To: CTEP Protocol and Information Office

From: Matthew Ingham, M.D.

Date: March 25, 2025

Re: Response to Disapproval of Amendment #8 of Protocol #10330: "A Phase 2 Study of Belinostat and SGI-110 (Guadecitabine) or ASTX727 for the Treatment of Unresectable and Metastatic Conventional Chondrosarcoma"

SUMMARY OF CHANGES – Protocol

I. Comments Requiring a Response– Administrative & Editorial Issues:

#	Section	Comments
1.	**ICD Risks**	Please add ASTX727 risk list (CAEPR version 2.2, December 19, 2024), as attached. **PI Response:** Risks updated in applicable consent. ASTX727 risks not described in SGI-110 consent.

II. Recommendations:

#	Section	Comments
2.	***8.1.4.1***	Agent Ordering and Agent Accountability Update the second paragraph to: Submit agent requests through the PMB AURORA application. Access to AURORA requires the establishment of credentials necessary to access secure NCI Clinical Oncology Research Enterprise (CORE) systems, maintenance of an "active" account status, a "current" password, and active person registration status. For questions about drug orders, transfers, returns, or accountability, call or email PMB any time or use the dialog function in AURORA to communicate with PMB staff. Refer to the PMB's website for specific policies and guidelines related to agent management. **PI Response: Updated**
3.	***8.1.4.2***	Agent Inventory Records In the first paragraph, replace "careful record" with "complete accountability". Add the following as a second paragraph: Product Quality Complaint (PQC): A product quality complaint is defined as any suspicion of a product defect related to a potential quality issue during manufacturing, packaging, release testing, stability monitoring, dose preparation,

#	Section	Comments
		storage or distribution of the product, or delivery system. Not all PQCs involve a study subject. Lot or batch numbers are of high significance and need to be provided where and when possible. PQC must be reported to the PMB as soon as the PQC is identified. Report PQC to PMB at PMBAfterHours@mail.nih.gov or by using the dialog function in AURORA to communicate with PMB staff. **PI Response: Updated and Added**
4.	***8.1.5***	Investigator Brochure Update the entire paragraph to: The current versions of the IBs for the agents will be accessible to site investigators and research staff through the PMB AURORA application. Access to AURORA requires the establishment of credentials necessary to access secure NCI Clinical Oncology Research Enterprise (CORE) systems, maintenance of an "active" account status, a "current" password and active person registration status. Questions about IB access may be directed to the PMB IB Coordinator via email. **PI Response: Updated**
5.	***8.1.6***	Useful Links and Contacts Change "PMB Online Agent Order Processing (OAOP) application:" to "PMB Agent Inventory Management System (AURORA) application: https://ctepcore.nci.nih.gov/aurora/login". **PI Response: Updated**
6.	***8.1.4***	Insert the following as a new subsection: Material Safety Data Sheets The current versions of the material safety data sheets (MSDS or SDS) for PMB-distributed agents will be accessible to site investigators and research staff through the PMB AURORA application. Questions about MSDS access may be directed to the PMB at PMBAfterHours@mail.nih.gov or by using the dialog function in AURORA to communicate with PMB staff. **PI Response: Added as section 8.1.4.2**
7.	***8.1.4***	Insert the following as a new subsection: Agent Shortages Specific guidance on how to address agent shortages for patients already enrolled on a clinical study as well as how to manage potential enrollment of new patients is provided at https://ctep.cancer.gov/branches/pmb/drug_shortages.htm. Treatment plan modifications being made to avoid immediate hazard to patients is

#	Section	Comments
		permissible under the Department of Health and Human Services (HHS) regulations at 45 CFR 46.103(b)(4)(iii). In accordance with HHS regulations, local investigators must promptly inform the IRB of record of this unanticipated problem and the management plan for the trial. **PI Response: Added as section 8.1.4.3**

III. Protocol changes in response to the Request for Rapid Protocol Amendment dated 02/25/25:

#	Section	Comments
1.	Header	Updated version date
2.	Title page	• Updated version date and added Amendment #8 to list of versions • Updated local protocol number • Updated ClinicalTrials.gov Identifier
3.	10.3.3 Expedited Reporting Guidelines	Specific Protocol Revisions to Address Risk Mitigation Plan • The updated AE Reporting Table

4.	10.1.1.2 CAEPR for ASTX727	Revision of the Protocol CAEPR
		• The SPEER grades have been updated.
		• Added New Risk:
		  • Also Reported on ASTX727 Trials But With Insufficient Evidence for Attribution: Confusion; Dehydration; Hypokalemia; Intracranial hemorrhage
		• Increase in Risk Attribution:
		  • Changed to Less Likely from Also Reported on ASTX727 Trials But With Insufficient Evidence for Attribution: Aspartate aminotransferase increased
		• Decrease in Risk Attribution:
		  • Changed to Less Likely from Likely: Fatigue
		  • Changed to Also Reported on ASTX727 Trials But With Insufficient Evidence for Attribution from Less Likely: Dizziness; Headache
		• Deleted Risk:
		  • Also Reported on ASTX727 Trials But With Insufficient Evidence for Attribution: Blood bilirubin increased; Colitis; Weight loss
		• Modified Specific Protocol Exceptions to Expedited Reporting (SPEER) reporting requirements:
		  • Added: Upper respiratory infection; Urinary tract infection
		• Provided Further Clarification:
		  • Infection is now reported as Lung infection/pneumonia, sepsis, tooth infection, upper respiratory infection, and urinary tract infection.

#	Section	Comments
		• Footnote #2 previously reported as, "Infection includes all 75 sites of infection under the INFECTIONS AND INFESTATIONS SOC" was deleted. • Footnote #2 is now reported as "The SPEER grade for febrile neutropenia should only be applied to cases of neutropenic fever • associated with hematologic malignancies and NOT for solid tumors." • Additional changes due to migrating from CAEPR version 2.0 to 2.2 • Replacing Cedazuridine with E7727

NCI Protocol #: 10330

Local Protocol #: 202008172

ClinicalTrials.gov Identifier: NCT04340843

TITLE: A Phase 2 Study of Belinostat and SGI-110 (Guadecitabine) or ASTX727 for the Treatment of Unresectable and Metastatic Conventional Chondrosarcoma

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NCI-Supplied Agents: Belinostat (PXD-101) (NSC # 726630), SGI-110 (Guadecitabine) (NSC # 780463), ASTX727 (NSC# 820631)

IND #: [redacted]
IND Sponsor: DCTD, NCI

Protocol Type / Version # / Version Date:
Original / December 12, 2019
Revision 1 / February 27, 2020
Revision 2a / May 1, 2020
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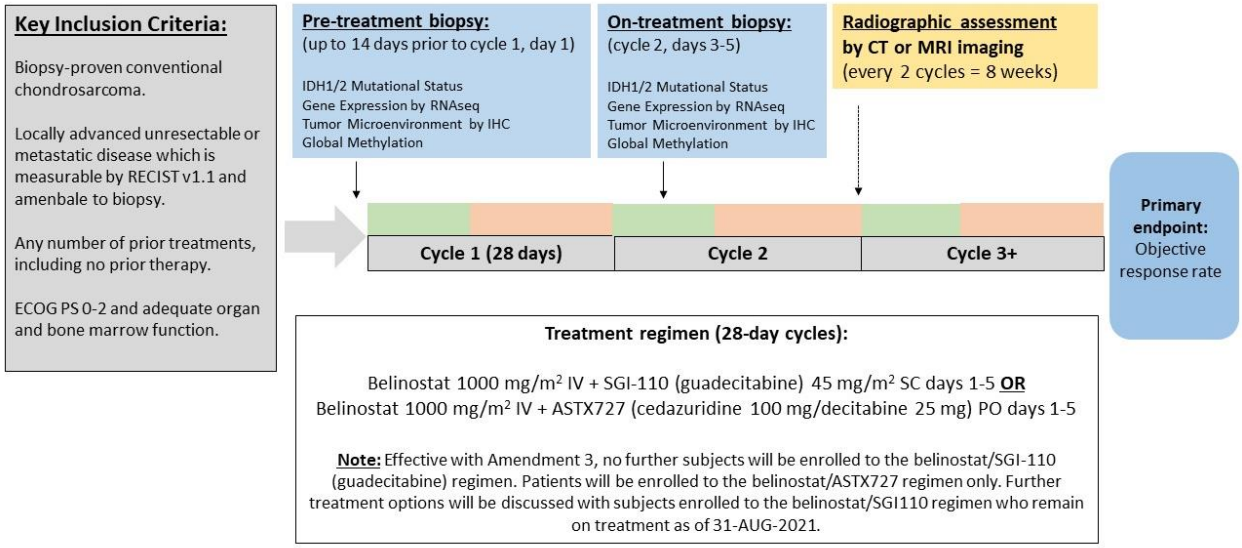
Amendment 2 / September 8, 2020
Amendment 5 / June 15, 2021
Amendment 6 / June 15, 2022
Amendment 7 / February 14, 2023
Amendment 8 / March 7, 2025
Amendment 9 / March 25, 2025

SCHEMA


Key Inclusion Criteria:
Biopsy-proven conventional chondrosarcoma.
Locally advanced unresectable or metastatic disease which is measurable by RECIST v1.1 and amenbale to biopsy.
Any number of prior treatments, including no prior therapy.
ECOG PS 0-2 and adequate organ and bone marrow function.
Pre-treatment biopsy:
(up to 14 days prior to cycle 1, day 1)
IDH1/2 Mutational Status
Gene Expression by RNAseq
Tumor Microenvironment by IHC
Global Methylation
On-treatment biopsy:
(cycle 2, days 3-5)
IDH1/2 Mutational Status
Gene Expression by RNAseq
Tumor Microenvironment by IHC
Global Methylation
Radiographic assessment by CT or MRI imaging
(every 2 cycles = 8 weeks)
Cycle 1 (28 days)
Cycle 2
Cycle 3+
Primary endpoint:
Objective response rate
Treatment regimen (28-day cycles):
Belinostat 1000 mg/m2 IV + SGI-110 (guadecitabine) 45 mg/m2 SC days 1-5 OR
Belinostat 1000 mg/m2 IV + ASTX727 (cedazuridine 100 mg/decitabine 25 mg) PO days 1-5
Note: Effective with Amendment 3, no further subjects will be enrolled to the belinostat/SGI-110 (guadecitabine) regimen. Patients will be enrolled to the belinostat/ASTX727 regimen only. Further treatment options will be discussed with subjects enrolled to the belinostat/SGI110 regimen who remain on treatment as of 31-AUG-2021.

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1. OBJECTIVES

1.1 Primary Objectives

1.1.1 To conduct a phase 2 clinical trial to evaluate whether combination treatment with belinostat and ASTX727 shows preliminary evidence of clinical activity in unresectable or metastatic conventional chondrosarcoma (CS) using an objective response rate endpoint.

1.2 Secondary Objectives

1.2.1 To evaluate the toxicity profile associated with belinostat and ASTX727.

1.2.2 To evaluate the progression free survival (PFS) associated with belinostat and ASTX727.

1.2.3 To evaluate the toxicity profile, objective response rate and progression free survival among the initial six patients treated with belinostat and SGI-110 (guadecitabine) prior to Amendment 5 in which ASTX727 was substituted for SGI-110 (guadecitabine).

1.3 Correlative Objectives

1.3.1 To determine the *IDH1/2* mutational status of subject's tumors and to evaluate for a relationship between presence of *IDH1/2* mutation and clinical benefit from study treatment.

1.3.2 To conduct RNAseq analysis using baseline and on-treatment tissue biopsies to study the effects of study treatment on CS gene expression patterns and identify candidate genes which may underlie treatment efficacy.

1.3.3 To evaluate for changes in global DNA methylation levels using baseline and on-treatment biopsies and correlate changes in global methylation with clinical benefit from study treatment.

1.3.4 To use multiplex immunohistochemistry to interrogate the immune microenvironment in baseline and on-treatment tissue biopsies to define changes in infiltrating immune cell subsets and PD-L1/MHC expression by immune and tumor cells associated with study treatment.

2. BACKGROUND

2.1 Study Disease

CS is a malignant bone tumor characterized by the production of cartilaginous matrix by neoplastic cells. CS accounts for 20-27% of primary malignant bone cancers and is the second most common primary bone tumor after osteosarcoma (Dorfman and Czerniak, 1995). CS most often occurs in the pelvis but can arise in the femur, humerus, ribs and at other sites. Distinct clinical and histologic subtypes have been described which have variable molecular characteristics, biologic behavior, and responsiveness to treatment (Polychronidou *et al.*, 2017). Conventional chondrosarcomas account for 90% of CS and are subdivided into primary (central) chondrosarcomas which form within the medullary cavity and much less common secondary (peripheral) chondrosarcomas which arise from the cartilage cap of pre-existing benign osteochondromas. The remaining 10% of CS are composed of the dedifferentiated, clear cell, and mesenchymal subtypes. Histologic grade is predictive of local recurrence and metastatic spread. Conventional CS are often low or intermediate grade and pursue an indolent course with low metastatic potential; however, a subset are high grade with a poor prognosis (Bovee *et al.*, 2005). Lower grade tumors often recur with higher histological grade and metastatic potential (Gelderblom *et al.*, 2008).

Surgical resection is the mainstay of CS treatment. CS are inherently resistant to chemotherapy and radiotherapy. Possible explanations for this resistance include low mitotic fraction, limited drug penetration due to poor vascularity and dense extracellular matrix, activation of multidrug resistance pumps, and increased expression of antiapoptotic factors (Polychronidou *et al.*, 2017). Currently, no FDA approved therapies exist for conventional CS. There is an unmet need for new treatment options for refractory, inoperable and metastatic CS. Studies evaluating cytotoxic agents and immunotherapy in CS have shown limited efficacy. In a retrospective study of 180 patients with advanced CS treated with various chemotherapeutic agents, the overall response rate was 15% (Italiano *et al.*, 2013). 73% of patients were treated with anthracycline-based regimens and 55% received combination cytotoxic therapy. Response rates for mesenchymal CS (31%) and dedifferentiated CS (21%) were significantly higher than for conventional CS (12%) (Italiano *et al.*, 2013). A phase 2 trial of gemcitabine with docetaxel in 25 patients with CS demonstrated a similarly low objective response rate of 8% (Fox *et al.*, 2012). Immunotherapy, which has shown promise in multiple cancers, was evaluated in dedifferentiated CS where PD-L1 expression is observed (Kostine *et al.*, 2016). A phase 2 trial of pembrolizumab, an anti-PD-1 antibody, included 5 patients with dedifferentiated CS, and one patient achieved an objective response while three showed progressive disease (Tawbi *et al.*, 2017). Conventional CS does not commonly express PD-1 and the efficacy of existing immunotherapy-based approaches is likely limited.

Mutations in the *IDH1* or *IDH2* genes are observed in 50-60% of conventional CS and are implicated in reprogramming the metabolic activity of CS cancer cells, similar to findings in AML, glioblastoma, and cholangiocarcinoma (Amary *et al.*, 2011). Normally, isocitrate dehydrogenase (IDH) catalyzes the oxidative decarboxylation of isocitrate to produce α-ketoglutarate (αKG), carbon dioxide, and NADPH in the Krebs cycle. Mutations in the *IDH* genes lead to conversion of αKG to the oncometabolite D-2-hydroxyglutarate (D-2HG). D-2HG

accumulation competitively inhibits αKG-dependent enzymes, which are involved in modulating chromatin structure, DNA methylation, cellular signaling, response to hypoxia, and collagen maturation. The dysregulation of these processes results in widespread epigenetic modifications and directly contributes to tumorigenesis (Molenaar *et al.*, 2018). Specifically, D-2HG inhibits the ten-eleven translocation (TET) family of demethylase enzymes, which leads to DNA hypermethylation causing transcriptional silencing of tumor suppressor genes. *In vitro*, inhibitors of DNA methyltransferases (DNMTi) reverse global hypermethylation, thereby restoring expression of differentiation genes and promoting cell differentiation in CS (Borodovsky *et al.*, 2013; Gagné *et al.*, 2017). The Jumonji C-domain lysine demethylases are another family of αKG-dependent dioxygenases that are inhibited by D-2HG. Inhibition causes increased lysine methylation of histone tails, altering chromatin structure and gene expression and blocking cell differentiation. Based on these findings and the high prevalence of *IDH1/IDH2* mutation among patients with CS, epigenetic dysregulation and impairment of differentiation play an important role in CS pathogenesis and represent an attractive target for novel therapeutic approaches. Impairment of differentiation through epigenetic dysregulation of various cell cycle and other genes appears relevant in *IDH* wild-type CS as well (Liu *et al.*, 2018).

2.2 CTEP IND Agents

2.2.1 Belinostat (PXD-101)

2.2.1.1 Mechanism of Action

Belinostat, FDA-approved in 2014 for relapsed/refractory peripheral T-cell lymphoma (PTCL), is a hydroxamate derivative that inhibits both class I and II HDACs (pan-HDACi) (Molife *et al*., 2011). HDAC inhibitors (HDACi) exert multiple biological effects in cancer cells, including induction of differentiation/apoptosis, cell cycle/mitotic arrest, and autophagic cell death. Malignant cells possess higher levels of HDACs than normal tissues, and normal cells are relatively resistant to DNA damage and cell death induced by HDACi (Ungerstedt *et al*., 2005; Lee *et al*., 2010). As noted above, HDACi can modify gene expression secondary to acetylation of histones and by altering the acetylation status of transcription factors and other proteins involved in transcription. One of the most important HDACi-induced genes is the cyclin-dependent kinase inhibitor CDKN1A (p21WAF1/CIP1) (Richon *et al*., 2000). HDACi-induced increases in the level of this protein (Rosato *et al*., 2003), as well as that of p16 (CDKN2 or INK4) and p27, decreased expression of cyclins A and D, and dephosphorylation of retinoblastoma (Rb) protein, all contribute to HDACi-induced cell cycle arrest. HDACi also induce telomerase activity (Bhalla, 2005). HDACi acetylate histone H3K9 in pericentromeric chromatin, thus interfering with assembly of the kinetochore. This leads to mitotic arrest at prometaphase, followed by aberrant mitosis with chromosome segregation defects and ultimately apoptosis (Quintas-Cardama *et al*., 2011). Furthermore, HDACi lethality has been related to disruption of various cell cycle checkpoints (Warrener *et al*., 2003) as well as induction of mitotic slippage (Stevens *et al*., 2008).

HDACi activate both the intrinsic and extrinsic pathways of apoptosis. They increase levels of both Fas/Fas ligand and DR5/TRAIL (Insinga *et al*., 2005, Nebbioso *et al*., 2005) and down-

regulate c-FLIP (Aron *et al*., 2003) in leukemic cells, but not in normal cells. HDACi increase levels of the pro-apoptotic Bcl-2 family proteins Bax, Bak, Bim (Chen *et al*., 2009), and Bmf; down-regulate the anti-apoptotic proteins Bcl-2, Bcl-xL, Mcl-1, XIAP (Rosato *et al*., 2006), and survivin; induce conformational change of Bax, and enhance the cleavage and subsequent activation of Bid (Bhalla, 2005; Quintas-Cardama *et al*., 2011; Ruefli *et al*., 2001).

HDACi induce DNA damage through increased generation of reactive oxygen species (ROS) (Rosato *et al*., 2003, Ruefli *et al*., 2001) and by impairing mechanisms of DNA repair (Bose *et al*., 2014). HDAC3 is essential for the maintenance of chromatin structure and genome stability (Bhaskara *et al*., 2010). HDACi decrease levels of the non-homologous end-joining (NHEJ) proteins Ku80 and Rad50 and acetylate Ku70 (Subramanian *et al.,* 2005), thereby decreasing its DNA binding. HDACi-mediated apoptosis is, in part, dependent on ROS generation, with subsequent DNA and mitochondrial damage and activation of the intrinsic apoptotic pathway (Bose *et al*., 2014). In addition, HDACi inhibit homologous recombination (HR) DNA repair (Kachhap *et al*., 2010). Recently, hypoacetylation of histone H3 lysine 56 has been shown to be necessary for efficient NHEJ (Miller *et al*., 2010), raising the possibility that HDACi may also disable this repair mechanism. Disabling certain cytoprotective pathways (*e.g*., NF-κB) promotes HDACi-mediated DNA damage and cell death in human leukemia cells (Dai *et al*., 2005).

Induction of autophagy is another important mechanism of cell death in response to HDACi (Robert *et al*., 2011; Shubassi *et al.,* 2012). Finally, HDACi, particularly HDAC6 inhibitors (Bali *et al.,* 2005), abrogate chaperone protein (Hsp90) function via hyperacetylation (Bhalla, 2005; Lane and Chabner, 2009; Quintas-Cardama *et al*., 2011). This leads to down-regulation, via polyubiquitinylation and proteasomal degradation, of misfolded Hsp90 client proteins such as Bcr-Abl (Nimmanapalli *et al*., 2003), FLT3, Akt, Raf (Bali *et al*., 2005) and checkpoint kinase 1 (Chk1) (Arlander *et al*., 2003; Brazelle *et al*., 2010). HDACi may also affect tumor cell survival by blocking tumor angiogenesis, and by inhibiting intracellular stress-response pathways (Lane and Chabner, 2009).

2.2.1.2 Summary of Clinical Experience

Although belinostat has mostly been administered intravenously (IV) in clinical trials, an oral formulation has also been tested (Steele *et al*., 2011). IV belinostat displays linear pharmacokinetics (PKs) with respect to the maximum concentration (C_{max}) and the area under the "concentration time" curve (AUC) (Steele *et al*., 2008), without significant accumulation (Yeo *et al*., 2012). The intermediate elimination half-life is 0.3 to 1.3 hrs and independent of dose (Steele *et al*., 2008). Belinostat PKs are unaltered by concurrent administration with carboplatin and paclitaxel (Lassen *et al*., 2010). Glucuronidation by UGT1A1 is by far the major route of belinostat elimination in humans (Wang *et al.,* 2013). The drug has 5 major metabolites, which are not expected to have anticancer effects, and the parent drug and metabolites can be quantified in human plasma by means of a validated liquid chromatography-tandem mass spectrometry assay (Kiesel *et al*., 2013). *In vitro* studies indicate that belinostat is a weak/moderate inhibitor of cytochrome P450 family 2 subfamily C member 8 (CYP2C8) and a moderate to strong inhibitor of CYP2C9.

In a phase 1 study in patients with advanced solid tumors, the maximum tolerated dose (MTD) of belinostat was 1000 mg/m^2 IV daily, administered over 30 minutes on Days 1-5 of a 21-day cycle (Steele *et al*., 2008). Dose-limiting toxicities (DLTs) included grade 3 fatigue, diarrhea, and atrial fibrillation, as well as grade 2 nausea/vomiting resulting in inability to complete a full 5-day cycle. A phase 1 study in patients with advanced hematologic neoplasms reported the same MTD (Gimsing *et al*., 2008). The most common treatment-related adverse events (AEs) (all grades) were nausea (50%), vomiting (31%), fatigue (31%), and flushing (31%). There was one case of grade 3 lymphopenia and two cases of grade 4 renal failure in the context of tumor lysis syndrome (TLS). The only related grade 3 events in more than one patient were fatigue and neurologic symptoms, and no cardiac events were noted. Oral belinostat was studied in 15 patients who were included in the phase 1 trial of IV belinostat in second or subsequent treatment cycles (Steele *et al*., 2011). High doses, up to 1000 mg/m^2 orally twice daily for 5 consecutive days, were found tolerable in this small study. In a phase 1 trial of oral belinostat in patients with lymphoma, the drug was found to be safe (no DLTs) at daily doses of 750-1250 mg on Days 1-14 every 3 weeks (Zain *et al*., 2009). In a phase 2 clinical trial, belinostat was administered IV at 1000 mg/m^2 daily on Days 1-5 every 21 days in patients with relapsed/refractory AML or newly diagnosed patients with AML over the age of 60 (Kirschbaum *et al.,* 2015). Belinostat was well tolerated; however, only 4 patients displayed stable disease (SD) for at least five cycles. A similar trial of belinostat at the same dose and schedule showed similar activity in myelodysplastic syndrome (MDS), with only one confirmed response (Cashen *et al*., 2012).

Belinostat has been found to be well tolerated in combination with carboplatin and paclitaxel in patients with solid tumors; in a phase 1 study, the maximal administered dose of belinostat was 1000 mg/m^2/d IV on Days 1-5 every 21 days, with carboplatin (AUC 5) and/or paclitaxel (175 mg/m^2) administered on Day 3 after belinostat (Lassen *et al*., 2010). Belinostat has also been evaluated in combination with 5-FU; cisplatin, doxorubicin, and cyclophosphamide (PAC, in advanced or recurrent thymic malignancies); cisplatin and etoposide; and 13-cis retinoic acid in phase 1 studies in patients with advanced solid tumors (Northfelt *et al*., 2007; Thomas *et al*., 2012; Balasubramaniam *et al*., 2013; Luu *et al*., 2013). In combination with a 96-hr infusion of 5-FU, 500 mg/m^2/d on Days 2-5, belinostat was well tolerated at doses up to 1000 mg/m^2 IV daily on Days 1-5 of a 21-day cycle (Northfelt *et al*., 2007). In combination with PAC (50/50/500 mg/m^2 IV every 3 weeks), the recommended phase 2 dose (RP2D) of belinostat was 1000 mg/m^2 over a 48-hr continuous IV infusion (Thomas *et al*., 2012). The RP2D of belinostat in combination with cisplatin 60 mg/m^2 on Day 1 and etoposide 80 mg/m^2/d on Days 1-3 of a 3-week cycle was 500 mg/m^2/24 h when administered by 48-hr continuous IV infusion (Balasubramaniam *et al*., 2013). In contrast, when combined with the non-cytotoxic 13-cis retinoic acid, 100 mg/m^2 daily on Days 1-14, the MTD of belinostat was not reached even at 2000 mg/m^2 daily on Days 1-5 every 21 days (Luu *et al*., 2013). The combination of belinostat, 1000 mg/m^2/d IV and azacitidine, 75 mg/m^2/d subcutaneously on Days 1-5 every 28 days has been found to be feasible and active in patients with advanced myeloid malignancies (Odenike *et al*., 2011). Furthermore, this dose of belinostat, administered on Days 1-5 and 8-12 of a 3-week cycle in conjunction with bortezomib (1.3 mg/m^2 on Days 1, 4, 8, and 11) was also found to be tolerable and active (1 complete response [CR], 2 partial responses [PRs], and 8 who have SD of 22 evaluable patients) in a phase 1 trial in patients with relapsed/refractory acute leukemia or MDS (Holkova *et al*., 2012). In this trial, the CR was attained in a heavily pretreated patient

with MLL-rearranged AML, while another patient with AML arising from JAK2-mutated myelofibrosis maintained SD for 2.5 years (Holkova *et al*., 2012).

2.2.2 SGI-110 (Guadecitabine)

2.2.2.1 Mechanism of Action

SGI-110 (guadecitabine; 2'-deoxy-5-azacytidylyl-(3'→5')-2'-deoxyguanosine sodium salt) is a next-generation hypomethylating agent (HMA) which inhibits DNA methyltransferase (DNMT) to reverse epigenetic aberrant DNA methylation characteristic of many cancer cells (SGI-110 Investigator's Brochure, 2019; Issa *et al.*, 2015). First-generation epigenetically targeted HMAs include azacitidine and decitabine, which are both approved in the U.S. for all myelodysplastic syndrome (MDS) subtypes and in Europe for acute myeloid leukemia (AML) (both) and MDS (azacitidine only).

SGI-110 (guadecitabine) is a dinucleotide of decitabine (its active metabolite) and deoxyguanosine linked with a 3'→5' phosphodiester bond and was rationally designed to be more stable than decitabine to provide enhanced PK and pharmacodynamic properties. In addition to SGI-110's designed resistance to cytidine deaminase, the main enzyme responsible for degradation of decitabine, the gradual cleavage of the 3'→5' phosphodiester bond by phosphodiesterase Type 1 and other enzymes results in continuous release of decitabine, prolonging its short half-life of less than 30 minutes and exposure window (Issa *et al.*, 2015). The prolonged exposure window and reduced peak plasma concentrations of decitabine formed after dosing with subcutaneous (SC) SGI-110 (guadecitabine) may result in better access to target tissues and is the proposed basis for potential increased efficacy (*i.e.*, more efficient S-phase dependent incorporation into DNA of cancer cells to cause DNA hypomethylation and subsequent cellular differentiation or apoptosis) and potential reduced off-target toxicity compared with IV decitabine (SGI-110 Investigator's Brochure, 2019).

2.2.2.2 Summary of Clinical Experience

As of June 30, 2019, a total of 1,388 subjects (AML, MDS, HCC, and ovarian cancer), 1,235 of which had AML or MDS, were treated with SGI-110 (guadecitabine) (SGI-110 Investigator's Brochure, 2019). Of the 1,235 subjects with AML/MDS treated with SGI-110 (guadecitabine) in Astex/Otsuka-sponsored studies, 966 subjects (78%) had serious adverse events (SAEs). The most common SAEs were febrile neutropenia (32%), pneumonia (26%), and sepsis (13%). Across all studies, SAEs that occurred in ≥2% of subjects included febrile neutropenia, pneumonia, sepsis, bacteremia, cellulitis, pyrexia, septic shock, anemia, thrombocytopenia, and urinary tract infection. Related SAEs occurred in 327 subjects (26.5%). Related SAEs that occurred in ≥0.5% of subjects included febrile neutropenia, pneumonia, sepsis, cellulitis, thrombocytopenia, anemia, bacteremia, infection, febrile bone marrow aplasia, neutropenia, pyrexia, septic shock, and stomatitis.

A total of 33 subjects (2.7%) died from related SAEs (more than half [24 subjects] died from infections, including 9 from pneumonia and 6 from sepsis; 4 died from general disorders including general physical health deterioration, multiorgan failure, and multiple organ

dysfunction syndrome; 2 died from febrile neutropenia; and the rest died from various other events).

Common related AEs (incidence ≥10%) observed with SGI-110 (guadecitabine) treatment include thrombocytopenia, neutropenia, anemia, febrile neutropenia, nausea, injection site reaction, fatigue, injection site pain, diarrhea, decreased appetite, and constipation.

2.2.3 ASTX727

2.2.3.1 Mechanism of Action

ASTX727 is an orally bioavailable hypomethylating agent which consists of two components: cedazuridine and decitabine. Decitabine is a cytidine-nucleoside analogue which incorporates into DNA during S-phase and inhibits the function of DNMT1 resulting in reduced methylation of CpG residues and DNA hypomethylation. The earlier generation DNMT inhibitor decitabine is administered intravenously. Decitabine is poorly bioavailable after oral administration due to rapid inactivation by cytidine deaminase (CDA) in the gut and liver. Tetrahydrouridine, a CDA inhibitor, has been shown to increase the oral bioavailability of decitabine. Cedazuridine (E7727, Astex Pharmaceuticals) is a novel, orally bioavailable CDA inhibitor which increases the exposure of decitabine up to 14-fold, and may therefore be used in combination with oral decitabine to allow that agent to achieve clinically active levels.

2.2.3.2 Summary of Clinical Experience

The oral cedazuridine and decitabine combination was initially studied in a multicenter, open-label, phase 1 dose escalation study using a 3+3 dose escalation design (Savona *et. al.* 2020). The objective of this study was to establish an oral combination of decitabine and cedazuridine that would achieve similar pharmacokinetic parameters as 20 mg/m^2 of decitabine administered intravenously. Eligible patients were age 18 or older with a diagnosis of MDS or AML and ECOG PS 0-2. Cedazuridine was dose-escalated first, and once maximum inhibition of CDA was achieved, the dose of decitabine was then escalated. Fixed doses of oral cedazuridine and decitabine were used without adjustment for body surface area. Dose escalation proceeded provided mean decitabine exposure from the oral cedazuridine decitabine combination was less than 90% of IV decitabine and no dose limiting toxicity was observed. The primary objective was to determine a fixed-dose oral combination of cedazuridine and decitabine that would closely approximate the pharmacokinetic and toxicity profile of intravenous decitabine 20 mg/m^2.

In this study, 44 patients were enrolled in five dose-escalation cohorts. The combination of cedazuridine 100 mg with decitabine 30 mg was found to approximate the pharmacokinetic parameters of decitabine 20 mg/m^2 (5-day AUC exposure 81% of IV decitabine) whereas decitabine 40 mg exceeded these parameters modestly (5-day AUC exposure 128% of IV decitabine). The elimination phase for oral cedazuridine and decitabine followed a similar trajectory to that of decitabine monotherapy. Drug-related adverse events seen with oral cedazuridine 100 mg plus decitabine 30 mg were thrombocytopenia (32%), fatigue (16%), neutropenia (21%) and nausea (16%). 14% of patients required dose reduction and 2%

discontinued treatment for drug-related adverse events. The overall toxicity profile and clinical efficacy appeared similar to decitabine 20 mg/m2 IV.

Subsequently, a phase 2 study, multi-center, open-label, crossover study was conducted using data from the phase 1 dose escalation to compare the pharmacokinetics, pharmacodynamics, safety and efficacy of oral cedazuridine and decitabine with IV decitabine (Garcia-Manero *et. al.* 2020). Here, most patients received a fixed-dose combination tablet (ASTX727) consisting of cedazuridine 100 mg and decitabine 35 mg. Intravenous decitabine was administered at 20 mg/m^2. Patients were randomized 1:1 to receive one of two possible treatment sequences during the first two 28-day cycles: ASTX727 daily for 5 days in cycle 1 followed by IV decitabine daily for 5 days in cycle 2, or the reverse. After the first 2 cycles of treatment, all patients continued treatment with ASTX727 alone. The study included patients 18 years or older with intermediate or high risk MDS and CMML. The primary endpoints were pharmacokinetic exposure, DNA demethylation and overall response rate. This study found that ASTX727 and decitabine have similar AUC profiles and both achieved peak plasma concentration at one hour post-dose. Both agents had comparable effects on LINE-1 demethylation. The efficacy and toxicity profiles of ASTX727 were very similar to historical controls for intravenous decitabine. On the basis of these studies, the FDA approved ASTX727 for use in MDS.

2.3 Rationale

DNMT inhibitors have been well characterized in the treatment of hematologic malignancies including myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). Currently, MDS represents the only FDA-approved indication for the hypomethylating agents 5-azacitidine and decitabine; however, these drugs are often used off-label in older adults with AML unfit for chemotherapy (Silverman *et al.*, 2002; Kantarjian *et al.*, 2006). SGI-110 (guadecitabine) is a second-generation hypomethylating agent with more favorable PK properties which has shown efficacy in phase 1/2 studies in older treatment-naïve patients with AML, relapsed-refractory AML, higher-risk MDS, and CMML (Montalban-Bravo *et al*., 2016; Kantarjian *et al.*, 2017).

Histone deacetylases are important epigenetic regulators that function through deacetylation of histone and non-histone proteins leading to gene silencing, regulation of cell cycle progression and proliferation (Bernhart *et al.*, 2017). Although the role of HDACs in cancer pathogenesis is not fully understood owing to their pleiotropic effects, these agents have shown clinical benefit when applied to various malignancies. Four HDAC inhibitors have received regulatory approval, including vorinostat and romidepsin for cutaneous T cell lymphoma, belinostat and romidepsin for peripheral T cell lymphoma, and panobinostat for multiple myeloma (Mann *et al.*, 2007; San-Miguel *et al.*, 2014; O'Connor *et al.*, 2015). The anti-tumor effect of HDAC inhibitors may be enhanced through combination use with a hypomethylating agent as shown in preclinical studies in multiple malignancies. A phase 1/2 trial of oral 5-azacitidine with romidepsin in patients with relapsed or refractory peripheral T cell lymphoma demonstrated an overall response rate of 80% and was well tolerated (O'Connor *et al.*, 2017).

In summary, CS represents a rare disease for which there is no effective or approved treatment. Patients are often treated with ineffective and toxic chemotherapy. Although insights into CS cancer biology are limited, epigenetic dysregulation appears central to pathogenesis. Previous

studies support this hypothesis and the potential efficacy of an epigenetic-based treatment strategy. The HDAC inhibitor vorinostat was shown to induce p21, result in G_1 cell cycle arrest, and effectively impair cell proliferation in preclinical CS models (Yamamoto *et al.*, 2008; Bernhart *et al.*, 2017). Similarly, treatment with the HDAC inhibitor desipeptide modulated expression of extracellular matrix genes and resulted in differentiation and chondrocytic maturation of CS cells with suppression of tumor growth *in vitro* and *in vivo* (Sakimura *et al.*, 2007). A particularly novel approach using HDAC-loaded bone cement for local cytotoxic activity demonstrated activity in CS models without toxicity on non-neoplastic cells (Tonak *et al.*, 2014). In addition, numerous studies have demonstrated activity for the DNMT inhibitor 5-azacitidine in CS models, through reversal of the hypermethylated state induced by *IDH1/2* mutation, suppression of Wnt/B-catenin signaling, induction of the cancer testis antigens NY-ESO-1 and PRAME, and various other mechanisms (Fitzgerald *et al.*, 2011; Pollack *et al.*, 2012; Sheng *et al.*, 2018). Among solid tumors, CS is an attractive target for an epigenetic treatment approach.

2.3.1 Preclinical Data

Preclinical studies were conducted in several conventional CS cell lines, including CH2879 (grade III; *IDH* wild type), JJ102 (grade II; *IDH1* mutant), CS1 (grade III; *IDH2* mutant), and SW1353 (grade II; *IDH2* mutant). Monotherapy treatment with either 5-azacitidine (DNMT inhibitor) or vorinostat (SAHA, an HDAC inhibitor) demonstrated minimal anti-proliferative effect with IC_{50} for cell viability >1 mcM across all CS cell lines tested in 5-day proliferation assays (**Figure 1A**). In contrast, combination treatment with 250 nM 5-azacitidine and 500 nM vorinostat significantly reduced cell viability as compared to no drug control and either monotherapy. In combination, 5-azacitidine and vorinostat reduced cell viability by approximately 70% as compared to untreated controls and was significantly more effective than either monotherapy in all CS cell lines tested, regardless of IDH mutational status (**Figure 1B**).

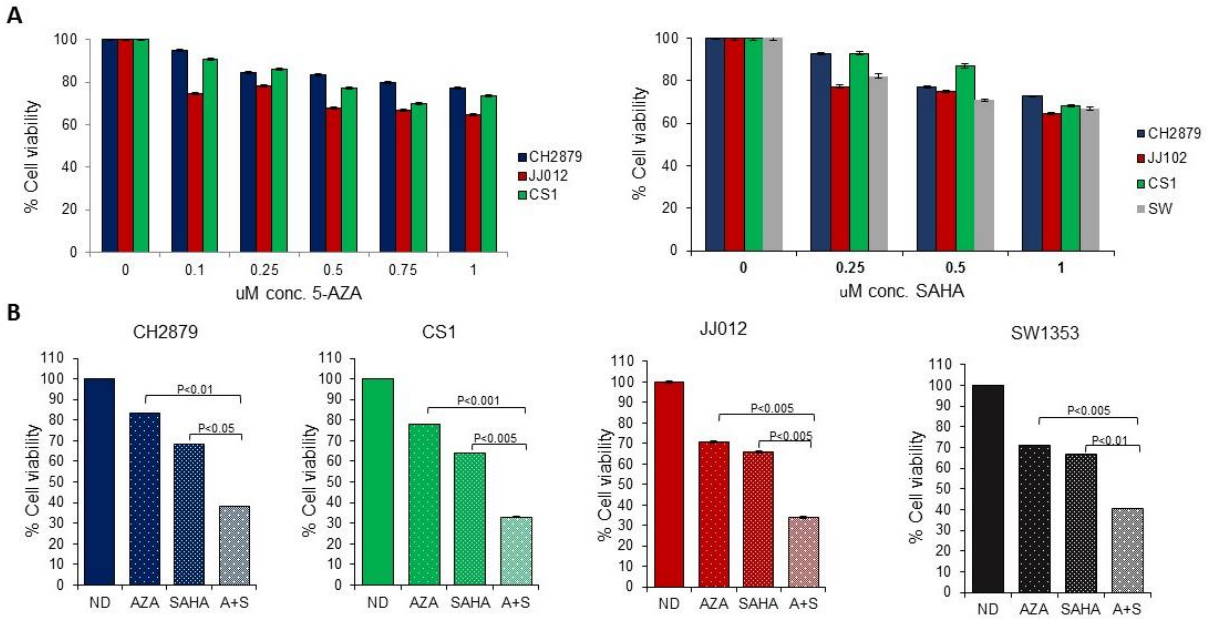


Figure 1: (A) 5-AZA (5-azacitidine) and SAHA (vorinostat) have limited anti-proliferative effect as monotherapy in CS cell lines. CS cell lines were treated with 5-AZA or SAHA monotherapy at doses from 0-1,000

nmol/L daily for 5 days and cell viability was assessed. A colorimetric cell proliferation assay using 96-well plates (Dojindo Molecular Technologies) was used to quantify the amount of formazan dye generated by the activity of dehydrogenases in the cells which is directly proportional to the number of living cells. The optical density at 450 nm was measured using Spectra Max 340 PC (Molecular Devices Corp) to determine cell viability. **(B) 5-AZA + SAHA combination treatment demonstrates significantly greater anti-proliferative effect than either monotherapy.** CS cell lines were treated with no drug (ND), 5-AZA 250 nM daily, SAHA 500 nM daily of the combination (A+S) for 5 days and cell viability was assessed as described above.

Western blots were performed to investigate the mechanisms of drug treatment. In western blots from cells lysed after 48-hour treatment, combination 5-azacitidine and vorinostat induced apoptosis as evidenced by PARP cleavage, which was not observed with either monotherapy (except for the CS1 cell line when treated with vorinostat alone) (**Figure 2A**). In all cell lines, the combination treatment induced greater DNA damage (H2AX), and in the CH2879, JJ012, and CS1 cell lines, the pro-apoptotic protein BIM was induced to a greater extent by combination treatment (**Figure 2A**).

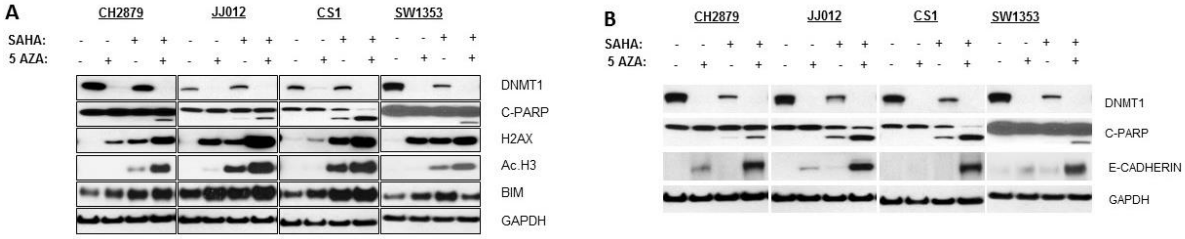


Figure 2: 5-azactidine (5-AZA) and vorinostat (SAHA) combination treatment induces apoptosis (cleaved PARP), acetylated histone H3, BIM, and E-cadherin in CS cell lines, regardless of IDH mutation status. Cell lines were treated with no drug (-/-), 5-AZA (250 nm), SAHA (500 nm), or the combination (+/+) for 48 hours. Cells were lysed and western blot analyses were performed using validated antibodies and GAPDH (Cell Signaling Technologies) as loading control.

Feinberg has proposed the concept of epigenetic modulators, modifiers and mediators as a framework for understanding epigenetic dysregulation in cancer (Feinberg *et al.*, 2016). Epigenetic modifiers are "genes whose products modify the epigenome directly through DNA methylation, post-translational modification of chromatin or alteration of the structure of chromatin" while epigenetic mediators are "genes whose products are the targets of epigenetic modifiers and which drive a tumor or progenitor cell towards a more stem-like state and prevent differentiation". Epigenetic modifiers include DNMTs and HDACs. Dysregulation of epigenetic modifiers is evident through changes in DNA methylation, histone modification (including acetylation), and chromatin organization, which affect the gene expression of epigenetic mediators. As one example of epigenetic modification, inactive genes show methylation at lysine 27 (H3K27me3), and permanently silenced genes carry methylation at lysine 9 (H3K9me3). Gain of H3K27 and H3K9 methylation has been associated with cancer through inactivation of tumor suppressors and impairment of differentiation.

In 50-60% of conventional CS cases, IDH1 or IDH2 mutations induce global DNA hypermethylation through production of the oncometabolite D-2HG which inhibits endogenous demethylases such as TET2. In the IDH1 mutant JJ012 cell line, 5-azacitidine and vorinostat each reduced global DNA methylation by approximately 40% as compared to non-treated controls, while combination treatment reduced global DNA methylation to a significantly greater extent than either monotherapy, suggesting more effective reversal of the aberrantly

hypermethylated state (**Figure 3A**). Furthermore, in western blot experiments, untreated CS cell lines expressed repressive methylation of H3K9 regardless of IDH mutational status. H3K9me3 levels were unaffected by monotherapy treatment with either 5-azacitidine or vorinostat. In contrast, the combination treatment markedly suppressed H3K9 methylation in all cell lines tested (**Figure 3B**).

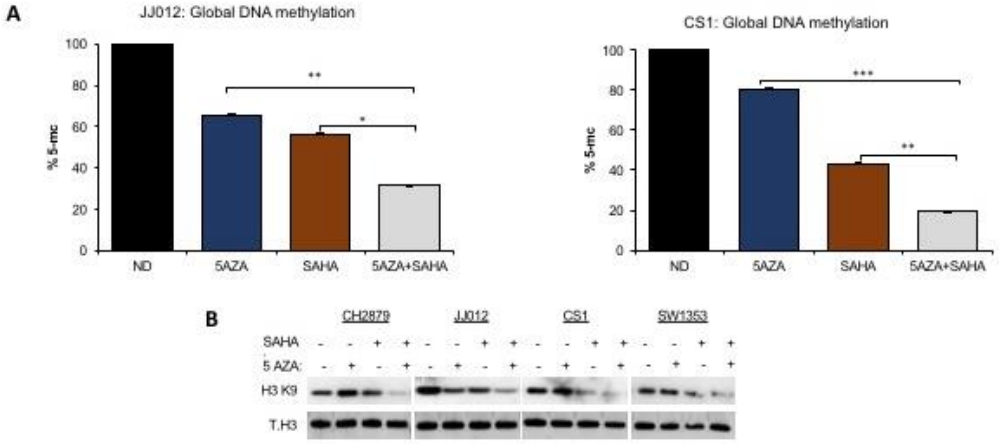


Figure 3: (A) Combination of 5-AZA and SAHA treatment effectively reverses the global DNA hypermethylation induced by IDH mutation in the JJ012 CS cell line. Cell lines were treated with no drug (-/-), 5-AZA (250 nm), SAHA (500 nM), or the combination (+/+) for 48 hours and methylation level using ELISA Easy kit (colorimetric). **(B) Combination of 5-AZA and SAHA treatment more effectively reduces repressive histone methylation at lysine 9 (H3K9me) while total histone H3 levels are unchanged.** Cell lines were treated with no drug (-/-), 5-AZA (250 nm), SAHA (500 nM), or the combination (+/+) for 48 hours and lysine 9 acetylated and total histone H3 levels were assessed by western blot using validated antibodies.

E-cadherin is a tumor suppressor gene which regulates cell migration, motility, and invasiveness. In many cancers, E-cadherin is epigenetically modified and represents an epigenetic mediator as described by Feinberg. In untreated CS cell lines evaluated by western blot, E-cadherin was not appreciably expressed regardless of IDH mutational status (**Figure 2B**). Monotherapy treatment with either 5-azacitidine or vorinostat was insufficient to re-express E-cadherin, although 5-azacitidine did have minimal effect in some CS cell lines (**Figure 2B**). In contrast, combination treatment with 5-azacitidine and vorinostat effectively induced E-cadherin expression in all CS cell lines (**Figure 2B**). The significance of E-cadherin expression for the anti-proliferative effect of combination 5-azacitidine and vorinostat treatment was explored in the JJ012 and CS1 cell lines using siRNA techniques (**Figure 4**). Following siRNA knockdown of E-cadherin gene expression, the additional activity of combination treatment over monotherapy was abrogated (**Figure 4)**. These findings suggest epigenetic re-expression of E-cadherin is partially responsible for the anti-proliferative effect of combination treatment. In additional studies, the combination treatment was shown to reduce levels of the transcription factors SNAIL1, SNAIL2 and ZEB1, which are known to regulate E-cadherin expression through epigenetic mechanisms involving H3K9 and H3K27 methylation (Cui *et al.*, 2018).

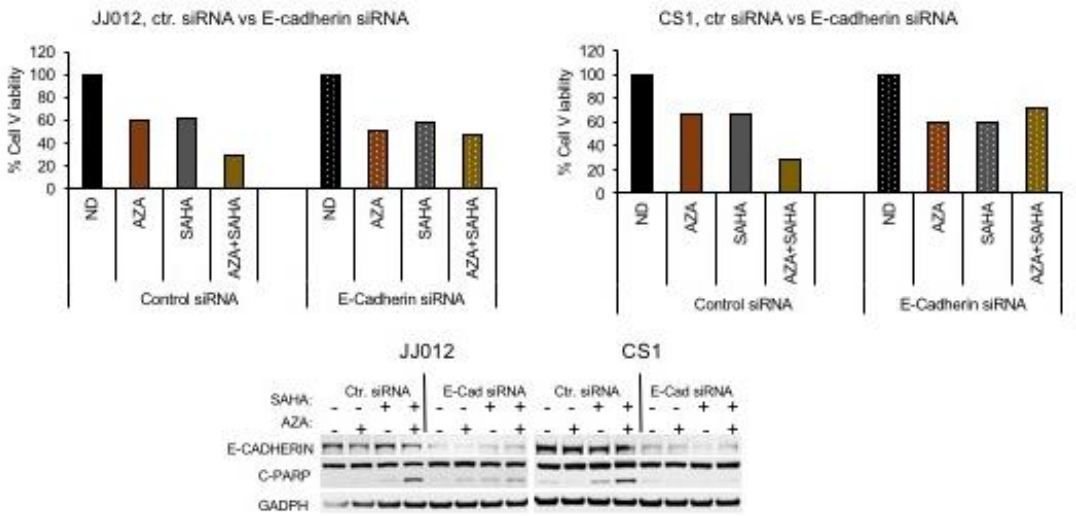


Figure 4: siRNA knockdown of E-cadherin gene expression partially abrogates the anti-proliferative effect of combination 5-azacitidine (5-AZA) and vorinostat (SAHA) treatment. Approximately 3-5 × 10^5 cells were plated and transfected with 100 nmol/L of indicated siRNAs (GE Dharmacon). 48 hours after transfection, cells were trypsinized, counted using a Nexcelom cell counter, and plated in 96-well plates (1,400 cells/well) in triplicate for cell viability assays or 60-mm plates (3-5 × 10^5 cells/plate) for western blotting analysis. Cells were also collected, lysed, and analyzed by western blot to check for knockdown of protein expression (shown below graphs). For western blotting, cells in 60-mm plates were treated for 24 hours, whereas, for cell viability assays, cells in 96-well plates were treated for 72 hours with no drug control (ND), 5-AZA (250 nm), SAHA (500 nM), or 5-AZA + SAHA. Cell viability was measured as described in Figure 2. Combined data from at least two independent experiments is shown.

Epigenetic modifications influence the tumor microenvironment by regulating expression of cytokines and checkpoint molecule expression on immune, stromal, and neoplastic cells. The suppression of interferon-responsive genes may assist tumors in evading the host immune response. At baseline, the CS1 and JJ012 CS cell lines expressed low levels of the interferon responsive genes IRF7, OASL, ISG15 and DDX58 (**Figure 5**). Treatment with either 5-azacitidine or vorinostat monotherapy had no effect on expression of these genes aside from modest induction of IRF7 in the JJ012 cell line (**Figure 5**). In contrast, combination treatment induced dramatic expression of IRF7 in both CS1 and JJ012, with up to 900-fold and 30,000-fold increase in expression over untreated controls, respectively (**Figure 5**). In the CS1 line, expression of OASL, ISG15, and DDX58 was also markedly increased with combination treatment (**Figure 5**). The relevance of IRF7 gene expression for the anti-proliferative effect of combination treatment was further evaluated using siRNA techniques (**Figure 6**). In the JJ012 cell line, siRNA knockdown of IRF7 gene expression partially abrogated the efficacy of combination treatment in proliferation assays (**Figure 6**). Similar effects were seen for the MDA5 gene, which is involved in the innate immune response, in the CS1 cell line. (**Figure 6**).

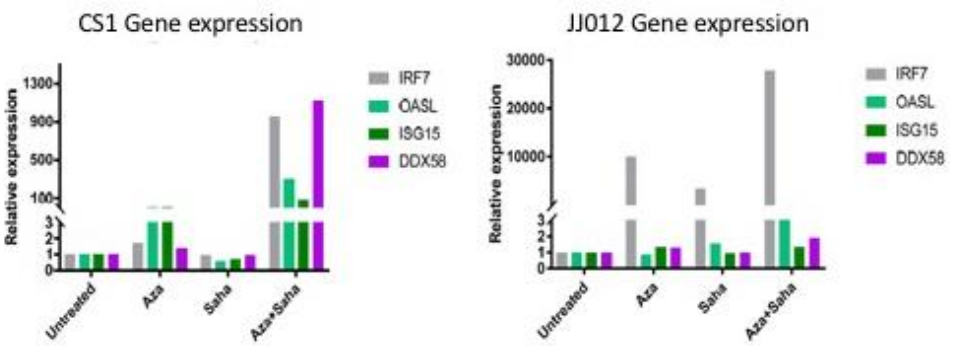


Figure 5: Combination of 5-azacitidine (5-AZA) + SAHA (vorinostat) treatment induces expression of the interferon-responsive gene IRF7 in the CS cell lines CS1 and JJ012. Cell lines were treated with no drug (-/-), 5-AZA (250 nM), SAHA (500 nM), or the combination (+/+) for 48 hours. Gene expression was measured using quantitative polymerase chain reaction (qPCR) and expression was reported relative to untreated control cell lines.

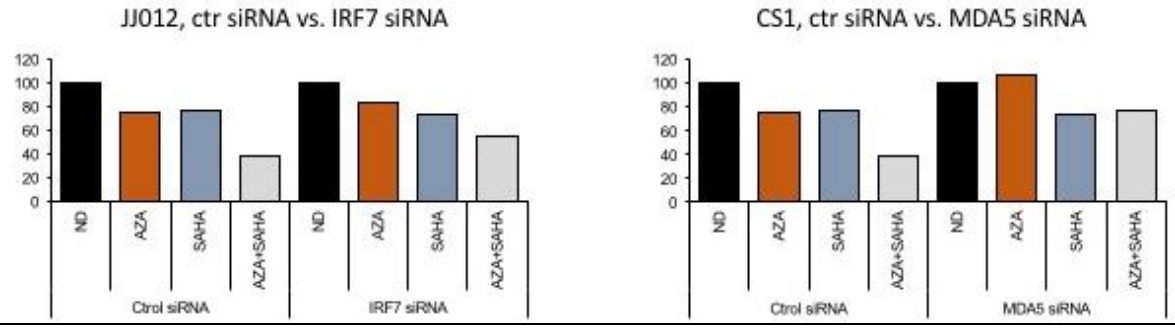


Figure 6: siRNA knockdown of IRF7 gene expression in the JJ012 cell line (left), and MDA5 gene expression in the CS1 cell line (right), partially abrogates the anti-proliferative effect of combination 5-azacitidine (5-AZA) and vorinostat (SAHA) treatment. Approximately 3-5 × 10^5 cells were plated and transfected with 100 nmol/L of indicated siRNAs (GE Dharmacon). 48 hours after transfections, cells were trypsinized, counted using a Nexcelom cell counter, plated in 96-well plates (1,400 cells/well) in triplicate, and treated for 72 hours with no drug control (ND), 5-AZA (250 nM), SAHA (500 nM), or 5-AZA + SAHA. Cell viability was measured as described in Figure 2. Combined data from at least two independent experiments is shown.

The use of combination DNMT and HDAC inhibitors for the treatment of CS was further assessed *in vivo* using a JJ012 mouse xenograft model. In this experiment, four groups of mice were treated for 4 weeks: group 1: vehicle IP daily for 5 days/wk; group 2: 5-azacitidine 2 mg/kg IP daily for 4 days/wk; group 3: vorinostat 50 mg/kg IP 4 days/wk; group 4: 5-azacitidine 2 mg/kg IP + vorinostat 50 mg/kg IP concurrently for 4 days/wk. Five mice were treated per group. In these experiments, 5-azacitidine or vorinostat monotherapy suppressed tumor growth modestly as compared to vehicle control, and when animals were sacrificed at 90 days, vorinostat appeared somewhat more active as monotherapy when compared with 5-azacitidine (**Figure 7A**). In contrast, at day 90, mice treated with combination 5-azacitidine and vorinostat treatment had experienced minimal tumor growth from baseline over the course of the experiment, and tumors were over 80% smaller by volume than vehicle control (**Figure 7A)**. The combination treatment was significantly more effective than either monotherapy (**Figure 7A)**. In western blots of cell lysates from the treated animals, combination treatment showed greater induction of E-cadherin, Ac.H3, and H2AX as compared to vehicle control or monotherapy treatment, and combination treatment showed some evidence of apoptosis, all consistent with the *in vitro* experiments described in **Figure 7B**.

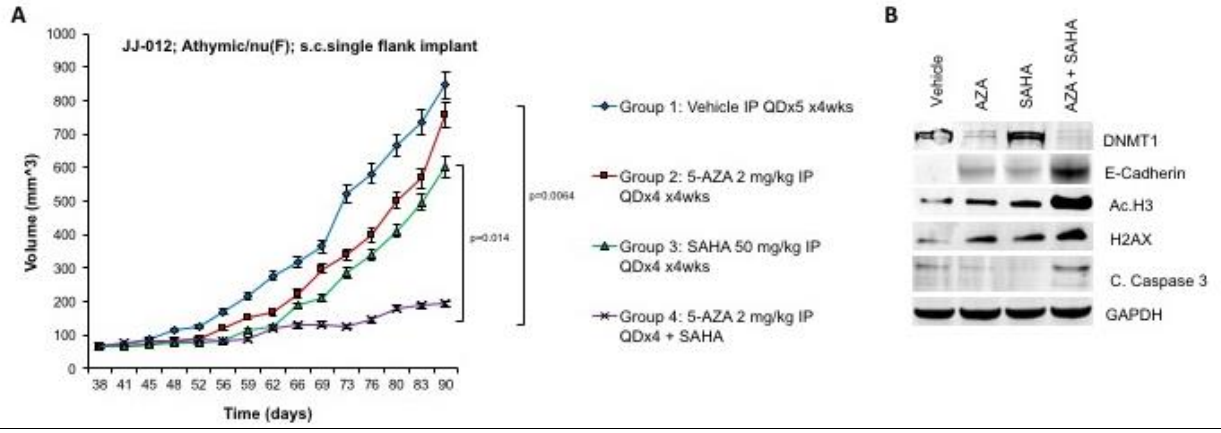


Figure 7: Combination treatment with 5-azacitidine (5-AZA) and vorinostat (SAHA) induces significantly greater suppression of tumor growth than either monotherapy *in vivo*. (A) Tumor growth of JJ012 mouse xenografts treated with the indicated drugs is shown (5 animals per group). (B) For western blots, 30 micrograms of RIPA lysates obtained using a sample grinding kit (GE Healthcare) from xenograft tissues at the end of 90-day treatments were loaded on SDS/PAGE and immunoblotted using the indicated antibodies with GAPDH as loading control.

Additional experiments were conducted using other combinations of DNMT and HDAC inhibitors to demonstrate class effect, including the second-generation DNMT inhibitor SGI-110 (guadecitabine) and the HDAC inhibitors romidepsin and belinostat. In summary, these studies showed equivalent efficacy for combinations of 5-azacitidine and romidepsin as compared to combinations of SGI-110 (guadecitabine) and romidepsin across all CS cell lines tested (**Figure 8**). In all cases, combination treatments were more effective than monotherapy with the respective DNMT and HDAC inhibitor (**Figure 8**). In these experiments, concurrent 6-day treatments, or sequential treatments (2 days hypomethylating agent followed by 4 days concurrent treatment) showed comparable efficacy except in the CH2879 cell line, where sequential treatment appeared modestly more active (**Figure 8**). In western blot studies from related cell lysates, combination treatment resulted in greater induction of PD-L1 and Ac.H3, and more evident apoptosis as demonstrated by PARP cleavage (**Figure 9**). Furthermore, combinations of 5-azacitidine and romidepsin appeared similar to guadecitabine and romidepsin western blot experiments (**Figure 9**).

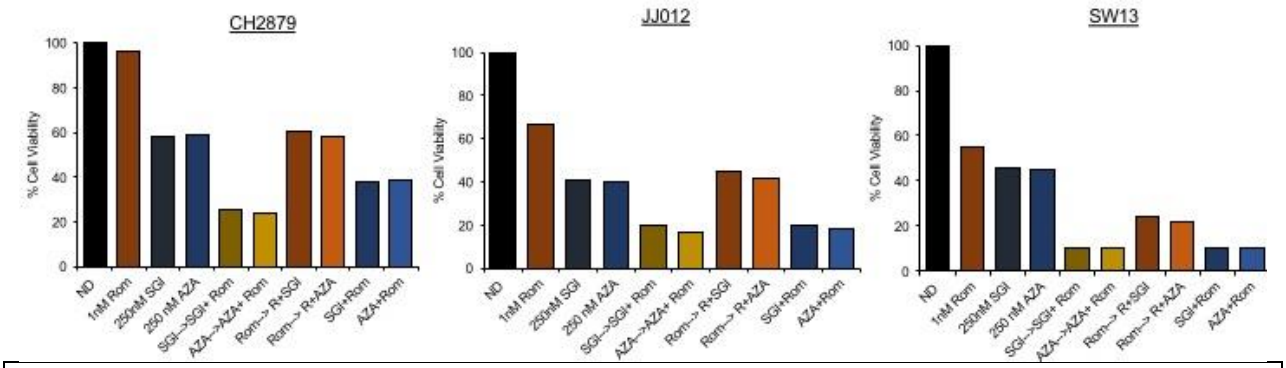


Figure 8: (A) SGI-110 and romidepsin exhibits comparable efficacy as 5-azacitidine (AZA) and romidepsin when given in combination as either concurrent or sequential treatment in CS cell lines. Romidepsin was given at 1 nanomolar, 5-azacitidine at 250 nM, and SGI-110 at 250 nM in all experiments. In concurrent treatments, both drugs were given together for 6 days. In sequential treatments, the hypomethylating agent was given alone for 2 days, followed by 4 days of concurrent treatment. Cell viability was assessed. A colorimetric cell proliferation assay using 96-well plates (Dojindo Molecular Technologies) was used to quantify the amount of formazan dye generated by the activity of dehydrogenases in the cells which is directly proportional to the number of living cells. The optical density at 450 nm was measured using Spectra Max 340 PC (Molecular Devices Corp) to determine cell viability.

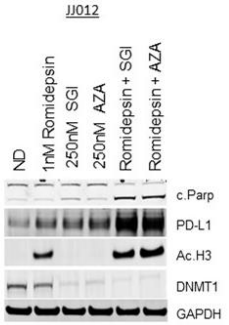


Figure 9: Combination treatment with 5-azacitidine (AZA) + romidepsin or SGI-110 + romidepsin effectively induce apoptosis, PD-L1, and acetylated histone H3. Cell lines were treated with no drug, 1 nM romidepsin, 250 nM SGI-110, 250 nM 5-AZA, combination 1 nM romidepsin + 250 nM SGI-110, or combination 1 nM romidepsin + 250 nM 5-AZA for 3 days. Cells were lysed and western blot analyses were performed using validated antibodies and GAPDH (Cell Signaling Technologies) as loading control.

Finally, additional studies were conducted with the CTEP IND agents belinostat and SGI-110 (guadecitabine). In cell viability assays, belinostat or SGI-110 (guadecitabine) appeared somewhat more active as monotherapy when compared with the other HDAC or DNMT inhibitors when used as monotherapy, as presented above (**Figure 10**). Nonetheless, the belinostat and SGI-110 (guadecitabine) combination remained significantly more active as compared to either monotherapy in all CS cell lines tested (**Figure 10**). In the IDH mutant cell lines, cell viability was reduced by approximately 90% as compared to no drug control (**Figure 10**). In western blots from cells lysed after 48-hour treatment, the combination treatment was more effective at inducing PD-L1, E-cadherin, and acetylated histone H3 than no drug treatment or either monotherapy. Evidence of PARP cleavage was seen both with the combination treatment and belinostat alone (**Figure 11**).

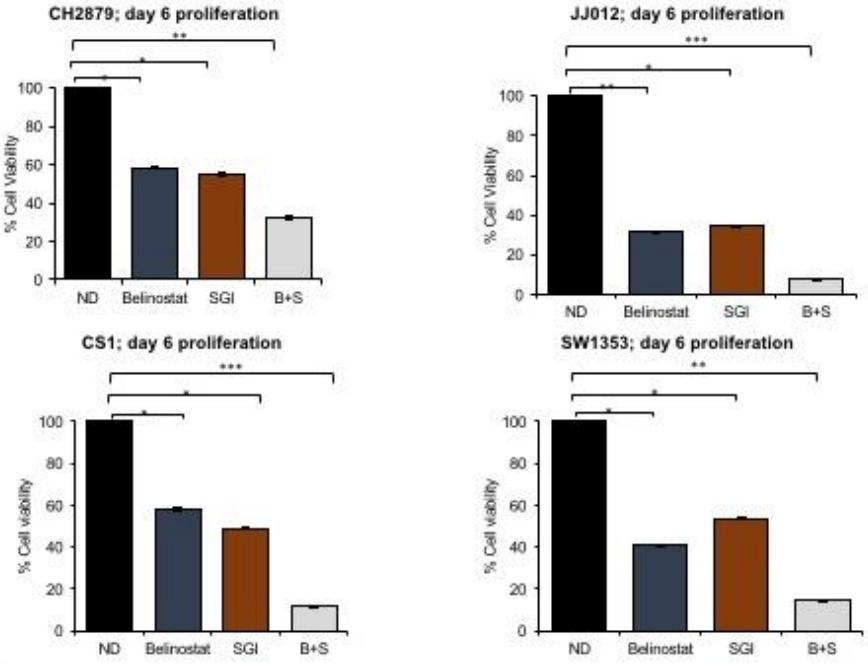


Figure 10: Belinostat and SGI-110 combination treatment is also effective in CS cell lines, regardless of IDH mutational status. CS cell lines were treated with no drug (ND), belinostat 300 nM daily, SGI-110 250 nM daily, or the combination, for 6 days and cell viability was assessed as described in Figure 2.

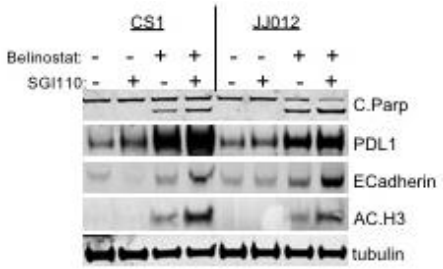


Figure 11: Belinostat and SGI-110 combination treatment induces apoptosis, PD-L1, E-cadherin, and acetylated histone H3 (AC.H3) in CS cell lines. Cell lines were treated with no drug (-/-), belinostat (300 nM), SGI-110 (250 nM), or the combination, for 48 hours. Cells were lysed and western blot analyses were performed using validated antibodies and tubulin (Cell Signaling Technologies) as loading control.

In summary, our preclinical work suggests combination treatment with a DNMT and HDAC inhibitor has marked efficacy for the treatment of CS *in vitro* and *in vivo* and regardless of IDH mutational status. Combining a DNMT inhibitor with an HDAC inhibitor demonstrates significantly greater efficacy than either agent used alone, which was particularly apparent *in vivo,* where the combination treatment resulted in near complete suppression of xenograft tumor growth. Efficacy was demonstrated with various combinations of DNMT and HDAC inhibitors. The mechanisms accounting for the observed efficacy are likely complex and multifactorial. However, our experiments suggest that reversal of the global hypermethylated state and differentiation block seen in CS is central to the treatment's efficacy, including re-expression of E-cadherin and IRF7, among others.

On the basis of these findings, we proposed a phase 2 clinical trial with the DNMT inhibitor SGI-110 (guadecitabine) and the HDAC inhibitor belinostat in patients with unresectable or metastatic conventional chondrosarcoma.

During the conduct of this study, further clinical development of the hypomethylating agent SGI-110 (guadecitabine) was discontinued by the drug manufacturer and further enrollment on the belinostat and SGI-110 treatment regimen will stop. ASTX727 is a novel, orally bioavailable hypomethylating agent which contains the same active agent (decitabine) and an analogous mechanism of action. Additional information on ASTX727 is provided in Section 2.2.3. Effective with Amendment 5, patients entering the study will be treated with belinostat and ASTX727, as described in Section 6.

2.4 Correlative Studies Background

2.4.1 Integrated Studies

2.4.1.1 Whole Exome Sequencing

CS is a rare disease. Currently, aside from recurrent mutations in *IDH1/2*, little is known regarding the genomic landscape of these tumors. A better understanding of genomic alterations in CS may inform the development of new therapeutic approaches for this disease. Only one major genomic profiling study has been performed in CS (Tarpey *et al*., 2013). In this study of 49 patients, IDH1/2 mutations were found in 59% of cases. Recurrent alterations in the collagen regulator gene COL2A1 were present in 37%, including both IDH mutant and wild-type cases. Mutations were also found in TP53 (20%), the Rb pathway (33%), and Hedgehog signaling (18%). In IDH wild-type cases, recurrent alterations in other epigenetic regulators were apparent, such as KDM6A, SETD2, TET, and DNMT3A, suggesting alternative mechanisms of epigenetic dysregulation may contribute to pathogenesis of IDH1/2 wild-type tumors. We hypothesize that recurrent IDH1/2 mutations will be found in CS, consistent with observations from prior studies, and that alterations in other genes may be responsible for epigenetic dysregulation in IDH1/2 wild-type tumors.

2.4.1.2 RNAseq

The efficacy of treatment with belinostat and SGI-110 (guadecitabine) or ASTX727 in an epigenetically-dysregulated malignancy such as CS is hypothesized to depend upon favorable changes in gene expression and reversal of aberrant hypermethylation of tumor suppressors imparted by effects of *IDH1/2* mutation and other biologic mechanisms. In our preclinical studies, combination treatment reduced global DNA methylation levels in CS cell lines. Furthermore, changes in expression of BIM, E-cadherin, PD-L1, MHC, and interferon-related genes such as IRF7 occurred with this treatment. siRNA studies showed that induction of E-cadherin and IRF7 expression was partially responsible for the anti-proliferative effect of combination treatment. In other preclinical studies, epigenetic repression of p16, E-cadherin, 3-OST (a heparin sulfate proteoglycan), and RUNX3 (cell cycle) appeared important for CS tumorigenesis. We hypothesize that belinostat and SGI-110 (guadecitabine) or ASTX727 will result in favorable changes in gene expression resulting in induction of differentiation and re-expression of tumor suppressors and endogenous retroviral elements with associated favorable effects on the tumor immune microenvironment.

2.4.2 Exploratory Studies

2.4.2.1 Tumor Immune Microenvironment

The effectiveness of many anti-cancer therapeutics depends upon activity of the immune system. Many cancers alter normal cellular processes to evade immune surveillance, and increasing evidence suggests that immune evasion is accomplished in part through epigenetic modification of genes involved in antigen presentation, expression of immunosuppressive cell surface molecules, suppression of candidate neoantigens, and other mechanisms. There is increasing evidence to support combining epigenetic-based therapies, including HDAC and DNMT inhibitors, with immune checkpoint inhibitors, and clinical trials with these combinations are ongoing (Weisenberger *et al.*, 2006). However, studies evaluating the effects of epigenetic treatments on the immune microenvironment using tissue from prospective clinical trials with HDAC and DNMT inhibitors is limited. We will analyze changes in densities of infiltrating immune cell subsets between pre-treatment and on-treatment specimens to evaluate changes induced by study treatment. We hypothesize that belinostat and SGI-110 (guadecitabine) or ASTX727 treatment will induce expression of MHC and PD-L1 by tumor cells and will increase infiltration of cytotoxic T-cells into chondrosarcoma tumors.

2.4.2.2 Global DNA Methylation Assay

Changes in global methylation represent one pharmacodynamic marker of epigenetic treatment activity in tumor tissue. In several clinical studies of HDAC and DNMT inhibitors in solid tumors, greater reduction in global methylation or higher levels of global methylation or methylation of specific genes at baseline correlated with clinical outcomes. In our preclinical studies, HDAC inhibitor and hypomethylating agent combinations effectively reduced global DNA methylation to a greater extent than either monotherapy in chondrosarcoma cell lines. We hypothesize that belinostat and SGI-110 (guadecitabine) or ASTX727 treatment will reduce global DNA methylation levels in CS tumors, which could serve as a relevant biomarker for the treatment's anti-cancer effects.

3. PATIENT SELECTION

3.1 Eligibility Criteria

3.1.1 Patients must have biopsy-proven conventional CS which is:

- either metastatic or locally advanced and unresectable, and
- measurable at study entry according to RECIST version 1.1 criteria, and
- amenable to biopsy with imaging guidance at no or acceptable risk to the patient as defined by institutional guidelines for research-related biopsies or the treating investigator's assessment.

In addition, the following criteria must be met:

- patients must have at least one lesion measurable by RECIST version 1.1 criteria which has not been previously irradiated.
- patients who have histologic evidence of grade 1 chondrosarcoma only must either be

symptomatic from their disease in the opinion of the treating investigator or demonstrate radiographic evidence of disease progression in the 3 months prior to initiation of study treatment.

Note: Pathology review and confirmation of diagnosis will occur at the site enrolling the patient on this study.

3.1.2 Patients may have been treated with any number of prior systemic therapies. Because there are no FDA-approved treatments for this disease, patients who have received no prior systemic therapy are also eligible. However, disease must be deemed surgically unresectable.

3.1.3 Age ≥18 years. Chondrosarcoma is rarely encountered in children and adolescents.

3.1.4 ECOG performance status ≤2 (Karnofsky ≥60%, see Appendix A).

3.1.5 Patients must have adequate organ and marrow function as defined below:

- absolute neutrophil count ≥1,000/mm^3
- hemoglobin 8 g/dL
- platelet count ≥75,000/mm^3
- total bilirubin ≤1.5 × institutional upper limit of normal (ULN)
- AST(SGOT)/ALT(SGPT) ≤2 × institutional ULN
- creatinine ≤1.5 × institutional ULN

  OR
- glomerular filtration rate (GFR*) ≥45 mL/min/1.73 m^2

  **Refer to Appendix B for calculation of GFR*

3.1.6 Human immunodeficiency virus (HIV)-infected patients on effective anti-retroviral therapy with undetectable viral load within 6 months are eligible for this trial.

3.1.7 For patients with evidence of chronic hepatitis B virus (HBV) infection, the HBV viral load must be undetectable on suppressive therapy, if indicated.

3.1.8 Patients with a history of hepatitis C virus (HCV) infection must have been treated and cured. For patients with HCV infection who are currently on treatment, they are eligible if they have an undetectable HCV viral load.

3.1.9 Patients with **treated brain metastases** are eligible if follow-up brain imaging after central nervous system (CNS)-directed therapy shows no evidence of progression, if patients have been clinically asymptomatic, and if patients have not received systemic corticosteroids for at least 28 days. Patients with brain metastases not meeting these criteria are not eligible.

3.1.10 Patients must be disease-free of prior invasive malignancies for >5 years, with the exception of curatively-treated basal cell or squamous cell carcinoma of the skin or

carcinoma *in situ* of the cervix.

NOTE: If there is a history of prior malignancy, patients must not be receiving other specific treatment for that cancer.

3.1.11 Patients with known history or current symptoms of cardiac disease, or history of treatment with cardiotoxic agents, should have a clinical risk assessment of cardiac function using the New York Heart Association Functional Classification. To be eligible for this trial, patients should be class 2B or better.

3.1.12 The effects of belinostat and SGI-110 (guadecitabine) or ASTX727 on the developing human fetus are unknown. For this reason, and because the DNA methyltransferase inhibitor decitabine, the active metabolite of SGI-110 (guadecitabine) and a component of ASTX727, is known to be teratogenic, and because belinostat may cause teratogenicity and/or embryo-fetal lethality by virtue of targeting actively dividing cells, 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 and for at least 6 months after the last dose of study drugs. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately. Men treated or enrolled on this protocol must also agree to use adequate contraception prior to the study, for the duration of study participation, and 3 months after completion of belinostat and SGI-110 (guadecitabine) or ASTX727 administration.

3.1.13 Patients must be able to understand and willing to sign a written informed consent document. Participants with impaired decision-making capacity (IDMC) who have a legally-authorized representative (LAR) and/or family member available will also be eligible.

3.2 Exclusion Criteria

3.2.1 Patients with dedifferentiated, mesenchymal, or clear cell chondrosarcoma are not eligible.

3.2.2 Patients who have not recovered from AEs (*i.e.*, have residual toxicities > Grade 1) due to prior anti-cancer therapy are not allowed, with the exceptions of alopecia and endocrinopathies from prior immunotherapy-based treatments that are well-controlled with hormone replacement.

In addition, the following time periods must elapse between the last dose of prior anti-cancer treatment and initiation of study treatment on this protocol:

- Cytotoxic chemotherapy or biologic, including immunotherapy: 28 days
- Small molecule targeted drug: 21 days or 5 half-lives, whichever is shorter. If 5 half-lives is shorter than 21 days, then 21 days applies.
- Radiation: 28 days, except for palliative radiation, for which 14 days applies.

3.2.3 Patients who are receiving any other investigational agents.

3.2.4 Patients with known history of allergic reactions or sensitivity attributed to compounds of similar chemical or biologic composition to SGI-110 (guadecitabine), its active metabolite decitabine, or ASTX727, or belinostat.

3.2.5 Chronic use of any medications or substances that are strong inhibitors of UGT1A1 is not allowed. Patients must switch to alternative medications 7-14 days before treatment with belinostat. Because the lists of these agents are constantly changing, it is important to regularly consult a frequently-updated medical reference. As part of the enrollment/informed consent procedures, the patient will be counseled on the risk of interactions with other agents, and what to do if new medications need to be prescribed or if the patient is considering a new over-the-counter medicine or herbal product.

3.2.6 Patients with any known *UGT1A1* polymorphism, heterozygous or homozygous, associated with reduced function (*UGT1A1*6*, *UGT1A1*28*, or *UGT1A1*60*).

3.2.7 Patients with uncontrolled intercurrent illness.

3.2.8 Patients with psychiatric illness/social situations that would limit compliance with study requirements.

3.2.9 Pregnant women are excluded from this study because SGI-110 (guadecitabine) is a derivative of decitabine, and ASTX727 contains the agent decitabine, which has the potential for teratogenic or abortifacient effects, and because belinostat may cause teratogenicity and/or embryo-fetal lethality by virtue of targeting actively dividing cells. Because there is an unknown but potential risk for AEs in nursing infants secondary to treatment of the mother with SGI-110 (guadecitabine), ASTX727 and belinostat, breastfeeding should be discontinued.

3.2.10 Prolongation of the heart-rate corrected QT (QTc) interval ≥450 ms (*i.e.,* grade 1 or higher) on the screening electrocardiogram (ECG) prior to initiation of study treatment.

If baseline QTc on screening ECG is ≥450 ms (*i.e.,* grade 1 or higher):

- Check potassium and magnesium serum levels, and
- Correct any identified hypokalemia and/or hypomagnesemia and repeat ECG to confirm a QTc interval <450 ms.

3.3 Inclusion of Women and Minorities

NIH policy requires that women and members of minority groups and their subpopulations be included in all NIH-supported biomedical and behavioral research projects involving NIH-defined clinical research unless a clear and compelling rationale and justification establishes to the satisfaction of the funding Institute & Center (IC) Director that inclusion is inappropriate with respect to the health of the subjects or the purpose of the research. Exclusion under other

circumstances must be designated by the Director, NIH, upon the recommendation of an IC Director based on a compelling rationale and justification. Cost is not an acceptable reason for exclusion except when the study would duplicate data from other sources. Women of childbearing potential should not be routinely excluded from participation in clinical research. Please see http://grants.nih.gov/grants/funding/phs398/phs398.pdf.

4. REGISTRATION PROCEDURES

4.1 Investigator and Research Associate Registration with CTEP

Food and Drug Administration (FDA) regulations and National Cancer Institute (NCI) policy require all individuals contributing to NCI-sponsored trials to register and to renew their registration annually. To register, all individuals must obtain a Cancer Therapy Evaluation Program (CTEP) Identity and Access Management (IAM) account at https://ctepcore.nci.nih.gov/iam. In addition, persons with a registration type of Investigator (IVR), Non-Physician Investigator (NPIVR), or Associate Plus (AP) (*i.e.*, clinical site staff requiring write access to Oncology Patient Enrollment Network (OPEN), Rave, or acting as a primary site contact) must complete their annual registration using CTEP's web-based Registration and Credential Repository (RCR) at https://ctepcore.nci.nih.gov/rcr.

RCR utilizes five person registration types.

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

RCR requires the following registration documents:

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

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

- Addition to a site roster,
- Assign the treating, credit, consenting, or drug shipment (IVR only) tasks in OPEN, and
- Act as the site-protocol Principal Investigator (PI) on the IRB approval.
- Assign the Clinical Investigator (CI) role on the Delegation of Tasks Log (DTL).

In addition, all investigators act as the Site-Protocol PI, consenting/treating/drug shipment, or as the CI on the DTL must be rostered at the enrolling site with a participating organization.

Additional information is located on the CTEP website at https://ctep.cancer.gov/investigatorResources/default.htm. For questions, please contact the **RCR Help Desk** by email at RCRHelpDesk@nih.gov.

4.2 Site Registration

Sites participating with the NCI Central Institutional Review Board (NCI CIRB) must submit the Study Specific Worksheet for Local Context (SSW) to the CIRB using IRBManager to indicate their intent to open the study locally. The NCI CIRB's approval of the SSW is automatically communicated to the CTSU Regulatory Office, but sites are required to contact the CTSU Regulatory Office at CTSURegPref@ctsu.coccg.org to establish site preferences for applying NCI CIRB approvals across their Signatory Network. Site preferences can be set at the network or protocol level. Questions about establishing site preferences can be addressed to the CTSU Regulatory Office by emailing the email address above or calling 1-888-651-CTSU (2878).

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

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

In addition, the Site-Protocol PI (*i.e.*, the investigator on the IRB/REB approval) must meet the following five criteria to complete processing of the IRB/REB approval record:

- Holds an Active CTEP status,
- Rostered at the site on the IRB/REB approval and on at least one participating roster,
- If using NCI CIRB, rostered on the NCI CIRB Signatory record,
- Includes the IRB number of the IRB providing approval in the Form FDA 1572 in the RCR profile, and
- Holds the appropriate CTEP registration type for the protocol.

Additional Requirements

Additional requirements to obtain an approved site registration status include:

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

4.2.1 Downloading Regulatory Documents

Download the site registration forms from the protocol-specific page located on the CTSU members' website. Permission to view and download this protocol and its supporting documents is restricted based on person and site roster assignment. To participate, the institution and its associated investigators and staff must be associated with the LPO or a Participating Organization on the protocol.

- Log on to the CTSU members' website (https://www.ctsu.org) using your CTEP-IAM username and password,
- Click on *Protocols* in the upper left of your screen
    - Enter the protocol number in the search field at the top of the protocol tree, or
    - Click on the By Lead Organization folder to expand, then select LAO-CT018, and protocol number 10330,
- Click on *Documents*, select *Site Registration*, and download and complete the forms provided. (Note: For sites under the CIRB initiative, IRB data will load automatically to the CTSU as described above.)

4.2.2 Protocol Specific Requirements For Protocol #10330 Site Registration

Upon site registration approval in RSS, the enrolling site may access OPEN to complete enrollments. The enrolling site will select their credentialed provider treating the subject in the OPEN credentialing screen and may need to answer additional questions related to treatment in the eligibility checklist.

- Specimen Tracking System Training Requirement:
    - All data entry users (Clinical Research Associate role) at each participating site will need to complete the Theradex-led training.
    - Theradex will provide a certificate of completion, which will need to be submitted to the CTSU through the Regulatory Submission Portal.
    - The training is a one-time only requirement per individual. If an individual has previously completed the training for another ETCTN study, the training does not need to be completed again nor does the certificate of completion need to be resubmitted to the CTSU. However, new versions of the Specimen Tracking System may require new training.
    - This training will need to be completed before the first patient enrollment at a given site.
    - Please contact STS Support at Theradex for the training

(STS.Support@theradex.com, Theradex phone: 609-799-7580).

4.2.3 Submitting Regulatory Documents

Submit required forms and documents to the CTSU Regulatory Office via the Regulatory Submission Portal on the CTSU website.

To access the Regulatory Submission Portal, log on to the CTSU members' website → Regulatory → Regulatory Submission.

Institutions with patients waiting that are unable to use the Regulatory Submission Portal should alert the CTSU Regulatory Office immediately at 1-866-651-2878 in order to receive further instruction and support.

Delegation of Tasks Log (DTL)

Each site must complete a protocol-specific DTL using the DTL application in the Delegation Log section on the CTSU members' website. The Clinical Investigator (CI) is required to review and electronically sign the DTL prior to the site receiving an Approved site registration status and enrolling patients to the study. To maintain an approved site registration status the CI must re-sign the DTL at least annually and when a new version of the DTL is released; and activate new task assignments requiring CI sign-off. Any individual at the enrolling site on a participating roster may initiate the site DTL. Once the DTL is submitted for CI approval, only the designated DTL Administrators or the CI may update the DTL. Instructions on completing the DTL are available in the Help Topics button in the DTL application and include a Master Task List, which describes DTL task assignments, CI signature, and CTEP registration requirements.

4.2.4 Checking Site Registration Status

You can verify your site's registration status on the members' side of the CTSU website.

- Log on to the CTSU members' website
- Click on *Regulatory* at the top of your screen
- Click on *Site Registration*
- Enter your 5-character CTEP Institution Code and click on Go

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

4.3 Patient Registration

4.3.1 OPEN / IWRS

The Oncology Patient Enrollment Network (OPEN) is a web-based registration system available

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

Requirements for OPEN access:

- A valid CTEP-IAM account.
- To perform enrollments or request slot reservations: Be on an LPO roster, ETCTN Corresponding roster, or Participating Organization roster with the role of Registrar. Registrars must hold a minimum of an AP registration type.
- If a DTL is required for the study, the registrar(s) must hold the OPEN Registrar task on the DTL for the site.
- Have an approved site registration for a protocol prior to patient enrollment.

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

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

1. Patient has met all eligibility criteria within the protocol stated timeframes, and
2. All patients have signed an appropriate consent form and HIPAA authorization form (if applicable).

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

Access OPEN at https://open.ctsu.org or from the OPEN link on the CTSU members' website. Further instructional information is in the OPEN section of the CTSU website at https://www.ctsu.org or https://open.ctsu.org. For any additional questions, contact the CTSU Help Desk at 1-888-823-5923 or ctsucontact@westat.com.

4.3.2 Special Instructions for Patient Enrollment

This Study will use the ETCTN Specimen Tracking System (STS).

- All biospecimens collected for this trial must be submitted using the ETCTN Specimen Tracking System (STS) unless otherwise noted.
- The system is accessed through special Rave user roles: "CRA Specimen Tracking" for data entry at the treating institutions and "Biorepository" for users receiving the specimens for processing and storage at reference labs and the Biorepository.
- Please refer to the Medidata Account Activation and Study Invitation Acceptance link on

the CTSU website under the Rave/DQP tab.

- **Important: Failure to complete required fields in STS may result in a delay in sample processing.** Any case reimbursements associated with sample submissions will not be credited if samples requiring STS submission are not logged into STS.

Detailed instructions can be found in Section 5.4.

4.3.3 OPEN/IWRS Questions?

Further instructional information on OPEN is provided on the OPEN link of the CTSU website at https://www.ctsu.org or at https://open.ctsu.org. For any additional questions contact the CTSU Help Desk at 1-888-823-5923 or ctsucontact@westat.com.

4.4 General Guidelines

Following registration, patients should begin protocol treatment within 7 days. 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 canceled. The Study Coordinator should be notified of cancellations as soon as possible.

5. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

5.1 Summary Table for Specimen Collection

Time Point	Specimen	Send Specimens To:
Pretreatment		
Within 21 days of C1D1	• 3-4 tumor cores in formalin* (mandatory) • 10 mL whole blood in EDTA K2 tube (mandatory)	EET Biobank
Cycle 2		
Day 3, 4, or 5 2-8 hours post dose	• 3-4 tumor cores in formalin* (mandatory)	EET Biobank
*For new biopsies, a copy of the radiology and operative reports from the tissue removal procedure *and* the diagnostic anatomic pathology report must be sent with the tissue to the EET Biobank.		

5.2 Summary Tables for Interventional Radiologist for Research Biopsies

Biopsy #: 1
Trial Time Point: Pretreatment (within 21 days of C1D1)
IR Biopsy Definition: Research – No Clinical Impact (All cores from a single biopsy procedure impact research goals, but do not directly impact patient care or benefit the patient.)

Core Priority	Use in the Trial	Biomarker Name(s)	Tumor Content Required	Post-Biopsy Processing
1	Integrated	WES/RNAseq in Tumor Tissue	>50%	formalin
2	Exploratory	Global Methylation Assay	>50%	formalin
3	Exploratory	Tumor Immune Microenvironment	>50%	formalin

Biopsy #: 2				
Trial Time Point: Cycle 2, Day 3, 4, or 5 (2-8 hours post treatment)				
IR Biopsy Definition: Research – No Clinical Impact (All cores from a single biopsy procedure impact research goals, but do not directly impact patient care or benefit the patient.)				
Core Priority	**Use in the Trial**	**Biomarker Name(s)**	**Tumor Content Required**	**Post-Biopsy Processing**
1	Integrated	WES/RNAseq in Tumor Tissue	>50%	formalin
2	Exploratory	Global Methylation Assay	>50%	formalin
3	Exploratory	Tumor Immune Microenvironment	>50%	formalin

Note: Pre-biopsy assessments will be reported and tracked through a trial-specific CRF within the CTEP Medidata Rave system (see Appendix C).

5.3 Specimen Procurement Kits and Scheduling

5.3.1 Specimen Procurement Kits

Kits for the collection and shipment of specimens to the ETCTN Biorepository can be ordered online via the Kit Management system:
(https://ricapps.nationwidechildrens.org/KitManagement).

Users at the clinical sites will need to set up an account in the Kit Management system and select a specific clinical trial protocol to request a kit. Please note that protocol may include more than one type of kit. Each user may order two kit types per protocol per day (daily max = 6 kits). Kits are shipped ground, so please allow 5-7 days for receipt. A complete list of kit contents for each kit type is located on the Kit Management system website.

Note: Kits or supplies are only provided for specimens shipped to the Biorepository. Institutional supplies must be used for all other specimen collection and processing.

5.3.2 Scheduling of Specimen Collections

5.3.2.1 Scheduling of Specimen Collections for the ETCTN Biorepository

Please adhere to the following guidelines when scheduling procedures to collect tissue:

- Tumor tissue specimens collected during biopsy procedures and fixed in formalin must be shipped on the same day of collection.
- Tissue in formalin can be collected Monday through Wednesday and shipped overnight for arrival on Tuesday through Thursday at the ETCTN Biorepository at Nationwide Children's Hospital.
- Fresh blood specimens may be collected and shipped Monday through Friday.

5.4 Specimen Tracking System Instructions

5.4.1 Specimen Tracking System Overview and Enrollment Instructions

For the ETCTN STS, the following information will be requested:

- Protocol Number
- Investigator Identification
  - Institution and affiliate name
  - Investigator's name
- Eligibility Verification: Patients must meet all the eligibility requirements listed in Section 3.
- Additional Requirements:
  - Patients must provide a signed and dated, written informed consent form.

Upon enrolling a patient, IWRS will communicate with OPEN, assigning two separate and unique identification numbers to the patient, a Universal patient ID (UPID) and a Treatment patient ID. The UPID is associated with the patient and used each and every time the patient engages with the portion of this protocol that uses the ETCTN Specimen Tracking System. The UPID contains no information or link to the treatment protocol. IWRS will maintain an association between the UPID for ETCTN biobanking and molecular characterization and any treatment protocols the patient participates in, thereby allowing analysis of the molecular characterization results with the clinical data.

Immediately following enrollment, the institutional anatomical pathology report for the diagnosis under which the patient is being enrolled must be uploaded into Rave. The report must include the surgical pathology ID (SPID), collection date, block number, and the IWRS-assigned UPID and patient study ID for this trial. For newly acquired biopsies, the radiology and operative report(s) must also be uploaded into Rave. **Important: Remove any personally identifying information, including, but not limited to, the patient's name, initials, medical record number, and patient contact information from the institutional pathology report prior to submission.**

Additionally, please note that the STS software creates pop-up windows when reports are generated, so you will need to enable pop-ups within your web browser while using the software.

For questions regarding the Specimen Tracking System, please contact STS Support at STS.Support@theradex.com.

A shipping manifest **must** be included with all sample submissions.

5.4.2 Specimen Labeling

5.4.2.1 Blood Specimen Labels

Include the following on blood specimens:

- Patient Study ID
- Universal Patient ID (UPID)
- Specimen ID (automatically generated by Rave)
- Time point
- Specimen type (*e.g.*, blood, serum)

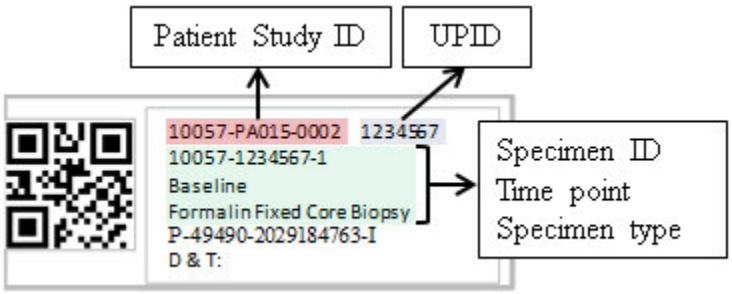
5.4.2.2 Tissue Specimen Labels

Include the following on all tissue specimens or containers (*e.g.*, formalin jar):

- Patient Study ID
- Universal Patient ID (UPID)
- Specimen ID (automatically generated by Rave)
- Time point
- Specimen type (*e.g.*, Formalin Fixed Tissue)
- Tissue type (P for primary, M for metastatic or N for normal)
- Surgical pathology ID (SPID) number, if applicable

5.4.2.3 Example of Specimen Label

The following image is an example of a tissue specimen label printed on a standard Avery label that is 1" high and 2.625" wide.



The QR code in the above example is for the Specimen ID shown on the second line.
NOTE: The QR code label is currently under development at Theradex as of 31-Aug-2018; therefore, labels generated by the STS for this study may not include a QR code.

The second line item from the end includes four data points joined together:

1. Tissue only: Primary (P), Metastatic (M), Normal (N) tissue indicated at the beginning of the specimen ID; this field is blank if not relevant (*e.g.*, for blood)

2. Block ID or blank if not relevant
3. SPID (Surgical Pathology ID) or blank if none
4. The last alpha-numeric code is protocol specific and is only included if the protocol requires an additional special code classification

The last line on the example label is for the handwritten date and optional time.

5.4.3 Overview of Process at Treating Site

5.4.3.1 OPEN Registration

All registrations will be performed using the Oncology Patient Enrollment Network (OPEN) system. OPEN communicates automatically with the Interactive Web Response System (IWRS) which handles identifier assignments, any study randomization, and any prescribed slot assignments. If specimen analysis is required to determine eligibility, the protocol will be set up with multi-step registration.

Registration without eligibility specimen analysis:

1. Site enters registration data into OPEN during one or more steps.
2. IWRS receives data from OPEN, generates the Patient Study ID and the Universal Patient ID, both of which are sent back to OPEN.
3. IWRS sends all applicable registration data directly to Rave at the end of the final registration step.

Any data entry errors made during enrollment should be corrected in Rave.

5.4.3.2 Rave Specimen Tracking Process Steps

Step 1: Complete the **Histology and Disease** form (but do not upload reports until a specimen label can be applied to them) and the Baseline forms regarding **Prior Therapies**. Enter the initial clinical specimen data:

- **Specimen Tracking Enrollment** CRF: Enter Time Point, Specimen Category, Specimen Type, Block number (if applicable), Tissue type, Surgical Path ID (if applicable), and number of labels needed (include extra labels to apply to reports to be uploaded). CRF generates unique Specimen ID.

Step 2: Print labels using report in EDC and collect specimen.

- Label specimen containers and write collection date and pre-treatment/on-treatment status on each label. After collection, store labeled specimens as described in Section 5.4.2.
- Apply an extra specimen label to *each* report before scanning. Return to the **Histology and Disease** form to upload any initial Pathology, Radiology, Molecular Reports (up to 4), Surgical (or Operative) reports and Pathology Verification form (when applicable). Return to **Specimen Tracking Enrollment** CRF to upload any molecular report (one per specimen) and/or specimen specific pathology or related report (one per specimen). Uploaded reports should have protected health information (PHI) data, like name,

mailing address, medical record number or social security number (SSN), redacted. Do not redact SPID, block number or relevant dates (such as collection date), and include the UPID and patient study ID on each document.

Step 3: Complete specimen data entry.

- **Specimen Transmittal** Form: Enter collection date and pre-treatment/on-treatment status and other required specimen details.

Step 4: When ready to ship, enter shipment information.

- **Shipping Status** CRF: Enter tracking number, your contact information, recipient, number of containers and ship date once for the first specimen in a shipment.
- **Copy Shipping** CRF: Select additional specimens to add to an existing shipment referenced by the tracking number.

Step 5: Print shipping list report and prepare to ship.

- Print two copies of the shipping list, one to provide in the box, the other for your own records.
- Print pathology or other required reports to include in the box. Be sure the printed copy includes the specimen label.

Step 6: Send email notification.

- For only one of the specimens in the shipment, click "Send Email Alert" checkbox on the **Shipping Status** CRF to email recipient.

Step 7: Ship the specimen(s).

5.5 Specimen Collection

5.5.1 Formalin-Fixed Tumor Biopsies

1. Label formalin-filled containers according to instructions in Section 5.4.2.
2. Obtain 3-4 16-gauge or 18-gauge core needle biopsy specimens, and place one core in each cassette.
3. Snap the cassette lids closed and place cassettes into a formalin-filled pre-labeled container as soon as possible after collection to prevent air drying. Up to two cassettes may be placed in one formalin jar.
4. Secure the container lids and package containers into the shipping kit according to instructions in Section 5.6. Keep tissue in formalin jars at room temperature until shipment to the EET Biobank.

5.5.2 Collection of Blood in EDTA Tubes for Ambient Whole Blood Shipment

1. Label EDTA tubes according to the instructions in Section 5.4.2.
2. Collect the specified amount of blood (10 mL) in EDTA tube(s) and gently invert tube to mix.
3. Ship on day of collection (whenever possible) according to instructions in Section 5.6.

4. If blood cannot be shipped on the day of collection (e.g., a late scheduled collection), then refrigerate until shipment.

5.6 Shipping Specimens from Clinical Site to the EET Biobank

5.6.1 General Shipping Information

Core biopsies that are fixed in formalin and fresh blood should be shipped as one shipment at ambient temperature, whenever possible. The shipping container sent with kit contents should be used to ship specimens to the EET Biobank. In winter months, please include extra insulation, such as bubble wrap, inside the shipping container.

For formalin-fixed biopsies, if the corresponding anatomical pathology report is not available at the time of shipment, then the surgical and/or radiology reports from the tissue removal procedure and the diagnostic anatomic pathology report must be included in the package, or the specimen will not be processed. Once completed, upload the corresponding pathology report to the ETCTN specimen tracking system and send a copy to the EET Biobank.

5.6.2 Specimen Shipping Instructions

Tissue in formalin must be shipped on the day of collection. Collect and ship on Monday through Wednesday.

Fresh blood may be shipped on Monday through Friday. Please select "Saturday Delivery" when shipping fresh blood on a Friday.

5.6.2.1 Shipping Ambient Blood in Your Own Container

If blood and tissue are ready to ship on the same day, then the blood may be packaged and shipped in the single chamber kit with the tissue. If blood is shipped separately then it must be shipped using institutional supplies. Packaging guidelines for sending blood in a separate box are provided below.

1. Before packaging specimens, verity that each specimen is labeled according to instructions in Section 5.4.1
2. Place the blood collection tube(s) into a zip-lock bag.
3. Place zip-lock bag into a biohazard envelope with absorbent material. Expel as much air as possible and seal the envelope securely.
4. Place the biohazard envelope into a Tyvek envelope. Expel as much air as possible and seal securely.
5. Place the specimen(s) and a copy of the shipping manifest into a sturdy shipping container. In winter months, please use an insulated container and include extra insulation, such as bubble wrap, inside the shipping container to prevent specimens from freezing.
6. Close the container and tape shut.
7. Attach a shipping label to the top of the shipping container.

8. Attach an Exempt Human Specimen sticker to the side of the container.
9. Ship specimens via overnight courier to the address below. FedEx Priority Overnight is strongly recommended to prevent delays in package receipt.

5.6.2.2 Shipping Ambient Tissue and Blood in a Single-Chamber Kit

If blood and tissue are ready to ship on the same day, then the blood may be packaged and shipped in the single chamber kit with the tissue. If blood is shipped separately then it must be shipped using institutional supplies. Packaging guidelines for sending both specimens in the kit box are provided below.

1. Before packaging specimens, verify that each specimen is labeled according to the instructions above and that the lids of all primary receptacles containing liquid are tightly sealed. The lids of formalin jars should be wrapped in parafilm. Absorbent material must be placed around each primary container that holds liquid.
2. Place the specimens in zip-lock bags. Use a separate bag for each specimen type.
3. Place specimens into the secondary pressure vessel surrounded by bubble wrap. Place the lid on the secondary pressure vessel and set it inside the kit chamber.
4. Place a copy of the shipping manifest and corresponding reports such as pathology, surgical, or radiology reports into the insulated shipping container.
5. Set the lid on top of the container. Close the outer flaps and tape shut.
6. Attach a shipping label to the top of the shipping container.
7. Attach an Exempt Human Specimen sticker to the side of the container.
8. Ship specimens via overnight courier to the address below. FedEx Priority Overnight is strongly recommended to prevent delays in package receipt.

5.6.3 Shipping Address

Ship to the address below. Ship formalin-fixed and fresh blood specimens the same day of specimen collection. Do not ship specimens the day before a holiday.

EET Biobank
The Research Institute at Nationwide Children's Hospital
700 Children's Drive, WA1340
Columbus, Ohio 43205
PH: (614) 722-2865
FAX: (614) 722-2897
Email: BPCBank@nationwidechildrens.org

FedEx Priority Overnight service is very strongly preferred.

NOTE: The EET Biobank FedEx Account will not be provided to submitting institutions.

5.6.4 Contact Information for Assistance

For all queries, please use the contact information below:

EET Biobank
Toll-free Phone: (800) 347-2486
E-mail: BPCBank@nationwidechildrens.org

5.7 Biomarker Plan

List of Biomarker Assays in Order of Priority

Priority	Biomarker Name	Assay (CLIA: Y/N)	Use in the Trial and Purpose	Specimens Tested	Collection Time Points	Mandatory or Optional	Assay Laboratory and Lab PI
Tissue-based Biomarkers							
1	Whole Exome Sequencing in Tumor Tissue	WES CLIA: N	Integrated To evaluate the genomic landscape of chondrosarcoma tumors, including IDH1/2 mutational status. To evaluate whether IDH1/2 mutational status is related to clinical benefit from study treatment.	DNA from FFPE tumor tissue	Pre-treatment (within 21 days of C1D1) Cycle 2 Day 3, 4, or 5 (2-8 hours post dose)	M	Molecular Characterization (MoCha) Laboratory, Frederick National Laboratory for Cancer Research (FNLCR) Mickey Williams, Ph.D.
2	RNAseq	RNAseq CLIA: N	Integrated To evaluate for changes in gene expression associated with study treatment to interrogate the mechanism of action. Specific hypotheses will be tested based upon the preclinical data.	RNA from FFPE tumor tissue	Pre-treatment (within 21 days of C1D1) Cycle 2 Day 3, 4, or 5 (2-8 hours post dose)	M	Molecular Characterization (MoCha) Laboratory, Frederick National Laboratory for Cancer Research (FNLCR) Mickey Williams, Ph.D.

Priority	Biomarker Name	Assay (CLIA: Y/N)	Use in the Trial and Purpose	Specimens Tested	Collection Time Points	Mandatory or Optional	Assay Laboratory and Lab PI
Tissue-based Biomarkers							
3	Tumor Immune Microenvironment	Multiplex IHC using the VECTRA and inFORM platforms CLIA: N	Exploratory To evaluate effects of study treatment on the immune microenvironment using multiplex immunohistochemistry to define changes in infiltrating immune cell subsets and expression of PD-L1 and MHC by tumor and immune cells.	Unstained slides from FFPE tumor tissue core	N/A (Collected as part of the WES/ RNAseq biopsies)	O	Columbia University Medical Center Immune Monitoring Core Yvonne Saenger, M.D. yms4@cumc.columbia.edu
4	Global DNA Methylation Assay	Infinium Human Methylation 450 Bead Chip Array CLIA: N	Exploratory To evaluate changes in global DNA methylation associated with study treatment.	Unstained slides from FFPE tumor tissue core	N/A (Collected as part of the WES/ RNAseq biopsies)	O	Columbia University Medical Center Herbert Irving Comprehensive Cancer Center Research Laboratory Chao Lu, Ph.D. cl3684@cumc.columbia.edu
Blood-based Biomarkers							
1	Companion Germline Sequencing	CLIA: N	Integrated To collect a blood sample for germline WES to complement the tumor tissue WES.	DNA from Blood in EDTA	Pre-treatment (within 21 days of C1D1)	M	Molecular Characterization (MoCha) Laboratory, Frederick National Laboratory for Cancer Research (FNLCR) Mickey Williams, Ph.D.

5.8 Integrated Correlative Studies

5.8.1 Whole Exome Sequencing in Tumor Tissue

5.8.1.1 Specimen Receipt and Processing at the EET Biobank

Tumor tissue received in formalin will be paraffin-embedded. All FFPE blocks will be sectioned to generate an initial hematoxylin and eosin (H&E)-stained slide and, for nucleic acid extractions, additional RNase-free slides. If needed, slides will be macrodissected to enrich tumor content before scraping for nucleic acid co-extraction.

DNA and RNA will be co-extracted from tumor tissue. The nucleic acids will be analyzed to determine concentration and quality. Aliquots of DNA will be shipped to the central sequencing laboratory for analysis.

DNA will be extracted from whole blood in EDTA. An aliquot of blood (germline) DNA will be shipped with the tumor DNA.

5.8.1.2 Site Performing Correlative Study

This assay will be performed at the Molecular Characterization (MoCha) Laboratory, Frederick National Laboratory for Cancer Research (FNLCR).

5.8.1.3 Shipment of Specimens

Specimens will be shipped from the EET Biobank to:

MoCha, Frederick National Laboratory for Cancer Research (FNLCR)
1050 Boyles St.
Bldg. 459, Rm. 125
Frederick, MD 21702
Attn: Alyssa Chapman or Ruth Thornton

5.8.1.4 Contact Information/Notification of Speicmen Shipment

Thomas Forbes, mochasamplereceiving@nih.gov

5.8.2 RNAseq

5.8.2.1 Specimen Receipt and Processing at the ETCTN Biorepository

Tumor tissue received in formalin will be paraffin-embedded. All FFPE blocks will be sectioned to generate an initial hematoxylin and eosin (H&E)-stained slide and, for nucleic acid extractions, additional RNase-free slides. If needed, slides will be macrodissected to enrich tumor content before scraping for nucleic acid co-extraction.

DNA and RNA will be co-extracted from tumor tissue. The nucleic acids will be analyzed to determine concentration and quality. Aliquots of RNA will be shipped to the central sequencing laboratory for analysis.

5.8.2.2 Site Performing Correlative Study

This assay will be performed at the Molecular Characterization (MoCha) Laboratory, Frederick National Laboratory for Cancer Research (FNLCR).

5.8.2.3 Shipment of Specimens

Specimens will be shipped from the EET Biobank to:

MoCha, Frederick National Laboratory for Cancer Research (FNLCR)
1050 Boyles St.
Bldg. 459, Rm. 125
Frederick, MD 21702
Attn: Alyssa Chapman or Ruth Thornton

5.8.2.4 Contact Information/Notification of Specimen Shipment

Thomas Forbes, mochasamplereceiving@nih.gov

5.9 Exploratory/Ancillary Correlative Studies

5.9.1 Tumor Immune Microenvironment

5.9.1.1 Specimen Receipt and Processing at the EET Biobank

Tumor tissue received in formalin will be paraffin-embedded. All FFPE blocks will be sectioned to generate an initial hematoxylin and eosin (H&E)-stained slide.

Ten (10) unstained, uncharged, 4-micron, air-dried slides are created from both the baseline (pre-treatment) and on-treatment biopsies, labeled appropriately, and sent under ambient conditions by overnight courier to the Immune Monitoring Core at Columbia University.

Shipping Address

Immune Monitoring Core Facility
Physicians and Surgeons Building, Room 9-428
630 West 168th Street
New York, NY 10032

Contact Information for Assistance

Name: Matthew Ingham, M.D.
Phone: 202-285-4944
Email: mi2337@cumc.columbia.edu

5.9.1.2 Site Performing Correlative Study

This assay will be performed at the Columbia University Medical Center Immune Monitoring Core.

5.9.2 Global DNA Methylation Assay

5.9.2.1 Specimen Receipt and Processing at the EET Biobank

Tumor tissue received in formalin will be paraffin-embedded. All FFPE blocks will be sectioned to generate an initial hematoxylin and eosin (H&E)-stained slide.

Ten (10) unstained, uncharged, 4-micron, air-dried slides are created from both the baseline (pre-treatment) and on-treatment biopsies, labeled appropriately, and sent under ambient conditions by overnight courier to the laboratory of Dr. Chao Lu at Columbia University.

Shipping Address

Chao Lu Laboratory
Irving Cancer Research Center
1130 St. Nicholas Avenue (ICRC Room 312)
New York, NY 10032

Contact Information for Assistance

Name: Chao Lu, PhD
Phone: 212-851-5243
Email: cl3684@cumc.columbia.edu

5.9.2.2 Site Performing Correlative Study

This assay will be performed at the laboratory of Dr. Chao Lu at Columbia University Medical Center.

6. TREATMENT PLAN

6.1 Agent Administration

Patients Receiving Belinostat and SGI-110 (guadecitabine)

Note: Effective with Amendment 5, no further subjects will be enrolled to the belinostat

and SGI-110 (guadecitabine) regimen. Patients enrolling on the study will be treated with belinostat and ASTX727 (see Section 6.2).

Patients enrolled prior to Amendment 5 who have been receiving belinostat and SGI-110 (guadecitabine) will continue to receive that treatment regimen. SGI-110 will no longer be available from the company as of 31-AUG-2021. Further treatment options will be discussed with subjects enrolled to the belinostat/SGI-110 regimen who remain on treatment as of 31-AUG-2021. Patients may be allowed to change treatment to ASTX727 and belinostat in August 2021 if the overall assessment of risks versus benefits support that change, primarily related to any observations of adverse safety and tolerability of ASTX727 and belinostat at that time.

Treatment will be administered on an outpatient basis. Patients will be evaluated in the outpatient clinic by the treating investigator and research team on day 1 of each cycle. Patients need not be evaluated by the investigator on the other treatment days in a cycle unless clinically warranted. The study agents will be administered by qualified nursing staff consistent with institutional guidelines for administration of subcutaneous and intravenous oncology agents of these classes.

SGI-110 (guadecitabine) is given first by subcutaneous injection. At least 10 minutes, but no longer than 60 minutes, after the SGI-110 (guadecitabine) injection is completed, the belinostat infusion may begin.

There is no clinical evidence to support the sequencing of one agent prior to the other in terms of efficacy or toxicity. SGI-110 (guadecitabine) will be given first considering this agent is administered subcutaneously and may take longer to absorb than belinostat. This approach is also consistent with published combination studies with SGI-110 (guadecitabine) in solid tumors. In addition, in our preclinical studies exploring a sequential approach to treatment, efficacy appeared greater when SGI-110 (guadecitabine) was given prior to concurrent treatment as compared to giving belinostat before concurrent treatment, providing further support for this approach (Figure 8).

Reported AEs and potential risks are described in Section 10. Appropriate dose modifications are described in Section 7. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

The proposed study is an open-label, single-arm, Simon 2-stage phase 2 clinical trial with a safety lead-in and a continuous toxicity monitoring rule. All patients will receive SGI-110 (guadecitabine) 45 mg/m^2 SC in combination with belinostat 1,000 mg/m^2 IV over 30 minutes on Days 1-5 in 28-day cycles. Treatment will continue until a criterion for removal from the study is met (see Section 6.3 Duration of Therapy).

The belinostat and SGI-110 (guadecitabine) combination has not been previously studied and there is no defined RP2D. The FDA-approved dose of belinostat in relapsed or refractory peripheral T-cell lymphoma is 1,000 mg/m^2 IV on Days 1-5 of a 21-day cycle. SGI-110 (guadecitabine) is not FDA-approved. This agent was initially evaluated in a phase 1 dose

escalation study in patients with AML or MDS where the drug was administered on Days 1-5 of a 28-day cycle (Issa *et al.*, 2015). DLT related to myelosuppression was observed at 125 mg/m^2; therefore, the MTD was established at 90 mg/m^2. Because pharmacodynamic studies demonstrated maximal biologic (demethylating) effect at 60 mg/m^2, the RP2D was 60 mg/m^2 SC on Days 1-5 of a 28-day cycle.

Although SGI-110 (guadecitabine) and belinostat have not been formally studied together, numerous published studies have evaluated various combinations of hypomethylating agents and histone deacetylase inhibitors and have found these combinations to be well tolerated. For example, in a phase 1 dose escalation study of azacitidine and belinostat in AML, no DLTs were observed at any dose level, and the RP2D was belinostat 1,000 mg/m^2 with azacitidine 75 mg/m^2 on Days 1-5 of a 28-day cycle (Odenike *et al.*, 2015). Thus, both agents were combined at their FDA-approved monotherapy doses. The most common toxicities were grade 1/2 nausea, vomiting, anorexia, and fatigue. Even in this heavily pretreated AML population, grade 3/4 hematologic events were uncommon. Similarly, in another phase 1 study in AML/MDS, decitabine and vorinostat were safe and tolerable in combination at their monotherapy doses (Kirschbaum *et al.*, 2014). Other studies evaluating azacitidine and panobinostat in myeloid malignancies and azacitidine and entinostat in both colorectal cancer and in breast cancer further support favorable tolerability of these combinations (Azad *et al.*, 2017; Connolly *et al.*, 2017).

SGI-110 (guadecitabine) has been studied as monotherapy and in combination with several other agents in solid tumors. In hepatocellular carcinoma, the RP2D as monotherapy was 45 mg/m^2 on Days 1-5 of a 28-day cycle. In colorectal cancer, SGI-110 (guadecitabine) was well tolerated at 45 mg/m^2 in combination with irinotecan with growth factor in a heavily pretreated population (Lee *et al.*, 2018). In melanoma, SGI-110 (guadecitabine) was well tolerated at 60 mg/m^2 in combination with checkpoint blockade (Di Giacomo *et al.*, 2019). Although a phase 1 study evaluating SGI-110 (guadecitabine) in combination with carboplatin in ovarian cancer arrived at a lower dose of SGI-110 (30 mg/m^2), this study included a heavily pre-treated population in which the median number of prior treatment lines was 7 and a myelosuppressive dose of carboplatin (AUC 5) was used (Matei *et al.*, 2018).

Considering there are no FDA-approved treatments for chondrosarcoma (that is, patients will not be heavily pretreated with chemotherapy) and noting that combination studies with hypomethylating agents and HDAC inhibitors have shown favorable tolerability as discussed above, a starting dose of 45 mg/m^2 appears warranted. To closely monitor for unexpected adverse events, both a safety lead-in and continuous toxicity monitoring rule are applied as further described in Section 9.

Regimen Description				
Agent	**Dose**	**Route**	**Schedule**	**Cycle Length**
SGI-110 (guadecitabine)	45 mg/m^2	Slow SC injection	Days 1-5	28 days (4 weeks)
Belinostat	1,000 mg/m^2	IV over 30 (±5) min*	Days 1-5	
*May extend belinostat infusion to 3 hours if infusion-related reactions occur.				

- SGI-110 (guadecitabine) is administered first, followed by belinostat. Separate SGI-110 (guadecitabine) and belinostat administration by at least 10 minutes but no more than 60 minutes.
- **Patients must receive a prophylactic 5-HT3 receptor antagonist prior to belinostat during the first two cycles of treatment – see Section 6.2.3.1.**
- **Patients must receive prophylactic use of growth factor– see Section 6.2.1.1.**

Patients Receiving Belinostat and ASTX727

Beginning with Amendment 5, subsequent patients enrolled on this study will receive treatment with belinostat and ASTX727. These patients will not receive SGI-110 (guadecitabine).

ASTX727 is an orally available DNMT inhibitor which consists of oral cedazuridine, a cytidine deaminase inhibitor, in combination with oral decitabine. ASTX727 is an oral tablet with a fixed dose combination of cedazuridine 100 mg and decitabine 35 mg. See Section 2.2.3 for additional information on ASTX727. Because ASTX727 has not been studied in combination with an HDAC inhibitor, we will repeat the safety lead-in portion of this study among the first 6 patients to receive belinostat and ASTX727. The safety lead-in and the effects of this change on study design and statistical considerations are further described in Section 9.

Treatment will be administered on an outpatient basis. Patients will be evaluated in the outpatient clinic by the treating investigator and research team on day 1 of each cycle. Patients need not be evaluated by the investigator on the other treatment days in a cycle unless clinically warranted. The study agents will be administered by qualified nursing staff consistent with institutional guidelines for administration of oral and intravenous oncology agents of these classes.

ASTX727 will be given first orally. At least 30 minutes, but no longer than 60 minutes, after ASTX727 is administered, the belinostat infusion may begin. Treatment will continue until a criterion for removal from the study is met (see Section 6.3 Duration of Therapy).

Regimen Description				
Agent	**Dose**	**Route**	**Schedule**	**Cycle Length**
ASTX727	1 tablet, which consists of cedazuridine 100 mg / decitabine 35 mg	Orally	Days 1-5	28 days (4 weeks)
Belinostat	1,000 mg/m^2	IV over 30 (±5) min*	Days 1-5	

*May extend belinostat infusion to 3 hours if infusion-related reactions occur.

- ASTX727 is administered first, followed by belinostat. Separate ASTX727 and belinostat administration by at least 30 minutes but no more than 60 minutes. Patients will be instructed to bring ASTX727 to clinic and take the medication in clinic to ensure the timeframe is observed.

- **Patients must receive a prophylactic 5-HT3 receptor antagonist prior to belinostat during the first two cycles of treatment – see Section 6.2.3.1.**
- **Patients must receive prophylactic use of growth factor– see Section 6.2.2.1.**

6.1.1 CTEP IND Agents

6.1.1.1 SGI-110 (Guadecitabine)

SGI-110 (guadecitabine) will be administered via slow SC injection, preferably in the abdominal area, upper thigh, or arm. The injection site should be rotated for the five injections in each cycle. Due to the viscosity of the SGI-110 (guadecitabine) solution, a 25-gauge, 5/8 inch needle is recommended for administration. SGI-110 (guadecitabine) should be administered in the clinic by a health professional. The total amount (in mg) of SGI-110 (guadecitabine) to be administered is determined by body surface area (BSA). In calculating BSA, use actual heights and weights. Do not adjust to "ideal" body weight. Utilize the dose calculated for the baseline BSA throughout the protocol unless weight changes by ±10% or more as assessed on Day 1 of each cycle, in which case it should be adjusted. For all dose calculations, sites can round the volume to the nearest tenth or follow institutional standards.

There may not be additional supply of SGI-110 (guadecitabine) after 31-AUG-2021 if the current batch of SGI-110 and its diluents expiry dates cannot be extended. Further treatment options will be discussed with subjects enrolled to the belinostat/SGI-110 regimen who remain on treatment with that regimen as of 31-AUG-2021. Patients may be allowed to change treatment to ASTX727 and belinostat in August 2021 if the overall assessment of risks versus benefits support that change, primarily related to any observations of adverse safety and tolerability of ASTX727 and belinostat at that time.

6.1.1.2 ASTX727

ASTX727 is an oral fixed-dose combination tablet and consists of cedazuridine 100 mg and decitabine 35 mg in one tablet. ASTX727 dosing is not based on weight or body surface area. All patients will receive the same dose of one tablet.

Patients should take the ASTX727 tablet at approximately the same time each day on an empty stomach. Do not eat, or drink milk or alcohol 2 hours before or 2 hours after taking ASTX727. Clear liquids, such as water, black coffee, or tea are allowed. Take whole tablets with 8 oz (240 mL) of water; do not crush, cut or chew the tablet.

Missed doses within 12 hours of the scheduled time can be made up. The patient needs to take the missed dose as soon as possible and resume the next day on schedule. If the patient fails to make up the dose within 12 hours, the dose should be resumed at the scheduled time the next day.

6.1.1.3 Belinostat (PXD-101)

Belinostat will be administered IV over 30 minutes (± 5 minutes) by central or peripheral access

through an in-line 0.22-micron filter. The total amount (in mg) of belinostat to be administered is determined by BSA. In calculating BSA, use actual heights and weights. Do not adjust to "ideal" body weight. Utilize the dose calculated for the baseline BSA throughout the protocol unless weight changes by ±10% or more as assessed on Day 1 of each cycle, in which case it should be adjusted. For all dose calculations, sites can round the volume to the nearest tenth or follow institutional standards.

Belinostat infusions may be prolonged up to 3 hours, if deemed necessary, to ameliorate acute infusion-related reactions (nausea, flushing, rhinitis, vomiting, *etc*.) that may occur during, or immediately following infusion. Once diluted in 250 mL of 0.9% sodium chloride, belinostat may be stored at ambient room temperature (15°-25°C) for up to 36 hours, including the infusion time.

6.2 General Concomitant Medication and Supportive Care Guidelines

Because there is a potential for interaction of SGI-110 (guadecitabine), ASTX727 and belinostat with other concomitantly administered drugs, the case report form must capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies. The Principal Investigator should be alerted if the patient is taking any agent known to affect or with the potential for drug interactions. The study team should check a frequently-updated medical reference for a list of drugs to minimize use of or avoid entirely. Appendix D (Patient Clinical Trial Wallet Card) should be provided to patients if available.

6.2.1 SGI-110 (Guadecitabine)

6.2.1.1 Concomitant medications and supportive guidelines

SGI-110 (guadecitabine) is associated with myelosuppression. **Patients must receive G-CSF prophylactically, with filgrastim (Neupogen®), pegfilgrastim (Neulasta®), or biosimilar.** The recommended dose of filgrastim is 5 mcg/kg SC per day, rounding off the dose to the nearest vial size, for 5-10 days. Start filgrastim the next day (and no later than 3 days) after completion of study treatment on day 5 of a given cycle. The recommended dose of pegfilgrastim is 6 mg SC once per cycle. Pegfilgrastim is given approximately 24 hours after completing Day 5 of study treatment (if treatment finishes on a Friday, pegfilgrastim may be given the following Monday). Biosimilars are acceptable. The duration of treatment with growth factor is per the treating investigator's discretion.

Patients should receive all supportive care appropriate for their medical condition, including, but not limited to, antibiotics, transfusions, and other agents deemed necessary for symptom management, unless specifically prohibited elsewhere in the protocol.

If patients develop injection site pain related to SGI-110 (guadecitabine) administration, pretreatment of topical or systemic analgesics can be considered.

6.2.1.2 Interactions with CYP proteins

In vitro studies in human hepatocytes suggest SGI-110 (guadecitabine) is unlikely to inhibit or

induce human cytochrome P450 (CYP) enzymes and is not a substrate for CYPs, indicating that CYP-mediated drug-drug interactions with SGI-110 (guadecitabine) are not anticipated. Neither SGI-110 (guadecitabine) nor the active metabolite decitabine inhibit major human drug transporters.

In human transporter studies, SGI-110 (guadecitabine) was noted to have some potential to be a substrate for concentrative nucleoside transporter (CNT) 1 and CNT2, and decitabine was a substrate for CNT1 and equilibrative nucleoside transporter (ENT) 1 (Study No. OPT-2012-062 and OPT-2013-070). SGI-110 (guadecitabine) minimally inhibited multidrug and toxin extrusion (MATE) 1 and decitabine minimally inhibited MATE1 and MATE2-K (Study No. OPT-2014-071). No drug-drug interactions with SGI-110 (guadecitabine) are expected. Overall, the nonclinical PK/toxicokinetic (TK) profile of SGI-110 (guadecitabine) has been well characterized to support its use as a therapeutic agent. However, patients receiving concomitant cytotoxic drugs are at greater risk of significant myelosuppression from SGI-110 (guadecitabine). Dose reductions and delays may be necessary to continue therapy.

6.2.1.3 Drug-drug interactions

Drug-drug interaction studies have not been conducted with SGI-110 (guadecitabine) or decitabine.

6.2.2 ASTX727

6.2.2.1 Concomitant medications and supportive guidelines

ASTX727 is associated with myelosuppression. **Patients must receive G-CSF prophylactically, with filgrastim (Neupogen®), pegfilgrastim (Neulasta®), or biosimilar.** The recommended dose of filgrastim is 5 mcg/kg SC per day, rounding off the dose to the nearest vial size, for 5-10 days. Start filgrastim the next day (and no later than 3 days) after completion of study treatment on day 5 of a given cycle. The recommended dose of pegfilgrastim is 6 mg SC once per cycle. Pegfilgrastim is given approximately 24 hours after completing Day 5 of study treatment (if treatment finishes on a Friday, pegfilgrastim may be given the following Monday). Biosimilars are acceptable. The duration of treatment with growth factor is per the treating investigator's discretion.

Patients should receive all supportive care appropriate for their medical condition, including, but not limited to, antibiotics, transfusions, and other agents deemed necessary for symptom management, unless specifically prohibited elsewhere in the protocol.

6.2.2.2 Drug-drug interactions

Drug-drug interaction studies have not been conducted with cedazuridine or decitabine. Nonclinical studies indicate that neither cedazuridine nor decitabine is likely to inhibit or induce major human cytochrome P450 enzymes. Thus, CYP450-mediated drug-drug interactions are not anticipated for cedazuridine or decitabine. Cedazuridine is not an inhibitor or a substrate of major human drug transporters. Cedazuridine is an inhibitor of CDA and as such, drugs known

to be metabolized by CDA should be avoided during treatment with ASTX727. Concomitant proton pump inhibitor medication usage was shown not to have a significant impact on decitabine, cedazuridine, and cedazuridine-epimer parameters and subsequent exposures, based on population PK analysis.

6.2.3 Belinostat

6.2.3.1 Concomitant medications and supportive guidelines

Belinostat is associated with nausea. **Patients must receive prophylactic treatment with a 5-HT3 receptor antagonist for the first two cycles of treatment.** Ondansetron (oral disintegrating tablet 8 mg PO or equivalent) may be administered once prior to each dose of belinostat. Alternatively, granisetron extended-release injection 10 mg SC may be administered once on day one prior to the first administration of belinostat in each treatment cycle. If nausea or vomiting occurs despite the use of a 5-HT3 receptor antagonist, consider the addition of dexamethasone 8-10 mg PO or IV prior to each dose of belinostat. Continuation of the 5-HT3 receptor antagonist after the first two cycles is at the treating investigator's discretion.

As discussed in Section 6.2.1.1 and 6.2.2.1, patients will be administered prophylactic growth factor.

Patients should receive all supportive care appropriate for their medical condition, including, but not limited to, antibiotics, transfusions, and other agents deemed necessary for symptom management, unless specifically prohibited elsewhere in the protocol.

6.2.3.2 Drug-drug interactions

Belinostat is primarily metabolized by hepatic UGT1A1, and to a lesser extent by CYP2A6, CYP2C9, and CYP3A4 enzymes. Patients with known UGT1A1 genetic polymorphisms, such as UGT1A1*28, may have reduced UGTA1A activity and may be at risk for increased belinostat exposure. **Subjects with known UGT1A1 genetic polymorphisms are not eligible for this study.**

Chronic use of any medications or substances that are strong inhibitors of UGT1A1 is not allowed. Patients must switch to alternative medications 7-14 days before treatment with belinostat. Because the lists of these agents are constantly changing, it is important to regularly consult a frequently-updated medical reference. As part of the enrollment/informed consent procedures, the patient will be counseled on the risk of interactions with other agents, and what to do if new medications need to be prescribed or if the patient is considering a new over-the-counter medicine or herbal product.

In vitro studies have shown belinostat and its metabolites are weak to moderate inhibitors of CYP2C8 and moderate to strong inhibitors of CYP2C9, although studies did not demonstrate effects when co-administered with warfarin. Avoid CYP2C8 and CYP2C9 substrates during belinostat treatment.

Avoid concomitant medications that may cause Torsade de Pointes.

6.3 Duration of Therapy

In the absence of treatment delays due to AEs, treatment may continue until one of the following criteria applies:

- Disease progression as defined by RECIST 1.1 criteria
- Intercurrent illness that prevents further administration of treatment
- Unacceptable AEs
- Patient decides to withdraw from the study
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator
- Clinical progression
- Patient non-compliance
- Pregnancy
    - All women of child-bearing potential should be instructed to contact the investigator immediately if they suspect they might be pregnant (*e.g.*, missed or late menstrual period) at any time during study participation.
    - The investigator must immediately notify CTEP in the event of a confirmed pregnancy in a patient participating in the study.
- Termination of the study by sponsor
- The drug manufacturer can no longer provide the study agent

The reason(s) for protocol therapy discontinuation, the reason(s) for study removal, and the corresponding dates must be documented in the Case Report Form (CRF).

6.4 Duration of Follow-Up

Patients will be followed for up to 24 months after removal from study or until death, whichever occurs first. During the follow-up period, patients will be evaluated in the clinic or contacted by telephone approximately once every 3 months to ascertain information related to disease status and subsequent anti-cancer treatment. Patients removed from study for unacceptable AEs will be followed until resolution or stabilization of the AE.

7. DOSING DELAYS/DOSE MODIFICATIONS

7.1 General Principles

The following general principles apply to management of study drug toxicities:

- Dosing for a given cycle will be based on AEs observed during the prior cycle(s). When multiple AEs occur, the modification that would require the patient to receive the lowest dose level of the study drug(s) is used.

- When toxicity requires a new treatment cycle to be delayed, both agents should be held until the toxicity resolves and then resumed simultaneously.

- If toxicity requires a study agent to be held during the dosing phase (Days 1-5) of a cycle, the other study agent may be either held or continued depending on the nature of the toxicity.

- A subject may incur up to 2 dose reductions of each study agent prior to discontinuation of that agent. SGI-110 (guadecitabine) should not be reduced below 15 mg/m^2. Belinostat should not be reduced below 500 mg/m^2. For ASTX727, dose modification involves reducing the number of days of treatment. The number of days of treatment should not be lowered below 3 days per treatment cycle. After discontinuation of one study agent, the other study agent may be continued.

- For both hematologic and non-hematologic AEs, a delay of up to four weeks (28 days) is permitted from the scheduled cycle start date to allow for toxicity to resolve such that criteria in Section 7.3 are met. If toxicity has not resolved by that time, study treatment should be discontinued.

- Patients who experience DLTs (as defined in Section 9) may continue study treatment after dose modification.

7.2 Dose Levels of Study Agents

The following dose levels for SGI-110 (guadecitabine), ASTX727 and belinostat will be used on this study and apply to the dose modifications recommended elsewhere in the protocol.

	SGI-110 (Guadecitabine)
Initial Dose	45 mg/m^2
Dose Level -1	30 mg/m^2
Dose Level -2	15 mg/m^2
Dose Level -3	Discontinue agent

	ASTX727
Initial Dose	5 day treatment (days 1-5)

Dose Level -1	4 day treatment (days 1-4)
Dose Level -2	3 day treatment (days 1-3)
Dose Level -3	Discontinue agent

	Belinostat
Initial Dose	1000 mg/m^2
Dose Level -1	750 mg/m^2
Dose Level -2	500 mg/m^2
Dose Level -3	Discontinue agent

7.3 Criteria for Initiation of a New Cycle

A new cycle of treatment may begin only if the following criteria are met:

- ANC ≥1,000/μL
- Hemoglobin ≥8 g/dL
- Platelets ≥75,000/μL

If transfusion has occurred, it should be evident that counts are stable or improving on their own (without the use of transfusion or growth factor) at the time treatment is resumed.

7.4 Dose Modifications for Hematologic Toxicity

Both study agents are associated with myelosuppression. In order to begin a new treatment cycle, criteria specified in Section 7.3 must be met. A delay of up to 4 weeks is permitted to allow toxicity to resolve and initiate a new cycle of treatment. If counts have not recovered despite a 4 week delay from the scheduled start date, the patient should be discontinued from the study. Because the agents are administered concurrently, it may be difficult to attribute hematologic toxicity to one of the two agents with certainty. In general, SGI-110 (guadecitabine) or ASTX727 (whichever agent the patient is receiving) is associated with more frequent myelosuppression than belinostat. Therefore, a stepwise approach to dose modification is recommended, beginning with SGI-110 (guadecitabine) or ASTX727, as shown in the table below. If the investigator believes a hematologic toxicity is clearly attributable to one of the two drugs, that drug may be preferentially modified.

Observed Hematologic Toxicity (at any time during cycle)	**Recommended Management**
ANC <500/μL Hemoglobin <7 g/dL Platelets <25,000/μL Grade 3 or 4 neutropenic fever Grade 3 thrombocytopenia with bleeding	Delay cycle. Monitor complete blood count at least weekly. Transfuse and/or use growth factor per institutional guidelines. When criteria for a new cycle are met, **resume at one dose level lower**, per the table immediately below.

Other hematologic AEs deemed clinically significant and for which dose modification is deemed necessary by the treating investigator.	
ANC 500-999/μL Hemoglobin 7.0-7.9 g/dL Platelets 25,000-74,999/μL	Delay cycle. Monitor complete blood count at least weekly. Transfuse and/or use growth factor per institutional guidelines. **When criteria for a new cycle are met, resume at the same dose if the delay was 14 days or less, otherwise resume at one dose level lower, per the table immediately below.** If hematologic toxicity requiring a dose reduction occurs, and a delay is also needed, only one dose reduction is applied.

Hematologic Toxicity Event	Recommended Management
First Event	Reduce SGI-110 (guadecitabine) or ASTX727 by one dose level
Second Event	Reduce belinostat by one dose level
Third Event	Reduce SGI-110 (guadecitabine) or ASTX727 by one dose level
Fourth Event	Reduce belinostat by one dose level

7.5 Dose Modifications for Non-Hematologic Toxicity

Toxicity Grade	Management of Agent Causing Toxicity
Diarrhea	
Grade 1	Continue treatment. Evaluate for infectious etiology if clinically indicated. Institute anti-diarrheal therapy with loperamide.
Grade 2	Continue treatment. Evaluate for infectious etiology if clinically indicated. Institute anti-diarrheal therapy with loperamide. If grade 2 diarrhea persists for more than 3 days despite supportive care, interrupt treatment or delay cycle and manage supportively until symptoms improve to grade ≤1. At next cycle, reinitiate treatment at same dose. For recurrent grade 2 diarrhea lasting more than 3 days despite supportive care, manage as above, but resume treatment when resolved to grade ≤1 at one dose level lower for the agent to which toxicity is attributed.
Grade 3 Grade 4	Interrupt treatment or delay cycle until symptoms improve to grade ≤1. Evaluate for infectious etiology if clinically indicated. Institute anti-diarrheal therapy. At next cycle, resume treatment at one dose level lower for the agent to which toxicity is attributed.
Fatigue	
Grade 1	Continue treatment. Evaluate for other causes of fatigue including infection or anemia.
Grade 2	Continue treatment. Evaluate for other causes of fatigue including

	infection or anemia. If grade 2 fatigue persists for more than 3 days and is deemed intolerable by the patient or treating investigator, interrupt treatment or delay cycle until resolved to grade ≤1. At next cycle, reinitiate treatment at same dose.
Grade 3	Interrupt treatment or delay cycle until grade ≤1. At next cycle, resume treatment at one dose level lower for the agent to which toxicity is attributed.
Vomiting	
Grade 1	Continue treatment. Escalate supportive care. Consider addition of dexamethasone prior to treatment.
Grade 2	Continue treatment. Escalate supportive care. Consider addition of dexamethasone and/or additional anti-emetics prior to treatment, per NCCN Guidelines for Supportive Care. If grade 2 vomiting persists for more than 3 days, interrupt treatment or delay cycle until resolved to grade ≤1. At next cycle, reinitiate treatment at same dose.
Grade 3	Interrupt treatment or delay cycle until improved to grade ≤1. Escalate supportive care. Consider addition of dexamethasone and/or additional anti-emetics prior to treatment, per NCCN Guidelines for Supportive Care. At next cycle, resume treatment at one dose level lower for the agent to which toxicity is attributed.
Grade 4	Discontinue offending agent.
Other Non-Hematologic Toxicity	
Other grade 3 AEs deemed clinically significant by the treating investigator*	Interrupt treatment or delay cycle until event resolves to grade ≤1 or baseline. Resume at one dose level lower for the study agent to which toxicity is attributed.
Grade 4 (except alopecia, clinically insignificant laboratory abnormalities or AEs described elsewhere in this section)	Discontinue offending agent.

*Treatment may also be interrupted for grade 2 non-hematologic toxicities deemed intolerable by the patient, until such toxicity is resolved to grade 1 or baseline. If intolerable toxicities persist >4 weeks despite supportive care, the agent responsible for the toxicity should be discontinued.

8. PHARMACEUTICAL INFORMATION

A list of the AEs and potential risks associated with the investigational agents administered in this study can be found in Section 10.1.

8.1 CTEP IND Agents

8.1.1 SGI-110 (Guadecitabine) (NSC # 780643)

There may not be additional supply of SGI-110 (Guadecitabine) after 31-AUG-2021 if the current batch of SGI-110 and its diluents expiry dates cannot be extended. Further treatment options will be discussed with subjects enrolled to the belinostat/SGI-110 regimen who remain on treatment with that regimen as of 31-AUG-2021. Patients may be allowed to change treatment to ASTX727 and belinostat in August 2021 if the overall assessment of risks versus benefits support that change, primarily related to any observations of adverse safety and tolerability of ASTX727 and belinostat at that time.

Chemical Name:
Sodium[(2*R*,3*S*,5*R*)-5-(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)-3-hydroxyoxolan-2-yl]methyl (2*R*,3*S*,5*R*)-5-(4-amino-2-oxo-1,3,5-triazin-1(2*H*)-yl)-2-(hydroxymethyl)oxolan-3-yl phosphate

Other Names: Guadecitabine

Classification: DNA methylation inhibitor

Molecular Formula: $C_{18}H_{23}N_9NaO_{10}P$ **M.W.:** 579.39 Da

Approximate Solubility*:* It is water soluble at about 30 mg/mL over a pH range of 6.0 to 7.0. It is unstable in aqueous solutions and is relatively more stable at neutral pH. SGI-110 is soluble in common organic solvent systems such as methanol, dimethylamine (DMA), and dimethyl sulfoxide (DMSO).

Mode of Action: SGI-110 is a potent inhibitor of DNA methylation. SGI-110 is a dinucleotide of decitabine and deoxyguanosine linked with a phosphodiester bond. Decitabine is the active metabolite. Guadecitabine is a new chemical entity that was designed to enhance pharmacokinetic (PK) properties compared with decitabine, with potential to improve pharmacodynamics (PD), clinical efficacy, and safety.

How Supplied: SGI-110 drug product as a two-vial system is supplied by Astex Pharmaceutical and distributed by the Pharmaceutical Management Branch, CTEP/DCTD/NCI as follows:

- **SGI-110 for Injection**: 100 mg dry powder lyophile containing free acid equivalent in a single-use vial;
- **Diluents for Reconstitution**: 1.2 mL single-use vial containing non-aqueous diluent of propylene glycol, glycerin, and ethanol.

All vials are packaged in clear glass vials with stoppers made of **latex-free rubber stoppe**r and capped with an aluminum flip-off seal.

Preparation:

- Allow SGI-110 vial(s) and diluent vial(s) to reach room temperature approximately 60 minutes.
- Next, loosen the lyophilized powder of SGI-110 by gently tapping and rotating the vial on a hard surface.
- Use a 1 mL syringe and withdraw 0.9 mL of diluent and add it to the SGI-110 vial resulting in a final concentration of 100 mg/mL. Manually shake or mechanically vortex the diluted vial(s).

- Manually shake: Intermittently shake the reconstituted vial vigorously (approximately 5 minutes). In general, the shaking process will take 5 to 10 minutes. Rotate the vial and inspect the vial contents to ensure that all lyophilized SGI-110 powder has dissolved. After dissolution, allow the vial to rest until all bubbles have dissipated (approximately 10 minutes).
- Mechanically shake (vortex): Before vortexing, rotate or shake the vial to ensure that the drug powder is thoroughly wetted with diluent. Then, vortex for 5-10 minutes at a speed setting of 5. Remove the vial from the holder and invert it 2-3 times. Place the vial on the vortex and mix for another 10 minutes. Ensure that all SGI-110 powder is completely dissolved. Repeat the process if partially dissolved. Once all drug is dissolved, allow the solution rest until all bubbles dissipate (approximately 10 minutes).
- The final solution of the diluted vial should be clear and colorless to pale yellow.
- Withdraw the diluted solution into a syringe. If two or more syringes are needed to deliver the total prescribed dose, all dispensed syringes should have equivalent volumes for consistency and ease of administration.
- If the local institutional standards require the use of a safety device, such as a PhaSeal™, then the extractable volume is reduced to around 60 mg (0.6 mL).

Note: If SGI-110 powder has not dissolved 60 minutes after completion of manually shaking or vortex, do not use the vial(s)**.**

Storage:
- Store SGI-110 lyophilized powder refrigerated between 2° and 8° C (36° and 46° F)
- Store 1.2 mL diluent between 2° and 8° C (36° and 46° F)

If a storage temperature excursion is identified, promptly return SGI-110 vials and the diluents to the recommended storage temperatures above and quarantine the supplies. Provide a detailed report of the excursion (including documentation of temperature monitoring and duration of the excursion) to PMBAfterHours@mail.nih.gov for determination of suitability.

Stability: Stability studies of SGI-110 intact vials are ongoing.

- Upon reconstitution, the diluted drug solution is good for 8 days (either in syringe or vial) when refrigerated between 2^0 and 8^0 C (36^0 and 46^0 F) or up to 24 hours at room temperature.

Route*(s)* of Administration: subcutaneous injection

Method of Administration: Administer by slow subcutaneous injection. If injection site pain occurs, give slow injection (over 60 seconds) and apply ice packs to the injection site both before and after injection for 5 – 10 minutes each; however, using icepack for the first dosing is not recommended (as it may not be needed). Pretreatment of topical or systemic analgesics can be considered.

Potential Drug Interactions*:* Drug-drug interaction studies have not been conducted with SGI-110 or decitabine. In vitro studies suggested that SGI-110 is unlikely to inhibit or induce major

P450 enzymes and is not a CYPs substrate. Furthermore, SGI-110 nor the active metabolite decitabine inhibit major human drug transporters.

Special Handling: SGI-110 is a cytotoxic agent. Use PPE when preparing SGI-110. If SGI-110 comes in direct contact with the skin, wash immediately with copious amount of water and soap (soap degrades the chemical rapidly). If SGI-110 comes in direct contact with the mucous membranes, flush the affected area thoroughly with water.

Drug spills can be inactivated by either using a 2 N sodium hydroxide solution or water and kericide CR Biocide B which consists of a blend of stabilized chlorine dioxide and a quaternary ammonium compound.

Availability: SGI-110 (guadecitabine) is an investigational agent supplied to investigators by the Division of Cancer Treatment and Diagnosis (DCTD), NCI.

SGI-110 (guadecitabine) is provided to the NCI under a Collaborative Agreement between Astex Pharmaceuticals, and the DCTD, NCI (see Section 13.5).

8.1.2 ASTX727 (NSC# 820631)

Chemical Name (Cedazuridine): (4*R*)-1-[(2*R*,4*R*,5*R*)-3,3-difluoro-4-hydroxy-5-(hydroxymethyl)oxolan-2-yl]-4-hydroxy-1,3-diazinan-2-one

Chemical Name (Decitabine): 4-amino-1-[(2*R*,4*S*,5*R*)-4-hydroxy-5-(hydroxymethyl)oxolan-2-yl]-1,3,5-triazin-2-(1*H*)-one

Other Names: Cedazuridine and decitabine; INQOVI®

Classification: Cytidine deaminase inhibitor and DNA hypomethylating agent

CAS Registry Number (Cedazuridine): 1141397-80-9

CAS Registry Number (Decitabine): 2353-33-5

Molecular Formula (Cedazuridine): $C_9H_{14}F_2N_2O_5$ **M.W**.: 268.21 Daltons

Molecular Formula (Decitabine): $C_8H_{12}N_4O_4$ **M.W**.: 228.21 Daltons

Approximate Solubility of Cedazuridine: Soluble in dimethylsulfoxide, sparingly soluble in water and 50 mM phosphate, slightly soluble in methanol, and insoluble in acetone, and acetonitrile. Not hygroscopic.

Approximate Solubility of Decitabine: Freely soluble in dimethyl sulfoxide, slightly soluble in water, very slightly soluble in methanol. Hygroscopic.

Mode of Action: Decitabine is a cytidine nucleoside analog that inhibits DNA methylation,

thereby causing hypomethylation of DNA and cellular differentiation and/or apoptosis. Cytidine deaminase (CDA) is an enzyme that catalyzes the degradation of cytidine, including the cytidine analog decitabine. High levels of CDA in the gastrointestinal tract and liver degrade decitabine and limit its oral bioavailability. Cedazuridine is a CDA inhibitor. Administration of cedazuridine with decitabine increases systemic exposure of decitabine.

How Supplied: Astex Pharmaceuticals Inc. supplies and PMB distributes, ASTX727 as a red, film-coated, oval, immediate-release, fixed dose combination (FDC) tablet containing the cedazuridine (100 mg) and decitabine (35 mg).

Inactive ingredients consist of lactose, hypromellose, croscarmellose sodium, colloidal silicon dioxide, magnesium stearate, and Opadry II 85F15458 Red. The Opadry is a tablet coating formula that contains titanium dioxide, polyvinyl alcohol, polyethylene glycol, talc, and iron oxide red. Tablets are plain faced on both sides. Future clinical supply may contain a debossed marking of "H35" on each tablet.

ASTX727 is packaged as 5 tablets per bottle. The bottles are white opaque high-density polyethylene (HDPE) with a child resistant closure containing 1 or 2 desiccant canisters for moisture absorption. Bottles have an induction seal.

Storage: Store intact/unopen bottles at 20°C to 25°C (68°F to 77°F); excursions permitted to 15°C to 30°C (59°F to 86°F).

> If a storage temperature excursion is identified outside of the permitted excursion 15°C to 30°C (59°F to 86°F), promptly return ASTX727 to 20°C to 25°C (68°F to 77°F) and quarantine the supplies. Provide a detailed report of the excursion (including documentation of temperature monitoring and duration of the excursion) to PMBAfterHours@mail.nih.gov for determination of suitability.

Stability: Stability studies are ongoing.

Route and Method of Administration: Oral. Take ASTX727 tablet approximately at the same time on an empty stomach. Do not eat, drink milk or alcohol 2 hours before and 2 hours after taking ASTX727. Clear liquids such as water, black coffee, or tea are allowed. Take whole tablets with 8 oz. (240 mL) of water; do not crush, cut, or chew the tablet.

Missed dose within 12 hours of the scheduled time can be made up. Patient needs to take as soon as possible and resume the next day on schedule. If the patient fails to make up the dose within 12 hours, the dose should be resumed at the scheduled time the next day.

Potential Drug Interactions: Decitabine had no clinically meaningful effect on the pharmacokinetics of cedazuridine. Cedazuridine increased the exposure of decitabine.

Cedazuridine is an inhibitor of cytidine deaminase (CDA) and as such, drugs known to be metabolized by CDA should be avoided during treatment with ASTX727.

CYP450: Cedazuridine is not a substrate of cytochrome P450 (CYP) enzymes. Cedazuridine does not induce CYP1A, CYP2B6, CYP2C9, or CYP3A or inhibit CYP1A, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A.

Protein Transporters: Cedazuridine is not a substrate of P-glycoprotein (P-gp), MATE1, MATE2-K, OAT1, OAT3, OATP1B1, OATP1B3, OATP2B1, OCT1, or OCT2, and does not inhibit P-gp, BCRP, MATE1, MATE2-K, OAT1, OAT3, OATP1B1, OATP1B3, or OCT2.

Concomitant use of proton pump inhibitor does not have significant impact on decitabine or cedazuridine exposure.

Protein binding: Plasma protein binding of decitabine is negligible (<1%) and for cedazuridine is low (~34% to 38%), therefore interactions due to displacement of more highly protein bound drugs from plasma proteins are not expected.

Patient Care: ASTX727 can cause fetal harm when administered to pregnant women. For that reason, women of childbearing potential must use highly effective contraception during treatment with ASTX727 and for at least 6 months after the last dose. Men with female partners of childbearing potential should be advised to practice highly effective contraceptive measures of birth control and not to father a child while receiving treatment with decitabine and for 3 months after the last dose. Breastfeeding is not allowed during the study and for 2 weeks after the last dose.

8.1.3 Belinostat (PXD-101) (NSC # 726630)

Chemical Name: (E)-*N*-hydroxy-3-(3-(N-phenylsulfamoyl)phenyl)acrylamide

Other Names: PXD101; Beleodaq®

Classification: Histone deacetylase (HDAC) inhibitor

CAS Registry Number: 414864-00-9; 866323-14-0

Molecular Formula: $C_{15}H_{14}N_2O_4S$ **M.W.:** 318.35

Approximate Solubility: Water 0.14 mg/mL; ethanol >200 mg/mL; polyethylene glycol 400 ~ 1.5 mg/mL; 1,2-propanediol ~ 0.2 mg/mL

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. Belinostat is a novel and potent HDAC inhibitor of the hydroxamate class. It alters acetylation levels of histone and non-histone proteins, thus influencing chromatin accessibility and ultimately gene transcription.

How Supplied: Acrotech Biopharma supplies and the Pharmaceutical Management

Branch, CTEP, DCTD, NCI distributes belinostat in single-use 30 mL clear glass vials with coated stoppers and aluminum crimp seals with "flip-off" caps containing 500 mg belinostat For Injection. The sterile yellow lyophilized product also contains 1000 mg arginine, Ph. Eur/USP.

Preparation: Reconstitute the lyophilized product with 9 mL Sterile Water for Injection to yield a final belinostat concentration of 50 mg/mL. Before intravenous administration, further dilute in 250 mL 0.9 % Sodium Chloride Injection.

Storage: Store intact vials of belinostat at controlled room temperature (20-25°C; 68- 77°F); brief excursions permitted (15-30°C; 59-86 °F). Leave intact vials of belinostat in the secondary packaging until use.

If a storage temperature excursion is identified, promptly return belinostat to controlled room temperature and quarantine the supplies. Provide a detailed report of the excursion (including documentation of temperature monitoring and duration of the excursion) to PMBAfterHours@mail.nih.gov for determination of suitability.

Stability: Shelf life stability studies of intact vials of belinostat are on-going; once the lyophilized product is reconstituted, the vialed solution is stable for up to 12 hours at ambient temperature (15-25°C).

Once further diluted in 250 mL of 0.9% sodium chloride, belinostat may be stored at ambient temperature (15-25°C) for up to 36 hours, including the infusion time.

Route and Method of Administration: Infuse belinostat intravenously over 30 minutes through a 0.22 micron in-line filter.

Potential Drug Interactions: Belinostat is primarily metabolized by hepatic UGT1A1, and to a lesser extent by CYP2A6, CYP2C9 and CYP3A4 enzymes. Concomitant use of strong inhibitors of UGT1A1 is not allowed. Use caution when co-administering agents which may compete for UGT1A1 metabolism, such as irinotecan. Patients with known UGT1A1 genetic polymorphisms, such as UGT1A1*28, can have reduced UGTA1A activity and may be at risk for increased belinostat exposure. If subjects with UGT1A1 genetic polymorphisms are not excluded from study participation, reduced doses are warranted.

In vitro studies have shown belinostat and its metabolites are weak to moderate inhibitors of CYP2C8 and moderate to strong inhibitors of CYP2C9; although, studies did not demonstrate effects when co-administered with warfarin. Avoid CYP2C8 and CYP2C9 substrates during belinostat treatment unless deemed medically necessary.

Avoid concomitant medications that may cause Torsade de Pointes.

Availability: Belinostat (PXD101) is an investigational agent supplied to investigators by the Division of Cancer Treatment and Diagnosis (DCTD), NCI.

Belinostat (PXD101) is provided to the NCI under a Collaborative Agreement between Acrotech

Biopharma and the DCTD, NCI (see Section 13.5).

8.1.4 Agent Ordering and Agent Accountability

8.1.4.1 NCI-supplied agents may be requested by eligible participating Investigators (or their authorized designee) at each participating institution. The CTEP-assigned protocol number must be used for ordering all CTEP-supplied investigational agents. The eligible participating investigators at each participating institution must be registered with CTEP, DCTD through an annual submission of FDA Form 1572 (Statement of Investigator), NCI Biosketch, Agent Shipment Form, 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 participating investigator at that institution.

Confirmation of patient enrollment is required for initial supply.

Submit agent requests through the PMB AURORA application. Access to AURORA requires the establishment of credentials necessary to access secure NCI Clinical Oncology Research Enterprise (CORE) systems, maintenance of an "active" account status, a "current" password, and active person registration status. For questions about drug orders, transfers, returns, or accountability, call or email PMB any time or use the dialog function in AURORA to communicate with PMB staff. Refer to the PMB's website for specific policies and guidelines related to agent management.

8.1.4.2 Material Safety Data Sheets

The current versions of the material safety data sheets (MSDS or SDS) for PMB-distributed agents will be accessible to site investigators and research staff through the PMB AURORA application. Questions about MSDS access may be directed to the PMB at PMBAfterHours@mail.nih.gov or by using the dialog function in AURORA to communicate with PMB staff.

8.1.4.3 Agent Shortages

Specific guidance on how to address agent shortages for patients already enrolled on a clinical study as well as how to manage potential enrollment of new patients is provided at https://ctep.cancer.gov/branches/pmb/drug_shortages.htm.

Treatment plan modifications being made to avoid immediate hazard to patients is permissible under the Department of Health and Human Services (HHS) regulations at 45 CFR 46.103(b)(4)(iii). In accordance with HHS regulations, local investigators must promptly inform the IRB of record of this unanticipated problem and the management plan for the trial.

8.1.4.4 Agent Inventory Records – The investigator, or a responsible party designated by the investigator, must maintain a complete accountability of the receipt, dispensing and

final disposition of all agents received from the PMB using the appropriate NCI Investigational Agent (Drug) Accountability Record (DARF) available on the CTEP forms page. Store and maintain separate NCI Investigational Agent Accountability Records for each agent, strength, formulation and ordering investigator on this protocol.

Product Quality Complaint (PQC): A product quality complaint is defined as any suspicion of a product defect related to a potential quality issue during manufacturing, packaging, release testing, stability monitoring, dose preparation, storage or distribution of the product, or delivery system. Not all PQCs involve a study subject. Lot or batch numbers are of high significance and need to be provided where and when possible. PQC must be reported to the PMB as soon as the PQC is identified. Report PQC to PMB at PMBAfterHours@mail.nih.gov or by using the dialog function in AURORA to communicate with PMB staff.

8.1.5 Investigator Brochure Availability

The current versions of the IBs for the agents will be accessible to site investigators and research staff through the PMB AURORA application. Access to AURORA requires the establishment of credentials necessary to access secure NCI Clinical Oncology Research Enterprise (CORE) systems, maintenance of an "active" account status, a "current" password and active person registration status. Questions about IB access may be directed to the PMB IB Coordinator via email.

8.1.6 Useful Links and Contacts

- CTEP Forms, Templates, Documents: http://ctep.cancer.gov/forms/
- NCI CTEP Investigator Registration: RCRHelpDesk@nih.gov
- PMB policies and guidelines: http://ctep.cancer.gov/branches/pmb/agent_management.htm

- PMB Agent Inventory Management System (AURORA) application: https://ctepcore.nci.nih.gov/aurora/login
- CTEP Identity and Access Management (IAM) account: https://ctepcore.nci.nih.gov/iam/
- CTEP IAM account help: ctepreghelp@ctep.nci.nih.gov
- IB Coordinator: IBCoordinator@mail.nih.gov
- PMB email: PMBAfterHours@mail.nih.gov
- PMB phone and hours of service: (240) 276-6575 Monday through Friday between 8:30 am and 4:30 pm (ET)

9. STATISTICAL CONSIDERATIONS

The proposed study is an open-label, single-arm, Simon 2-stage phase 2 clinical trial. Neither

the combination of belinostat and SGI-110 (guadecitabine) nor the combination of belinostat and ASTX727 have been formally evaluated in a phase 1 dose-finding study and therefore a safety lead-in and continuous safety monitoring rule will be applied to monitor for excessive or unexpected toxicity as described in this section.

There is no approved or accepted standard therapy for CS. Chemotherapy is generally ineffective, and no drug has shown clinical efficacy to date. Conventional CS patients are commonly enrolled on clinical trials in the first-line setting. Although chemotherapy is sometimes employed, efficacy is marginal and toxicity is significant. We expect that many sarcoma oncologists would find a chemotherapy control arm problematic, and this would create a barrier to accrual. For these reasons, a single-arm (non-randomized) design is proposed in this rare disease.

With Amendment 5, the study was modified to substitute ASTX727 for SGI-110 (guadecitabine). These are similar agents with the same mechanism of action. At the time of this modification, 6 patients were enrolled and treated with belinostat and SGI-110 (guadecitabine). We will report safety and efficacy endpoints separately for these 6 patients. These 6 patients will be replaced for the purposes of the original study design. The Simon 2-stage design described below will be conducted among patients treated with belinostat and ASTX727. A new safety lead-in will be performed among the first 6 patients treated with belinostat and ASTX727. The continuous toxicity monitoring rule will also apply. The total sample size for the study will increase from 26 patients to 32 patients, accounting for the 6 patients that will be replaced.

9.1 Study Design/Endpoints

9.1.1 Primary Endpoint

The primary endpoint for this phase 2 study is the objective response rate. Noting that chemotherapy is associated with objective response rates of 8-12% in conventional chondrosarcoma (Fox *et al.*, 2012; Italiano *et al.*, 2013), and most clinical trials with targeted agents have shown response rates of 0%, we will consider an objective response rate of 8% as reflecting the activity of chemotherapy and an objective response rate of 28% suggesting promising activity warranting further study. A Simon optimal 2 stage design is employed. The design calls for 26 patients. In stage one, 13 patients will be enrolled. If 2 or more responses are observed, the study will proceed to full accrual. If 5 or more responses are seen among the 26 patients, the study treatment is considered promising for further study. This design has 85% power with alpha of 0.054 to test for a response rate of 8% (null hypothesis) *versus* 28% (alternative hypothesis). The probability of early stopping is 72%.

With Amendment 5, the study was modified to substitute AST727 for SGI-110 (guadecitabine). At that time, 6 patients had been enrolled and initiated study treatment. We will report safety and efficacy separately for these 6 patients. We will report the objective response rate, progression-free survival and safety and tolerability for this group of patients. These 6 patients will be replaced. The Simon optimal 2 stage design described above will be applied to patients receiving the belinostat and ASTX727 combination.

9.1.2 Secondary Endpoints

Secondary endpoints are the presence of treatment related AEs, the occurrence of DLTs, and PFS. Adverse events will be recorded at each clinical visit and will be categorized according to NCI CTCAE version 5.0. The attribution of AEs to each of the study drugs will also be recorded.

Secondary endpoints will be reported separately for the population of patients receiving belinostat and SGI-110 (guadecitabine) and the population of patients receiving belinostat and ASTX727.

The study will use a continuous toxicity monitoring rule as described in Section 9.4. DLTs are defined in Section 9.2 and to qualify as DLT must be attributed to the study drugs and not to disease, and occur (or first become evident) during the first four cycles of treatment for the purposes of continuous toxicity monitoring.

PFS is defined as the time from first treatment with the study drug to the earliest of either disease progression or death from any cause. Patients who are alive and progression free will be censored at the time of their last follow-up.

9.2 Dose-Limiting Toxicity

The following definitions of dose-limiting toxicity (DLT) apply to both the safety lead-in (Section 9.3) and the continuous toxicity monitoring rule (Section 9.4). DLTs are defined as the following toxicities which are attributed to the study drug(s) and not to disease, and which occur (or first become evident) during a prespecified timeframe. For the purposes of the safety lead-in, this timeframe is the first two cycles of treatment. For the purposes of the continuous toxicity monitoring rule, this timeframe is the first four cycles of treatment.

1. Any grade ≥3 non-hematologic toxicity, except grade ≥3 fatigue, nausea, or vomiting which must persist >5 days despite maximal supportive care to be considered dose limiting.
2. Any grade ≥4 hematologic toxicity lasting >14 days, except grade 4 lymphopenia, which is not considered a DLT.
3. Grade ≥3 neutropenia with fever
4. Grade ≥3 thrombocytopenia with clinically significant bleeding
5. Any laboratory abnormality not otherwise addressed above which is associated with clinical sequelae and which fails to resolve to grade ≤1 within 5 days of occurrence, or any laboratory criteria meeting Hey's law for liver toxicity.
6. Any event leading to dose reduction or permanent discontinuation of any study drug during cycles 1-2 (applies to safety lead-in subjects only).

9.3 Safety Lead-In

Recognizing that SGI-110 (guadecitabine) and belinostat have not been formally evaluated together, but that previously completed studies have shown DNA hypomethylating agents and

HDAC inhibitors are generally well tolerated in combination, we will perform a safety lead-in among six patients to confirm safety and tolerability of the proposed dose and schedule.

The safety lead-in will apply to the first six patients treated for two cycles (8 weeks). These patients will undergo safety assessments as specified in the study calendar. The study will not proceed to further accrual until these six patients have completed two cycles or 8 weeks of study treatment; however, patients who end study treatment during this period for reasons other than toxicity, *i.e.*, disease progression, will not be replaced. At that time, the study investigators, CTEP, and the DSMB (if applicable) will review the safety data. If two or more patients in the safety lead-in population experience DLTs as defined in Section 9.2, the study team, CTEP, and the DSMB (if applicable) will consider terminating the study, amending the study to evaluate a lower dose level, and/or further evaluating safety at the current dose level among a limited number of additional patients, and the amended protocol and rationale for modification will be submitted to the CIRB for review. The study team, CTEP, and the DSMB (if applicable) may also take such action if safety concerns exist even in the absence of 2 or more DLTs occurring during the safety lead-in. Formal documentation of the study team's review of toxicity data from the safety lead-in will be provided prior to resuming accrual.

With Amendment 5, the study was modified to substitute AST727 for SGI-110 (guadecitabine). The safety lead-in period will be repeated as described among the first 6 patients treated with ASTX727 and belinostat. However, the safety lead-in period for belinostat and ASTX727 will use a DLT monitoring period of 4 weeks (instead of 8 weeks). The continuous toxicity monitoring rule discussed below will continue to monitor for later-onset toxicity. Definitions of DLT remain the same.

Investigators are expected to report any DLT events directly to the study statistician and the Data Coordinating Center within 24 hours of their knowledge of such an event.

9.4 Continuous Toxicity Monitoring Rule

In addition to the safety lead-in, a continuous toxicity monitoring rule will be applied to this study to monitor for excessive or unexpected toxicity associated with study treatment (Ivanova *et al.*, 2005). Patients will be subjected to continuous toxicity monitoring during the first four cycles of study treatment. Sequential boundaries will be used to monitor the DLT rate. DLTs are defined in Section 9.2 above. According to this model, shown in the table below, enrollment will be halted if the number of DLTs is equal to or exceeds b_n out of n patients with completed DLT follow-up. This is a Pocock-type stopping boundary which yields a probability of crossing the boundary (early stopping) of at most 30% stopping when then the rate of DLT is equal to the acceptable rate of 20% using a pre-defined sample size of 26 patients. This boundary is equivalent to testing the null hypothesis, after each patient, that the event rate is equal to 0.20, using a one-sided level 0.14 test.

With Amendment 5, the study was modified to substitute ASTX727 for SGI-110 (guadecitabine). The continuous toxicity monitoring rule will apply as defined above to patients treated with belinostat and ASTX727. Toxicities experienced by the initial cohort of 6 patients treated with SGI-110 (guadecitabine) and belinostat will not be considered in the model as it

applies to subsequent patients treated with ASTX727 and belinostat.

If the early stopping event occurs, the study will be halted and the investigators, CTEP, and the DSMB (if applicable) will discuss whether to close the study early for toxicity or modify the protocol to address the observed events. In addition, if any grade 5 event at least possibly related to study treatment occurs, the study will also be halted for review in this fashion.

Number of patients (n)	1	2	3	4	5	6	7	8	9	10	11	12	13
Boundary, b_n	-	2	2	3	3	3	4	4	4	4	5	5	5
Number of patients (n)	14	15	16	17	18	19	20	21	22	23	24	25	26
Boundary, b_n	5	6	6	6	6	7	7	7	7	8	8	8	8

Investigators are expected to report any DLT events directly to the study statistician and the Data Coordinating Center within 24 hours of their knowledge of such an event.

9.5 Sample Size/Accrual Rate

The study was initially planned to enroll 26 patients at a rate of approximately 1-2 patients per month across all participating centers. Thus, assuming the study meets the stage 1 endpoint, we anticipated full accrual after approximately 13-26 months. We intend to open the proposed study throughout the ETCTN, which includes several sarcoma referral centers.

With Amendment 5, the study was modified to substitute AST727 for SGI-110 (guadecitabine). The initial cohort of 6 patients who received SGI-110 (guadecitabine) and belinostat will be evaluated separately. The study will accrue a subsequent 13-26 patients (depending on whether the stage 1 efficacy endpoint is met) who will be treated with ASTX727 and belinostat. That is, the 6 patients initially accrued and treated with SGI-110 (guadecitabine) and belinostat will not count towards the Simon 2-stage design. Therefore, total accrual over the entire study will be 26 + 6 patients = 32 patients.

PLANNED ENROLLMENT REPORT

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/ Alaska Native	0	0	0	0	**0**
Asian	2	2	0	0	**4**

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
Native Hawaiian or Other Pacific Islander	0	0	0	0	**0**
Black or African American	3	3	0	0	**6**
White	11	11	2	2	**26**
More Than One Race	1	1	0	0	**2**
Total	**17**	**17**	**2**	**2**	**32**

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9.6 Analysis of Secondary Endpoints

9.6.1 Evaluation of Toxicity

Adverse event rates that are possibly, probably, or definitely related to treatment will be reported as counts and percentages per AE by grade and the number of patients with a given maximal grade of toxicity. We will also report the frequency and percentage of DLTs.

9.6.2 Evaluation of Response

PFS is defined as the time from first treatment with the study drug to the earliest of either disease progression or death from any cause. Patients who are alive and progression-free will be censored at the time of their last follow-up. The Kaplan-Meier method will be used to evaluate time to event endpoints. Median PFS will be reported with a 95% confidence interval. Data will be presented as Kaplan Meier plots.

9.7 Analysis of Correlative Endpoints

9.7.1 Relationship Between *IDH1/2* Mutational Status and Response to Study Treatment

We will compare the difference in the objective response rate among patients with *IDH1/2* mutations as compared those without *IDH1/2* mutations using Fisher's exact test.

9.7.2 Changes in Expression of CS genes and Candidate Genes Affecting Gene Efficacy

Data from RNAseq will be analyzed in collaboration with the MoCha lab, differential expression profiles between baseline and on-treatment samples will be analyzed at the gene level and gene

expression changes defined as significant based on absolute log_2 fold changes >2 and adjusted p values <0.005 for each comparison with consideration of the false discovery rate.

9.7.3 Changes in Global DNA Methylation

A Wilcoxon signed-rank test with continuity correction will be used to calculate P values for comparing methylation level distribution at the different time points.

9.7.4 Changes in Tumor Microenvironment

For quantitative immune multiplexing, data is derived from inForm software, and changes in defined immune cell subsets and expression between baseline and on-treatment samples will be assessed using paired T-tests.

9.8 Reporting and Exclusions

9.8.1 Evaluation of Toxicity

All patients will be evaluable for toxicity from the time of their first treatment with SGI-110 (guadecitabine) and/or belinostat.

9.8.2 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 to have a treatment failure (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. Subanalyses 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 subanalyses 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.

10. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

AE monitoring and reporting is a routine part of every clinical trial. The following list of AEs (Section 10.1) and the characteristics of an observed AE (Sections 10.2 and 10.3) will determine whether the event requires expedited reporting via the CTEP Adverse Event Reporting System (CTEP-AERS) **in addition** to routine reporting.

10.1 Comprehensive Adverse Events and Potential Risks Lists (CAEPRs)

The Comprehensive Adverse Events 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 (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.
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.

10.1.1 CAEPRs for CTEP IND Agents

10.1.1.1 CAEPR for SGI-110 (Guadecitabine)

Frequency is provided based on 757 patients. Below is the CAEPR for SGI-110 (Guadecitabine).

Comprehensive Adverse Events and Potential Risks list (CAEPR)
for
SGI-110 (Guadecitabine, NSC 780463)

The Comprehensive Adverse Events 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 (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 757 patients.* Below is the CAEPR for SGI-110 (Guadecitabine).

> **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 2.1, May 8, 2019[1]

Adverse Events with Possible Relationship to SGI-110 (Guadecitabine) (CTCAE 5.0 Term) [n= 757]			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)***
	Febrile neutropenia		***Febrile neutropenia (Gr 2)***
EYE DISORDERS			
		Periorbital edema	
GASTROINTESTINAL DISORDERS			
	Constipation		***Constipation (Gr 2)***
	Diarrhea		***Diarrhea (Gr 2)***
	Mucositis oral		***Mucositis oral (Gr 2)***
	Nausea		***Nausea (Gr 2)***
	Vomiting		***Vomiting (Gr 2)***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
		Edema face	
Fatigue			***Fatigue (Gr 2)***
	Fever		
Injection site reaction			***Injection site reaction (Gr 2)***
	Pain		
INFECTIONS AND INFESTATIONS			
	Infection[2]		
INJURY, POISONING AND PROCEDURAL COMPLICATIONS			
	Bruising		***Bruising (Gr 1)***
INVESTIGATIONS			
	Lymphocyte count decreased		***Lymphocyte count decreased (Gr 4)***
Neutrophil count decreased			***Neutrophil count decreased (Gr 2)***
Platelet count decreased			***Platelet count decreased (Gr 2)***
	White blood cell decreased		***White blood cell decreased (Gr 2)***
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		***Anorexia (Gr 2)***
		Tumor lysis syndrome	
NERVOUS SYSTEM DISORDERS			
	Dizziness		
	Headache		
PSYCHIATRIC DISORDERS			
	Insomnia		
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Dyspnea		
	Epistaxis		
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
	Purpura		
	Rash maculo-papular		

[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 may include any of the 75 infection sites under the INFECTIONS AND INFESTATIONS SOC.

Adverse events reported on SGI-110 (Guadecitabine) trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that SGI-110 (Guadecitabine) caused the adverse event:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Blood and lymphatic system disorders - Other (agranulocytosis); Blood and lymphatic system disorders - Other (coagulopathy); Blood and lymphatic system disorders - Other (febrile bone marrow aplasia); Blood and lymphatic system disorders - Other (histiocytosis hematophagic); Blood and lymphatic system disorders - Other (lymphadenopathy); Blood and lymphatic system disorders - Other (pancytopenia); Bone marrow hypocellular; Leukocytosis; Thrombotic thrombocytopenic purpura
CARDIAC DISORDERS - Atrial fibrillation; Cardiac disorders - Other (atrial tachycardia); Cardiac disorders - Other (bradycardia); Chest pain - cardiac; Heart failure; Left ventricular systolic dysfunction; Palpitations; Sinus tachycardia
CONGENITAL, FAMILIAL AND GENETIC DISORDERS - Congenital, familial and genetic disorders - Other (phimosis)
EAR AND LABYRINTH DISORDERS - Ear pain; Vertigo
ENDOCRINE DISORDERS - Adrenal insufficiency
EYE DISORDERS - Blurred vision; Eye disorders - Other (eye/retinal/conjunctival hemorrhage)
GASTROINTESTINAL DISORDERS - Abdominal distension; Abdominal pain; Belching; Cheilitis; Colitis; Dry mouth; Dyspepsia; Dysphagia; Enterocolitis; Esophagitis; Flatulence; Gastritis; Gastroesophageal reflux disease; Gastrointestinal disorders - Other (small intestinal hemorrhage); Gastrointestinal disorders - Other (tongue discoloration); Gastrointestinal pain; Gingival pain; Hemorrhoids; Ileus; Lip pain; Lower gastrointestinal hemorrhage; Oral hemorrhage; Oral pain; Periodontal disease; Rectal hemorrhage; Rectal pain; Small intestinal mucositis; Small intestinal obstruction; Toothache; Typhlitis; Upper gastrointestinal hemorrhage
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Chills; Edema limbs; Gait disturbance; General disorders and administration site conditions - Other (exercise tolerance decreased); General disorders and administration site conditions - Other (general physical health deterioration); General disorders and administration site conditions - Other (systemic inflammatory response syndrome [SIRS]); Infusion site extravasation; Localized edema; Malaise; Multi-organ failure; Non-cardiac chest pain
HEPATOBILIARY DISORDERS - Cholecystitis
IMMUNE SYSTEM DISORDERS - Allergic reaction
INVESTIGATIONS - Activated partial thromboplastin time prolonged; Alanine aminotransferase increased; Alkaline phosphatase increased; Aspartate aminotransferase increased; Blood bilirubin increased; Creatinine increased; Ejection fraction decreased; Electrocardiogram QT corrected interval prolonged; GGT increased; INR increased; Investigations - Other (elevated c-reactive proteins [c-reactive protein increased]); Investigations - Other (klebsiella test positive); Investigations - Other (reticulocyte count decreased); Investigations - Other (thrombocytosis); Lymphocyte count increased; Weight loss
METABOLISM AND NUTRITION DISORDERS - Acidosis; Alkalosis; Dehydration; Hypercalcemia; Hyperglycemia; Hyperkalemia; Hyperphosphatemia; Hyperuricemia; Hypoalbuminemia; Hypocalcemia; Hypoglycemia; Hypokalemia; Hypomagnesemia; Hyponatremia; Hypophosphatemia; Metabolism and nutrition disorders - Other (failure to thrive); Metabolism and nutrition disorders - Other (gout)
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Arthralgia; Arthritis; Back pain; Bone pain; Flank pain; Generalized muscle weakness; Joint range of motion decreased; Muscle cramp; Musculoskeletal and connective tissue disorder - Other (joint contracture); Musculoskeletal and connective tissue disorder - Other (musculoskeletal stiffness); Musculoskeletal and connective tissue disorder - Other (pain in jaw); Myalgia; Neck pain; Pain in extremity
NERVOUS SYSTEM DISORDERS - Ataxia; Dysgeusia; Lethargy; Paresthesia; Presyncope; Syncope
PSYCHIATRIC DISORDERS - Anxiety; Delirium; Depression
RENAL AND URINARY DISORDERS - Acute kidney injury; Hematuria; Urinary frequency; Urinary tract pain; Urinary urgency
REPRODUCTIVE SYSTEM AND BREAST DISORDERS - Penile pain; Vaginal hemorrhage

RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Adult respiratory distress syndrome; Atelectasis; Bronchopulmonary hemorrhage; Cough; Hypoxia; Nasal congestion; Oropharyngeal pain; Pleural effusion; Pleuritic pain; Pneumonitis; Respiratory failure; Respiratory, thoracic and mediastinal disorders - Other (oropharyngeal blistering); Rhinorrhea; Sneezing; Wheezing
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Alopecia; Bullous dermatitis; Dry skin; Hyperhidrosis; Lipohypertrophy; Pruritus; Rash acneiform; Skin and subcutaneous tissue disorders - Other (dermal cyst); Skin and subcutaneous tissue disorders - Other (granuloma annulare); Skin and subcutaneous tissue disorders - Other (onychoclasis); Skin induration; Skin ulceration
VASCULAR DISORDERS - Hematoma; Hot flashes; Hypertension; Hypotension; Thromboembolic event

Note: SGI-110 (Guadecitabine) 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.

10.1.1.2 CAEPR for ASTX727

Comprehensive Adverse Events and Potential Risks list (CAEPR)
for
ASTX727 (E7727 and Decitabine, NSC 820631)

The Comprehensive Adverse Events 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 (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 ASTX727 (E7727 and Decitabine).

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 2.2, December 19, 2024[1]

Adverse Events with Possible Relationship to ASTX727 (E7727 and Decitabine) (CTCAE 5.0 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)***
	Febrile neutropenia[2]		***Febrile neutropenia[2] (Gr 3)***
GASTROINTESTINAL DISORDERS			
	Constipation		***Constipation (Gr 2)***
	Diarrhea		***Diarrhea (Gr 2)***
	Mucositis oral		

Adverse Events with Possible Relationship to ASTX727 (E7727 and Decitabine) (CTCAE 5.0 Term) [n= 702]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
	Nausea		***Nausea (Gr 2)***
	Vomiting		
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
	Fatigue		***Fatigue (Gr 2)***
INFECTIONS AND INFESTATIONS			
	Lung infection/pneumonia		
	Sepsis		
	Tooth infection		
	Upper respiratory infection		***Upper respiratory infection (Gr 2)***
	Urinary tract infection		***Urinary tract infection (Gr 2)***
INJURY, POISONING AND PROCEDURAL COMPLICATIONS			
	Bruising		
INVESTIGATIONS			
	Alanine aminotransferase increased		
	Aspartate aminotransferase increased		
Neutrophil count decreased			***Neutrophil count decreased (Gr 2)***
Platelet count decreased			***Platelet count decreased (Gr 2)***
	White blood cell decreased		***White blood cell decreased (Gr 2)***
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		

[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]The SPEER grade for febrile neutropenia should only be applied to cases of neutropenic fever associated with hematologic malignancies and NOT for solid tumors.

Adverse events reported on ASTX727 (E7727 and Decitabine) trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that ASTX727 (E7727 and Decitabine) caused the adverse event:

CARDIAC DISORDERS - Heart failure; Myocarditis
GASTROINTESTINAL DISORDERS - Abdominal pain
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Chills; Edema limbs; Fever
IMMUNE SYSTEM DISORDERS - Allergic reaction
INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Fall
INVESTIGATIONS - Creatinine increased
METABOLISM AND NUTRITION DISORDERS - Dehydration; Hypoalbuminemia; Hypokalemia; Hypomagnesemia; Tumor lysis syndrome
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Arthralgia; Back pain; Myalgia

NERVOUS SYSTEM DISORDERS - Dizziness; Headache; Intracranial hemorrhage
PSYCHIATRIC DISORDERS - Confusion; Insomnia
RENAL AND URINARY DISORDERS - Acute kidney injury
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Adult respiratory distress syndrome; Cough; Dyspnea; Epistaxis; Oropharyngeal pain; Pharyngeal hemorrhage; Respiratory failure
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Pruritus; Rash maculo-papular; Skin and subcutaneous tissue disorders - Other (angioedema)

Note: ASTX727 (E7727 and Decitabine) 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.

10.1.1.3 CAEPR for Belinostat (PXD-101)

Frequency is provided based on 583 patients. Below is the CAEPR for Belinostat (PXD-101).

Comprehensive Adverse Events and Potential Risks list (CAEPR)
For
Belinostat (PXD-101, NSC 726630)

The Comprehensive Adverse Events 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 (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 583 patients.* Below is the CAEPR for Belinostat (PXD-101).

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, October 29, 2018[1]

Adverse Events with Possible Relationship to Belinostat (PXD-101) (CTCAE 5.0 Term) [n= 583]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
	Anemia		***Anemia (Gr 3)***
GASTROINTESTINAL DISORDERS			
	Abdominal pain		
	Constipation		***Constipation (Gr 2)***
Diarrhea			***Diarrhea (Gr 3)***
	Dry mouth		***Dry mouth (Gr 2)***
Nausea			***Nausea (Gr 3)***

Adverse Events with Possible Relationship to Belinostat (PXD-101) (CTCAE 5.0 Term) [n= 583]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
Vomiting			***Vomiting (Gr 3)***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
	Edema limbs		***Edema limbs (Gr 2)***
Fatigue			***Fatigue (Gr 3)***
	Fever		***Fever (Gr 2)***
	Injection site reaction		
INFECTIONS AND INFESTATIONS			
	Infection[2]		***Infection[2] (Gr 3)***
INVESTIGATIONS			
	Alanine aminotransferase increased		
	Aspartate aminotransferase increased		
	Creatinine increased		
	Electrocardiogram QT corrected interval prolonged		
	Lymphocyte count decreased		***Lymphocyte count decreased (Gr 4)***
	Neutrophil count decreased		
	Platelet count decreased		***Platelet count decreased (Gr 4)***
	Weight loss		***Weight loss (Gr 2)***
	White blood cell decreased		
METABOLISM AND NUTRITION DISORDERS			
Anorexia			***Anorexia (Gr 2)***
	Dehydration		
		Tumor lysis syndrome	
NERVOUS SYSTEM DISORDERS			
	Dizziness		
	Dysgeusia		
	Headache		***Headache (Gr 2)***
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Dyspnea		***Dyspnea (Gr 2)***
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
	Rash maculo-papular		
VASCULAR DISORDERS			
	Flushing		***Flushing (Gr 2)***

[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 may include any of the 75 infection sites under the INFECTIONS AND INFESTATIONS SOC.

Adverse events reported on Belinostat (PXD-101) trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that Belinostat (PXD-101) caused the adverse event:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Febrile neutropenia
CARDIAC DISORDERS - Atrial fibrillation; Cardiac disorders - Other (bundle branch block left); Chest pain - cardiac; Heart failure; Left ventricular systolic dysfunction; Myocardial infarction; Palpitations; Sinus bradycardia; Sinus tachycardia; Supraventricular tachycardia; Ventricular fibrillation
EYE DISORDERS - Eye disorders - Other (visual loss); Vision decreased
GASTROINTESTINAL DISORDERS - Abdominal distension; Dyspepsia; Gastroesophageal reflux disease; Mucositis oral; Rectal hemorrhage; Small intestinal obstruction; Upper gastrointestinal hemorrhage
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Chills; Flu like symptoms; General disorders and administration site conditions - Other (general physical health deterioration); Malaise; Multi-organ failure; Non-cardiac chest pain; Pain
HEPATOBILIARY DISORDERS - Hepatic failure; Hepatobiliary disorders - Other (hepatic cirrhosis)
IMMUNE SYSTEM DISORDERS - Allergic reaction; Anaphylaxis; Cytokine release syndrome
INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Infusion related reaction; Tracheal hemorrhage
INVESTIGATIONS - Alkaline phosphatase increased; Blood bilirubin increased; CPK increased; Cardiac troponin I increased; Cholesterol high; Ejection fraction decreased; Electrocardiogram T wave abnormal; INR increased; Investigations - Other (prothrombin time shortened); Investigations - Other (total protein decrease); Lipase increased; Weight gain
METABOLISM AND NUTRITION DISORDERS - Hypercalcemia; Hyperglycemia; Hypermagnesemia; Hypertriglyceridemia; Hypoalbuminemia; Hypocalcemia; Hypokalemia; Hyponatremia; Hypophosphatemia
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Arthralgia; Back pain; Bone pain; Chest wall pain; Generalized muscle weakness; Muscle cramp; Myalgia; Pain in extremity
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS) - Tumor pain
NERVOUS SYSTEM DISORDERS - Ataxia; Depressed level of consciousness; Dysesthesia; Encephalopathy; Lethargy; Nervous system disorders - Other (apraxia); Nervous system disorders - Other (burning sensation); Peripheral sensory neuropathy; Reversible posterior leukoencephalopathy syndrome; Seizure; Stroke; Syncope
PSYCHIATRIC DISORDERS - Confusion; Depression; Insomnia; Psychosis
RENAL AND URINARY DISORDERS - Acute kidney injury; Renal and urinary disorders - Other (azotemia); Urinary frequency
REPRODUCTIVE SYSTEM AND BREAST DISORDERS - Genital edema; Vaginal inflammation
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Cough; Hiccups; Hypoxia; Nasal congestion; Pneumonitis; Pulmonary hypertension
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Hyperhidrosis; Pruritus; Urticaria
VASCULAR DISORDERS - Hematoma; Hypertension; Hypotension; Phlebitis; Thromboembolic event; Vasculitis

Note: Belinostat (PXD-101) 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.

10.2 Adverse Event Characteristics

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

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

- **For expedited reporting purposes only:**
  - AEs for the agent that are ***bold and italicized*** in the CAEPR (*i.e.*, those listed in the SPEER column, Section 10.1) should be reported through CTEP-AERS only if the grade is above the grade provided in the SPEER.

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

10.3 Expedited Adverse Event Reporting

10.3.1 Rave-CTEP-AERS Integration

The Cancer Therapy Evaluation Program Adverse Event Reporting System (CTEP-AERS) integration enables evaluation of post-baseline AEs entered in Rave to determine whether they require expedited reporting, and facilitates entry in CTEP-AERS for those AEs requiring expedited reporting.

All AEs that occur after baseline are collected in Medidata Rave using the Adverse Event form, which is available for entry at each treatment or reporting period, and used to collect AEs that start during the period or persist from the previous reporting period. The Clinical Research Associate (CRA) will enter AEs that occur prior to the start of treatment on a baseline form that is not included in the Rave-CTEP-AERS integration. AEs that occur prior to enrollment must begin and end on the baseline Adverse Event form and should not be included on the standard Adverse Events form that is available at treatment unless there has been an increase in grade.

Prior to sending AEs through the rules evaluation process, site staff should verify the following on the Adverse Event form in Rave:

- The reporting period (course/cycle) is correct, and
- AEs are recorded and complete (no missing fields) and the form is query-free (fields added to the form during study build do not need to be query-free for the integration call with CTEP-AERS to be a success).

The CRA reports AEs in Rave at the time the Investigator learns of the event. If the CRA modifies an AE, it must be re-submitted for rules evaluation.

Upon completion of AE entry in Medidata Rave, the CRA submits the AE for rules evaluation by completing the Expedited Reporting Evaluation form. Both NCI and

protocol-specific reporting rules evaluate the AEs submitted for expedited reporting. A report is initiated in CTEP-AERS using information entered in Medidata Rave for AEs that meet reporting requirements. The CRA completes the report by accessing CTEP-AERS via a direct link on the Medidata Rave Expedited Reporting Evaluation form.

In the rare occurrence that Internet connectivity is lost, a 24-hour notification is to be made to CTEP by telephone at 301-897-7497. Once Internet connectivity is restored, the 24-hour notification that was phoned in must be entered immediately into CTEP-AERS using the deep link from Medidata Rave.

Additional information about the CTEP-AERS integration is available on the CTSU website:

- Study specific documents: Protocols > Documents > Education and Promotion, and
- Expedited Safety Reporting Rules Evaluation user guide: Resources > CTSU Operations Information > User Guides.

NCI requirements for SAE reporting are available on the CTEP website:

- NCI Guidelines for Investigators: Adverse Event Reporting Requirements is available at https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf.

10.3.2 Distribution of Adverse Event Reports

CTEP-AERS is programmed for automatic electronic distribution of reports to the following individuals: Principal Investigator and Adverse Event Coordinator(s) (if applicable) of the Corresponding Organization or Lead Organization, the local treating physician, and the Reporter and Submitter. CTEP-AERS provides a copy feature for other e-mail recipients.

10.3.3 Expedited Reporting Guidelines

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

Note: A death on study requires both routine and expedited reporting, regardless of causality as long as the death occurred within 30 days after the last administration of the investigational agent. Attribution to treatment or other cause must be provided.

Death due to progressive disease should be reported as **Grade 5 "Disease progression"** in the system organ class (SOC) "General disorders and administration site conditions." Evidence that

the death was a manifestation of underlying disease (*e.g.*, radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted.

Phase 1 and Early Phase 2 Studies: Expedited Reporting Requirements for Adverse Events that Occur on Studies under an IND/IDE within 30 Days of the Last Administration of the Investigational Agent/Intervention [1, 2]

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

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

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

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

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

Grade 1-2 Timeframes	Grade 3-5 Timeframes
24-Hour notification, 10 Calendar Days	24-Hour notification, 5 Calendar Days

NOTE: Protocol-specific exceptions to expedited reporting of SAEs are found in the Specific Protocol Exceptions to Expedited Reporting (SPEER) portion of the CAEPR.

Expedited AE reporting timeframes are defined as:

- "24-Hour notification, 5 Calendar Days" - The SAE must initially be reported via CTEP-AERS within 24 hours of learning of the SAE, followed by a complete expedited report within 5 calendar days of the initial 24-hour report.
- "24-Hour notification, 10 Calendar Days" - The SAE must initially be reported via CTEP-AERS within 24 hours of learning of the SAE, followed by a complete expedited report within 10 calendar days of the initial 24-hour report.

[1]SAEs that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:
Expedited 24-Hour notifications are required for all SAEs followed by a complete report

- Within 5 calendar days for Grade 3-5 SAEs
- Within 10 calendar days for Grade 1-2 SAEs

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

Effective Date: August 30, 2024

10.4 Routine Adverse Event Reporting

All Adverse Events **must** be reported in routine study data submissions. **AEs reported expeditiously through CTEP-AERS must also be reported in routine study data submissions.**
Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of patients enrolled in the studies as well as those who will enroll in future studies using similar agents. AEs are reported in a routine manner at scheduled times during the trial using Medidata Rave. For this trial the Adverse Event CRF is used for routine AE reporting in Rave.

10.5 Pregnancy

Although not an adverse event in and of itself, pregnancy as well as its outcome must be documented via **CTEP-AERS**. In addition, the ***Pregnancy Information Form*** included within the NCI Guidelines for Adverse Event Reporting Requirements must be completed and submitted to CTEP. Any pregnancy occurring in a patient or patient's partner from the time of consent to 90 days after the last dose of study drug must be reported and then followed for outcome. Newborn infants should be followed until 30 days old. Please see the "NCI Guidelines for Investigators: Adverse Event Reporting Requirements for DCTD (CTEP and CIP) and DCP INDs and IDEs" (at http://ctep.cancer.gov/protocolDevelopment/adverse_effects.htm) for more details on how to report pregnancy and its outcome to CTEP.

10.6 Secondary Malignancy

A *secondary malignancy* is a cancer caused by treatment for a previous malignancy (*e.g.*, treatment with investigational agent/intervention, radiation or chemotherapy). A secondary malignancy is not considered a metastasis of the initial neoplasm.

CTEP requires all secondary malignancies that occur following treatment with an agent under an NCI IND/IDE be reported expeditiously via CTEP-AERS. Three options are available to describe the event:

- Leukemia secondary to oncology chemotherapy (*e.g.*, acute myelocytic leukemia [AML])
- Myelodysplastic syndrome (MDS)
- Treatment-related secondary malignancy

Any malignancy possibly related to cancer treatment (including AML/MDS) should also be reported via the routine reporting mechanisms outlined in each protocol.

10.7 Second Malignancy

A second malignancy is one unrelated to the treatment of a prior malignancy (and is **NOT** a metastasis from the initial malignancy). Second malignancies require **ONLY** routine AE reporting unless otherwise specified.

11. STUDY CALENDAR

	Pre-Treatment Screening[L]	Cycle 1[M]				Cycle 2[M]				Cycle 3+[M]				Off Study[N]
		Wk 1	Wk 2	Wk 3	Wk 4	Wk 1	Wk 2	Wk 3	Wk 4	Wk 1	Wk 2	Wk 3	Wk 4	
Belinostat[A]		X				X				X				
SGI-110 (guadecitabine) or ASTX727[A]		X				X				X				
Informed consent	X													
Demographics	X													
Medical history	X													
Concurrent meds	X	X				X				X				X
Physical exam[B]	X	X		X		X				X				X
Vital signs[C]	X	X		X		X				X				X
Height	X													X
Weight	X	X				X				X				X
Performance status	X													
CBC[D]	X	X		X		X		X		X				X
Serum chemistry[E]	X	X		X		X		X		X				X
Coagulation[F]	X													X
Pregnancy test[G]	X													X
ECG[H]	X													X
Adverse events		Throughout study												X
Pill Diary		X				X				X				X
Imaging evaluation of disease status[I]	X									X				X
Tumor biopsy for research correlatives[J]	X					X								
Blood collection for research correlatives[K]	X													

A Belinostat: 1000 mg/m^2 IV on days 1-5 of each 28 day cycle; SGI-110 (guadecitabine): 45 mg/m^2 SC on days 1-5 of each 28 day cycle; ASTX727: cedazuridine 100 mg/decitabine 35 mg PO on days 1-5 of each 28 day cycle. Patients will be instructed to bring the pill bottle for ASTX727 to clinic where they will take the drug. Patients are assessed by the treating investigator on day 1 of each cycle. During cycle 1, an additional clinic assessment occurs at week 3, day 1 (± 3 days). Dosing window of ± 3 days is allowed; however, both agents must begin dosing on the same day.

B A complete physical exam is performed at screening and end of study. A limited, symptom based physical exam is performed at other timepoints.

C Vital signs include measurements of temperature, heart rate, blood pressure, respiratory rate, and oxygen saturation.

D CBC with differential includes: hemoglobin, RBC, platelets, MCV, WBC, absolute differential (neutrophils, lymphocytes, monocytes, eosinophils, and basophils). Local laboratory testing is permitted.

	Pre-Treatment Screening[L]	Cycle 1[M]				Cycle 2[M]				Cycle 3+[M]				Off Study[N]
		Wk 1	Wk 2	Wk 3	Wk 4	Wk 1	Wk 2	Wk 3	Wk 4	Wk 1	Wk 2	Wk 3	Wk 4	

E Serum chemistry must include: albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, magnesium, phosphorus, potassium, total protein, SGOT (AST), SGPT (ALT), sodium. Local laboratory testing is permitted.

F Coagulation factors include: aPTT, INR; required pre-study and as clinically indicated. Local laboratory testing is permitted.

G Serum or urine pregnancy test (for women of childbearing potential) must be completed at screening. If results are positive, the patient is ineligible and must be discontinued from the study.

H A 12-lead ECG is performed at screening and end of study. Additional ECGs are performed as clinically indicated.

I MRI or CT imaging to evaluate disease status is performed at baseline and once every 2 cycles (8 weeks) ± 5 days. Imaging should be performed prior to the initiation of the next cycle of study treatment whenever possible.

J Pre-treatment biopsy is performed within 21 days of C1D1. **The pre-treatment biopsy may only be collected after informed consent is obtained.** On-study biopsy is performed on Day 3, 4, or 5 of cycle 2, 2-8 hours post-treatment with the study agents whenever possible. If the start of C2 is delayed, the biopsy should be delayed accordingly. **The on-study biopsy should not be obtained if the patient has not begun dosing for C2.**

K Blood collection for germline whole exome sequencing. Pre-treatment collection is performed within 21 days of C1D1. **The pre-treatment research blood collection may only be collected after informed consent is obtained.**

L **All screening assessments must be performed within 21 days of C1D1.**

M **All assessments occur on day 1 of the specified week, unless otherwise noted.** Start of cycle laboratory assessments may be performed up to 3 days prior to initiating treatment for a new cycle. Between cycle laboratory assessments may be performed ± 3 days from the scheduled date. **Local laboratory testing is permitted.**

N Off-study evaluations must be performed within 21 days of the last dose of the study drug.

12. MEASUREMENT OF EFFECT

12.1 Antitumor Effect – Solid Tumors

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

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

12.1.1 Definitions

Evaluable for toxicity. All patients will be evaluable for toxicity from the time of their first treatment with SGI-110 (guadecitabine) and/or belinostat.

Evaluable for objective response. All patients will be evaluable for objective response from the time of their first treatment with SGI-110 (guadecitabine) and belinostat.

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.

12.1.2 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 (≥2 cm) by chest x-ray or as ≥10 mm (≥1 cm) with CT scan, MRI, or calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Note: Tumor lesions that are situated in a previously irradiated area might or might not be considered measurable.

Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm (≥1.5 cm) in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm [0.5 cm]). 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 [<1 cm] or pathological lymph nodes with ≥10 to <15 mm [≥1 to <1.5 cm] 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.

12.1.3 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 (≥1 cm) 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 (0.5 cm) or less. If CT scans have slice thickness greater than 5 mm (0.5 cm), 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.

12.1.4 Response Criteria

12.1.4.1 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 (<1 cm).

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 (0.5 cm). (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.

12.1.4.2 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 [<1 cm] 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).

12.1.4.3 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

12.1.5 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.

12.1.6 Progression-Free Survival

PFS is defined as the duration of time from start of treatment to time of progression or death, whichever occurs first.

12.1.7 Response Review

Not applicable.

13. STUDY OVERSIGHT AND DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 10 (Adverse Events: List and Reporting Requirements).

13.1 Study Oversight

This protocol is monitored at several levels, as described in this section. The Protocol Principal Investigator is responsible for monitoring the conduct and progress of the clinical trial, including the ongoing review of accrual, patient-specific clinical and laboratory data, and routine and serious adverse events; reporting of expedited adverse events; and accumulation of reported adverse events from other trials testing the same drug(s). The Protocol Principal Investigator and statistician have access to the data at all times through the CTMS web-based reporting portal.
Click or tap here to enter text.
All Study Investigators at participating sites who register/enroll patients on a given protocol are responsible for timely submission of data via Medidata Rave and timely reporting of adverse events for that particular study. This includes timely review of data collected on the electronic CRFs submitted via Medidata Rave.

All studies are also reviewed in accordance with the enrolling institution's data safety monitoring plan.

13.2 Data Reporting

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

- Site staff will need to be registered with CTEP and have a valid and active CTEP-IAM account, and
- Assigned one of the following Rave roles on the relevant Lead Protocol Organization (LPO) or Participating Organization roster at the enrolling site: Rave CRA, Rave Read Only, Rave CRA (LabAdmin), Rave SLA, or Rave Investigator. Refer to https://ctep.cancer.gov/investigatorResources/default.htm for registration types and documentation required.
    1. To hold Rave CRA or Rave CRA (Lab Admin) role, site staff must hold a minimum of an AP registration type,
    2. To hold Rave Investigator role, the individual must be registered as an NPIVR or IVR, and
    3. To hold Rave Read Only role, site staff must hold an Associates (A) registration type.

If the study has a DTL, individuals requiring write access to Rave must also be assigned the appropriate Rave tasks on the DTL.

Upon initial site registration approval for the study in Regulatory Support System (RSS), all persons with Rave roles assigned on the appropriate roster will be sent a study invitation e-mail from iMedidata. To accept the invitation, site staff must log in to the Select Login (https://login.imedidata.com/selectlogin) using their CTEP-IAM username and password, and click on the *accept* link in the upper right-corner of the iMedidata page. Site staff will not be able to access the study in Rave until all required Medidata and study specific trainings are completed. Trainings will be in the form of electronic learnings (eLearnings), and can be accessed by clicking on the link in the upper right pane of the iMedidata screen. If an eLearning is required and has not yet been taken, the link to the eLearning will appear under the study name in iMedidata instead of the *Rave EDC* link; once the successful completion of the eLearning has been recorded, access to the study in Rave will be granted, and a *Rave EDC* link will display under the study name.

Site staff that have not previously activated their iMedidata/Rave account at the time of initial site registration approval for the study in RSS will also receive a separate invitation from iMedidata to activate their account. Account activation instructions are located on the CTSU website in the Rave section under the Rave resource materials (Medidata Account Activation and Study Invitation Acceptance). Additional information on iMedidata/Rave is available on the CTSU members' website in the Data Management > Rave section at www.ctsu.org/RAVE/ or by contacting the CTSU Help Desk at 1-888-823-5923 or by e-mail at ctsucontact@westat.com.

13.2.1 Method

This study will be monitored by the Clinical Trials Monitoring Service (CTMS). Data will be submitted to CTMS at least once every two weeks via Medidata Rave (or other modality if approved by CTEP). Information on CTMS reporting is available at: http://www.theradex.com/clinicalTechnologies/?National-Cancer-Institute-NCI-11. On-site audits will be conducted on an 18-36 month basis as part of routine cancer center site visits. More frequent audits may be conducted if warranted by accrual or due to concerns regarding

data quality or timely submission. For CTMS monitored studies, after users have activated their accounts, please contact the Theradex Help Desk at (609) 619-7862 or by email at CTMSSupport@theradex.com for additional support with Rave and completion of CRFs.

13.2.2 Responsibility for Data Submission

For ETCTN trials, it is the responsibility of the PI(s) at the site to ensure that all investigators at the ETCTN Sites understand the procedures for data submission for each ETCTN protocol and that protocol specified data are submitted accurately and in a timely manner to the CTMS via the electronic data capture system, Medidata Rave.

Data are to be submitted via Medidata Rave to CTMS on a real-time basis, but no less than once every 2 weeks. The timeliness of data submissions and timeliness in resolving data queries will be tracked by CTMS. Metrics for timeliness will be followed and assessed on a quarterly basis. For the purpose of Institutional Performance Monitoring, data will be considered delinquent if it is greater than 4 weeks past due.

Data from Medidata Rave and CTEP-AERS is reviewed by the CTMS on an ongoing basis as data is received. Queries will be issued by CTMS directly within Rave. The queries will appear on the Task Summary Tab within Rave for the CRA at the ETCTN to resolve. Monthly web-based reports are posted for review by the Drug Monitors in the IDB, CTEP. Onsite audits will be conducted by the CTMS to ensure compliance with regulatory requirements, GCP, and NCI policies and procedures with the overarching goal of ensuring the integrity of data generated from NCI-sponsored clinical trials, as described in the ETCTN Program Guidelines, which may be found on the CTEP (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/adverse_events.htm) and CTSU websites.

CTMS will utilize a core set of eCRFs that are Cancer Data Standards Registry and Repository (caDSR) compliant (http://cbiit.nci.nih.gov/ncip/biomedical-informatics-resources/interoperability-and-semantics/metadata-and-models). Customized eCRFs will be included when appropriate to meet unique study requirements. The PI is encouraged to review the eCRFs, working closely with CTMS to ensure prospectively that all required items are appropriately captured in the eCRFs prior to study activation. CTMS will prepare the eCRFs with built-in edit checks to the extent possible to promote data integrity.

CDUS data submissions for ETCTN trials activated after March 1, 2014, will be carried out by the CTMS contractor, Theradex. CDUS submissions are performed by Theradex on a monthly basis. The trial's lead institution is responsible for timely submission to CTMS via Rave, as above.

Further information on data submission procedures can be found in the ETCTN Program Guidelines (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/adverse_events.htm).

13.3 Data Quality Portal

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

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

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

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

Note: Some Rave protocols may not have delinquent form details or reports specified on the DQP. A protocol must have the Calendar functionality implemented in Rave by the Lead Protocol Organization (LPO) for delinquent form details and reports to be available on the DQP. Site staff should contact the LPO Data Manager for their protocol regarding questions about Rave Calendaring functionality.

13.4 CTEP Multicenter Guidelines

N/A

13.5 Collaborative Agreements Language

The agents supplied by CTEP, DCTD, NCI used in this protocol is/are provided to the NCI under Collaborative Agreements (CRADA, CTA, CSA) between the Pharmaceutical Companies (hereinafter referred to as "Collaborators") 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" (http://ctep.cancer.gov/industryCollaborations2/intellectual_property.htm) contained within the terms of award, apply to the use of the Agents in this study:

1. Agents may not be used for any purpose outside the scope of this protocol, nor can Agents be transferred or licensed to any party not participating in the clinical study. Collaborators' data for Agents are confidential and proprietary to Collaborators and shall be maintained as such by the investigators. The protocol documents for studies utilizing 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 or patient's family member

participating on the study, 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 another Agents, 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 will provide all Collaborators with prior written notice regarding the existence and nature of any agreements governing their collaboration with NCI, 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 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 Agent.

3. Clinical Trial Data and Results and Raw Data developed under a Collaborative Agreement will be made available to Collaborators, the NCI, and the FDA, as appropriate and unless additional disclosure is required by law or court order as described in the IP Option to Collaborator (http://ctep.cancer.gov/industryCollaborations2/intellectual_property.htm). Additionally, all Clinical 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 Collaborators 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 by the Group office for Cooperative Group studies or by the principal investigator for non-Cooperative Group studies for immediate delivery to Collaborators for advisory review and comment prior to submission for publication. Collaborators will have 30 days from the date of receipt for review. Collaborators shall have the right to request that publication be delayed for up to an additional 30 days in order to ensure that Collaborators' confidential and proprietary data, in addition to Collaborators' intellectual property rights, are protected.

Copies of abstracts must be provided to CTEP for forwarding to Collaborators 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:

Email: ncicteppubs@mail.nih.gov

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

13.6 Genomic Data Sharing Plan

Not applicable.

13.7 Incidental/Secondary Findings Disclosure Procedure

In the event that a germline genomic mutation/alteration of potential clinical significance is noted, the Study Chair will discuss the findings with the treating investigator. The treating investigator will be responsible for discussing such findings with the patient according to institutional procedures at the treating site. A genetic counselor should be involved in the discussion whenever possible. Germline genomic alterations will be confirmed at the treating site at the direction of the treating investigator. Disclosure must be documented in the electronic medical record at the treating institution.

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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 FORMULA TO ESTIMATE RENAL FUNCTION USING SERUM CREATININE

Formulas to estimate renal function using serum creatinine provided by the NCI's Investigational Drug Steering Committee (IDSC) Pharmacological Task Force in table below.

1. Estimated glomerular filtration rate (eGFR) using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) (Levey *et al.*, 2009).

Formulae:

Race and Sex	Serum Creatinine (SCr), *µmol/L (mg/dL)*	Equation
Black		
Female	≤62 (≤0.7)	$GFR = 166 \times (SCr/0.7)^{-0.329} \times (0.993)^{Age}$
	>62 (>0.7)	$GFR = 166 \times (SCr/0.7)^{-1.209} \times (0.993)^{Age}$
Male	≤80 (≤0.9)	$GFR = 163 \times (SCr/0.9)^{-0.411} \times (0.993)^{Age}$
	>80 (>0.9)	$GFR = 163 \times (SCr/0.9)^{-1.209} \times (0.993)^{Age}$
White or other		
Female	≤62 (≤0.7)	$GFR = 144 \times (SCr/0.7)^{-0.329} \times (0.993)^{Age}$
	>62 (>0.7)	$GFR = 144 \times (SCr/0.7)^{-1.209} \times (0.993)^{Age}$
Male	≤80 (≤0.9)	$GFR = 141 \times (SCr/0.9)^{-0.411} \times (0.993)^{Age}$
	>80 (>0.9)	$GFR = 141 \times (SCr/0.9)^{-1.209} \times (0.993)^{Age}$

SCr in mg/dL; Output is in mL/min/1.73 m^2 and needs no further conversions.

2. eGFR using the Modification of Diet in Renal Disease (MDRD) Study (Levey *et al.*, 2006).

$175 \times SCr^{-1.154} \times age^{-0.203} \times 0.742$ (if female) $\times$ 1.212 (if black)
Output is in mL/min/1.73 m^2 and needs no further conversions.

3. Estimated creatinine clearance (ClCr) by the Cockcroft-Gault (C-G) equation (Cockcroft and Gault, 1976).

$$CLcr\ (mL/min) = \frac{[140 - age\ (years)] \times weight\ (kg)}{72 \times serum\ creatinine\ (mg/dL)} \{\times 0.85\ for\ female\ patients\}$$

Followed by conversion to a value normalized to 1.73 m^2 with the patient's body surface area (BSA).

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APPENDIX C PRE-BIOPSY ASSESSMENT

A pre-biopsy lesion assessment can increase trial safety and efficiency. By agreement between all investigators, an attempt at biopsy will be made if the clinical trial team determines that a biopsy poses minimal relative risk, provides potential clinical gain to the participant, and will likely yield sufficient tissue for analysis.

Pre-biopsy assessments will be reported and tracked through a trial-specific CRF within the CTEP Medidata Rave system. Additional information can be found in the Investigational Radiology SOP available at:
https://ctep.cancer.gov/initiativesPrograms/docs/ETCTN_IR_Research_Biopsy_SOP.pdf.

Individual Patient Pre-Biopsy Assessment. IR co-investigators are encouraged to apply this pre-biopsy scoring and correlation system to assist in the determination of biopsy appropriateness.

- IR co-investigators assign a subjective score of 1-3 based on likelihood of success due to lesion characteristics.

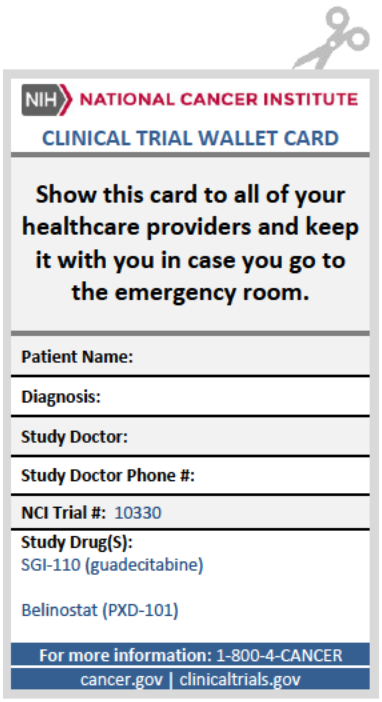
    1. Biopsy should not be done
        A. Due to safety concerns
        B. Due to lack of suitable lesion for biopsy

    2. Uncertainty about success
        A. Due to access path to lesion
        B. Due to lesion characteristics

    3. Likely successful

- Lesion characteristics to be considered
    - Size (small) (<2 cm)
    - Location/path to lesion
    - Morphologic features (necrosis, sub-solid, sclerosis, ill-defined/infiltrative)
    - PET (+/-), avidity
    - Organ/site (sclerotic bone is low yield; fine needle aspiration to be used)

APPENDIX D PATIENT CLINICAL TRIAL WALLET CARD: BELINOSTAT AND SGI-110 (GUADECITABINE)



NIH NATIONAL CANCER INSTITUTE

CLINICAL TRIAL WALLET CARD

Show this card to all of your healthcare providers and keep it with you in case you go to the emergency room.

Patient Name:

Diagnosis:

Study Doctor:

Study Doctor Phone #:

NCI Trial #: 10330

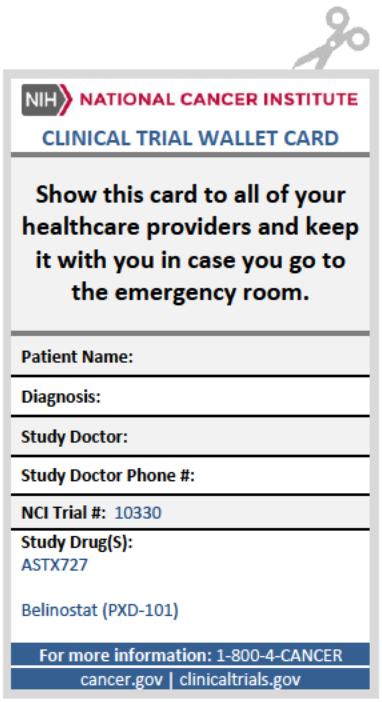
Study Drug(S):
SGI-110 (guadecitabine)

Belinostat (PXD-101)

For more information: 1-800-4-CANCER
cancer.gov | clinicaltrials.gov

INITIALS:_____ DATE: _____

APPENDIX E PATIENT CLINICAL TRIAL WALLET CARD: BELINOSTAT AND ASTX727



NIH NATIONAL CANCER INSTITUTE

CLINICAL TRIAL WALLET CARD

Show this card to all of your healthcare providers and keep it with you in case you go to the emergency room.

Patient Name:

Diagnosis:

Study Doctor:

Study Doctor Phone #:

NCI Trial #: 10330

Study Drug(S):
ASTX727

Belinostat (PXD-101)

For more information: 1-800-4-CANCER
cancer.gov | clinicaltrials.gov

INITIALS:_____ DATE: _____

APPENDIX F PATIENT MEDICATION DIARY – ASTX727

CTEP-assigned Protocol # 10330

Local Protocol # _____

PATIENT'S MEDICATION DIARY

Today's date _____ **Agent** _____**ASTX727**_____

Patient Name (*initials acceptable)* **Patient Study ID**

INSTRUCTIONS TO THE PATIENT:

1. Complete one form every four weeks.
2. You will take 1 tablet each day during the first 5 days of a 28-day cycle as shown below, unless instructed otherwise. You will bring the ASTX727 tablets to the clinic and take them at the clinic under the direction of a doctor or nurse.
3. Do not eat, drink milk or alcohol 2 hours before and 2 hours after taking ASTX727. Clear liquids such as water, black coffee, or tea are allowed. Take whole tablets with 8 oz. (240 mL) of water; do not crush, cut, or chew the tablet.
4. If you miss or forget about a dose, you can still take that day's medication if you remember within 12 hours of the scheduled time you were supposed to take that dose. If you miss or forget about a dose and it is beyond 12 hours from the time you were supposed to take it, you should skip that dose, and resume at the scheduled time the next day. Always tell your nurse or doctor if you miss, forget about, or vomit up a dose..
6. Record the date and time the tablet is taken below.
7. If you have any comments or notice any side effects, please record them in the Comments column.
8. Please return the forms to your doctor when you go for your next appointment.

Day	Date	What time was dose taken?	# of tablets taken	Comments
1				
2				
3				
4				
5				
6 through 28	Do not take ASTX727 on days 6 through 28.			

Physician's Office will complete this section:

Date patient started protocol treatment: _____ Date patient was removed from study_____

Patient's planned total daily dose_____ Total number of pills taken this month (each size):_____

Physician/Nurse/Data Manager's Signature _____

Patient's Signature:_____