)
• creatinine	within normal institutional limits
OR	
creatinine clearance	≥60 mL/min/1.73 m^2 for patients with creatinine levels above institutional normal

3.1.8 Eligibility of patients receiving any medications or substances known to affect or with the potential to affect the activity or pharmacokinetics of vorinostat will be determined following review of their case by the Principal Investigator.

3.1.9 No other diagnosis of malignancy unless non-melanoma skin cancer, carcinoma in situ of the cervix, or a malignancy diagnosed ≥ 5 years previously and currently with no evidence of disease. HOWEVER - if the patient has had a previously diagnosed Stage I/II malignancy of another type, consideration for recruitment may be made by the CTEP senior investigator after discussion with local PI and patient's physician

3.1.10 Confirmed availability of tumor tissue (either fresh or from paraffin block) from the primary tumor or metastatic site to be available to use on correlative studies (see Section 8 for details).

3.1.11 The effects of vorinostat on the developing human fetus at the recommended therapeutic dose are unknown. For this reason and because HDAC inhibitors are known to be teratogenic, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately.

3.1.12 Ability to understand and the willingness to sign a written informed consent document.

3.1.13 If the patient's tumor can be easily accessed, a pre-treatment biopsy will be mandatory.

3.2 Exclusion Criteria

3.2.1 Patients who have had chemotherapy or radiotherapy within 4 weeks (6 weeks for nitrosoureas or mitomycin C) prior to entering the study or those who have not recovered from adverse events due to agents administered more than 4 weeks earlier. More than 21 days from major surgery should have elapsed before the first dose of the study drug.

3.2.2 Patients may not be receiving any other investigational agents or have received vorinostat in the past. Patients should not have taken valproic acid for at least 4 weeks prior to enrollment.

3.2.3 Inability to take oral medications on a continuous basis.

3.2.4 Patients with active brain metastases should be excluded from this clinical trial because of their poor prognosis and because they often develop progressive neurologic dysfunction that would confound the evaluation of neurologic and other adverse events. Patients with previous brain metastases will be eligible if condition is treated and stable for ≥ 1 month

3.2.5 History of allergic reactions attributed to compounds of similar chemical or biologic composition to SAHA.

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

3.2.7 Pregnant women are excluded from this study because SAHA is a HDAC inhibitor agent with the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with vorinostat, breastfeeding should be discontinued if the mother is treated with vorinostat.

3.2.8 Patient is unable or unwilling to abide by the study protocol and to cooperate fully with the investigator or designee.

3.2.9 Patient on current therapy with enzyme-inducing anticonvulsants (see Appendix G)

3.3 Inclusion of Women and Minorities

Both men and women and members of all races and ethnic groups are eligible for this trial.

4. REGISTRATION PROCEDURES

4.1 General Guidelines

Eligible patients will be entered on study centrally at the Karmanos Cancer Institute by the Study Coordinator. All sites should call the Study Coordinator at 313-576-9369 to verify agent availability.

Following registration, patients should begin protocol treatment within 72 hours. Issues that would cause treatment delays should be discussed with the Principal Investigator. If a patient does not receive protocol therapy following registration, the patient's registration on the study may be cancelled. The Study Coordinator should be notified of cancellations as soon as possible.

Except in very unusual circumstances, each participating institution will order DCTD-supplied agents directly from CTEP. Agents may be ordered by a participating site only after the initial IRB approval for the site has been forwarded by the Coordinating Center to the CTEP PIO (PIO@ctep.nci.nih.gov) except for Group studies.

4.2 Registration Process

To register a patient, the following documents should be completed by the research nurse or data manager and faxed to (313) 576-8365 or e-mailed (casettal@karmanos.org) to the Study Coordinator:

- Copy of required laboratory tests
- Signed patient consent form
- HIPAA authorization form (signed by patient)
- Eligibility screening worksheet
- Registration form

The research nurse or data manager at the participating site will then call (313) 576-9369 or e-mail (casettal@karmanos.org) the Study Coordinator to verify eligibility. To complete the registration process, the Coordinator will

- assign a patient study number
- register the patient on the study
- fax or e-mail the patient study number and dose to the participating site
- call the research nurse or data manager at the participating site and verbally confirm registration.

5. TREATMENT PLAN

5.1 Vorinostat Administration

Treatment will be administered on an outpatient basis. Reported adverse events and potential risks are described in Section 7. Appropriate dose modifications for vorinostat 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 patient's malignancy.

On Day 1 of the first cycle of vorinostat will be initiated on a continuous daily oral regimen at 400mg PO. The dose may be reduced for individual patients in subsequent cycles depending on toxicity (See Section 6.2.2). Each treatment cycle will consist of 28 days of therapy.

Vorinostat can be taken with food. Patients need to swallow each capsule whole with a full glass of water. Capsules should not be chewed or opened. If a capsule is broken and the powder of the capsules gets on skin, patients should be instructed to wash the exposed area with copious amounts of water and to inform investigator or nurse of the fact.

Patients are required to record their daily dose in a "pills diary". Missed/ skipped pills or vomited doses should not be re-taken. Patients should be instructed to record those pills as missed doses in the diary and to inform investigator or nurse if that happens.

5.2 Supportive Care Guidelines

Patients will be permitted to receive appropriate supportive care measures as deemed necessary by the treating physician. The use of prophylactic granulocyte growth factors is not permitted for the first cycle of therapy.

Patients should maintain adequate fluid and food intake because the dose-limiting toxicities related to vorinostat are anorexia, dehydration, diarrhea, and fatigue. In the setting of dysgeusia, popsicles and Gatorade have been found to be useful by some investigators to maintain adequate hydration. Antiemetics, particularly after the first 3 days of therapy, may be required. Patients should also be encouraged to seek a nutritional consult.

Diarrhea: Treat diarrhea promptly with appropriate supportive care, including loperamide. Instruct patients to begin taking loperamide at the first signs of: 1) poorly formed or loose stool, 2) occurrence of more bowel movements than usual in one day, or 3) unusually high volume of stool. Loperamide should be taken in the following manner: 4 mg at first onset of diarrhea, then 2 mg after each unformed stool. Daily dose should not exceed 16 mg/day. Loperamide should not be taken prophylactically. Advise patients to drink plenty of clear fluids to help prevent dehydration caused by diarrhea. Avoid loperamide if there is the presence of blood or mucus in the stool or if diarrhea is accompanied by fever. If grade 3 or 4 diarrhea develops, discontinue further treatment with vorinostat.

5.3 Duration of Therapy

In the absence of treatment delays due to adverse event(s), treatment may continue for until one of the following criteria applies:

- Disease progression,
- Intercurrent illness that prevents further administration of treatment,
- Unacceptable adverse event(s),
- Patient decides to withdraw from the study, or
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.
- If a female patient gets pregnant during the study

Patients will be removed from study when any of the criteria listed above applies. The reason for study removal and the date the patient was removed must be documented in the Case Report Form.

5.4 Duration of Follow Up

Patients will be followed for 180 days after removal from study or until death, whichever occurs first. Patients removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event.

6. DOSING DELAYS/DOSE MODIFICATIONS

6.1 Retreatment Criteria

Prior to administration of each new cycle following cycle one, patients must have recovered the following organ function:

- absolute neutrophil count ≥1.5 x 109/L
- platelets ≥100 x 109/L
- creatinine ≤1.5 x upper limit of normal (ULN)
- other drug related toxicity must have recovered to ≤ grade 1 (excluding alopecia, controllable hypertension, leukopenia and lymphopenia)
- total bilirubin WNL
- AST(SGOT)/ALT(SGPT) ≤2.5 X ULN

Patients not fulfilling these criteria should have treatment delayed by 1 week to allow for recovery of organ function. Patients who cannot be retreated within 3 weeks (21 days) of the end of the previous cycle should be removed from study, unless they are deriving benefit. In the later case, a discussion with the PI and/or CTEP senior investigator will ensue.

6.2 Dose Modification Guidelines

6.2.1 During the Course of a Cycle:

The dose of vorinostat will be held during the course of the cycle for one or more of the following events:

- Grade 4 neutropenia lasting > 7 days
- Grade 4 thrombocytopenia
- Any grade 4 non-hematological toxicity
- Grade 3 non-hematological toxicity that cannot be controlled with appropriate supportive care measures.

Treatment can be restarted with a reduction in dose (refer to table 3) once the toxicity resolves to severity of grade 1 or less. Inability to start the next cycle within 3 weeks (21 days) of the scheduled start due to toxicity will result in removal of the patient from the study, unless the patient is deriving benefit. In the later case, a discussion with the PI and/or CTEP senior investigator will ensue.

6.2.2 Dose Modification for Subsequent Cycles

Laboratory parameters should be obtained within 72 hours prior to initiation of the next cycle of therapy.

Table 3- Dose Reduction for Toxicity (based on events from previous cycle)

Toxicity	**Dose Reduction**[*,†]
Grade 3/4 Neutropenia associated with fever and/or Grade 4 neutropenia and/or Grade 4 thrombocytopenia	1st occurrence: Reduce by 100 mg/day – 300mg/day (D1-7) 2nd occurrence: 300mg/day (D1-5), with 2 days of rest 3rd occurrence: Off study
Grade 3 nausea, emesis or diarrhea (not controlled by appropriate supportive care measures)	1st occurrence: Reduce dose by 100 mg/day- 300mg/day (D1-7) 2nd occurrence: 300mg/day (D1-5), with 2 days of rest 3rd occurrence: Off study
Other drug-related grade 3/4 non-hematological toxicity**	1st occurrence: Reduce dose by 100 mg/day – 300mg/day (D1-7) 2nd occurrence: 300mg/day (D1-5), with 2 days

	of rest 3rd occurrence: Off study

* If a patient is experiencing benefits from vorinostat and needs further dose reduction, a discussion will occur between CTEP and the PI.

** Since fatigue can be a symptom of underlying malignancy, dose reductions will only be done if fatigue can be attributed to vorinostat. No dose reductions will be done for alopecia.

† Note: "D1-7" means for 7 contiguous days in a week for ALL the weeks that the patient will be on treatment (i.e, 300mg/day everyday from D1-28, without any days of rest). "D1-5" means for 5 days out of 7 days (i.e., 5 days on treatment/ 2 days of rest in a week) for a cycle of 28 days.

7. PHARMACEUTICAL INFORMATION

7.1 Suberoylanilide Hydroxamic Acid (NSC 701852)

Chemical Name: N-hydroxy-N'-phenyl-octane-1,8-diotic acid diamide;
N-hydroxyl-N'-phenyl (9CI) octanediamide

Other Names: SAHA, Vorinostat, L-001079038, WIN 64652, MSK390, AP390, Zolinza™.

Classification: Antineoplastic

CAS Registry Number: 149647-78-9

Molecular Formula: $C_{14}H_{20}N_2O_3$ **M.W.:** 264

Approximate Solubility: Water $\leq$ 5 mg/mL

Description: Histone deacetylase (HDAC) inhibitor

Mode of Action: Histone deacetylases (HDACs) are a family of enzymes that regulate chromatin remodeling and gene transcription via the dynamic process of acetylation and deacetylation of core histones.

Suberoylanilide hydroxamic acid, a potent inhibitor of HDAC activity, binds directly to the catalytic pocket of HDAC enzymes. SAHA causes G1 or G2 phase cell-cycle arrest, apoptosis, or differentiation in cultured transformed cells.

How Supplied: Suberoylanilide hydroxamic acid is supplied by Merck and Co., Inc. and distributed by the CTEP, DCTD, NCI. Suberoylanilide hydroxamic acid is supplied in the following strength:

1. a white, opaque gelatin capsule containing 100 mg (size 3 capsule) of suberoylanilide hydroxamic acid

The inactive ingredients contained in each capsule are microcrystalline cellulose, sodium croscarmellose, and magnesium stearate.

Suberoylanilide hydroxamic acid 100 mg capsules are supplied in bottles of 120 capsules.

Storage: Store suberoylanilide hydroxamic acid capsules at room temperature, 15 to 30 ºC (59 to 86 ºF). Do not store above 30 ºC .Avoid exposure to excessive moisture.

Stability: Shelf life stability studies of the intact bottles are ongoing.

Route of Administration: Orally

Method of Administration: Unless otherwise stated in the protocol, suberoylanilide hydroxamic acid capsules must be administered whole. The absolute bioavailability of suberoylanilide hydroxamic acid under fasted or fed conditions is approximately 46%. Oral administration with food does not appear to substantially alter the rate or extent of absorption.

Potential Drug Interactions: Vorinostat has a low propensity to cause or be affected by drug-drug interactions. In vivo animal models and in vitro human systems have demonstrated that the major pathways of metabolism of vorinostat involve glucuronidation, and hydrolysis followed by β- oxidation. As vorinostat is not eliminated via cytochrome P450 (CYP) pathways, it is anticipated that vorinostat will not be subject to drug-drug interactions when co-administered with drugs that are known to be CYP inhibitors or inducers. Additionally, vorinostat is not recovered intact in urine to any appreciable extent. Therefore, compounds known to affect renal elimination are not expected to affect the pharmacokinetics of vorinostat. Prothrombin time and INR prolongation have been reported in patients taking vorinostat concomitantly with coumadin derivatives anticoagulants. Monitor these patients more frequently in their coagulation parameters.

Special Handling: SAHA is an anticancer drug. Powder spills from SAHA

capsules due to damaged or broken capsules should be cleaned up carefully so as to minimize inhalation. The affected area must be washed with copious amounts of water.

Patient Care Implications: Because vorinostat's dose limiting toxicities are anorexia, dehydration, diarrhea, and fatigue, patients should maintain adequate fluid and food intake. Encourage patients to seek a nutritional consult.

Comprehensive Adverse Events and Potential Risks list (CAEPR) for Vorinostat (SAHA, Zolinza, NSC 701852)

The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with bold and italicized text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI via CTEP-AERS (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification. *Frequency is provided based on 702 patients.* Below is the CAEPR for vorinostat (SAHA, Zolinza).

NOTE: Report AEs on the SPEER **ONLY IF** they exceed the grade noted in parentheses next to the AE in the SPEER. If this CAEPR is part of a combination protocol using multiple investigational agents and has an AE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

Version 2.7, February 28, 2013[1]

Adverse Events with Possible Relationship to Vorinostat (SAHA, Zolinza) (CTCAE 4.0 Term) [n= 702]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
Anemia			***Anemia (Gr 2)***
GASTROINTESTINAL DISORDERS			
	Abdominal pain		
	Constipation		***Constipation (Gr 2)***
Diarrhea			***Diarrhea (Gr 2)***
	Dry mouth		***Dry mouth (Gr 2)***
	Dyspepsia		***Dyspepsia (Gr 2)***
Nausea			***Nausea (Gr 2)***
Vomiting			***Vomiting (Gr 2)***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
Fatigue			***Fatigue (Gr 2)***
	Fever		

INFECTIONS AND INFESTATIONS			
	Infection[2]		
INVESTIGATIONS			
	Alanine aminotransferase increased		***Alanine aminotransferase increased (Gr 2)***
	Alkaline phosphatase increased		
	Aspartate aminotransferase increased		***Aspartate aminotransferase increased (Gr 2)***
	Blood bilirubin increased		
	Creatinine increased		***Creatinine increased (Gr 2)***
	Lymphocyte count decreased		***Lymphocyte count decreased (Gr 2)***
	Neutrophil count decreased		***Neutrophil count decreased (Gr 2)***
Platelet count decreased			***Platelet count decreased (Gr 2)***
	Weight loss		***Weight loss (Gr 2)***
	White blood cell decreased		***White blood cell decreased (Gr 2)***
METABOLISM AND NUTRITION DISORDERS			
Anorexia			***Anorexia (Gr 2)***
	Dehydration		***Dehydration (Gr 2)***
	Hyperglycemia		***Hyperglycemia (Gr 2)***
	Hypoalbuminemia		
	Hypocalcemia		
	Hypokalemia		
	Hyponatremia		
	Hypophosphatemia		***Hypophosphatemia (Gr 2)***
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Musculoskeletal and connective tissue disorder - Other (muscle spasms)		
	Muscle weakness[3]		***Muscle weakness[3] (Gr 2)***
NERVOUS SYSTEM DISORDERS			
	Dizziness		***Dizziness (Gr 2)***
	Dysgeusia		***Dysgeusia (Gr 2)***
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Cough		***Cough (Gr 2)***
	Dyspnea		
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
	Alopecia		
		Skin and subcutaneous tissue disorders - Other (skin necrosis)	

[1]This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting PIO@CTEP.NCI.NIH.GOV. Your name, the name of the investigator, the protocol and the agent should be included in the e-mail.

[2]Infection includes all 75 sites of infection under the INFECTIONS AND INFESTATIONS SOC.

[3]Muscle weakness includes Generalized muscle weakness, Muscle weakness left-sided, Muscle weakness lower limb, Muscle weakness right-sided, Muscle weakness trunk, and Muscle weakness upper limb under the MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS SOC.

[4]Prolongation of prothrombin time and International Normalized Ratio have been observed in patients using vorinostat concomitantly with coumarin-derivative anticoagulants.

Also reported on vorinostat (SAHA, Zolinza) trials but with the relationship to vorinostat (SAHA, Zolinza) still undetermined:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Febrile neutropenia
CARDIAC DISORDERS - Atrial fibrillation; Atrial flutter; Chest pain - cardiac; Left ventricular systolic dysfunction; Myocardial infarction; Palpitations; Pericardial effusion; Sinus bradycardia; Sinus tachycardia; Ventricular fibrillation
EAR AND LABYRINTH DISORDERS - Tinnitus; Vertigo
EYE DISORDERS - Blurred vision
GASTROINTESTINAL DISORDERS - Abdominal distension; Anal hemorrhage; Bloating; Cheilitis; Colitis; Dysphagia; Esophageal hemorrhage; Esophagitis; Flatulence; Gastric hemorrhage; Gastritis; Gingival pain; Lower gastrointestinal hemorrhage; Mucositis oral; Oral hemorrhage; Small intestinal obstruction; Stomach pain; Upper gastrointestinal hemorrhage
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Chills; Death NOS; Edema limbs; Gait disturbance; General disorders and administration site conditions - Other (angioedema); General disorders and administration site conditions - Other (failure to thrive); Malaise; Multi-organ failure; Non-cardiac chest pain; Pain
HEPATOBILIARY DISORDERS - Hepatic failure
INFECTIONS AND INFESTATIONS - Infections and infestations - Other (Herpes zoster)
INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Bruising; Vascular access complication; Wound dehiscence
INVESTIGATIONS - Activated partial thromboplastin time prolonged[4]; Cardiac troponin I increased; Electrocardiogram QT corrected interval prolonged; GGT increased; INR increased[4]; Investigations - Other (elevated LDH); Lipase increased
METABOLISM AND NUTRITION DISORDERS - Acidosis; Hypercalcemia; Hyperkalemia; Hypermagnesemia; Hypernatremia; Hypoglycemia; Hypomagnesemia; Tumor lysis syndrome
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Arthralgia; Back pain; Chest wall pain; Myalgia; Neck pain; Pain in extremity
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS) - Neoplasms benign, malignant and unspecified (incl cysts and polyps) - Other (tumor hemorrhage); Tumor pain
NERVOUS SYSTEM DISORDERS - Ataxia; Cognitive disturbance; Depressed level of consciousness; Dysphasia; Encephalopathy; Facial muscle weakness; Facial nerve disorder; Headache; Intracranial hemorrhage; Ischemia cerebrovascular; Lethargy; Memory impairment; Nervous system disorders - Other (Guillain-Barre syndrome); Nervous system disorders - Other (head injury); Nervous system disorders - Other (polyneuropathy); Paresthesia; Peripheral motor neuropathy; Peripheral sensory neuropathy; Seizure; Syncope; Tremor
PSYCHIATRIC DISORDERS - Agitation; Anxiety; Confusion; Depression; Insomnia; Personality change; Psychosis
RENAL AND URINARY DISORDERS - Acute kidney injury; Hematuria; Proteinuria; Urinary frequency; Urinary incontinence; Urinary retention; Urinary tract pain
REPRODUCTIVE SYSTEM AND BREAST DISORDERS - Pelvic pain; Uterine hemorrhage
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Bronchopulmonary hemorrhage; Epistaxis; Hypoxia; Nasal congestion; Pharyngeal mucositis; Pharyngolaryngeal pain; Pleural effusion; Pleuritic pain; Pneumonitis
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Dry skin; Hyperhidrosis; Nail loss; Palmar-plantar erythrodysesthesia syndrome; Pruritus; Purpura; Rash maculo-papular
VASCULAR DISORDERS - Flushing; Hematoma; Hot flashes; Hypertension; Hypotension; Thromboembolic event; Vascular disorders - Other (arterial thrombosis); Vasculitis

Note: Vorinostat (SAHA, Zolinza) in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

7.2 Agent Availability

Vorinostat is an investigational agent supplied to investigators by the Division of Cancer Treatment and Diagnosis (DCTD), NCI.

Vorinostat is provided to the NCI under a Collaborative Agreement between Merck and Co., Inc. and the DCTD, NCI (see Section 11.4).

7.3 Agent Ordering

NCI supplied agents may be requested by the Principal Investigator (or their authorized designee) at each participating institution. Pharmaceutical Management Branch (PMB) policy requires that agent be shipped directly to the institution where the patient is to be treated. PMB does not permit the transfer of agents between institutions (unless prior approval from PMB is obtained.) The CTEP assigned protocol number must be used for ordering all CTEP supplied investigational agents. The responsible investigator at each participating institution must be registered with CTEP, DCTD through an annual submission of FDA form 1572 (Statement of Investigator), Curriculum Vitae, Supplemental Investigator Data Form (IDF), and Financial Disclosure Form (FDF). If there are several participating investigators at one institution, CTEP supplied investigational agents for the study should be ordered under the name of one lead investigator at that institution.

Active CTEP-registered investigators and investigator-designated shipping designees and ordering designees can submit agent requests through the PMB Online Agent Order Processing (OAOP) application < https://eapps-ctep.nci.nih.gov/OAOP/pages/login.jspx >. Access to OAOP requires the establishment of a CTEP Identity and Access Management (IAM) account < https://eapps-ctep.nci.nih.gov/iam/ > and the maintenance of an "active" account status and a "current" password. For questions about drug orders, transfers, returns, or accountability, call (240) 276-6575 Monday through Friday between 8:30 am and 4:30 pm (ET) or email PMBAfterHours@mail.nih.gov anytime.

7.4 Agent Accountability

The Investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of all agents received from DCTD using the NCI Drug Accountability Record Form. See the CTEP web site for Policy and Guidelines for Accountability and Storage of Investigational Drugs (http://ctep.cancer.gov).

8. CORRELATIVE STUDIES

A detailed description of all the planned/optional (**pending funding**) correlative studies is described in appendicies D, E, and F.

Archival paraffin blocks of formalin-fixed diagnostic tumor biopsy specimens will be requested for each patient from the participating centers. If a paraffin block cannot be provided, twenty-five slides containing 5µm sections from an archival block, mounted onto Superfrost slides suitable for immunostaining can alternatively be submitted. If twenty-five slides are not available, a lesser amount may be acceptable after discussion with the study Principal Investigator, Dr. Patricia LoRusso.

Methods

Samples

Barbara Ann Karmanos Cancer Institute (KCI) has a set of formalin-fixed paraffin embedded (FFPE) tumor blocks or slides from individual ACC patients who were treated with SAHA. A high number of patients had stable disease on treatment over 6 months (median and range), and we observed a partial response in 2 of the patients in this trial. These FFPE samples are well annotated with clinical follow-up and represent highly favorable samples to exploit for advancing improved outcomes in ACC.

This study will use previously collected FFPE tissue blocks or slides from 10 patients (all available samples may be used at a later point) who achieved some benefit from SAHA and patients whose tumors did not demonstrate a therapeutic response. If an entire FFPE block is not available, PI's will request up to 20 unstained slides (10 microns). A deidentified, coded copy of the pathology report will also be sent with the FFPE block or slides.

Whole blood was also collected on most of the patients at the time of enrollment in the original study. Karmanos will send white blood cells from the blood to TGen to serve as patient matched germline DNA. If no blood exists from a selected patient, or the blood was of poor quality, Karmanos study personnel will recontact the patient to obtain new informed consent and draw a new blood sample.

Blood samples will be spun down to remove the white blood cells from the red blood cells. The white blood cell pellet will be snap frozen using liquid nitrogen and stored at -80° until shipping on dry ice to TGen.

All samples and data will be de-identified and coded with a unique study code assigned by Karmanos study personnel. A key linking the patient name to the study code will be maintained and stored by Karmanos study personnel. TGen will not receive any patient identifiable information before, during or after this study is conducted.

Sample Shipment

Initial sample were shipped as specified below to Karmanos Cancer Institute.

Shipping of paraffin blocks/slides should be done with a padded envelope. Paraffin blocks should be wrapped in a plastic bag before placement in the envelope. Slides

should be put in an appropriate slide case before placement in the envelope.

Attention: Richard Wiegand
Barbara Ann Karmanos Cancer Institute
Wayne State University
4100 John R, 531 HWCRC
Detroit, MI 48201
Office: 313-576-8244
Fax: 313-576-8266
Email: wiegandr@karmanos.org

All samples will be shipped to: **The TGen Barrett Laboratory**
C/O Elizabeth Lenkiewicz
13208 E. Shea Blvd.
Scottsdale, Arizona 85259
602- 358-8839

FFPE Blocks or Slides

When shipping the FFPE block, place the block in a ziplock bag and wrap in bubble wrap or another type of padded material prior to shipping. The zip lock bag can be placed into a FedEx envelope or small shipping box.

If sending slides, pack the labeled slides into plastic slide cassette(s). Tape plastic slide cassettes shut and wrap in bubble wrap or another type of padded material prior to shipping. Place the slide cassettes into an outer shipping box.

White Blood Cell Pellets
Place container with frozen specimen in a ziplock bag. Place 2-3 inches of dry ice in the bottom of a Styrofoam cooler. Place bag in the center of the cooler on top of the dry ice, and then fill the cooler the rest of the way with dry ice (preferably pelleted). Place a single paper-towel or piece of paper across top of ice, then put lid on the cooler and tape the lid tightly to the cooler, sealing all the way around the lid. **Ship Monday through Wednesday only unless prior notification is made is made with TGen. Do not ship the day before a U.S. Holiday.**

Laboratory

Each 50 μm section will be dewaxed and the nuclei extracted in the presence of DAPI. These nuclei suspensions will then be sorted with an Influx flow cytometer equipped with a UV and a 488 nm laser. For each sample, we will simultaneously collect each diploid, aneuploid, and tetraploid population present in the tissue. Our sorting techniques can collect up to 4 simultaneous streams, thus optimizing the use of clinical samples for genomic studies. Sorting

gates out cellular and tissue debris that is typically present in FFPE samples and provides highly purified tumor material for genomic analysis. The DNA from each sorted fraction will be extracted and then amplified using our established protocols. This linear amplification extends the use of each purified sample and allows deep genomic profiling including NGS of each tumor population of interest. In addition, we will save aliquots of unamplified genomic DNA from each sample for PCR-based validation studies. For each of the tumors in this study, we will initially profile sorted populations of interest with high-density aCGH to identify the tumor population(s) in each FFPE sample that will be processed for NGS. These data also will define the landmarks (focal amplicons, homozygous deletions, and breakpoints) of each tumor genome. We will then interrogate the DNA from each sorted tumor cell population in parallel with patient matched germline DNA with whole exome NGS.

Site(s) Performing Correlative Studies

Dr. Michael Barrett
13208 E. Shea Blvd.
Scottsdale, Arizona 85259
mbarrett@tgen.org
602- 358-8383
602- 358-8360 FAX

Volumetric Tumor Assesment (refer to appendix I for details)_
RECIST criteria uses 2D-proxies (single maximum diameter on axial images) to estimate tumor volumes. RECIST is limited in that it only assess tumor response in terms of change in maximal axial diameter of selected target lesions.

Recent studies in metastatic cancer have demonstrated the added value of accounting for tumor density and volume using size and attenuation CT (SACT), mass attenuation, size and structure (MASS), or modified versions of the original Choi CT response criteria, beyond RECIST.

We propose a retrospective comprehensive assesment of tumor response, using total viable tumor volume (VTV) to quantify the change in viable versus necrotic tumor volumes across all lesions through volumetric characterization of density distribution throughout all parts of measurable tumors.

In this retrospective study, imaging already obtained (CT and/or MRI) from patients with locally advanced, recurrent or metastastic adenoid cystic carcinoma treated on this trial will be re-reviewed.

Target lesions will be volumetric segmented by a research assistant blinded to clinical status withing PACS (Carestream VuePACS v12.0, Rochester, NY).

RECIST imaging from selected CT and/or MRIs from all the patients treated on this trial will be compared to volumetric size and viable tumor volume (VTV).

No patient-related decisions will be made based on this assesment. Off-study patients will not be reconsented for retrospective data analysis.

CT and/or MRI will be re-reviewed at the National Cancer Institutes by Dr. Les Folio team.

9. STUDY CALENDAR

Baseline evaluations are to be conducted within 1 week prior to administration of protocol therapy. Scans and x-rays must be done 2 weeks prior to the start of therapy. In the event that the patient's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy.

	Pre-Study (D-21 to D-1)	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Wk 7	Wk 8	Wk 9	Wk 10-12	Wk 13	Off Study
Vorinostat[a]		X	X	X	X	X	X	X	X	X	X	X	
Informed consent	X[b]												
Demographics	X[b]												
Medical history[n]	X[b, c]		X[e]		X[e]	X[e]		X[e]		X[e]		X[e]	X
Concurrent meds[n]	X[b, c]		X[e]		X[e]	X[e]		X[e]		X[e]		X[e]	X
Physical exam[n]	X[b, c]		X[e]		X[e]	X[e]		X[e]		X[e]		X[e]	X
Vital signs[n]	X[b, c]		X[e]		X[e]	X[e]		X[e]		X[e]		X[e]	X
Height	X[b]												
Weight[n]	X[b, c]		X[e]		X[e]	X[e]		X[e]		X[e]		X[e]	X
Performance status (ECOG)[n]	X[b, c]		X[e]		X[e]	X[e]		X[e]		X[e]		X[e]	X
Hematology labs[d,o]	X[c]		X[e]		X[e]	X[e]		X[e]		X[e]		X[e]	X
Serum chemistry[f,o]	X[c]		X[e]		X[e]	X[e]		X[e]		X[e]		X[e]	X
EKG	X[b]												
Urinalysis	X[c]												
B-HCG[g]	X[c]												
Adverse events evaluation[n]			X[e]		X[e]	X[e]		X[e]		X[e]		X[e]	X
Tumor measurements[p]	X[c]	Tumor measurements are repeated every 8 weeks (± 1 week) for the first 6 cycles. After 6 months on study, can be performed every 12 weeks (± 1 week). Documentation (radiologic) must be provided for patients removed from study for progressive disease. Objective responses should be confirmed within 4 weeks.											X[h]
Archival tumor tissue collected	X[b]												
Blood for correlative studies[j, m]		X			X								

PET/CT [L]	X[c]				X[i]				X[i]					

a- Vorinostat: continuous oral dose 400mg/day (or other dosage if drug reduction required).
b- Days -21 to day -1: informed consent, physical examination and medical history; vital signs including weight and height; review of concurrent medication; ECG; collection of archival tumor tissue.
c- Days -14 to day -1: limited physical examination with vital signs (including weight); review of new events on history; review of medication; performance status; tumor measurement (by CT or MRI) and PET/CT (optional). Laboratory tests, pregnancy test (if appropriate) and urinalysis should be done within 7 days from first drug intake.
d- CBC with differential and platelets; PTT, PT and INR.
e- Study visits assessments to be done on weeks 2 and 4 (±1 day) for the first cycle, then for the second cycle on week 5 and 7, (±1 day); then from week 9 on, every four weeks (±1 day) thereafter.
f- Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, phosphorus, potassium, total protein, AST, ALT, sodium, magnesium.
g- Serum pregnancy test (women of childbearing potential).
h- Off-study evaluation.
i- PET/CT to be done on days -14 to -1; D28±3 days and D56±3 days (optional).
j- Blood for correlative studies on days 1 (prior to first dose of vorinostat), 7 (+-1day) and 28 (+-1day)
k- A tumor biopsy will be done if the tumor can be easily accessed.
L – PET/CT scans are optional and will be performed based on the availability of funding
m- If no blood exists from a selected patient, or the blood was of poor quality, Karmanos study personnel will recontact the patient to obtain new informed consent and draw a new blood sample.
n- For patients that have been on study longer than 2 years, office visits can be done every 12 weeks (±7 days), with medical history, concurrent meds, physical exam, vital signs, weight, performance status, and adverse events evaluation done also at that time
o- For patients that have been on study longer than 2 years, hematology labs and serum chemistry can be done every 6 weeks (±7 days)
p- For patients that have been on study longer than 2 years, tumor measurements can be done every 24 weeks (±10 days)

10. MEASUREMENT OF EFFECT

For the purposes of this study, patients should be re-evaluated for response every 8_ weeks. Patients may receive scans every 3 cycles/12 weeks (± 1 week) after completing 6 months of treatment on study. Patients that have been on study longer than 2 years can have tumor measurements (CT scans or MRI) done every 24 weeks (±10 days). In addition to a baseline scan, confirmatory scans should also be obtained 4 (not less than 4) weeks following initial documentation of objective response.

10.1 Definitions

Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) (58). 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.

10.1.1 **Definitions**

Evaluable for toxicity. All patients will be evaluable for toxicity from the time of their first treatment with vorinostat.

Evaluable for objective response. Only those patients 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 response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

Evaluable Non-Target Disease Response. Patients 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.

10.1.2 **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, as ≥10 mm with CT scan, or ≥10 mm with 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.

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, and abdominal masses (not followed by CT or MRI), are considered as 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 patient, 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.

10.1.3 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. 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 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.

Chest x-ray Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

Conventional CT and MRI This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for 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.

Ultrasound Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

Endoscopy, Laparoscopy The utilization of these techniques for objective tumor evaluation is not advised. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response (CR) or surgical resection is an endpoint.

Tumor markers Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response. Specific guidelines for both CA-125 response (in recurrent ovarian cancer) and PSA response (in recurrent prostate cancer) have been published [*JNCI* 96:487-488, 2004; *J Clin Oncol* 17, 3461-3467, 1999; *J Clin Oncol* 26:1148-1159, 2008]. In addition, the Gynecologic Cancer Intergroup has developed CA-125 progression criteria which are to be integrated with objective tumor assessment for use in first-line trials in ovarian cancer [*JNCI* 92:1534-1535, 2000].

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.

FDG-PET While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

a. Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a

sign of PD based on a new lesion.

b. No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.
c. FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

Note: A 'positive' FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

10.1.4 Response Criteria

10.1.4.1 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

10.1.4.2 **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).

10.1.4.3 **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 Patients 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: Patients 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 Patients 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

10.1.5 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).

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.

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.

10.1.6 Progression-Free Survival

Progression-Free Survival (PFS) is defined as the duration of time from start of treatment to time of progression or death, whichever occurs first.

10.2 Other Response Parameters

10.2.1 Time to Response (TTR)

TTR is measured from the start of the treatment until the RECIST measurement criteria are met for CR or PR (whichever is first recorded). TTR only applies to the patients achieving either PR or CR, and involves no censoring.

10.2.2 Overall Survival (OS)

OS is measured from the start of treatment until death from any cause. OS is subject to censoring.

10.3 Response Review

We do not routinely mark scans and will encourage all sites that submit radiographic images to submit unmarked images. After the initial patient recruitment, we will use an outside radiologist to review the scans prior to going to the second phase. If the decision is made to proceed to the second phase, an outside radiologist will look at all images at protocol completion.

11. REGULATORY AND REPORTING REQUIREMENTS

Expedited adverse event (AE) reporting for this study is via CTEP-AERS (CTEP Adverse Event Reporting System), accessed via the secure CTEP web site(https://eapps-ctep.nci.nih.gov/ctepaers). The reporting procedures to be followed are presented in the "CTEP, NCI Guidelines: Adverse Event Reporting Requirements"" which can be downloaded from the *CTEP web site* (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/adverse_events.htm).

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for adverse event reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov).

A list of adverse events that have occurred or might occur (Comprehensive Adverse Events and Potential Risks list) can be found in Section 7 (Pharmaceutical Information). A copy of the

current Agent-Specific Adverse Event List (ASAEL) is sent with LOI approval letters. The ASAEL is updated periodically; copies of the updated list are available on request via e-mail from aemd@tech-res.com.

11.1 Expedited Adverse Event Reporting
(AE; formerly known as Adverse Drug Reaction)

Expedited reports are submitted to CTEP via the secure CTEP-AERS application accessed via the CTEP web site (https://eapps-ctep.nci.nih.gov/ctepaers). Those AEs that do not require expedited reporting must be reported in routine (CDUS) study data submissions. AEs reported through CTEP-AERS must **also** be reported in routine study data submissions. In the rare event when Internet connectivity is disrupted a 24-hour notification is to be made to NCI by telephone at: 301-897-7497, or 301-897-7402 for CIP studies. An electronic report MUST be submitted immediately upon re-establishment of internet connection. Please note that all paper CTEP-AERS forms have been removed from the CTEP website and will NO LONGER be accepted.

11.1.1 Expedited Reporting Guidelines –
Phase 2 studies with investigational agents (including hospitalization defined in bullet 1 below)

CTEP-AERS Reporting Requirements for Adverse Events that occur within 30 Days[1] of the Last Dose of the Investigational Agent on Phase 2 and 3 Trials

Phase 2 and 3 Trials									
	Grade 1	Grade 2	Grade 2	Grade 3		Grade 3		Grades 4 & 5[2]	Grades 4 & 5[2]
	Unexpected and Expected	Unexpected	Expected	Unexpected with Hospitalization	Unexpected without Hospitalization	Expected with Hospitalization	Expected without Hospitalization	Unexpected	Expected
Unrelated Unlikely	Not Required	Not Required	Not Required	10 Calendar Days	Not Required	10 Calendar Days	Not Required	10 Calendar Days	10 Calendar Days
Possible Probable Definite	Not Required	10 Calendar Days	Not Required	10 Calendar Days	10 Calendar Days	10 Calendar Days	Not Required	24-Hour; 5 Calendar Days	10 Calendar Days

[1] **Adverse events with attribution of possible, probable, or definite that occur greater than 30 days after the last dose of treatment with an agent under a CTEP IND require reporting as follows:**
CTEP-AERS 24-hour notification followed by complete report within 5 calendar days for:
- Grade 4 and Grade 5 unexpected events

CTEP-AERS 10 calendar day report:
- Grade 3 unexpected events with hospitalization or prolongation of hospitalization
- Grade 5 expected events

[2] Although an CTEP-AERS 24-hour notification is not required for death clearly related to progressive disease, a full report is required as outlined in the table.

December 15, 2004

Note: All deaths on study require both routine and expedited reporting regardless of causality. Attribution to treatment or other cause must be provided.

- Expedited AE reporting timelines defined:
  - "24 hours; 5 calendar days" – The investigator must initially report the AE via CTEP-AERS within 24 hours of learning of the event followed by a complete CTEP-AERS report within 5 calendar days of the initial 24-hour report.
  - "10 calendar days" - A complete CTEP-AERS report on the AE must be submitted within 10 calendar days of the investigator learning of the event.

- Any medical event equivalent to CTCAE grade 3, 4, or 5 that precipitates hospitalization (or prolongation of existing hospitalization) must be reported regardless of attribution and designation as expected or unexpected with the exception of any events identified as protocol-specific expedited adverse event reporting exclusions.

- Any event that results in persistent or significant disabilities/incapacities, congenital anomalies, or birth defects must be reported via CTEP-AERS if the event occurs following treatment with an agent under a CTEP IND.

- Use the NCI protocol number and the protocol-specific patient ID assigned during trial registration on all reports.

11.1.2 Expedited Adverse Event Reporting Exclusions

N/A

Note: All deaths on study must be reported using expedited reporting regardless of causality. Attribution to treatment or other cause should be provided.

11.1.3 Secondary AML/MDS

Investigators are required to report cases of secondary AML/MDS occurring on or following treatment on NCI-sponsored chemotherapy protocols through CTEP-AERS. If Please refer to the CTEP, NCI Guidelines (available at htpp://ctep.cancer.gov) for additional information about secondary AML/MDS reporting.

Second malignancies and non-AML/MDS secondary malignancies (*e.g*., endometrial cancer in a breast cancer patient receiving tamoxifen) should NOT be reported via CTEP-AERS but should be submitted as part of the study results via routine CDUS reporting.

11.2 Data Reporting

This study will be monitored by the Clinical Data Update System (CDUS) version 3.0. Cumulative CDUS data will be submitted quarterly to CTEP by electronic means. Reports are

due January 31, April 30, July 31, and October 31.

11.3 CTEP Multicenter Guidelines

If an institution wishes to collaborate with other participating institutions in performing a CTEP sponsored research protocol, then the following guidelines must be followed.

Responsibility of the Protocol Chair

- The Protocol Chair will be the single liaison with the CTEP Protocol and Information Office (PIO). The Protocol Chair is responsible for the coordination, development, submission, and approval of the protocol as well as its subsequent amendments. The protocol must not be rewritten or modified by anyone other than the Protocol Chair. There will be only one version of the protocol, and each participating institution will use that document. The Protocol Chair is responsible for assuring that all participating institutions are using the correct version of the protocol.
- The Protocol Chair is responsible for the overall conduct of the study at all participating institutions and for monitoring its progress. All reporting requirements to CTEP are the responsibility of the Protocol Chair.
- The Protocol Chair is responsible for the timely review of Adverse Events (AE) to assure safety of the patients.
- The Protocol Chair will be responsible for the review of and timely submission of data for study analysis.

Responsibilities of the Coordinating Center

- Each participating institution will have an appropriate assurance on file with the Office for Human Research Protection (OHRP), NIH. The Coordinating Center is responsible for assuring that each participating institution has an OHRP assurance and must maintain copies of IRB approvals from each participating site.
- Prior to the activation of the protocol at each participating institution, an OHRP form 310 (documentation of IRB approval) must be submitted to the CTEP PIO.
- The Coordinating Center is responsible for central patient registration. The Coordinating Center is responsible for assuring that IRB approval has been obtained at each participating site prior to the first patient registration from that site.
- The Coordinating Center is responsible for the preparation of all submitted data for review by the Protocol Chair.
- The Coordinating Center will maintain documentation of AE reports. There are two options for AE reporting: (1) participating institutions may report directly to CTEP with a copy to the Coordinating Center, or (2) participating institutions report to the Coordinating Center who in turn report to CTEP. The Coordinating Center will submit AE reports to the Protocol Chair for timely review.
- Audits may be accomplished in one of two ways: (1) source documents and research records for selected patients are brought from participating sites to the Coordinating Center for audit, or (2) selected patient records may be audited on-site at participating

sites. If the NCI chooses to have an audit at the Coordinating Center, then the Coordinating Center is responsible for having all source documents, research records, all IRB approval documents, NCI Drug Accountability Record forms, patient registration lists, response assessments scans, x-rays, etc. available for the audit.

Inclusion of Multicenter Guidelines in the Protocol

- The protocol must include the following minimum information:
- The title page must include the name and address of each participating institution and the name, telephone number and e-mail address of the responsible investigator at each participating institution.
- The Coordinating Center must be designated on the title page.
- Central registration of patients is required. The procedures for registration must be stated in the protocol.
- Data collection forms should be of a common format. Sample forms should be submitted with the protocol. The frequency and timing of data submission forms to the Coordinating Center should be stated.
- Describe how AEs will be reported from the participating institutions, either directly to CTEP or through the Coordinating Center.

Agent Ordering

- Except in very unusual circumstances, each participating institution will order DCTD-supplied investigational agents directly from CTEP. Investigational agents may be ordered by a participating site only after the initial IRB approval for the site has been forwarded by the Coordinating Center to the CTEP PIO.

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

The agent(s), supplied by CTEP, DCTD, NCI, used in this protocol is/are provided to the NCI under a Collaborative Agreement (CRADA, CTA) between the Merck Pharmaceutical Company [hereinafter referred to as Collaborator] and the NCI Division of Cancer Treatment and Diagnosis. Therefore, the following obligations/guidelines, in addition to the provisions in the Intellectual Property Option to Collaborator contained within the terms of award, apply to the use of Agent(s) in this study:

1. Agent(s) may not be used for any purpose outside the scope of this protocol, nor can Agent(s) be transferred or licensed to any party not participating in the clinical study. Collaborator(s) data for Agent(s) are confidential and proprietary to Collaborator(s) and shall be maintained as such by the investigators. The protocol documents for studies utilizing investigational agents contain confidential information and should not be shared or distributed without the permission of the NCI. If a copy of this protocol is requested by a patient participating on the study or patient's family member, the individual should sign a confidentiality agreement. A suitable model agreement can be downloaded from http://ctep.cancer.gov.

2. For a clinical protocol where there is an investigational Agent used in combination with (an)other investigational Agent(s), each the subject of different collaborative agreements, the access to and use of data by each Collaborator shall be as follows (data pertaining to such combination use shall hereinafter be referred to as "Multi-Party Data".):

a. NCI must provide all Collaborators with prior written notice regarding the existence and nature of any agreements governing their collaboration with NIH, the design of the proposed combination protocol, and the existence of any obligations that would tend to restrict NCI's participation in the proposed combination protocol.

b. Each Collaborator shall agree to permit use of the Multi-Party Data from the clinical trial by any other Collaborator solely to the extent necessary to allow said other Collaborator to develop, obtain regulatory approval, or commercialize its own investigational agent.

c. Any Collaborator having the right to use the Multi-Party Data from these trials must agree in writing prior to the commencement of the trials that it will use the Multi-Party Data solely for development, regulatory approval, and commercialization of its own investigational agent.

3. .Clinical Trial Data and Results and Raw Data developed under a Collaborative Agreement will be made available exclusively to Collaborator(s), the NCI, and the FDA, as appropriate and unless additional disclosure is required by law or court-order. Additionally, all Clinical Trial Data and Results and Raw Data will be collected, used and disclosed consistent with all applicable federal statutes and regulations for the protection of human subjects, including, if applicable, the Standards for Privacy of Individually Identifiable Health Information set forth in 45 C.F.R. Part 164.

4. When a Collaborator wishes to initiate a data request, the request should first be sent to the NCI, who will then notify the appropriate investigators (Group Chair for Cooperative Group studies, or PI for other studies) of Collaborator's wish to contact them.

5. Any data provided to Collaborator(s) for phase 3 studies must be in accordance with the guidelines and policies of the responsible Data Monitoring Committee (DMC), if there is a DMC for this clinical trial.

6. Any manuscripts reporting the results of this clinical trial must be provided to CTEP for immediate delivery to Collaborator(s) for advisory review and comment prior to submission for publication. Collaborator(s) will have 30 days from the date of receipt for review. Collaborator shall have the right to request that publication be delayed for up to an additional 30 days in order to ensure that Collaborator's confidential and proprietary data, in addition to Collaborator(s)'s intellectual property rights, are protected. Copies of abstracts must be provided to CTEP for forwarding to Collaborator(s) for courtesy review as soon as possible and preferably at least three (3) days prior to submission, but in any case, prior to presentation at the meeting or publication in the proceedings. Press releases and other media presentations must also be forwarded to CTEP prior to release. Copies of any manuscript, abstract, and/or press release/ media presentation should be sent to:

Regulatory Affairs Branch, CTEP, DCTD, NCI
6130 Executive Boulevard, Suite 7111
Rockville, MD 20852
FAX 301-402-1584
E-mail: ncicteppubs@mail.nih.gov

The Regulatory Affairs Branch will then distribute them to Collaborator(s). No publication, manuscript or other form of public disclosure shall contain any of Collaborator's confidential/proprietary information.

12. STATISTICAL CONSIDERATIONS

12.1 Study Design/Endpoints

12.1.1. Study Design:

We will use a 2-stage Simon (1989) optimal design to minimize the number of patients to be treated. Complete + partial (CR+PR) rate will be the primary statistical endpoint. We wish to distinguish response rate regions of at most 5% vs. at least 20%. For this 2-stage design its Stage 1 will require 12 response-evaluable (r-e) patients. At least 1 responder in Stage 1 will be needed to justify continuing to Stage 2, which will require 17 more r-e patients (i.e., maximum of N=29 r-e patients for the study). If ≥ 3 responders are observed among the final 29 r-e patients, then vorinostat will be considered promising. If < 3, conclude that vorinostat has insufficient efficacy to justify further study. This design specifies significance level = 0.15, and power = 0.90, and has a probability of early termination (PET) = 0.540 under the null hypothesis. Alpha is relaxed in this design since there are no effective therapies available, but beta is limited to 0.10 to better ensure that we identify vorinostat as a promising therapy, if it truly is one.

In ACC, late responses (by RECIST) may occur; hence we anticipate the need to suspend accrual after 12 r-e patients have been enrolled for Stage 1. Among stable disease patients still on vorinostat treatment, responses could occur as long as one year after treatment started. Should vorinostat have little efficacy, disease progression among the Stage 1 patients will be more rapid, thus limiting the time until study termination can be justified based on the Stage 1 response results.

12.1.2 Study Endpoints:

The primary endpoint is objective response based on RECIST 1.1 criteria. Response criteria and categories are defined in detail in Sections 10.1.1-10.1.4.

Secondary endpoints include toxicity, time to response (TTR), response duration (RD), progression free survival (PFS), and overall survival (OS). Definition, assessment, and reporting of toxicity (adverse events) may be found in Sections 6.1, or 10. TTR, RD, PFS, and OS are defined in Sections 10.2.1, 10.1.5, 10.1.6, and 10.2.2, respectively.

Exploratory endpoints include metabolic response by PET/CT scan, and several laboratory biomarkers listed in Section 1.3 (Exploratory Objectives), and described in Section 8 and in the Appendix D.

12.2 Sample Size/Accrual Rate

We anticipate an accrual rate of 1-2 ACC patients/month from the 10 organ dysfunction working group institutions combined. That would yield an expected combined accrual rate of 12-24 patients/year. At that rate, 12 r-e patients should be accrued in 6-12 months. Allowing for up to 12 additional months for the discovery of late responders, a Stage 1 interim analysis result should be obtainable within 18-24 months from the time the first patient begins treatment.

If Stage 2 is needed (i.e., an additional 17 r-e patients), that should take an additional 9-17 months of accrual, plus up to 12 additional months for the discovery of late responders. Thus, a final analysis result at the end of Stage 2 (if Stage 2 is needed) should be obtainable within 39-54 months from the time the first patient begins treatment.

12.3 Stratification Factors

None, as this is a single sample Phase II trial. Any stratification of interest can be performed at the time of statistical analysis, after the study data have been collected.

12.3.1 Gender and Minority Accrual Estimates

Phase II Study: Stage 1 / Stage 2 Accrual Estimates

Ethnic Category	Sex/Gender				
	Females		Males		Total
Hispanic or Latino	0	+	0	=	0
Not Hispanic or Latino	7 / 18	+	5 / 11	=	12 / 29
Ethnic Category: Total of all subjects	7 / 18 (A1)	+	5 / 11 (B1)	=	12 / 29 (C1)
Racial Category					
American Indian or Alaskan Native	0	+	0	=	
Asian	0	+	0	=	
Black or African American	1 / 2	+	0	=	1 / 2
Native Hawaiian or other Pacific Islander	0	+	0	=	
White	6 / 16	+	5 / 11	=	11 / 27
Racial Category: Total of all subjects	7 / 18 (A2)	+	5 / 11 (B2)	=	12 / 29 (C2)
	(A1 = A2)		(B1 = B2)		(C1 = C2)

12.4 Analysis of Secondary Endpoints

There are 5 Secondary Objectives:

1) To characterize the safety and tolerability of vorinostat in this patient population
2) To assess the time to response (TTR)
3) To assess the response duration (RD)
4) To evaluate progression free survival (PFS)
5) To assess overall survival (OS)

Secondary Objective 1 concerns toxicity and adverse events, hence will involve categorical variables. They will be tabulated via frequency distributions, and also dichotomized to report the proportion (and percentage) of patients experiencing a specified level (e.g., Grade 3-4) of toxicity. Analysis of these proportions (i.e., rates) will include both point and 90% confidence interval (CI) estimates, with the CI estimates calculated via Wilson's method. The 90% confidence level is appropriate for either the Stage 1 sample size (n=12) or even the Stage 2 sample size (n=29) planned for this Phase II trial.

Secondary Objective 2 concerns TTR, which is a continuous variable not subject to censoring. It will be summarized descriptively, reporting N, median, mean, standard deviation (SD), standard error (SE), minimum, maximum, and 90% CI for the mean calculated from the SE and asymptotic Normal distribution theory.

Secondary Objectives 3-5 concern censored time-to-event (TTE) endpoints. Their distribution will be estimated using standard survival analysis techniques, and the Kaplan-Meier (K-M) method. From the K-M life tables, both point and 90% CI estimates of various statistics of interest can be calculated (e.g., median, 6-month event-free rate, 12-month event-free rate, etc.). Statistical graphs of each K-M curve (with 90% CI lines) will be generated for visual display of these censored TTE endpoints.

There are 7 Exploratory Objectives:

1) To assess the association between a metabolic response by PET/CT after one cycle of chemotherapy and subsequent best tumor response according to standard anatomic response evaluation criteria (RECIST).

2) To assess the association between a metabolic response by PET/CT after the first and second chemotherapy cycle and PFS.

3) Flow sort diploid, aneuploid, and tetraploid populations of tumor cells from FFPE tissue blocks from patients who benefited from SAHA therapy and from patients who did not demonstrate a durable benefit.

4) Profile the genomes of each cell population using oligonucleotide CGH arrays. This will confirm the identification of tumor populations in each sample and provide genomic landmarks

for NGS analysis.

5) Perform whole exome analysis of the sorted tumor population and matching germ line sample for each of the patients. The combined aCGH and exome data will be mined to identify genes and pathways that are targeted by select somatic events in each of the patient subsets

6) To assess stable disease duration (SDD).

7) To assess the association between response to vorinostat treatment and HR23B on tumor paraffin blocks.

Exploratory Objective 1 concerns the potential association of PET/CT response (magnitude of decrease in SUV after 1 treatment cycle) with clinical response by RECIST 1.1 criteria. Percent change in SUV (ΔSUV) is a continuous variable, whereas objective response is a categorical (typically, binary) variable. First, we will use receiver operator characteristic (ROC) curve analysis to determine the optimal cutoff in the distribution of ΔSUV, in relationship to objective response (yes/no). That will allow us to define "PET response". We will also compare the descriptive statistics of the ΔSUV distribution in the RECIST responders vs non-responders. At Stage 1 (n=12), the association of ΔSUV with subsequent best tumor response will be explored in a simple 2 x 2 contingency table, with point and 90% CI estimates of objective response rates among the patients with vs. without PET response. Descriptively only, we can then examine the point and CI estimates to see if there is a hint of any association. At Stage 2 (n=29), we would repeat the same descriptive, exploratory investigation, and then determine what, if any, additional statistical analysis might be appropriate. For example, that might involve exact logistic regression to model the log-odds of later objective response as a function of only one predictor: early PET response (yes/no). This exact logistic modeling investigation would still be viewed as exploratory only, but would yield a point and 90% CI estimate of the odds ratio for response.

Exploratory Objective 2 concerns the potential association of PET/CT response (magnitude of decrease in SUV after 1 treatment cycle) with censored PFS. "PET response" (yes/no) will be determined as just described above for Exploratory Objective 1. At Stage 1 (n=12), to explore the potential association of PET/CT response with length of censored PFS, we will use the Cox proportional hazards model. This analysis will focus on the length of PFS *after* the second PET/CT scan, and will be exploratory only. The goal would be to determine if there is any hint of a potential positive association in the hypothesized *direction*, i.e., is the hazard ratio (HR) < 1 or not for patients with an early PET/CT response. At Stage 2 (n=29), we would repeat the same Cox model investigation, but pay more attention to the magnitude of (HR), its precision (i.e., half-width of it 90% CI), but not its statistical significance (i.e., p-value). Again, this Cox modeling investigation would still be viewed as exploratory only. A similar analysis will be performed utilizing the third PET/CT scan, taken after 2 treatment cycles, at day 56.

Exploratory Objectives 3, 4 and 5 are strictly exploratory and will be reported as descriptive results

Exploratory Objective 6 concerns yet another time-to-event endpoint (SDD), which is defined in Section 10.1.5. The analysis of the censored SDD distribution will use the same approach as described above for Secondary Objectives 3-5.

Exploratory Objective 7 concerns a biomarker (HR23B), measured by immunohistochemistry (IHC). Its expression level will be recorded as: 0, 1+, 2+, or 3+. In light of the small to modest sample sizes for this Phase II trial (either n=12 after Stage 1, or n=29 after Stage 2), we will dichotomize the total staining score (TSS) of HR23B into ≥ 6 ("positive") or < 6 ("negative").

At Stage 1 (n=12), the association of HR23B's dichotomized expression level (absence/presence) to objective response (yes/no) can be explored via a simple 2 x 2 contingency table. From the table we can compute the point and 90% CI estimates of response rates among the patients who do vs. those who do not "express" HR23B. The most basic exploratory observation will be the *direction* of the association, i.e., whether the point-estimate of response rate is higher (or lower) among expressers of HR23B. We can also observe the precision in the estimate of the response rate via its CI.

At Stage 2 (n=29), we would repeat the same descriptive, exploratory investigation, and then determine what, if any, additional statistical analysis might be appropriate. For example, that might involve exact logistic regression to model the log-odds of later objective response as a function "expression" of HR23B (yes/no). This exact logistic modeling investigation would still be viewed as exploratory only, but would yield a point and 90% CI estimate of the odds ratio for response.

12.5 Reporting and Exclusions

12.5.1 Evaluation of Toxicity.

All patients will be evaluable for toxicity from the time of their first treatment with vorinostat.

12.5.2 Evaluation of Response.

All patients included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible. Each patient will be assigned one of the following categories: 1) complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death because of other cause, or 9) unknown (not assessable, insufficient data). [Note: By arbitrary convention, category 9 usually designates the "unknown" status of any type of data in a clinical database.]

All of the patients who met the eligibility criteria (with the possible exception of those who received no study medication) should be included in the main analysis of the response rate. Patients in response categories 4-9 should be considered as failing to respond to treatment (disease progression). Thus, an incorrect treatment schedule or drug administration does not

result in exclusion from the analysis of the response rate. Precise definitions for categories 4-9 will be protocol specific.

All conclusions should be based on all eligible patients. Sub-analyses may then be performed on the basis of a subset of patients, excluding those for whom major protocol deviations have been identified (e.g., early death due to other reasons, early discontinuation of treatment, major protocol violations, etc.). However, these sub-analyses may not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding patients from the analysis should be clearly reported. The 95% confidence intervals should also be provided.

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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
PET Imaging Acquisition Parameters and Image Data Analysis

Additional information for PET Imaging Acquisition Parameters and Analysis are available on the ACRIN web site at (www.acrin.org/6678_protocol.aspx).(76)

Acquisition and Analysis of FDG-PET/CT Scans

FDG-PET/CT scans will be performed according to the guidelines for NCI-sponsored studies as recently published by Shankar et al. (77).

1. Participant Preparation

Participants must fast for a minimum of 4 hours prior to the injection of FDG for the PET scan. However, they will be encouraged to drink water to ensure adequate hydration.

- Upon arrival at the PET facility, the participant's weight and height will be measured and recorded. Serum glucose should be measured to determine that the blood glucose concentration is within the normal range.
- If the serum glucose concentration is found to be greater than 150 mg/dL, the study should be rescheduled. The referring oncologist or the primary physician of the patient will be contacted to optimize blood glucose control.
- The participant should be placed in a comfortable position, either supine or semi-recumbent. A large-bore intravenous line (21-gauge or greater) should be placed in an arm or hand vein. The room should be kept warm to avoid shivering and temperature effects that may increase muscular or fat uptake. The participant should move as little as possible and should not talk more than necessary in the first 30 min following FDG injection.
- Prior to positioning the participant on the PET scanner the participant should be asked to urinate.

2. Injection of [^{18}F] Fluorodeoxyglucose (FDG)

- The dose of FDG to be administered should be 10 to 20 millicuries (mCi), adjusted according to weight as suggested by the scanner manufacturer.
- FDG will be synthesized and prepared in accordance with USP compendial standards.
- The exact time of calibration of the dose should be recorded and the exact time of injection noted to permit correction of the administered dose for radioactive decay. In addition, the dose remaining in the tubing or syringe, or that was spilled during injection should be recorded. The injection should be performed through an intravenous catheter.

3. FDG-PET/CT Imaging

- PET scanning must begin 60 ± 10 minutes after FDG injection. The time between injection and the start of the PET scanning for the second and third scans should be matched as closely as possible to that for the first scan (less than 10 min difference in uptake times).
- Participants will generally be positioned in the PET/CT scanner with their arms raised above the head. If participants cannot tolerate this position for the duration of the PET/CT study, a different participant positioning may be chosen. However, arms should be positioned in the same way at the baseline and the follow-up studies.
- A low-dose CT scan will be acquired for attenuation correction and anatomical localization of findings in the PET scan.
- The acquisition parameter for the low-dose CT scan for attenuation correction should be: kV = 120-140; effective mAs = 30-120 (patient dependent); gantry rotation time ≤ 0.8 sec; maximum reconstructed width = 3-5 mm without overlap; standard reconstruction algorithm, minimum reconstruction diameter = outer arm to outer arm; and without iodinated contrast (78).
- The axial field of view of the CT scan for attenuation correction will range from the mid thighs to the base of the skull. Arm positioning will be the same as for the PET scan, typically above the head.
- The CT scan will be performed during "shallow breathing" as described previously [67]. No respiratory gating will be applied.
- After the CT scan, a PET scan covering the same axial field of view will be performed. This scan will start at the mid thighs. The number of bed positions and the acquisition time per bed position will be scanner specific. Typical parameters are 6 bed positions and an acquisition of 2-5 min per bed position.

4. Image Reconstruction

- The PET data will be corrected for dead time, scatter, randoms and attenuation using standard algorithms provided by the scanner manufacturers.
- Image reconstruction will be performed.

5. Image Analysis

- Activity concentrations in the attenuation-corrected PET images will be converted to standardized uptake values (SUVs) by dividing the activity concentrations by the decay-corrected injected dose and multiplying with the body weight of the participant.
- A circular region of interest (ROI) with a diameter of 0.75 to 1.5 cm will be centered at the site of maximum FDG uptake within this lesion. ROIs of the same size will be placed in the slices immediately above and below, at the same transverse location.

- The average SUV within the volume encompassed by these three ROIs will be determined and recorded. This approach for definition of ROIs has been successfully used for assessment of tumor response in patients with advanced NSCLC [63].
- In most patients the primary tumor will show the highest FDG uptake. However, in a small subgroup of participants the primary tumor may be small (< 2cm) and show only low FDG uptake in PET or it may have been resected. In this case, the metastatic lesion with the highest FDG uptake will be used for analysis.
- For quality control purposes, a large circular ROI (diameter >= 5 cm) will be placed in normal liver tissue. The mean SUV in this ROI will be recorded.
- In the case of multiple liver metastases, it may not be feasible to place one large ROI in normal liver tissues. In this case, several small ROIs, including the same number of pixels as one 5-cm ROI, may be placed in normal liver tissue. FDG uptake within these lesions will be averaged and used for further analysis.
- If the mean SUV within the liver changes by more than 1.0 between two scans, the SUV calculation will be checked for errors. Specifically, the scanner cross calibration, the decay correction of the injected activity, and the participant's body weight will be checked (see checklist below for details)

6. Image Interpretation

- Describe the location of the primary tumor and of metastatic lesions if present.
- Measure and record the maximum SUV in the primary tumor and most active metastatic lesion.
- If the diameter of the primary tumor is less than 2 cm or the primary tumor demonstrates only low FDG uptake, measure the maximum SUV of the lesion with the highest FDG uptake.
- Measure and record the mean SUV in the liver as described in Section 5 above.

APPENDIX C
CT Acquisition Parameters and Image Data Analysis

Additional Information for the CT Acquisition Parameters and Volumetric Analyses are also available on the ACRIN web site at (www.acrin.org/6678_protocol.aspx).

Acquisition and Analysis of the CT Scans
The CT scans serve three purposes: (a) attenuation correction and registration with PET scans for anatomic localization of FDG uptake; (b) response assessment using RECIST; and (c) high spatial quality data sets for central volumetric analyses of lung lesions. Depending upon considerations of workflow and the need for intravenous contrast, individual institutions may have different processes for completing the chest CT scans used for RECIST and tumor volumetric assessments. The following are potential scenarios:

- **Single Platform**: fusion PET/CT scanner: All studies are performed on a fusion PET/CT scan, including the PET/CT scanner, and one single inspiratory CT without contrast for both RECIST criteria and tumor volumetry.
- **Single Platform**: fusion PET/CT scanner: All studies are performed on a fusion PET/CT scan, including the PET/CT scanner, an inspiratory CT with contrast for RECIST criteria, and an inspiratory CT without contrast for tumor volumetry.
- **Dual Platforms**: Fusion PET/CT scanner used to perform a PET/CT scan and an inspiratory CT without contrast for tumor volumetry as well as a dedicated, standalone CT scanner to perform a CT with contrast for RECIST criteria.

1. CT Volumetry
1.1 Image Acquisition
To ensure the level of spatial quality necessary for tumor volumetric analyses, the scanner platforms must be able to perform prospective reconstructions of a single helical sequence at different slice thicknesses. The following are representative PET/CT systems:

- Siemens Biograph PET/CT (CT scanners 6,16, 64-slice); Siemens Medical Solutions | Malvern, PA.
- GE Discovery PET/CT scanners; GE Healthcare Technologies | Waukesha, WI, USA
- Philips Gemini PET/CT scanners (Brilliance CT: 6, 10, 16, 40-slice; 40 and 64 channel); Philips Medical Systems | Andover, MA, USA

All CT scans for an individual participant should be performed on the same platform throughout the trial. In the rare instance of equipment malfunction, follow-up scans on an individual participant can be performed on the *same type* of platform.

Each scanner platform has slightly different technical specifications and user inputs for imaging parameters. The following table provides specifications for the acquisition and reconstruction parameters for the CT series performed for tumor volumetry.

Table 1. Parameters for the CT scans used to measure tumor volume.

Parameter	Diagnostic CT Scan for Tumor Volumetry
KV	120
Gantry rotation time	≤ 0.5 sec
Scanner Effective mAs (Regular-large size patient)	100-260
Maximum scan | breath-hold time (40 cm long thorax)	≤ 15 sec
Number of active channels (N)	≥ 6
Maximum Reconstructed slice width	1-1.5 mm
Reconstruction interval	0 – 20% overlap
Reconstruction algorithm	Standard
Reconstruction diameter (dFOV)	outer rib to outer rib
Intravenous contrast media	None
Arm positioning	Above the head

Parameters for tumor volumetry should provide an in-plane voxel size of 0.55-0.75 mm.

2. Tumor Response Evaluation According To RECIST

2.1 Image Acquisition

Response assessment using RECIST criteria will be performed on all CT scans during the trial. Scans will be performed after administration of oral and IV contrast agents according to institutional practices.

Iodinated intravenous (IV) contrast media may be administered for diagnostic CT scans per standard institutional practice, unless contraindicated. The decision not to use IV contrast for CT is at the discretion of the performing radiologist. If IV contrast is *not* administered for these scans, then these non-contrast CT series can be used for both RECIST and volumetric measurements by prospectively reconstructing the image data into both thick (2.5 to 5 mm) and thin-section (1 to 1.25 mm) series, respectively..

The CT scan with contrast is obtained with arms elevated above the head and at suspended maximal inspiration to provide optimal relative contrast of lung lesions within aerated lung. Typical parameters for image acquisition and reconstruction are shown in Table 2.

Table 2: Parameters for the CT scans used to assess tumor response according to RECIST

Parameter	Dedicated CT for RECIST
KV	120
Gantry rotation time	≤ 0.5 sec
Scanner Effective mAs (Regular-large size patient)	100-260
Maximum scan | breath-hold time (40 cm long thorax)	≤ 15 sec
Number of active channels (N)	≥ 6
Maximum Reconstructed slice width	3 mm[1]
Reconstruction interval	0-20% overlap
Reconstruction algorithm	Standard
Reconstruction diameter (dFOV)	outer rib to outer rib
Intravenous contrast media	Per institution practice | indication[2]
Arm positioning	Above the head

1 **If the inspiratory scan of the PET/CT is non-contrast, it *also* should be used for tumor volumetry, and prospectively reconstructed at 1-1.25 (volumetry) and 2.5-5 mm (diagnostic interpretation using RECIST) slice thicknesses.**

2. **Intravenous contrast should be administered according to standard practices with respect to amount, flow rates, and timing with respect to image acquisition, depending upon the body parts imaged. The same method of contrast administration should be followed with all subsequent scans.**

2.2 Image Data Analysis

2.2.1 The following categories of disease will be used in determining response to treatment by RECIST:

- **Measurable disease:** The presence of at least one measurable lesion. If the measurable disease is restricted to a solitary lesion, its neoplastic nature should be confirmed by cytology | histology.
- **Measurable lesions**: Lesions that can be accurately measured in at least one dimension with longest diameter ≥ 10 mm with helical CT.
- **Non-measurable lesions**: all other lesions, including small lesions (longest diameter < 20 mm with conventional techniques or < 10 mm with helical CT scan), i.e., bone lesions, leptomeningeal disease, Ascites, pleural/pericardial effusion, inflammatory breast disease, lymphangitic cutis | pulmonis, cystic lesions, and also abdominal masses that are not confirmed and followed by imaging techniques.

All measurements will be taken and recorded in metric notation on a calibrated diagnostic imaging workstation at full resolution using electronic calipers. The baseline measurements will be performed within one week of the start of treatment; measurements obtained following chemotherapy cycles will be obtained on scans performed within 1-3 days of the next cycle of treatment. The same method of assessment and the same technique will be used to characterize each identified and reported lesion at baseline and during follow-up. Lung lesions will be

evaluated on lung windows; mediastinal and or soft tissue lesions will be evaluated on soft tissue windows. The acquisition parameters provided above serve as guidelines for CT technique.

"Target" and "Non-Target" lesions will be documented according to the following guidelines:

- All measurable lesions up to a maximum of five lesions per organ and 10 lesions 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 and their suitability for accurate repeated measurements.
- A sum of the longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference by which to characterize the objective tumor response.
- All other lesions (or sites of disease) should be identified as non-target lesions and should also be recorded at baseline. Measurements of these lesions are not required, but the presence or absence of each should be noted throughout follow-up.

The following summarizes the categories of response:

Response Criteria	**Evaluation of Target Lesions**
Complete Response (CR)	Disappearance of all target lesions.
Partial Response (PR)	At least a 30% decrease in the sum of the LD of target lesions, taking as reference the baseline sum LD.
Progressive Disease (PD)	At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions.
Stable Disease (SD)	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since treatment start
	Evaluation of Non-Target Lesions
Complete Response (CR)	Disappearance of all non-target lesions and normalization of tumor marker level.
Incomplete Response; Stable Disease (SD)	Persistence of one or more non-target lesions(s) or/and maintenance of tumor marker level above the normal limits
Progressive disease	Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions (1)
(1) Although a clear progression of non-target lesions only is exceptional, in such circumstances, the opinion of the treating physician should prevail and the progression status should be confirmed later on by follow-up imaging.	

Evaluation of Best Overall Response: The best overall response recorded from the start of the treatment until disease progression | recurrence (taking as reference for PD the smallest measurements recorded since the treatment started). In general, the participant's best response assignment will depend on the achievement of both measurement and confirmation criteria.

Target Lesions	Non-Target Lesions	Evaluation of Non-Target Lesions	Overall Response
CR	CR	No	CR
CR	Incomplete Response / SD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

Confirmation:

- To be assigned the status of PR or CR, changes in tumor measurements must be confirmed by repeat assessments that should be performed no less than 4 weeks after the criteria for response are first met. Longer intervals as determined by the study protocol may also be appropriate.
- To be assigned the status of SD, follow-up measurements must have met the SD criteria at least once after study entry at a minimum interval (in general, not less than 6-8 weeks) that is defined in the study protocol.

Duration of Overall Response: This is measured from the time measurement criteria are met for CR or PR (whichever status is recorded first) until the first date that recurrence or PD is objectively documented, taking as reference for PD the smallest measurements recorded since the treatment started.

Duration of Stable Disease: Stable disease is measured from the start of the treatment until the criteria for disease progression are met, taking as reference the smallest measurements recorded since the treatment started. The clinical relevance of the duration of SD varies for different tumor types and grades.

APPENDIX D -Correlative Studies

Background

The mainstay of treatment for patients with adenoid cystic carcinomas (ACC) has unfortunately not advanced over the last several years. Outside of local disease control, which includes surgery and radiotherapy, systemic therapy has not demonstrated significant benefit in terms of either response rate or overall survival. Studies have shown that ACC genomes are characterized by multiple copy number aberrations and recurring gene translocations (79). In addition recent studies have started to catalog the mutational landscape of these genomes (80). Despite these recent advances, there is an urgent need to study the genomic basis of therapeutic benefit (response and stable disease rates) and clinical behaviors of this disease. In order to address this current unmet need, we are proposing a novel study of ACC genomes in patients who have been treated with the histone deacetylase inhibitor, suberoylanilide hydroxamic acid (SAHA).

Dr. Michael Barrett (TGen) has recently validated the use of DNA content-based sorting strategies with clinical FFPE samples. These methods allow the identification and purification of distinct and pure populations of tumor cells from each sample of interest. In addition, TGen has optimized methods to use the DNA from each sorted population for whole genome and whole exome analysis, and we have established a highly efficient pipeline to process next generation sequencing (NGS) data and to extract clinically actionable results from patient samples during clinical trials.

HR23B was identified as a candidate cancer biomarker through a genome-wide loss-of-function screen (81). It sensitizes osteosarcoma cells to HDAC inhibitor-dependent cell death. Depletion of HR23B in CTCL (cutaneous T cell lymphoma) cell lines reduced sensitivity to apoptosis of these cells upon SAHA treatment (add pearsson ref). Skin biopsies from patients with CTCL enrolled in a SAHA phase 2 study were evaluated for pre and post treatment quantitative HR23B levels (82) The total staining score (TSS) was compared to clinical response. The positive predictive value of HR23B in this clinical setting was 68.8%. Lack of response or progression of disease also correlated with initial absence or disappearance of HR23B in pre and post-treatment biopsies respectively.

Specific Aims

The fundamental hypothesis of our study is that distinct genomic aberrations including amplifications, mutations, and gene translocations that target specific genes and signaling pathways will distinguish those tumors that had a meaningful response to SAHA. We propose that our comprehensive study of the genomic landscape of this patient population, specifically looking at the differences in therapeutic benefit, will provide a unique data set that can be exploited for advancing effective targeted therapy for patients with disease. To accomplish our study we will complete the following Aims:

SPECIFIC AIMs

- Flow sort diploid, aneuploid, and tetraploid populations of tumor cells from FFPE tissue blocks from each of 5 patients who benefited from SAHA therapy and from each of 5 patients who did not demonstrate a durable benefit.

- Profile the genomes of each cell population using oligonucleotide CGH arrays. This will confirm the identification of tumor populations in each sample and provide genomic landmarks for NGS analysis.

-Perform whole exome analysis of the sorted tumor population and matching germ line sample for each of the 10 patients. The combined aCGH and exome data will be mined to identify genes and pathways that are targeted by select somatic events in each of the patient subsets.

Methods *FFPE Sample Preparation and Flow Sorting:* Excess paraffin is removed with a scalpel from individual 40-60µm scrolls which are then washed with 1ml Xylene for 5 minutes to remove remaining paraffin. Each sample is rehydrated in sequential ethanol washes (100% 5 minutes x2, then 95%, 70%, 50% and 30% ethanol) and washed 2 times in 1ml 1mM EDTA pH 8.0. A 1ml aliquot of 1mM EDTA pH 8.0 is added to the samples which are then incubated at 95^{o}C for 80 minutes to facilitate the removal of protein cross-links. Samples are cooled to room temperature for $\geq$ 5 minutes, followed by addition of 300µl PBS 7.4 and gentle centrifugation for 2 minutes. The pellet is washed 3 times with 1ml PBS 7.4/0.5mM CaCl2 to remove EDTA. Each sample is digested overnight (6-17 hours) in 1ml of enzymatic cocktail containing 50 units/ml of collagenase type 3, 80 units/ml of purified collagenase, and 100 units/ml of hyaluronidase in PBS ph7.4/0.5mM CaCl2 buffer. Following overnight digestion 500µl NST buffer is added and samples are centrifuged for 5 minutes at 3000rcf, after which pellets are resuspended in 750µl of NST/10% fetal bovine serum and then passed through a 25g needle 10-20 times. The samples are filtered through a 35µm mesh and collected into a 5ml polypropylene round bottom tube. The mesh is rinsed with an additional 750µl of NST/10% fetal bovine serum and placed on ice while processing remaining samples. An equal volume of 20µg/ml DAPI is added to each tube to achieve a final concentration of 10µg/ml DAPI prior to flow sorting with an Influx cytometer with ultraviolet excitation (Becton-Dickinson, San Jose, CA). Optimal settings for sorting FFPE samples are: Drop formation is achieved with piezzo amplitude of 6-10 volts and a drop frequency of 30khertz. The sort mode is set to purity yield with a drop delay of 31.5-32. Sheath fluid pressure is typically 17-18psi with a 100µm nozzle. For single parameter DNA content assays DAPI emission is collected at > 450nm. DNA content and cell cycle are then analyzed using the software program MultiCycle (Phoenix Flow Systems, San Diego, CA).

DNA Extraction and Amplification: DNA from sorted nuclei is extracted using an amended protocol for QIAamp® DNA Micro Kit from Qiagen. Briefly each sorted sample is resuspended in 180µl buffer ATL and 20µl proteinase K, and then incubated for 3 hours at 56°C for complete lysis. Samples are bound and washed according to supplier's instructions, eluted into 50µl of H_20, and then precipitated overnight with 5ul sodium acetate and 180µl 100% EtOH. Each sample is then centrifuged for 30 minutes at 20,000 x g, washed in 1ml of 70% EtOH for 30 minutes at 20,000 x g. The DNA pellet is dried then resuspended in a small volume (e.g. 10-

50µl) of H_20 for final concentrations suitable for accurate quantization. Genomic DNA from sorted FFPE samples is amplified using Ovation® WGA FFPE System (NuGEN® Technologies, San Carlos CA) with an alternate fragmentation step. Resulting amplified products are used as template for aCGH. In all cases the amplification product quality is assessed by gel electrophoresis.

Array CGH: For each flow sorted tumor sample of interest we will label 1 µg of tumor DNA and 1 µg of reference DNA according to our published protocols (83). These will be hybridized to Agilent 60mer oligonucleotide CGH arrays. All microarray slides will be scanned using an Agilent 2565C DNA microarray scanner. Microarray images will be analyzed using Agilent Feature Extraction software version 11.0 (FE 11.0) using default settings according to the supplier's recommendations. Log_2 ratios of fluorescent signals and corresponding log_2 ratio errors, as calculated from the log_{10} output of FE 11.0, will be analyzed in Genome Workbench 6.7. All aCGH experiments will be evaluated using a series of quality control (QC) metrics. These include background noise, signal intensities and signal to noise ratios for each dye-specific channel, the reproducibility of a series of replicate control probes on the arrays, and a measure of the spread of the distribution of the log_2 ratios reported in each experiment. The data from arrays that pass the QC metrics will then be analyzed using an aberration detection algorithm (ADM2) (84). The latter identifies all aberrant intervals in a given sample with consistently high or low log ratios based on the statistical score derived from the average normalized log ratios of all probes in the genomic interval multiplied by the square root of the number of these probes. Aberration calling thresholds for ADM2 will be determined based on hybridizations with normal 46XY and 46XX DNA derived from sorted control tissues. We will use the distribution of log_2 ratios for autosome probes and the corresponding chromosome X probes from control experiments to determine the error rates in our experiments and select the statistical threshold for calling aberrant genomic intervals in the PDA samples.

NGS Template and Exome Sampling: For each sample of interest we will generate double stranded (ds) DNA template using our published methods and the Ovation® WGA FFPE System (85). Each sample selected for NGS will be assayed for final QC metrics including size range and concentration of dsDNA prior to exome sampling and sequencing. Patient matched germline DNAs will be processed in parallel. All exome sampling and NGS data generation will be done in TGen's Collaborative Sequencing Center (CSC).

NGS Analyses Workflow: Once exome sequencing data is generated, we will leverage our substantial bioinformatics and statistics skills, experience, and large-scale computational capabilities to carry out research-objective-specific customized data-analyses. We have several automated workflows that consist of both published and internally developed data analyses tools for processing sequenced data to generate interpretation-ready results. This includes germline results, such as detecting variants, comparisons with a variety of publically available variants datasets (e.g., dbSNP, 1000 genomes, etc.), functional annotation, Ti/Tv ratios, target sequencing efficiency, etc. Further, when both tumor and normal sequencing data is available, we have well-tested sophisticated workflows for somatic variant detection with detailed comparisons with publically available datasets (e.g., COSMIC). Both germline and somatic variants are comprehensively annotated for functional consequences, such as non-synonymous mutations,

frame-shift insertion-deletions. Our workflows are compatible with standard, platform-independent input sequencing data format (e.g., FASTQ format), generate detailed QC and several additional statistical metrics at various stages of data processing, and deliver results that conform to standard output formats, such as BAM (alignments), VCF (variant calls), functional annotation, etc.

HR23B protein levels will be measured by immunohistochemistry (IHC) using published protocols (86). We will use a single thin section slide for all IHC experiments. All assays will be done through TGen's Macromolecular Analyte and Processing Center (MAPC) pending additional funding. In addition a second slide from each patient will be used for further validation of the recurring t(6:9) MYB-NFIB translocation using conventional fluorescent in situ hybridization (FISH) (87). A semiquantitative analysis based upon intensity and frequency of HR23B staining and the type of dermal and epidermal infiltrate will be performed on each biopsy(87). A total staining score (TSS) will be determined out of 13, where a score of greater than or equal to 6 will be considered to be strong HR23B staining. The threshold for differentiating between positive and negative immunostaining will be set at at TSS of equal or higher than 6. This optimal cutoff was determined by the receiver operating characteristic curve (ROC) distribution analysis.

Samples

Barbara Ann Karmanos Cancer Institute (KCI) has a set of formalin-fixed paraffin embedded (FFPE) tumor slides/blocks from individual ACC patients who were treated with SAHA. A high number of patients had stable disease on treatment over 1 year (median and range), and we observed partial responses in two of the patients in this trial. These FFPE samples are well annotated with clinical follow-up and represent highly favorable samples to exploit for advancing improved outcomes in ACC.

This study will use previously collected FFPE tissue blocks from 10 patients; 5 patients who achieved durable benefit from SAHA and 5 patients whose tumors did not demonstrate therapeutic response. If an entire FFPE block is not available, PI's will request up to 20 unstained slides (10 microns). One or two 5 micron slides will be requested and used for validations. of the recurring t(6:9) MYB-NFIB translocation and measurement of HR23B protein levels A deidentified, coded copy of the pathology report will also be sent with the FFPE block or slides.

Whole blood was also collected on most of the patients at the time of enrollment in the original study. Karmanos will send white blood cells from the blood to TGen to serve as patient matched germline DNA. If no blood exists from a selected patient, or the blood was of poor quality, Karmanos study personnel will recontact the patient to obtain informed consent and to draw a new blood sample.

Blood samples will be spun down to remove the white blood cells from the red blood cells (see Blood Sample Collection below). The white blood cell pellet will be snap frozen using liquid nitrogen and stored at -80° until shipping on dry ice to TGen.

Blood Sample Collection

Peripheral Blood Mononuclear Cells (PBMCs) for White Blood Cell Pellets

The procedures for Processing Peripheral Blood Mononuclear Cells (PBMC) are described below:

Equipment

- One BD Vacutainer CPT Cell Preparation Tube with sodium heparin
- 15 ml size plastic conical centrifuge tubes with caps
- Transfer pipettes
- Temperature-controlled centrifuge with swinging bucket rotor and adapters for 16 X 125 mm tube size
- Coulter counter or hemocytometer

Reagents

- Phosphate Buffered Saline (PBS) without Ca^{++} or Mg^{++}
- 0.4% Trypan blue
- BioRad BioPlex Cell Lysis Kit (BioRad, Cat # 171-304011)

Procedures

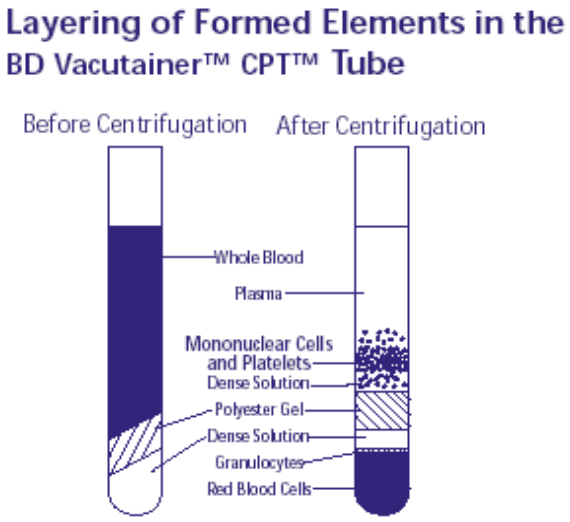
Blood Collection

1. Samples will be collected into 1BD Vacutainer CPT tube with sodium heparin. These will be kept at room temperature (18-26^{o}C) and properly labeled with patient information and study number. .
2. Once blood is collected, the tubes will be inverted approximately 8-10 times to ensure that the whole blood is mixed thoroughly with the anticoagulant.
3. The tubes will be centrifuged within 2 hr of collection at room temperature for 30 min at 1700 RCF (relative centrifugal force).

   Note: The tube will be checked to be in the proper centrifuge adapter. Excessive centrifuge speed (over 2000 RCF) may cause tube breakage, exposure to blood and possible injury. To calculate the correct centrifuge speed for a given RCF, the following formula is used:

$$\text{RPM Speed Setting} = \sqrt{\frac{(\text{RCF}) \times (100{,}000)}{(1.12) \times (r)}}$$

   The variable "r" is the radial distance in centimeters from the centrifuge center post to the tube bottom, when the tube is positioned horizontally. RCF is the desired relative centrifugal force, 1700 in this case. (2.5cm = 1 inch).
4. After centrifugation, mononuclear cells and platelets will be in a whitish layer just under the plasma layer (see below figure).



5. The plasma layer will be removed, taking care not to disturb the PBMC layer, and discarded.
6. The PBMCs will be washed with ice-cold PBS, centrifuging at 300RCF for 10 min. Before centrifuging, an aliquot of 20uL will be taking for counting in a cell counter or by hemocytometer.
7. The cells will be counted and the cell number will be recorded on the label which is to be placed on the cryovial. When the centrifugation is finished, the supernatant will be taken off and discarded.
8. The bottom of the centrifuge tube will be flicked to resuspend the cells and 1 mL of BioRad BioPlex cell wash buffer will be added. The tube will be centrifuged again at 300 RCF for 10 minutes. The supernatant will be removed by transfer pipet and the last bit of supernatant removed using a micropipetter.
9. The lysates will be prepared by adding BioRad BioPlex lysis buffer. The cell counts will be used to determine the quantity to use. For example, if the cell counts ar $> 10 \times 10^6$, 75 uL will be used. If the cell number is $> 2.5 \times 10^6$ but $< 10 \times 10^6$, 50 uL will be used; and if the cell number is $< 2.5 \times 10^6$, 25 uL will be used. The cells will be incubated with the lysis buffer for 30 minutes at 4 ^{o}C.
10. The cells will be sonicated on ice for 3 seconds at setting 6 and the cell lysate will be frozen at -70^{o}C or -80 ^{o}C.

All samples and data will be de-identified and coded with a unique study code assigned by Karmanos study personnel. A key linking the patient name to the study code will be maintained and stored by Karmanos study personnel. TGen will not receive any patient identifiable information before, during or after this study is conducted.

Sample Shipment

All samples will be shipped to: **The TGen Barrett Laboratory**
C/O Elizabeth Lenkiewicz
13208 E. Shea Blvd.
Scottsdale, Arizona 85259
602- 358-8839

FFPE Blocks or Slides

When shipping the FFPE block, place the block in a ziplock bag and wrap in bubble wrap or another type of padded material prior to shipping. The zip lock bag can be placed into a FedEx envelope or small shipping box.

If sending slides, pack the labeled slides into plastic slide cassette(s). Tape plastic slide cassettes shut and wrap in bubble wrap or another type of padded material prior to shipping. Place the slide cassettes into an outer shipping box.

White Blood Cell Pellets
Place container with frozen specimen in a ziplock bag. Place 2-3 inches of dry ice in the bottom of a Styrofoam cooler. Place bag in the center of the cooler on top of the dry ice, and then fill the cooler the rest of the way with dry ice (preferably pelleted). Place a single paper-towel or piece of paper across top of ice, then put lid on the cooler and tape the lid tightly to the cooler, sealing all the way around the lid. **Ship Monday through Wednesday only unless prior notification is made is made with TGen. Do not ship the day before a U.S. Holiday.**

APPENDIX E- PD Generic SOP: Storage and Shipping

PD Sample Storage

Archival Tissue:

- Shipping of paraffin blocks/slides should be done with a padded envelope and shipped to KCI. Paraffin blocks should be wrapped in a plastic bag before placement in the envelope. Slides should be put in an appropriate slide case before placement in the envelope.

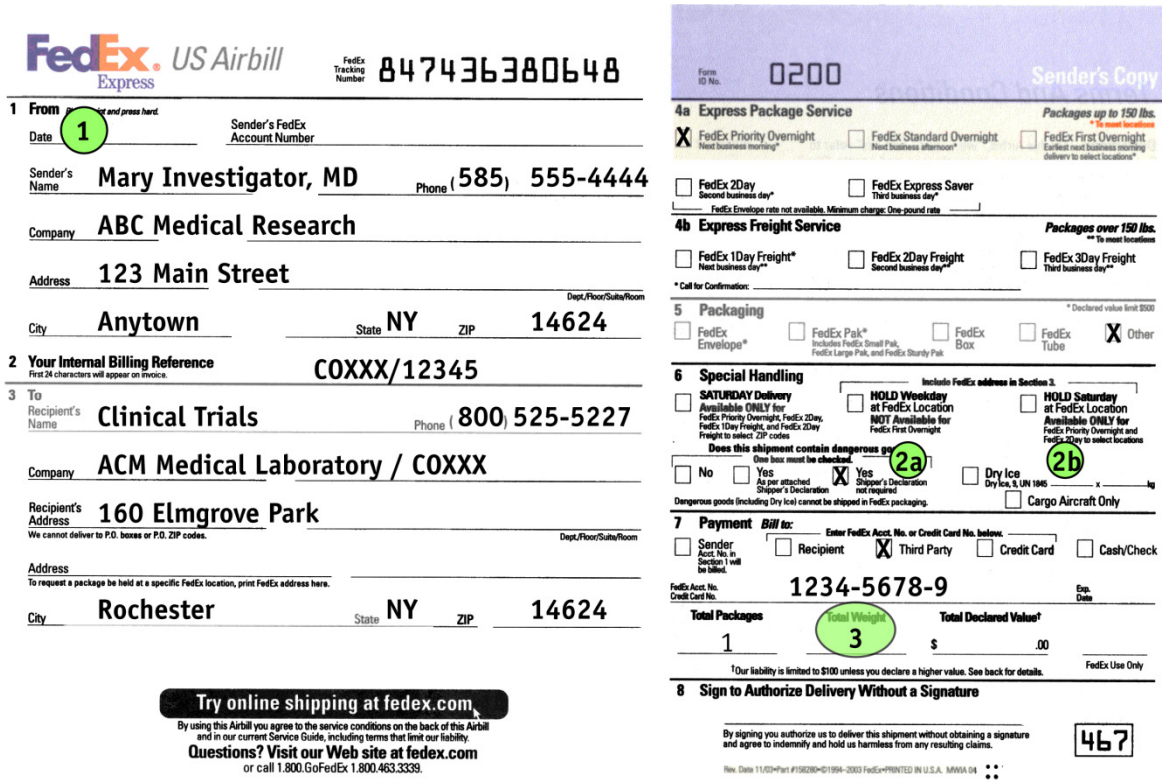
PD Sample Shipping

- **When to ship out the sample?**
- **How to pack the frozen sample?**
  - Verify each specimen is labeled properly.
  - Verify spread sheet for the box contents is accurate, so that individual samples can rapidly identified.
  - Place box into a large biohazard bag with absorbent strip. Seal bag completely.
  - Insert sealed biohazard bag into white "Saf-T-Pak" envelope.
  - Place copies of spread sheet of box contents into the "Saf-T-Pak" envelope, between the plastic biohazard bag and envelope. Place the envelopes containing storage box inside cooler
  - Fill cooler completely with dry ice.
  - Place cover on the cooler. (Do Not Tape Cooler Closed).
  - Place cooler in the fiberboard container, close the cardboard flaps and secure with tape.
  - Complete Shipper's Information on the outside of kit box (name, address, and phone).
  - Affix completely filled-out airbill on top of kit box in designated area.

Ship Frozen Samples no Later Than Wednesday

- **Complete the following items on the airbill:**
  - 1. Address as provided in the study protocol
  - 2.a Shipment date
  - 2.b Verify the box is checked for "**Yes. Shippers Declaration not required**"
  - 2.c Check the box for "dry ice", record the # of packages and record the weight of dry ice.
  - 3. Enter the total weight of the package- this cannot be pre-printed and **must** be filled in by the sender.

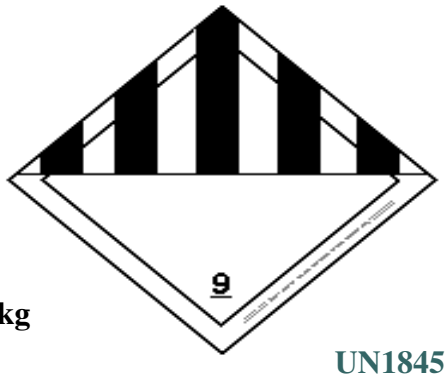
- **Affix completely filled-out air bill on top of the box in designated area.**



Completing Dry Ice Information

- Complete and affix the dry ice label on the outside of the shipping container.
- Contact the local Federal Express office to arrange for diagnostic specimen pick up.
- **Pick-up must be the same day as packaging. Do not leave the box for pick-up the next day**, as this may severely compromise testing.

Shippers declaration not required
Part B is required
Dry ice amount must be in kilograms
Note 2.2 lbs = 1 kg



Airbills must have the following:

- **"Dangerous Goods shippers declaration not required"**
- **Dry Ice; 9; UN1845**

DRY ICE _____kg

UN1845

Shippers name and address
_____
_____
_____

Recipient address

Notify the Recipient about the Shipment

- Please email the respective recipient when the samples are shipped
  - WSU Pharmacology Laboratory Contact:
    - Richard Wiegand (wiegandr@karmanos.org)
  - TGen Contact: (602) 343 8839
  - **Elizabeth Lenkiewicz** (Elenkiewicz@tgen.org)

- Inform which samples that are included in the shipment and FedEx tracking number in the email

APPENDIX F- Enzyme inducing anti-convulsants

The following agents are potential hepatic enzyme inducing antiepileptic drugs (EIAEDs):
Carbamazepine (Tegretol, Tegretol XR, Carbatrol)
Oxcarbazepine (Trileptal)
Phenytoin (Dilantin, Phenytek)
Fosphenytoin (Cerebyx)
Phenobarbital
Pentobarbital
Primidone (Mysoline)

The following agents are non (non- IAEDs):
Valproic acid (Depakote, Depakene, Depacon)
Gabapentin (Neurontin)
Lamotrigine (Lamictal)
Topiramate (Topamax)
Tiagabine (Gabitril)
Zonisamide (Zonegran)
Levetriacetam (Keppra)
Clonazepam (Klonopin)
Clonozam (Frisium)

APPENDIX G- PATIENT'S MEDICATION DIARY (300 mg)

300mg Suberoylanilide Hydroxamic Acid (SAHA)/Vorinostat
Today's Date _____

CTEP-assigned Protocol #_____
Local Protocol # _____

Patient Name_____(*initials acceptable)* **Patient Study ID** _____

INSTRUCTIONS

1. Complete one form for each cycle of treatment.
2. You will take **three 100mg** vorinostat capsules each day (300 mg total dose), once a day, at approximately the same time, continuously over a 28 day period.
3. Swallow each capsule whole with a full glass of water. Do not chew or open the capsules. If capsule is broken and the powder of the capsules gets on skin, wash the exposed area with as much water as necessary. Inform investigator or nurse if that occurs.
4. Record the date and time you took the capsule.
5. If you have any comments or notice any side effects, please record them in the Comments column.
6. Please bring this form and your bottle of vorinostat when you return for your Day 28 appointment.
7. In case of errors, please place a single slash mark through the error and initial it. Please do not white out any error or scribble it out with ink. Please do not write the correct information directly over the error, but on a separate line next to the error.

Day	Date	Time of Dose	Comments
1			
2			
3			
4			
5			
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11			
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28			

Patient's signature: _____

APPENDIX H- PATIENT'S MEDICATION DIARY (400 mg)

400mg Suberoylanilide Hydroxamic Acid (SAHA)/Vorinostat
Today's Date _____

CTEP-assigned Protocol #_____
Local Protocol # _____

Patient Name_____(*initials acceptable)* **Patient Study ID** _____

INSTRUCTIONS

8. Complete one form for each cycle of treatment.
9. You will take **four 100mg** vorinostat capsules each day (400 mg total dose), once a day, at approximately the same time, continuously over a 28 day period.
10. Swallow each capsule whole with a full glass of water. Do not chew or open the capsules. If capsule is broken and the powder of the capsules gets on skin, wash the exposed area with as much water as necessary. Inform investigator or nurse if that occurs.
11. Record the date and time you took the capsule.
12. If you have any comments or notice any side effects, please record them in the Comments column.
13. Please bring this form and your bottle of vorinostat when you return for your Day 28 appointment.
14. In case of errors, please place a single slash mark through the error and initial it. Please do not white out any error or scribble it out with ink. Please do not write the correct information directly over the error, but on a separate line next to the error.

Day	Date	Time of Dose	Comments
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4			
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Patient's signature: _____

APPENDIX I: THERAPEUTIC RESPONSE ASSESSMENT USING VOLUMETRIC DENSITY

Department of Radiology and Imaging Sciences investigator Dr. Les Folio and colleagues will retrospectively evaluate CT and/or MRI of patients treated in the current trial. These cross sectioning imaging (CT/MR) have already been obtained. No new imaging will be performed.

All the CTs and/or MRI will be evaluated at the National Cancer Institute, under Dr. Folio and colleagues.

All pre-specified target lesions will be assessed at specific time-points: baseline, date of best response, a mid-treatment time point and most recent imaging.

The specific aims are to:

1. Retrospectively compare VTV (all lesions; volume/density) vs. pre-determined RECIST (axial only)

2. Correlate VTV, RECIST, and treatment response (partial response, progressive disease, stable disease and stable disease over 6 months)

Registration and Lesion Management Application (algorithm/ software):
Automated registration and semi-automatic segmentation of all target lesions will be performed on serial CT scans or MRI using PACS (Picture Archiving and Communication System) .

Lesion Management Algorithm radiology imaging software (Carestream vue PACS v12, Rochester, NY).
First, each scan is manually opened and globally registered to align axial planes between the two scans. The global registration algorithm employs a fully automated volumetric voxel-based rigid registration algorithm.1 Second, tumors are manually located, which is performed by singly clicking on tumors, or by extending a measurement markup across conglomerated or abutting lesions. The PACS software then performs a refined registration by modifying the initial global

registration dataset to enhance localization of the selected metastatic tumor across serial exams.2 Next, the software automatically segments the target tumor on both CT and/or MR scans using enhanced tumor edge detection (not necessarily defining separate lesions). 3 Finally, the PACS software automatically couples segmentation and tumor measurement, providing longest axial tumor length, perpendicular to longest axial length, short axis, mean HU density, and tumor volume of interest (VOI) in the segmented area. Observers then edit the typical segmentation outline using a freeform segmentation tool to exclude non-tumoral components.

1 Hawkes DJ. Algorithms for radiological image registration and their clinical application. J. Anat. (1998) 193, pp. 347-361.
2 Hong H, Lee J, Yim Y. Automatic lung nodule matching on sequential CT images. Comput Biol Med. 2008 May;38(5):623-34. Epub 2008 Apr 15.
3 Maintz JBA, Viergever MA. A survey of medical image registration. Med Image Anal 1998; 2:1–36.
All volume, size, and volumetric density measurements of the target tumors will be recorded on both the baseline and follow-up CT scans. All measurements will be performed by two observers (college graduates with no formal medical training) under other authors with over 10 years of experience in CT as body radiologists. The radiologists will verify observer segmentations, in consensus, when needed.