

## PROTOCOL SUMMARY OF CHANGES

**RPCI # I 145208 / NCI 7870**

**Amendment:** RPCI #31 / CTEP #32 Protocol Dated: 5/29/19

**Study Title:** Phase I / II Study of High Dose Interleukin 2, Aldesleukin, in Combination with the Histone Deacetylase Inhibitor Entinostat in Patients with Metastatic Renal Cell Carcinoma

Section	Page	Rationale	Previous Text	New Text
<a href="#">Title Page</a>	1	Updated for submission	<b>Amendment # 30 10/11/18 (CTEP version 10/9/18 Amendment # 31)</b>	<b>Amendment # 31 05/29/19 (CTEP version 10/9/18 Amendment # 32)</b>
<a href="#">Footer</a>	All	Updated for Submission	<b>10/11/18</b>	<b>5/29/19</b>
<a href="#">4.3</a>	17	Updated to add in termination language once primary endpoint is met.	<p><b>Duration of Follow Up</b></p> <p><b>Follow up safety evaluations will occur 30 days (+/- 4 days) after last dose of study drug or until resolution of any drug related toxicity (telephone contact is acceptable). Follow up will include review of concomitant medications and adverse events.</b></p> <p><b>Patients (both Phase I and Phase II) will be followed up after completion of therapy every three months for survival assessment (telephone contact is acceptable).</b></p>	<p><b>Duration of Follow Up</b></p> <p><b>Follow up safety evaluations will occur 30 days (+/- 4 days) after last dose of study drug or until resolution of any drug related toxicity (telephone contact is acceptable). Follow up will include review of concomitant medications and adverse events.</b></p> <p><b>Patients (both Phase I and Phase II) will be followed up after completion of therapy every three months for survival assessment (telephone contact is acceptable) or until completion of primary endpoint/ termination of study.</b></p>
ICF Changes	ICF Addendum-separate document	ICF Addendum	10/11/18	5/29/19

**Title: Phase I/II Study of High Dose Interleukin 2, Aldesleukin, in Combination with the Histone Deacetylase Inhibitor Entinostat in Patients with Metastatic Renal Cell Carcinoma**

**RPCI Study No.: I 145208**

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**NCI Protocol #: 7870**

Protocol Version Date 5/29/19

page 2 of 78

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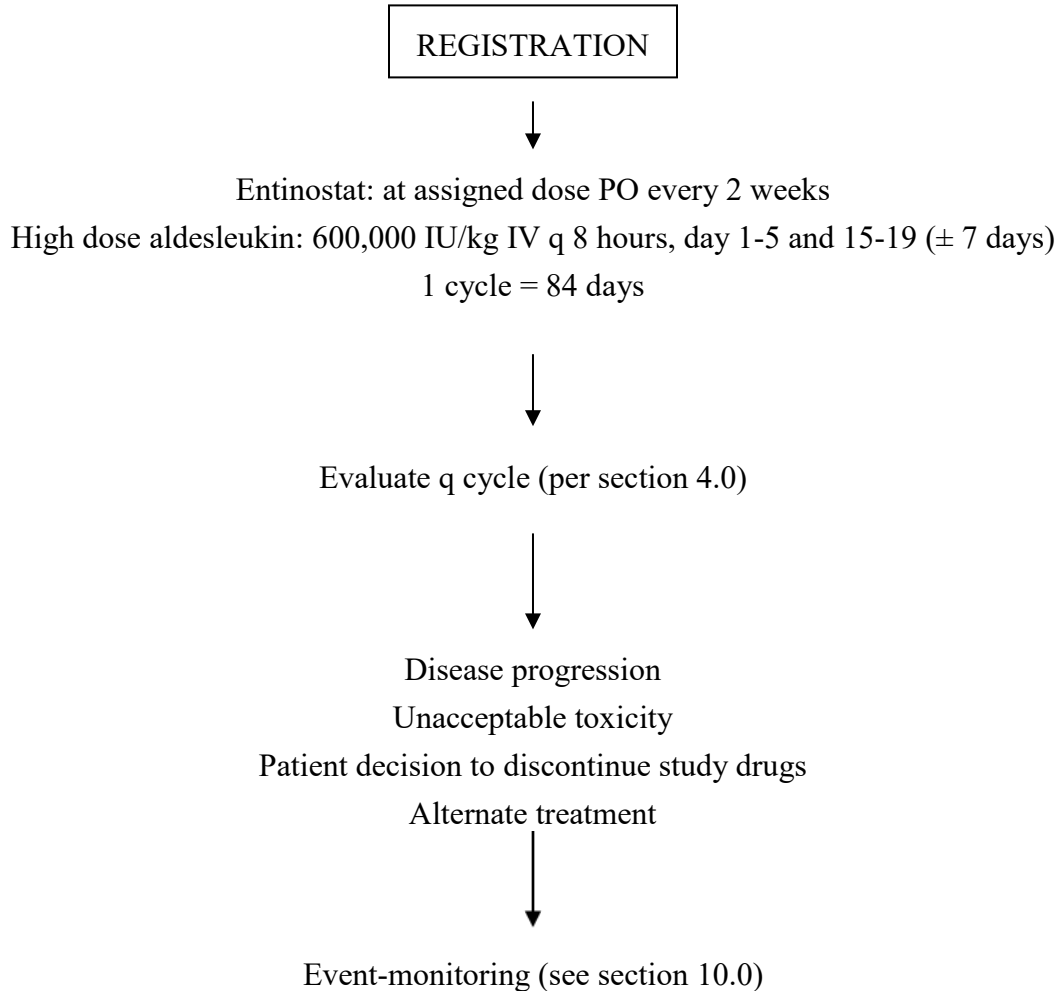
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**NCI Supplied Agent:** Entinostat (MS-275, SNDX-275), NSC 706995, IND 61198

**SCHEMA****Metastatic renal cell carcinoma patients meeting eligibility criteria in section 3.0****Drug Names/Abbreviations**

Generic name: Entinostat	Generic name: Interleukin 2 Brand name: Aldesleukin/Proleukin
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**TABLE OF CONTENTS**

	Page
<b>TITLE: PHASE I/II STUDY OF HIGH DOSE INTERLEUKIN 2, ALDESLEUKIN, IN COMBINATION WITH THE HISTONE DEACETYLASE INHIBITOR ENTINOSTAT IN PATIENTS WITH METASTATIC RENAL CELL CARCINOMA .....</b>	<b>1</b>
<b>RPCI STUDY NO.: I 145208.....</b>	<b>1</b>
<b>PRINCIPAL INVESTIGATOR: SABY GEORGE, MD .....</b>	<b>2</b>
<b>CO-INVESTIGATORS:.....</b>	<b>2</b>
<b>SCHEMA.....</b>	<b>4</b>
<b>1. OBJECTIVES .....</b>	<b>8</b>
1.1 Phase I Primary Objectives.....	8
1.2 Phase II Primary Objectives .....	8
1.3 Phase II Secondary Objectives .....	8
<b>2. BACKGROUND .....</b>	<b>8</b>
2.1 Renal cell carcinoma.....	8
2.2 Interleukin 2 (Aldesleukin).....	8
2.3 Histone deacetylase inhibitors .....	9
2.4 Entinostat .....	10
2.5 Rationale for the combination of entinostat and high dose aldesleukin .....	10
2.6 Rationale for entinostat dose and schedule selection .....	12
<b>3. PATIENT SELECTION .....</b>	<b>12</b>
3.1 Eligibility Criteria.....	12
3.2 Exclusion Criteria .....	14
3.3 Inclusion of Women and Minorities .....	15
<b>4. TREATMENT PLAN.....</b>	<b>15</b>
4.1 Entinostat / aldesleukin administration and definition of Dose-Limiting Toxicity (DLT)	16
4.2 Duration of Therapy .....	17
4.3 Duration of Follow Up.....	17

<b>5.</b>	<b>DOSING DELAYS/DOSE MODIFICATIONS</b>	<b>17</b>
5.1	Entinostat Treatment Modification for Adverse Events	18
5.2	Adverse Events with entinostat	18
5.3	Aldesleukin Treatment Modification for Adverse Events	19
5.4	Specific aldesleukin Toxicity Management	20
5.5	Management of Grade 4 Toxicity	21
5.6	Toxicity Criteria for Discontinuing Treatment	22
<b>6.</b>	<b>PHARMACEUTICAL INFORMATION</b>	<b>22</b>
6.1	Entinostat (NSC 706995)	22
6.2	Interleukin-2 (Aldesleukin)	29
<b>7.</b>	<b>CORRELATIVE/SPECIAL STUDIES</b>	<b>30</b>
7.1	Pharmacodynamic Studies	30
7.2	Pharmacokinetic studies (Phase I only)	31
7.3	Non invasive imaging studies	32
7.4	Optional tumor Samples	32
<b>8.</b>	<b>STUDY CALENDAR</b>	<b>35</b>
<b>9.</b>	<b>MEASUREMENT OF EFFECT</b>	<b>37</b>
9.1	Methods of Measurement	37
9.2	Response Criteria	38
<b>10</b>	<b>REGULATORY AND REPORTING REQUIREMENTS</b>	<b>41</b>
10.1	Protocol Amendments	41
10.2	Informed Consent	41
10.3	Adverse Events	42
10.4	Data and Safety Monitoring	48
10.5	CTEP Multicenter Guidelines	48
<b>11.</b>	<b>STATISTICAL CONSIDERATIONS</b>	<b>51</b>
11.1	Study Design-	51
11.2	Phase I	51
11.3	Phase II	52
11.4	Toxicity monitoring	54
11.5	Patient Compliance	55

11.6	Data quality assurance .....	56
11.7	Reporting and Exclusion.....	57
<b>12.</b>	<b>ETHICS .....</b>	<b>58</b>
12.1	Institutional Review Board.....	58
12.2	Evaluation of benefits and risks/discomforts.....	58
12.3	Risks/benefits analysis.....	58
12.4	Patient information and consent .....	58
12.5	Data handling and record keeping .....	59
<b>13.</b>	<b>REFERENCES.....</b>	<b>61</b>
<b>APPENDIX A: PROTOCOL FOR QUANTITATIVE ANALYSIS OF TUMOR METABOLISM AND TUMOR BLOOD FLOW USING 18FDG–PET/CT IMAGING. ...</b>		<b>63</b>
<b>APPENDIX B: PERFORMANCE STATUS CRITERIA.....</b>		<b>67</b>
<b>APPENDIX C: HDIL-2 (HIGH DOSE ALDESLEUKIN) ADMINISTRATION GUIDELINES .....</b>		<b>68</b>
<b>APPENDIX D: INSTRUCTIONS FOR NETWORK SITES .....</b>		<b>77</b>



## 1. OBJECTIVES

### 1.1 Phase I Primary Objectives

1.1.1 To evaluate the safety and tolerability of high dose interleukin 2 (aldesleukin) in combination with entinostat in patients with metastatic renal cell carcinoma (RCC).

### 1.2 Phase II Primary Objectives

1.2.1 To monitor toxicity and estimate the efficacy of high dose aldesleukin combined with entinostat in patients with metastatic RCC.

### 1.3 Phase II Secondary Objectives

1.3.1 To compare the time-to-tumor progression, progression-free survival and overall survival of patients with metastatic RCC treated with high dose aldesleukin combined with entinostat to the historical data of patients treated with high dose aldesleukin alone.

1.3.2 To assess the toxicity of high dose aldesleukin combined with entinostat.

1.3.3 To evaluate entinostat pharmacodynamics (PD) in blood and tumor samples.

1.3.4 To measure the association between baseline laboratory parameters (e.g. CD4+, CD8+, CD4+/Foxp3), tumor blood metabolism, and a variety of response variables (e.g. toxicity, response and survival).

1.3.5 To explore the relationship between entinostat exposure with PD endpoints (e.g. toxicity and histone acetylation in peripheral blood mononuclear cells or PBMNCs and changes in T cell subset population).

1.3.6 To evaluate the modulation of tumor metabolism by FDG PET/CT scan.

## 2. BACKGROUND

### 2.1 Renal cell carcinoma

More than 51,000 new cases of kidney cancer and approximately 13,000 cancer specific deaths in the United States have been predicted for 2007<sup>1</sup>. One third of patients have metastatic disease at the time of diagnosis. Two randomized studies in metastatic RCC patients have shown survival benefit of nephrectomy and cytokine therapy over cytokine therapy alone. Many patients undergo nephrectomy as a component of their standard care. The prognosis for recurrent or metastatic renal cell carcinoma is poor, as median survival is 10-13 months and 5-year survival is less than 5%. These figures underscore the need for effective systemic therapy in this disease.

In 1992, high-dose bolus aldesleukin was approved by the FDA for the treatment of patients with metastatic renal cell cancer based on data presented on 255 patients entered into 7 phase II clinical trials<sup>2-4</sup>. HD bolus aldesleukin is superior in terms of response rate and response quality to regimens involving either intermediate or low dose aldesleukin or subcutaneous interferon alpha (IFN $\alpha$ )<sup>5,6</sup>.

### 2.2 Interleukin 2 (Aldesleukin)

The Cytokine Working Group performed a phase III trial in which patients were randomized to

NCI Protocol #: 7870

receive either outpatient aldesleukin (5 MIU/m<sup>2</sup> subcutaneously every 8 hours x 3 doses on day 1 then daily 5 days/week for 4 weeks) and IFN $\alpha$  (5 MIU/m<sup>2</sup> subcutaneously thrice weekly for 4 weeks) every 6 weeks or standard high dose inpatient aldesleukin (600,000 IU/kg/dose every 8 hours intravenously, days 1-5 and 15-19 [max 28 doses]) every 12 weeks<sup>5</sup>. The response rate for high-dose aldesleukin was 23% (22/95) versus 10% (9/91) for aldesleukin / IFN $\alpha$  (p=0.018). Eight patients achieved a complete response on high dose aldesleukin versus only 3 on low dose aldesleukin / IFN $\alpha$ . The median response durations were 24 months for high dose aldesleukin and 15 months for aldesleukin / IFN $\alpha$  (p=0.18). Median overall survivals were 17 and 13 months (p=0.21), favoring high dose aldesleukin. Ten patients on high dose aldesleukin were progression free at 3 years versus 3 on aldesleukin / IFN $\alpha$  (p=0.08). Durable CRs favored HD aldesleukin (7 vs. 0). Median progression free survival was 3 months for each treatment arm. Another three-arm randomized study compared response rates and overall survival for patients with metastatic RCC receiving high-dose or one of two low-dose aldesleukin regimens<sup>7</sup>. Major tumor regressions, as well as complete responses, were seen with all regimens tested but high dose aldesleukin was more clinically active, although this did not produce an overall survival benefit. Taken together these data suggests that high dose aldesleukin should remain the preferred therapy for appropriately selected patients with access to such therapy before or after the use of the recently FDA approved receptor tyrosine kinase inhibitors sorafenib and sunitinib<sup>8,9</sup>. However, given the toxicity and limited efficacy of high dose aldesleukin therapy, additional efforts should be directed to increase the efficacy of immunotherapy.

### 2.3 Histone deacetylase inhibitors

Histone deacetylases (HDACs) are critically important in the regulation of gene expression and in the field of target-specific anticancer drug.<sup>10-12</sup> Inhibitors of HDAC present an exciting, novel approach to the treatment of solid tumors, many of which are refractory to current therapies. Several chemical groups of HDAC inhibitors have been identified: 1) *short-chain fatty acids* – butyrates 2) *hydroxamic acids* – trichostatin A (TSA), suberoylanilide hydroxamic acid (SAHA or vorinostat), oxamflatin and LBH-589; 3) *cyclic tetrapeptides containing a 2-amino-8-oxo-9,10-epoxy-decanoyl (AOE) moiety* – trapoxin A; 4) *cyclic peptides not containing the AOE moiety* – depsipeptide and apicidin and 5) *benzamides* – entinostat.

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

## 2.4 Entinostat

The synthetic benzamide, entinostat has demonstrated induction of chromatin hyper-acetylation and antitumor activity by inhibition of HDAC enzyme activity. Entinostat has also demonstrated inhibition of tumor cell growth in nude mice that was comparable or superior to conventional cytotoxic agents<sup>13</sup>. The results from the Phase I testing of entinostat have been recently published<sup>14</sup>. Patients with advanced solid tumors or lymphoma were treated with entinostat orally initially on a once daily x 28 every 6 weeks (daily) and later on once every-14-days (q14-day) schedules. The starting dose was 2 mg/m<sup>2</sup> and the dose was escalated in three- to six-patient cohorts based on toxicity assessments. With the daily schedule, the MTD was exceeded at the first dose level. PK analysis suggested the half-life of entinostat in humans was 39 to 80 hours, substantially longer than predicted by preclinical studies. With the q14-day schedule, 28 patients were treated. The MTD was 10 mg/m<sup>2</sup> and dose-limiting toxicities were nausea, vomiting, anorexia, and fatigue. Exposure to entinostat was dose dependent, suggesting linear PK. Increased histone H3 acetylation in peripheral-blood mononuclear-cells was apparent at all dose levels by immunofluorescence analysis. Ten of 29 patients remained on treatment for > or = 3 months. The entinostat oral formulation on the daily schedule was intolerable at a dose and schedule explored. The q14-day schedule is reasonably well tolerated. Histone deacetylase inhibition was observed in peripheral-blood mononuclear-cells. Based on PK data from the q14-day schedule, more frequent dosing schedules such as weekly x 4, repeated every 6 weeks have been tested. In a Phase I study (Berlex 306121) the MTD for entinostat dosed on D1, D8, D15 of 4 weeks cycle was established at 4mg/m<sup>2</sup> dose level. Most common toxicities hypophosphatemia (grade 3 in 48% of patients) bone marrow suppression, gastrointestinal events such as nausea and vomiting (grade 1 + 2 frequent) fatigue, asthenia (grade 1 +2 frequent, hypoalbuminemia, hypoproteinemia.

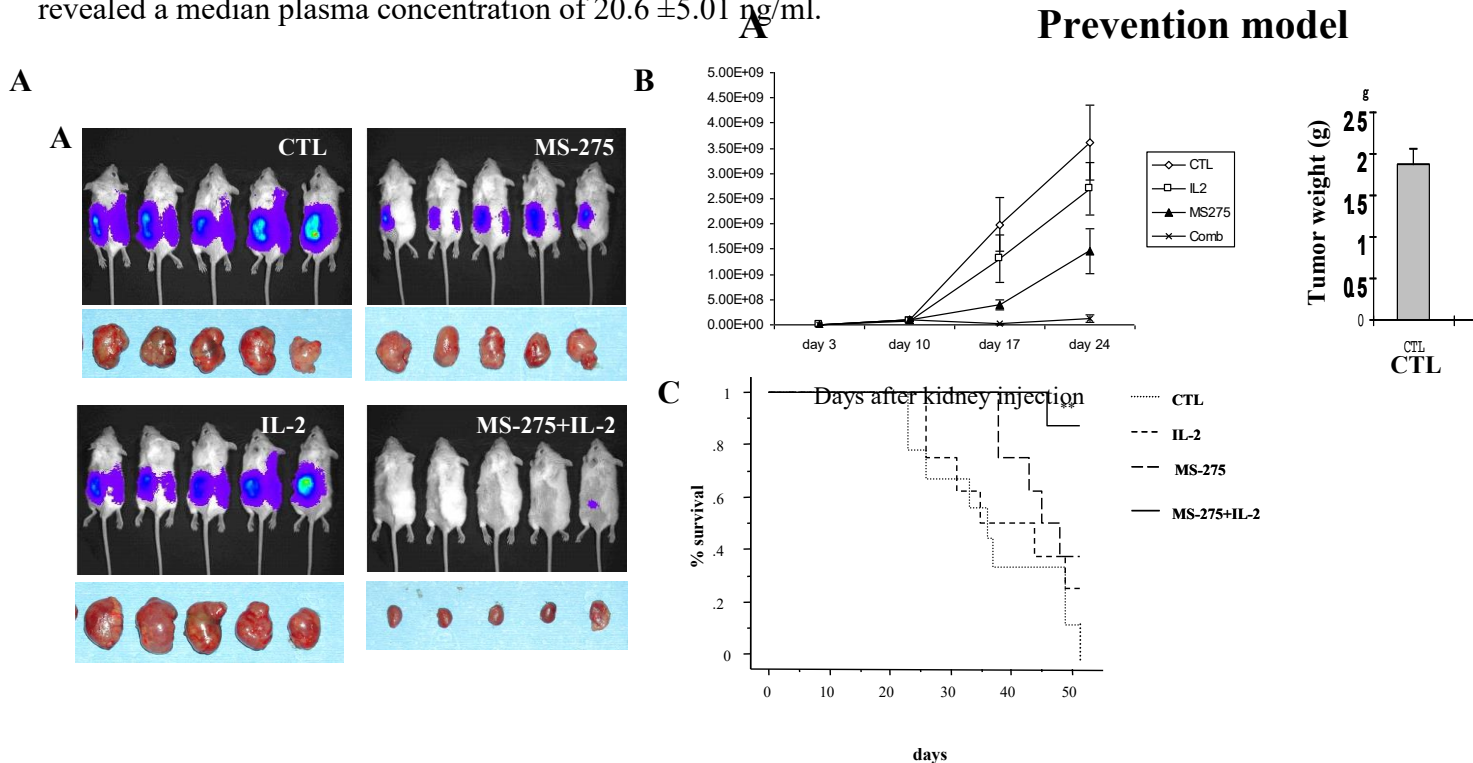
## 2.5 Rationale for the combination of entinostat and high dose aldesleukin

HDAC inhibitors induce cell-cycle arrest, differentiation or apoptosis *in vitro*, and have potent anti-tumor activities *in vivo*. We have previously showed HDAC inhibitor entinostat has a significant antitumor activity in a renal cell carcinoma model<sup>15</sup>. HDAC inhibitors have been also shown to have immunodulatory properties including activation of major histocompatibility complex (MHC) class I and II proteins, and co-stimulatory molecules CD40, CD80 and CD86. The results from the clinical trials with HDAC inhibitors in cutaneous T cell lymphoma and large cell lymphoma patients suggest that the antitumor activity these agents may be in part due to the modulation of the immune response.

In our lab, we have recently tested the effectiveness of combination therapy of aldesleukin with the HDAC inhibitor entinostat in a murine renal cell carcinoma (RENCA) model<sup>16</sup>. RENCA-luciferase expressing cells were implanted in the left kidney of BALB/C mice. Animals were randomly divided in four groups and treated with either vehicle, 150,000 IU aldesleukin twice daily by i.p injections (twice weekly for two weeks), 5 mg/kg entinostat daily by oral gavage (5 days/week for two weeks), or combination. Treatment was started 3 days following tumor cell injection. Weekly luciferase images and tumor weight after 3 weeks treatment showed significant tumor inhibition (>80%) in the as compared to aldesleukin (no significant inhibition) or entinostat (~40% inhibition) (see Panel A and B). Spontaneous lung metastases were also

**NCI Protocol #: 7870**

inhibited in the combination treatment (>90 % inhibition) as compared to single treatment. Kaplan-Meier analyses showed statistically significant increase in survival in the combination group as compared with control and single agents (Panel C). The percentage of CD4<sup>+</sup> 25<sup>+</sup> and Fox-p3<sup>+</sup> T cells was increased or reduced, respectively, in lymph nodes from tumor bearing animals treated with the combination of entinostat and aldesleukin as compared to control and single agents (Panel D). Splenocytes from mice treated with combination treatment showed greater lysis than splenocytes from mice treated with single agents (Panel E). These results suggest that combination of aldesleukin and entinostat has a synergistic antitumor effect in vivo in an immunocompetent murine model of renal cell carcinoma. The antitumor effect was associated with decreased number of regulatory T cells and increased antitumor cytotoxicity by splenocytes. Lower dose aldesleukin (15,000 IU) also induced a greater antitumor effect as compared to single agents (Panel F). However, microscopic examination revealed the presence of tumor in all the samples suggesting a dose-dependent effect of aldesleukin in this combination (Panel G). Blood samples collected after two hours from entinostat administration revealed a median plasma concentration of  $20.6 \pm 5.01$  ng/ml.



In summary, these results suggest that combination of aldesleukin and entinostat has a synergistic antitumor effect in vivo in an immunocompetent murine model of renal cell carcinoma. The antitumor effect was associated with decreased number of regulatory T cells and increased antitumor cytotoxicity by splenocytes. A dose dependent effect for aldesleukin but not for entinostat was observed. In conclusion, these data provide the rationale for clinical testing of the combination of high dose aldesleukin and HDAC inhibitors in the treatment of renal cell carcinoma patients. The immunomodulatory activity of entinostat and its direct antitumor effect may increase the response rate to high dose aldesleukin, further delay disease progression and increase progression-free survival in patients with metastatic RCC.

## 2.6 Rationale for entinostat dose and schedule selection

Entinostat has been evaluated in vitro, in non-clinical in vivo studies, and in phase 1 studies in patients with various solid tumors and hematological malignancies at doses between 2 and 12 mg/m<sup>2</sup> and at dosing frequencies ranging from once daily to every 2 weeks. Increased histone acetylation was observed at the lowest dose evaluated with the effect persisting at least 48 hours post-dose. Pharmacokinetic studies of entinostat have indicated a long half-life of entinostat, **Patients may have received up to two prior therapies including VEGF, mTOR and PD-1/PDL1 inhibitors** ranging from 40 hours to 120 hours, with concentrations detectable 168 hours post-dose at doses of 6 mg/m<sup>2</sup> to 12 mg/m<sup>2</sup>, consistent with this long half-life.

The MTD for single agent in non-hematologic indications has been established as 4mg/m<sup>2</sup> weekly x 3 and one week rest, or 10 mg/m<sup>2</sup> every second week continuously. In combination with 13-*cis*-retinoic acid the MTD is also 4 mg/m<sup>2</sup> weekly x 3 with one week of rest. For hematologic indications the MTD is 6 mg/m<sup>2</sup> weekly x 3 and one week rest.

Approximately 40% variability in clearance of entinostat was noted. When clearance was adjusted for body surface area, the inter-patient variability was similar. A linear regression analysis on factors that may contribute to this variability, such as ideal body weight, lean body mass, body weight, and body mass index, were not significant covariates. As a result of this analysis, fixed dosing is considered to be as accurate as dosing based on body surface area<sup>17</sup>. Therefore, based on the clinical experience with entinostat, a dose of 3 and 5 mg given every 2 weeks has been selected as the regimen for this study. Combination studies with flat every 2 weeks dose of entinostat are currently conducted in breast and lung cancer. Antitumor response has been reported in patients with melanoma treated with 3 mg every 2 weeks dose.

Our preclinical data suggest that the immunomodulatory effect of entinostat is observed 7-14 days after administration. The every 2 week schedule will allow the pharmacodynamic assessment of entinostat as single agent and eventually prime the immune response to high dose aldesleukin administration.

## 3. PATIENT SELECTION

### 3.1 Eligibility Criteria

- 3.1.1 Patients must have pathological diagnosis of renal cell carcinoma that is metastatic or surgically unresectable. The histology must be clear cell carcinoma or predominant clear cell carcinoma.
- 3.1.2 Patients may have received up to two prior therapies including VEGF, mTOR and PD-1/PDL1 inhibitors. Prior palliative radiation to metastatic lesion(s) is permitted, provided there is at least one measurable and/or evaluable lesion(s) that has not been irradiated.
- 3.1.3 Patients must have measurable or evaluable disease.

- 3.1.4 Age  $\geq$  18 years.
- 3.1.5 ECOG performance status 0 (see Appendix B).
- 3.1.6 Life expectancy of greater than 6 months.
- 3.1.7 Patients must have normal organ and marrow function as defined below:
- Hemoglobin  $\geq$ 12 g/dL
  - Leukocytes  $\geq$ 3,000/mm<sup>3</sup>
  - Absolute neutrophil count  $\geq$ 1,500/mm<sup>3</sup>
  - Platelets  $\geq$ 100,000/mm<sup>3</sup>
  - Total bilirubin  $\leq$ 1.5 x laboratory upper limit of normal
  - AST(SGOT)/ALT(SGPT)  $\leq$ 2.5 x laboratory upper limit of normal
  - Creatinine  $\leq$ 1.5 x laboratory upper limit of normal or Calculated Creatinine clearance of  $\geq$ 50ml/min (please use institutional formula)
  - LDH WNL
  - Corrected calcium  $\leq$ 10 mg/dL
  - PT/INR  $\leq$ 1.5
  - Urine protein  $<$ 1+; if  $\geq$ 1+, 24 hour urine protein should be obtained and should be  $<$ 1000 mg
- 3.1.8 Pulmonary: FEV1  $\geq$ 2.0 liters or  $\geq$ 75% of predicted for height and age. (PFTs are required for patients over 50 or with significant pulmonary or smoking history).
- 3.1.9 Cardiac: No evidence of congestive heart failure, symptoms of coronary artery disease, myocardial infarction less than 6 months prior to entry, serious cardiac arrhythmias, or unstable angina. Patients who are over 40 or have had previous myocardial infarction greater than 6 months prior to entry will be required to have a negative or low probability cardiac stress test for cardiac ischemia.
- 3.1.10 CNS: No history of cerebrovascular accident or transient ischemic attacks.
- 3.1.11 The effects of entinostat on the developing human fetus at the recommended therapeutic dose are unknown. For this reason women of child-bearing potential must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately. Men with female partners of child bearing potential must also agree to use adequate contraception.

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

## **3.2 Exclusion Criteria**

3.2.1 Patients who have received more than two prior therapies.

3.2.2 Concurrent use of valproic acid is not allowed.

3.2.3 Patients may not be receiving any other investigational agents.

3.2.4 Patients with untreated CNS metastases. Patients should have a head CT/MRI within 28 days prior to treatment initiation. Patients with previously excised/gamma knifed solitary or oligometastases and controlled disease are eligible.

3.2.5 Any medical condition that would preclude adequate evaluation of the safety and toxicity of the study combination.

3.2.6 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure (New York Association Class II, III, or IV), angina pectoris requiring nitrate therapy, recent myocardial infarction (< the last 6 months), cardiac arrhythmia, history of CVA within 6 months, hypertension (defined as blood pressure of >160 mmHg systolic and/or >90 mmHg diastolic on medication) history of peripheral vascular disease, or psychiatric illness/social situations that would limit compliance with study requirements.

3.2.7 Patients with a history of allergy to entinostat or other medications that have a benzamide structure (i.e. tiapride, remoxipride, and clebopride).

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

3.2.9 HIV-positive patients receiving combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with entinostat. In addition, these patients are at increased risk of lethal infections when treated with marrow-suppressive therapy. Appropriate studies will be undertaken in patients receiving combination anti-retroviral therapy when indicated.

3.2.10 Serious or non-healing wound, ulcer or bone fracture.

3.2.11 Major surgical procedure, open biopsy, or significant traumatic injury within 28

days prior to day 1 therapy.

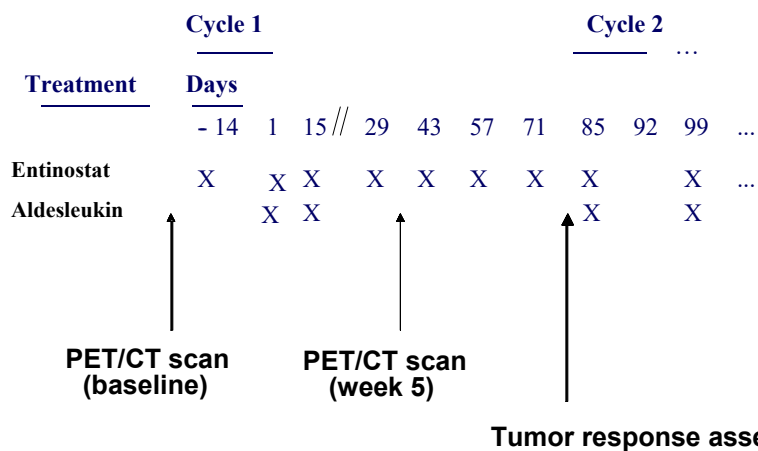
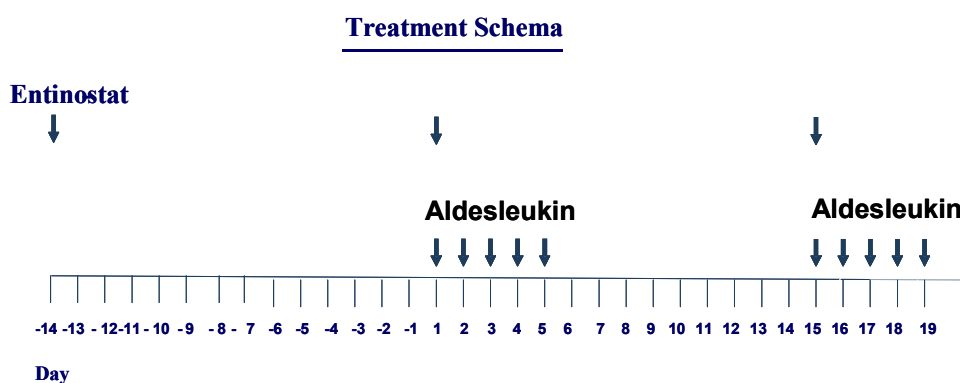
3.2.12 Anticipation of need for major surgical procedures during the course of the study

3.2.13 Left ventricular ejection function <45%.

### 3.3 Inclusion of Women and Minorities

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

## 4. TREATMENT PLAN



Treatment will be administered on an inpatient/outpatient basis. Patients will be admitted to the hospital unit with intensive care capabilities for the administration of high dose aldesleukin. Reported adverse events and potential risks are described in Section 5. Appropriate dose modifications for entinostat and aldesleukin are described in Section 5. 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 case report form will capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies.

#### **4.1 Entinostat / aldesleukin administration and definition of Dose-Limiting Toxicity (DLT)**

Patients with metastatic renal cell carcinoma and no prior therapies will be eligible for treatment. One cycle of treatment (84 days) will consist of 2 courses of high dose aldesleukin 600,000 units/kg administered IV every 8 hrs. on day 1-5 and day 15-19 ( $\pm$  7 days) (maximum 28 doses), and entinostat orally (1-2 hrs. prior to aldesleukin infusion) given every 2 weeks starting on day-14, continuously. Entinostat is to be taken on an empty stomach, at least 1 hour before or 2 hours after a meal. Tumor response assessment will be performed on week 11 ( $\pm$  7 days). In the event of clinical benefit (stable disease or tumor shrinkage) patients will receive a second cycle of therapy.

Entinostat will continue every 2 weeks ( $\pm$  7 days dependent on adjustments necessary for aldesleukin dosing) until documented disease progression or 8 weeks following documented complete response. Patients with evidence of tumor shrinkage may receive up to 3 cycles of high dose aldesleukin therapy. Cycle 2 will start on or within 2 weeks following day 85 (or last day of cycle dependent on dosing adjustment) as in cycle 1. Cycle 3 can start within 2 weeks after completion of cycle 2. Patients with stable disease by RECIST V.1.0 criteria, but without evidence of tumor shrinkage after two cycles will receive only entinostat until disease progression is documented.

In order to assess the effect of entinostat versus the combination on the proposed correlative pharmacodynamic parameters, initial treatment will be with entinostat monotherapy, followed by combination with high dose aldesleukin. The Phase I starting dose level of entinostat will be 3 mg PO every 2 weeks. The first dose level will have a minimum of 3 patients treated unless the first 2 patients experience dose-limiting toxicity(s) (DLT) before 3<sup>rd</sup> patient is enrolled. DLTs are defined as extended grade 4 toxicity (duration of one week or more) during the first 45 days of treatment. If DLTs occur in 1 patient treated at the starting dose level, a minimum of 3 or 4 patients will be treated at this dose level. If DLT occurs in more than 1 patient in the first 6 patients, patients will be treated at a lower dose level (entinostat 1 mg PO every 2 weeks, dose level 0) and DLT will be evaluated similarly. If DLTs occur in  $\leq$  1/6 patients, dose level 1 will be considered the recommended Phase II dose. If no DLTs occur at the starting dose level 1, 3 or 4 patients will be treated at the next dose level (level 2) with 5 mg PO every 2 weeks (wait 45 days for DLT assessment on first 2 patients before 3<sup>rd</sup> patient is enrolled). If no DLTs occur at dose level 2, this dose level will be recommended for the phase II portion of the study. If DLTs occur in  $\geq$  1 patient, another 3 patients will be treated at this dose level. If DLT occurs in  $\geq$  2 patients in the first 6 patients, dose level 1 will be the recommended for the Phase II. If DLTs occur in  $\leq$  1/6 patients, dose level 2 will be considered the recommended Phase II dose. Patients who experience extended grade 4 toxicity (duration of one week or more) and recover to  $\leq$  Grade 1 (or to pretreatment baseline level toxicity) may continue treatment at the next entinostat lower level. Once the entinostat has been dose reduced, patients must remain at the reduced dose for the rest of the study. Patients may have their dose de-escalated twice or up to dose level 0 before being removed from the treatment. Patients will be allowed to remain on the therapy provided that they are tolerating the treatment and do not develop progressive disease. No dose de-

escalation for aldesleukin will be performed.

#### Dosing levels

Dose Level	Entinostat (mg, every 2 weeks, PO)	Aldesleukin (IU/kg, every 8hrs, IV)
0	1	600,000
1	3	600,000
2	5	600,000

Patients will receive only entinostat during the “maintenance” (between cycles) period. During Phase II, patients receiving 5 mg entinostat during the “maintenance” period will be given the option to dose escalate entinostat to 10 mg PO if tolerated except two weeks before and two weeks after administration of aldesleukin.

The recommended dose of 5 mg PO every 2 weeks was determined in the Phase I portion of the study. This cohort will be expanded in the phase II portion so that 36 new patients will be treated at this dose level. The evaluations for the phase II component of the study will be identical to the phase I portion.

Compliance will be assessed via patient diaries Also, pill containers will be returned to the study doctor or nurse at the end of each cycle.

#### 4.2 Duration of Therapy

Patients may continue on therapy until one of the following criteria applies:

- Disease progression,
- Unacceptable toxicity.
- Patient decides to withdraw from the study.
- General or specific changes in the patient’s condition that renders the patient unacceptable for further treatment in the judgment of the investigator.
- The study is closed.

#### 4.3 Duration of Follow Up

Follow up safety evaluations will occur 30 days (+/- 4 days) after last dose of study drug or until resolution of any drug related toxicity (telephone contact is acceptable). Follow up will include review of concomitant medications and adverse events.

Patients (both Phase I and Phase II) will be followed up after completion of therapy every three months for survival assessment (telephone contact is acceptable) or until completion of primary endpoint/ termination of study.

### 5. DOSING DELAYS/DOSE MODIFICATIONS

The CTEP Active Version of the NCI Common Terminology Criteria for Adverse Events (CTCAE) [[http://ctep.cancer.gov/protocolDevelopment/electronic\\_applications/ctc.htm](http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm)] will be used to classify toxic effects of therapy. Patients with life-threatening or persistent, severe toxic reactions to aldesleukin will receive no further treatment with this agent. Specific toxicities, which require special consideration, are discussed below. Previously unknown or severe toxicities will be reported to the PI as Adverse Drug Reactions.

## 5.1 Entinostat Treatment Modification for Adverse Events

**Patients who experience any Grade 3/4 toxicity related to entinostat during the period off aldesleukin should stop the offending drug up to one dose. If the patients recover to  $\leq$  Grade 1 (or to pretreatment baseline level of toxicity), they may continue treatment with entinostat at the next lower dose.** Once the dose has been reduced, patients must remain at the reduced dose for the remainder of the study. Patients may have their dose reduced down to the lowest dose level 0 (1mg). If the patient misses or vomits a dose of entinostat he/she will skip that dose and notify the research nurse. If a patient has to miss more than one dose of entinostat due to grade 3/4 toxicity during the period off aldesleukin he/she will discontinue entinostat.

## 5.2 Adverse Events with entinostat

Risks to patients receiving entinostat have been minimized with careful monitoring by trained and experienced investigators and evaluation of laboratory safety parameters at appropriate intervals. The following points should be considered for the prevention or minimization of potential adverse events and other risks related to entinostat:

- *Nausea and vomiting:* Antiemetics may be administered to prevent or control nausea or vomiting.
- *Diarrhea:* Antidiarrheal agent(s) may be used in the event of diarrhea.
- *Neutropenia:* The patient should be closely followed for fever, focal signs of infection, and neutrophil nadir. Standard growth factor support may be considered unless excluded in the individual clinical protocols.
- *Anemia:* RBC transfusion support may be given for anemia, particularly when hemoglobin falls below 8 g/dL or as instructed in the individual clinical protocols.
- *Thrombocytopenia:* If bleeding develops or invasive procedures are planned, platelet transfusions may be administered in accordance with standard practice or as instructed in the individual clinical protocol.
- *Hypophosphatemia and other electrolyte abnormalities:* Careful monitoring of electrolytes during treatment with entinostat is recommended and electrolyte supplementation may be warranted. In clinical studies, abnormalities in most patients were successfully managed with supplementation, allowing continued treatment without interruption.

- *Renal abnormalities:* Based on mild increases in creatinine seen in a few patients and results of nonclinical studies, monitoring of renal function is recommended during treatment with entinostat. If clinically significant changes in renal function occur, modification of entinostat dose should be considered.

Entinostat may cause fatigue or malaise; advise patient to exercise caution while driving a vehicle or operating machinery.

Administration of entinostat is contraindicated in patients with a history of allergy to entinostat or other medications that have a benzamide structure (e.g., tiapride, remoxipride, clebopride).

Careful monitoring of patients for signs of infection or reactivation of past infections is recommended, as reactivation of infection has been reported in patients treated with entinostat, in some cases without evidence of neutropenia. The clinical significance of this finding and the potential association with entinostat is unknown.

### 5.3 Aldesleukin Treatment Modification for Adverse Events

Modification of the treatment protocol will occur by withholding doses of aldesleukin rather than continuing therapy at a reduced dose (see Appendix C). Missed doses of aldesleukin will not be made up. Dose of aldesleukin will be withheld for:

- 5.3.1 Hypotension refractory to fluids and pressors or requiring unacceptably high pressor doses
- 5.3.2 Anuria for >24 hours and unresponsive to fluid replacement and low-dose dopamine
- 5.3.3 Respiratory distress requiring oxygen >4 liters to maintain O<sub>2</sub> saturation >95%.
- 5.3.4 Confusion (mental status changes can progress to paranoia despite discontinuation of aldesleukin; it is imperative that the aldesleukin be stopped at any sign of persistent confusion or disorientation).
- 5.3.5 Sustained ventricular tachycardia or any sign or symptom of myocardial ischemia or myocarditis. Patients experiencing sustained ventricular tachycardia or myocardial ischemia should not receive further treatment with aldesleukin
- 5.3.6 Metabolic acidosis with HCO<sub>3</sub><sup>-</sup> <18, despite attempts to correct with IV HCO<sub>3</sub>.
- 5.3.7 Atrial fibrillation or myocarditis.
- 5.3.8 Documented systemic infection.
- 5.3.9 Any other serious toxicity that is not controlled at time of next dose.

## 5.4 Specific aldesleukin Toxicity Management

Several treatment-related toxicities have been uniquely associated with the administration of high-dose aldesleukin. The frequency of Grade 3 and Grade 4 toxicities in Cycle 1 ranges, for example, from 56.8% hypotension to 14.7% neurological, and 13.7% hematological, neurological, pulmonary, renal/electrolytes. Antihypertensive medication should be stopped prior to aldesleukin therapy.

Recommendations for management of the more significant toxicities typically seen with high-dose bolus aldesleukin are as follows (the following are only optional guidelines for toxicity management):

### 5.4.1 Fluid Replacement.

Excessive fluid replacement will increase the patient's likelihood of developing pulmonary edema. It is suggested that when intravenous access has been established; begin administration of normal saline at 75cc/hour IV. Fluid replacement should be given to allow patients to gain approximately 1kg/day. Once patients have gained greater than 5% of baseline weight, additional fluid boluses should not be given to maintain blood pressure. Furosemide should not be given unless symptomatic fluid retention develops and blood pressure is adequate.

### 5.4.2 Hypotension

Administration of high dose aldesleukin leads to decreased peripheral vascular resistance and consequent hypotension. In order to manage this toxicity, the physician may utilize the following guidelines:

1. Monitor patients in a setting capable of providing ICU level care.
2. Prior to starting aldesleukin therapy determine a minimum tolerated blood pressure (MTBP). For patients under age 40 and with no prior history of ischemic or valvular heart disease, the MTBP can be a systolic blood pressure (SBP) of 80 mmHg, while the MTBP for all other patients should be a SBP of 85-90 mmHg based on perceived risk of cardiac toxicity.
3. When a patient's systolic BP falls below the MTBP, suggested therapy may involve (in the following order):
  - a. Begin fluid boluses (250cc normal saline IV over 15 min. may repeat x 2) until SBP is > MTBP.
  - b. Should fluid boluses fail make the SBP > MTBP, phenylephrine should also be instituted (0.1-2 mcg/kg/min) to maintain SBP.
  - c. If blood pressure cannot be maintained on phenylephrine alone, or if unacceptable tachycardia develops, treatment with dopamine (1-6 mcg/kg/min) should be added to sustain blood pressure.

#### 5.4.3 Management of Arrhythmias/Myocarditis.

If significant arrhythmias occur at any stage in the patient's treatment (whether on pressor agents or not), the possibility of myocardial ischemia/infarction must be excluded by both EKG and cardiac enzyme assessment. Patients who develop atrial fibrillation should have aldesleukin doses held. Therapy may resume when the patient converts to normal sinus rhythm and is hemodynamically stable. If a significant supraventricular arrhythmia occurs while a patient is on dopamine therapy; phenylephrine should also be substituted for dopamine as initial blood pressure support. Patients experiencing sustained ventricular tachycardia or documented myocardial ischemic episodes during therapy should not receive further treatment with aldesleukin. Patients with myocarditis may resume treatment in subsequent courses if CPK returns to normal. A cardiac MUGA stress test documenting normal cardiac function should be performed prior to restarting therapy.

#### 5.4.4 Management of Neurotoxicity

Doses are held rather than reduced for neurotoxicity. If Grade 4 neuro-cortical toxicity is encountered and is not reversible within 48 hours, no further treatment should be given and the patient should be removed from treatment. If Grade 4 toxicity is reversible to Grade 1 within 48 hours, future treatment may be considered (in subsequent courses) if the patients shows any evidence of tumor regression.

#### 5.4.5 Metabolic Acidosis

In the course of aldesleukin therapy, when a patient's  $\text{HCO}_3$  falls to below 20,  $\text{NaHCO}_3$  should be added to the maintenance IV infusion. Should the  $\text{HCO}_3$  level fall below 18, aldesleukin therapy should be held, and bolus infusions of  $\text{NaHCO}_3$  should be instituted. Aldesleukin therapy may resume if repeat  $\text{HCO}_3 > 18$ .

#### 5.4.6 Other Toxicities

Increases in the serum creatinine to 2.0-3.5 mg/dl and total bilirubin to 3.0-10.0 mg/dl are common and reversible upon cessation of treatment. Doses of aldesleukin have not generally been withheld for renal and hepatic dysfunction alone. Although toxicity may become severe, recovery usually occurs following cessation of aldesleukin and vigorous supportive care is warranted.

### 5.5 Management of Grade 4 Toxicity

Patients with Grade 4 (Life Threatening) toxicity may be treated with dexamethasone 4 mg qid until side effects improve to an acceptable level. In the clinical trials with aldesleukin, dexamethasone has been used to treat patients with pulmonary edema requiring assisted ventilation, although many patients may be managed successfully without steroids.

## 5.6 Toxicity Criteria for Discontinuing Treatment

Patients will not be considered for further therapy if the following toxicities are encountered:

- 5.6.1 Pulmonary toxicity requiring endotracheal intubation
- 5.6.2 Renal dysfunction requiring dialysis
- 5.6.3 Grade 4 cardiac dysrhythmia or Grade 2 or 3 dysrhythmia not easily controlled with medical management
- 5.6.4 Myocardial ischemia (Grade 3 or 4) or infarction or symptomatic myocarditis (note: asymptomatic CPK or CPK-MB band elevations without EKG changes are not a contraindication to further treatment).
- 5.6.5 Coma
- 5.6.6 Life-threatening sepsis
- 5.6.7 Pericardial tamponade
- 5.6.8 Bowel ischemia or perforation
- 5.6.9 Grade 4 hypertension or hemorrhage, symptomatic grade 4 venous thromboembolic event, nephritic syndrome
- 5.6.10 Any grade arterial thromboembolic event
- 5.6.11 Inability of subject to comply with study requirements
- 5.6.12 Any other severe or life-threatening toxicity which, in the opinion of the investigator, would preclude further treatment with these agents, or has not resolved to baseline 8 weeks after treatment.

## 6. PHARMACEUTICAL INFORMATION

### 6.1 Entinostat (NSC 706995)

**Chemical name:** 3-Pyridylmethyl N-{4-[(2-aminophenyl)carbamoyl]benzyl} carbamate

**Other names:** MS-27-275, MS-275, SNDX-275

**Classification:** Histone deacetylase inhibitor (HDACi)

**Molecular formula:**  $C_{21}H_{20}N_4O_3$

**M.W.:** 376.41

**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. Entinostat inhibits histone deacetylases, changes chromatin configuration, and induces differentiation and apoptosis of cancer cells through an epigenetic mechanism.

**How Supplied:** Entinostat is supplied by the Syndax Pharmaceuticals, Inc. and distributed by DCTD, NCI as 1 mg (pink to light red, in bottles of 40), or 5 mg (yellow, in bottles of 40) film-coated tablets (round-biconvex). Each tablet also contains mannitol, sodium starch glycolate, hydroxypropyl cellulose, potassium bicarbonate, and magnesium stearate. The film coating consists of hypromellose, talc, titanium dioxide, and ferric oxide pigments (red and yellow) as colorants.

Matching placebo for entinostat has the same appearance as the corresponding active tablets and contains the same inactive ingredients and film coating.

**Storage:** Store the bottles at controlled room temperature (15-25°C), and protect from light. Entinostat is not to be exposed to extremes of temperature (greater than 30°C or less than 5°C).

**Stability:** Shelf life stability studies of the intact bottles are on-going.

**Route of Administration:** Oral, on an empty stomach, at least 1 hour before or 2 hours after a meal. Entinostat tablets should not be split, crushed, or chewed.

#### 6.1.1 Availability

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

Entinostat is provided to the NCI under a Collaborative Agreement between Syndax Pharmaceuticals, Inc. and the DCTD, NCI.

#### 6.1.2 Agent Ordering

NCI-supplied agents may be requested by the Principal Investigator (or their authorized designees) at each participating institution. Pharmaceutical Management Branch (PMB) policy requires that the agent be shipped directly to the institution where the patient is to



be treated. PMB does not permit the transfer of agents between institutions (unless prior approval from PMB is obtained). The CTEP-assigned protocol number must be used for ordering all CTEP-supplied investigational agents. The responsible investigator at each participating institution must be registered with CTEP, DCTD through an annual submission of FDA Form 1572 (Statement of Investigator), Curriculum Vitae, Supplemental Investigator Data Form (IDF), and Financial Disclosure Form (FDF). If there are several participating investigators at one institution, CTEP-supplied investigational agents for the study should be ordered under the name of one lead investigator at that institution.

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

#### 6.1.3 Agent Inventory Records

The Investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of all agents received from DCTD using the NCI Oral Drug Accountability Record Form (Oral DARF). (See the NCI Investigator’s Handbook for Procedures for Drug Accountability and Storage.)

#### 6.1.4 Comprehensive Adverse Events and Potential Risks list (CAEPR) for Entinostat (SNDX-275, MS-275, NSC 706995)

The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with **bold** and *italicized* text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI via CTEP-AERS (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' [http://ctep.cancer.gov/protocolDevelopment/electronic\\_applications/docs/aeguidelines.pdf](http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf) for further clarification. *Frequency is provided based on 215 patients.* Below is the CAEPR for entinostat (SNDX-275, MS-275).

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 2.5, September 10, 2018<sup>1</sup>

Adverse Events with Possible Relationship to MS-275 (SNDX-275, entinostat) (CTCAE 5.0 Term) [n= 221]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	Less Likely (<=20%)	Rare but Serious (<3%)	
<b>BLOOD AND LYMPHATIC SYSTEM DISORDERS</b>			
Anemia			<i>Anemia (Gr 3)</i>
<b>GASTROINTESTINAL DISORDERS</b>			
	Abdominal pain		<i>Abdominal pain (Gr 2)</i>
	Constipation		<i>Constipation (Gr 2)</i>
	Diarrhea		<i>Diarrhea (Gr 3)</i>
	Dyspepsia		<i>Dyspepsia (Gr 2)</i>
Nausea			<i>Nausea (Gr 3)</i>
Vomiting			<i>Vomiting (Gr 3)</i>
<b>GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS</b>			
	Edema limbs		<i>Edema limbs (Gr 2)</i>
Fatigue			<i>Fatigue (Gr 3)</i>
	Fever		<i>Fever (Gr 2)</i>
<b>INFECTIONS AND INFESTATIONS</b>			
	Infection <sup>2</sup>		<i>Infection<sup>2</sup> (Gr 3)</i>
<b>INVESTIGATIONS</b>			
	Alkaline phosphatase increased		<i>Alkaline phosphatase increased (Gr 2)</i>
	Lymphocyte count decreased		<i>Lymphocyte count decreased (Gr 4)</i>
Neutrophil count decreased			<i>Neutrophil count decreased (Gr 4)</i>
Platelet count decreased			<i>Platelet count decreased (Gr 4)</i>
	White blood cell decreased		<i>White blood cell decreased (Gr 3)</i>
<b>METABOLISM AND NUTRITION DISORDERS</b>			
Anorexia			<i>Anorexia (Gr 3)</i>
	Dehydration		<i>Dehydration (Gr 2)</i>

Adverse Events with Possible Relationship to MS-275 (SNDX-275, entinostat) (CTCAE 5.0 Term) [n= 221]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	Less Likely (<=20%)	Rare but Serious (<3%)	
	Hyperglycemia		<i>Hyperglycemia (Gr 2)</i>
Hypoalbuminemia			<i>Hypoalbuminemia (Gr 2)</i>
	Hypocalcemia		<i>Hypocalcemia (Gr 2)</i>
	Hypokalemia		<i>Hypokalemia (Gr 2)</i>
	Hyponatremia		<i>Hyponatremia (Gr 3)</i>
Hypophosphatemia			<i>Hypophosphatemia (Gr 3)</i>
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Myalgia		<i>Myalgia (Gr 2)</i>
NERVOUS SYSTEM DISORDERS			
	Dysgeusia		<i>Dysgeusia (Gr 2)</i>
Headache			<i>Headache (Gr 2)</i>
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Cough		<i>Cough (Gr 2)</i>
	Dyspnea		<i>Dyspnea (Gr 3)</i>
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
		Erythema multiforme	

<sup>1</sup>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](mailto:PIO@CTEP.NCI.NIH.GOV). Your name, the name of the investigator, the protocol and the agent should be included in the e-mail.

<sup>2</sup>Infection includes all 75 sites of infection under the INFECTIONS AND INFESTATION SOC.

<sup>3</sup>Gastrointestinal hemorrhage includes Anal hemorrhage, Cecal hemorrhage, Colonic hemorrhage, Duodenal hemorrhage, Esophageal hemorrhage, Esophageal varices hemorrhage, Gastric hemorrhage, Hemorrhoidal hemorrhage, Ileal hemorrhage, Intra-abdominal hemorrhage, Jejunal hemorrhage, Lower gastrointestinal hemorrhage, Oral hemorrhage, Pancreatic hemorrhage, Rectal hemorrhage, Retroperitoneal hemorrhage, and Upper

**NCI Protocol #: 7870**

Protocol Version Date 5/29/19

page 26 of 78

gastrointestinal hemorrhage under the GASTROINTESTINAL DISORDERS SOC.

**Adverse events reported on MS-275 (SNDX-275, entinostat) trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that MS-275 (SNDX-275, entinostat) caused the adverse event:**

**BLOOD AND LYMPHATIC SYSTEM DISORDERS** - Febrile neutropenia; Hemolysis; Leukocytosis

**CARDIAC DISORDERS** - Atrial fibrillation; Atrioventricular block complete; Cardiac disorders - Other (transient right-side heart failure with worsening tricuspid regurgitation); Chest pain - cardiac; Conduction disorder; Heart failure; Left ventricular systolic dysfunction; Palpitations; Pericardial effusion; Pericarditis; Sinus tachycardia; Supraventricular tachycardia; Ventricular fibrillation

**EAR AND LABYRINTH DISORDERS** - Hearing impaired

**EYE DISORDERS** - Blurred vision

**GASTROINTESTINAL DISORDERS** - Anal mucositis; Colitis; Dysphagia; Enterocolitis; Esophageal pain; Esophagitis; Flatulence; Gastrointestinal disorders - Other (hyperdefecation); Gastrointestinal hemorrhage<sup>3</sup>; Hemorrhoids; Mucositis oral; Pancreatitis; Periodontal disease; Rectal mucositis; Rectal pain; Small intestinal mucositis; Typhlitis; Visceral arterial ischemia

**GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS** - Chills; Edema face; Generalized edema; Injection site reaction; Multi-organ failure; Non-cardiac chest pain; Pain

**IMMUNE SYSTEM DISORDERS** - Allergic reaction; Anaphylaxis; Autoimmune disorder

**INJURY, POISONING AND PROCEDURAL COMPLICATIONS** - Bruising

**INVESTIGATIONS** - Activated partial thromboplastin time prolonged; Alanine aminotransferase increased; Aspartate aminotransferase increased; Blood bilirubin increased; CPK increased; Creatinine increased; GGT increased; INR increased; Investigations - Other (coagulopathy); Investigations - Other (vitamin D deficiency); Lipase increased; Serum amylase increased; Weight loss

**METABOLISM AND NUTRITION DISORDERS** - Acidosis; Hypercalcemia; Hyperkalemia; Hypermagnesemia; Hyponatremia; Hypertriglyceridemia; Hyperuricemia; Hypoglycemia; Hypomagnesemia; Tumor lysis syndrome

**MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS** - Arthralgia; Back pain; Bone pain; Chest wall pain; Generalized muscle weakness; Muscle cramp; Musculoskeletal and connective tissue disorder - Other (thorax pain); Myositis; Pain in extremity

**NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS)** - Tumor pain

**NERVOUS SYSTEM DISORDERS** - Ataxia; Depressed level of consciousness; Dizziness; Dysphasia;

**NCI Protocol #: 7870**

Protocol Version Date 5/29/19

page 27 of 78

Intracranial hemorrhage; Neuralgia; Olfactory nerve disorder; Peripheral motor neuropathy; Peripheral sensory neuropathy; Seizure; Syncope; Tremor

**PSYCHIATRIC DISORDERS** - Anxiety; Confusion; Depression; Insomnia; Libido decreased

**RENAL AND URINARY DISORDERS** - Acute kidney injury; Proteinuria; Renal and urinary disorders - Other (bladder distension); Renal calculi; Renal hemorrhage; Urinary frequency; Urinary retention

**RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS** - Allergic rhinitis; Atelectasis; Epistaxis; Hypoxia; Laryngeal mucositis; Pharyngeal mucositis; Pleural effusion; Pleuritic pain; Pulmonary edema; Respiratory failure; Tracheal mucositis

**SKIN AND SUBCUTANEOUS TISSUE DISORDERS** - Alopecia; Hyperhidrosis; Nail loss; Photosensitivity; Pruritus; Purpura; Rash maculo-papular; Skin and subcutaneous tissue disorders - Other (hyperkeratotic lesions/squamous cell carcinoma); Urticaria

**SURGICAL AND MEDICAL PROCEDURES** - Surgical and medical procedures - Other (packed RBC transfusion)

**VASCULAR DISORDERS** - Flushing; Hypertension; Hypotension; Thromboembolic event

**Note:** MS-275 (SNDX-275, entinostat) 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.

### 6.1.5 Potential Drug Interactions

Metabolism: Data from *in vitro* metabolism experiments in human tissues demonstrated that entinostat is not metabolized by CYP enzymes (Acharya 2006), but UGT 1A4 did metabolize entinostat to its M2 glucuronide metabolite. No metabolites could be detected after incubation of entinostat in human liver microsomes (Acharya 2006). While inhibition of CYP enzymes 2B6 and 3A4 was seen, the data show that the degree of the inhibition makes it unlikely that any *in vivo* systemic interactions would occur. Intestinal CYP 3A4 may be inhibited by entinostat. However, entinostat did not inhibit any UGT enzymes tested. Entinostat was found to induce CYP 1A2, CYP 2C6, and CYP 2B8 as well as UGT 1A4. Finally, entinostat was found to be a substrate for P-gp and BCRP transporters, but did not inhibit either of these transport proteins.

### 6.1.6 Patient Care Implications

**Entinostat may cause fatigue or malaise; advise patient to exercise caution while driving a vehicle or operating machinery.**

Administration of entinostat is contraindicated in patients with a history of allergy to entinostat or other medications that have a benzamide structure (eg, tiapride, remoxipride, clobopride).

Careful monitoring of patients for signs of infection or reactivation of past infections is recommended, as reactivation of infection has been reported in patients treated with entinostat, in some cases without evidence of neutropenia. The clinical significance of this finding and the potential association with entinostat is unknown.

Entinostat must not be used during pregnancy or while breast-feeding. Women and men participating in entinostat clinical studies must agree to use acceptable contraceptive methods, as indicated in the clinical study protocol, during treatment and for 3 months thereafter.

## 6.2 Interleukin-2 (Aldesleukin)

**Description:** Aldesleukin is recombinant formulation of interleukin-2. Aldesleukin is a non-glycosylated biosynthetic interleukin-2 (also known as T-cell growth factor), which differs only slightly in amino acid sequence from the natural compound. Aldesleukin is commercially available.

**Mechanism of Action:** Aldesleukin's effects are essentially identical to those of endogenous interleukin-2. Aldesleukin interacts with the high-affinity interleukin 2 receptor expressed on cells of the immune system and stimulates a cytokine cascade involving various interferons, interleukins, and tumor necrosis factors. Aldesleukin along with other cytokines induce proliferation and differentiation of B and T-cells, monocytes, macrophages, and cytotoxic lymphocytes which include natural killer (NK) cells, cytotoxic T-cells, tumor-infiltrating lymphocytes (TIL), and lymphokine-activated killer (LAK) cells. Aldesleukin's antitumor activity is believed to result from activation of cytotoxic lymphocytes; however, the exact mechanism is unknown. Whether aldesleukin acts directly or through second messengers is also unclear, however, aldesleukin does elevate production of interleukin-1, tumor necrosis factors alpha and beta, interferon gamma, and interleukin-6.

**Pharmacokinetics:** Aldesleukin is administered parenterally. Following a short IV infusion, the drug is rapidly distributed to the extravascular and extracellular space as well as to the liver, spleen, kidneys, and lungs. Approximately 30% of an administered dose is distributed within the plasma. The pharmacokinetics of aldesleukin may be affected by sodium dodecyl sulfate, the solubilizing agent in the commercial formulation. In addition, subcutaneous administration with albumin produces slightly higher and more prolonged serum concentrations of aldesleukin. Following distribution, aldesleukin is cleared from the systemic circulation by the kidneys through both glomerular filtration and peritubular extraction. The drug is then metabolized to amino acids by renal cells lining the proximal convoluted tubules. Very little drug is excreted unchanged in the urine. Following a 5 minute IV infusion, the serum distribution and elimination half-life in cancer patients was 13 and 85 minutes, respectively.

**Toxicities:** Abdominal pain ,alopecia, anemia, angina, anorexia, anuria, arthralgia, ascites, atrial fibrillation, azotemia, back pain, chills, cholestasis, conjunctivitis, constipation, diarrhea,

**NCI Protocol #: 7870**

drowsiness, dysgeusia, dyspepsia, dyspnea, dysuria, edema, elevated hepatic enzymes, eosinophilia, erythema, exfoliative dermatitis, fatigue, fever, GI bleeding, GI perforation, glomerulonephritis, hallucinations, headache, hematuria, hepatomegaly, hyperbilirubinemia, hyperkalemia, hypoalbuminemia, hypocalcemia, hypokalemia, hypomagnesemia, hyponatremia, hypophosphatemia, hypotension, hypovolemia, infection, injection site reaction, interstitial nephritis, jaundice, leukocytosis, leukopenia, lymphopenia, malaise, metabolic acidosis, metabolic alkalosis, myalgia, myocardial infarction, nausea/vomiting, oliguria, pancreatitis, paranoia, petechiae, pleural effusion, premature atrial contractions (PACs), premature ventricular contractions (PVCs), proteinuria, pruritus, pulmonary edema, purpura, sinus bradycardia, sinus tachycardia, splenomegaly, stomatitis, supraventricular tachycardia (SVT), syncope, tachypnea, thrombocytopenia, thrombosis, urticaria, visual impairment, weakness, weight gain, weight loss, wheezing, xerosis.

## 7. CORRELATIVE/SPECIAL STUDIES

### 7.1 Pharmacodynamic Studies

#### **PBMCs:**

Relationships between entinostat and aldesleukin exposure and pharmacodynamic effects (e.g. histone acetylation in peripheral blood mononuclear cells or PBMCs and changes in T cell subset population) will be characterized.

Collect four 8 ml of peripheral blood in CPT tubes for mononuclear cell fraction. Mononuclear cell fractions will be obtained in CPT tubes (Beckon Dickinson) sodium citrate using manufacture's guidelines.

Collection time points will be:

- Prior to treatment on Day -14 and Day-7,
- Cycle 1 – pre-dose on Day 1 and at 8 hrs. and 24 hrs. after the dose of entinostat,
- Cycle 1 - pre-dose Day 15
- Cycle 2 and 3 - pre-dose Day 1
- Cycle 2 and 3 - pre-dose Day 15
- At the time of progression.

Samples will be processed, PBMCs isolated and histone acetylation status determined. Blood samples will be also analyzed for immune cell sub populations. CD4+, CD4+/Foxp3, CD8+ cells, NK cells will be quantitated by FACS analysis. Foxp3 gene expression will be evaluated by quantitative RTPCR. Specimens must be shipped at ambient temperature.

#### **Serum for microRNA profiling:**

Collect a serum sample in one 10 ml red top tube to perform microRNA profiling to identify predictive biomarkers for response or resistance to therapy and prognostic biomarkers for survival.

Collection time points will be:

**NCI Protocol #: 7870**

- Day-14 pre-dose
- Cycle 1 – pre-dose on Day 1
- Cycle 1- pre-dose day 15
- Cycle 2 and 3 - pre-dose Day 1
- At the time of progression.

Centrifuge the tube (within 30 min after blood draw) at 1000 g for 10 min at room temperature. Remove supernatant (serum) and aliquot ~ 1 ml into 3 microcentrifuge tubes. Shipped ambient same day of collection Monday through Thursday to Dr. Pili's lab for analysis. If collected on Friday, weekends, or holiday store refrigerated and shipped [ambient](#) the next business day.

### **Sample Shipment**

All specimens should be correctly labeled with patient initials, study-specific subject ID number, protocol number, time point, and date and time of collection. NOTE: In the event that baseline specimens are available but the patient is not successfully registered to the protocol, do not submit the patient's specimens. Specimens must be shipped for overnight delivery Monday through Thursday.

**Address shipments and any question regarding specimen processing to:**

**Notification of sample shipment should be sent by email to:**

Roberto Pili and Ashley Orillion at [rpili@iupui.edu](mailto:rpili@iupui.edu) and [aurillio@iu.edu](mailto:aurillio@iu.edu).

Attn: Ashley Orillion  
Laboratory of Dr. Roberto Pili  
Division of Hematology and Oncology,  
Department of Medicine,  
Indiana University School of Medicine  
Joseph E. Walther hall  
[Pharmacokinetic studies \(Phase I only\)](#)  
980 W. Walnut Street, R3 C550  
Indianapolis, IN 46202

## **7.2 Pharmacokinetic studies (Phase I only)**

Pharmacokinetic analysis will be performed by Michelle A. Rudek, PharmD, PhD. at the Analytical Pharmacology Core Laboratory at the SKCCC at Johns Hopkins. Patients in the phase I component of the study will have plasma samples collected to assess entinostat pharmacokinetics. Entinostat concentrations will be assessed pretreatment on Day -14 and at 2 and 6 hrs., Day -7, pretreatment on Day 1 and at 2 and 6 hrs. and Day 15 during the first cycle and at the time of progression. These pharmacokinetic parameters will be used to explore the relationship between entinostat exposure with pharmacodynamic (PD) endpoints such as toxicity and histone acetylation in peripheral blood mononuclear cells or PBMCs and changes in T cell subset populations.

**NCI Protocol #: 7870**

Protocol Version Date 5/29/19

page 31 of 78



**Blood sample preparation**

Blood will be collected for entinostat pharmacokinetics in sodium heparin vacutainer tubes. Each sample will require 5 mL of patient blood. Samples will be placed on ice immediately and centrifuged within 30 minutes of collection at 1,000 xg for 10 minutes in a refrigerated centrifuge. Plasma will be stored at -20°C until analysis. Entinostat concentrations will be determined using a validated LC/MS/MS method that will be developed by the Analytical Pharmacology Core Laboratory (Zhao M, Rudek MA, Mnasakanyan A, Hartke C, Pili R, Baker SD. A liquid chromatography/tandem mass spectrometry assay to quantitate entinostat in human plasma. *J Pharm Biomed Anal.* 2007 Jan 17;43(2):784-7.).

**Sample Shipment**

All specimens should be correctly labeled with patient initials, study-specific subject ID number, protocol number, and date and time of collection. NOTE: In the event that baseline specimens are available but the patient is not successfully registered to the protocol, do not submit the patient's specimens. Overnight shipments should occur on Monday through Wednesday except when the following day is a holiday. A fax or call should be placed to the Analytical Pharmacology Core Laboratory prior to shipment providing the shipment tracking information.

**Address shipments and any question regarding specimen processing to:**

Analytical Pharmacology Core Laboratory  
Attn: Entinostat/IL-2 Study Samples  
Bunting-Blaustein Cancer Research Building 1  
1650 Orleans Street  
Room 184  
Baltimore, MD 21231-1000  
Phone: 410-955-1129  
Phone with voicemail: 410-502-7192  
Fax: 410-502-0895

**7.3 Non invasive imaging studies**

Non invasive imaging studies assessing changes in tumor metabolism will be performed both at RPCI and JHU. This study will evaluate changes in tumor metabolism in all patients undergoing PET/CT utilizing <sup>18</sup>FDG. Patients will have PET/CT studies before starting entinostat administration and during week 5.

**7.4 Optional tumor Samples**

All Patients with accessible tumors will be asked to undergo an optional tumor biopsy within 21 days before starting treatment and on Day 30 of Cycle 1 +/- 2 days. This tumor biopsy is not required for patients to take part in this protocol.

### 7.4.1. Formalin-Fixed Paraffin-Embedded Tissue

#### Sample Collection Time Points

*Samples may be obtained from any time prior to drug dosing.*

Sample	Screening Visit
Original diagnostic material	X
Any subsequent biopsy material prior to dosing	X

#### Sample Collection Procedure

Per institutional procedures, obtain a whole formalin-fixed paraffin block for patient. Paraffin blocks should contain a tumor specimen measuring at least 0.5 cm x 0.5 cm x 100 microns. Paraffin blocks may be from original diagnosis or optional biopsy any time prior to first drug treatment.

#### Packaging of Paraffin Block

- Place the paraffin block in a specimen container.

**NOTE: If the entire paraffin block cannot be submitted, please submit 10 unstained slides to:**

Attn: Ashley Orillion  
 Laboratory of Dr. Roberto Pili  
 Division of Hematology and Oncology,  
 Department of Medicine,  
 Indiana University School of Medicine  
 Joseph E. Walther hall  
 980 W. Walnut Street, R3 C550  
 Indianapolis, IN 46202

#### Shipment of Paraffin-embedded Tissue

**Notification of sample shipment should be sent by email to**

**Roberto Pili and Ashley Orillion at [rpili@iupui.edu](mailto:rpili@iupui.edu) and [aorillio@iu.edu](mailto:aorillio@iu.edu).**

#### Analysis

These samples will be analyzed for microvessel density (anti-CD34 staining), HIF-1  $\alpha$  staining, acetylated Histone and CD4+/FOXP3+ T cells. All IHC studies will be done with established protocols in Dr. Roberto Pili's lab.

### 7.4.2 Optional fresh tumor samples

#### Sample Collection

- Optional tumor biopsy samples will be collected from patients with accessible tumor per institutional procedure.

- The specific biopsy samples and time points for collection of fresh tumor samples are given below:

<b>Time Points</b>	<b>Number of Tumor Biopsy Samples per Time Point</b>
<i>Pretreatment</i>	1
<i>On treatment</i> *	1

\* The on-treatment samples will be collected 2 hours following the most recent dose of entinostat on Day 30 Cycle 1 +/- 2 days. To ensure that these samples are obtained at the correct time, patients are urged to take their entinostat dose in the clinic on the day of on-treatment sample collection. The time of the entinostat dose and sample collection must be recorded.

Attn: Ashley Orillion

Laboratory of Dr. Roberto Pili  
Division of Hematology and Oncology,  
Department of Medicine,  
Indiana University School of Medicine  
Joseph E. Walther hall  
980 W. Walnut Street, R3 C550  
Indianapolis, IN 46202

Notification of sample shipment should be sent by email to  
Roberto Pili and Ashley Orillion at [rpili@iupui.edu](mailto:rpili@iupui.edu) and [aorillio@iu.edu](mailto:aorillio@iu.edu).

These samples will be also analyzed for microvessel density (anti-CD34 staining), HIF-1  $\alpha$  staining, acetylated histone, CD4+, CD4+/FOXP3+, and CD8+ T cells. All IHC studies will be done with established protocols in Dr. Roberto Pili's lab.

**8. STUDY CALENDAR**

**Pre-study evaluations are to be performed within 4 weeks prior to start of therapy.**

Treatments and Evaluations	Pre-Study	Cycle 1 and subsequent cycles																				Every 4 weeks between cycles <sup>8</sup>	End of Treatment <sup>9</sup>	Follow-Up <sup>9a</sup>			
		Week -2						Week 1							Week 3												
		D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D						
		-14	-13	-12	-11	-10	-9	-7	1	2	3	4	5	6	7	15	16	17	18	19	20				21		
Entinostat dosing		√							√								√										
IL-2 dosing									√	√	√	√	√			√ <sup>10</sup>	√	√	√	√							
Informed Consent	√																										
History and Physical	√								√							√								√ <sup>8a</sup>	√		
Vital Signs and Weight	√	√ <sup>11</sup>					√	√	√							√								√ <sup>8a</sup>	√		
ECOG Performance Status	√	√ <sup>11</sup>						√	√							√								√ <sup>8a</sup>	√		
CBC w/diff., plts	√	√ <sup>11</sup>						√	√							√								√	√		
Urine analysis, (CMP) <sup>1</sup>	√							√	√							√								√	√		
PT/PTT/INR	√							√	√							√								√	√		
Serum Pregnancy test <sup>2</sup>	√																										
Head MRI or CT	√																										
EKG,MUGA, cardiac stress test <sup>3</sup>	√																										
PFT	√																										
Tumor Measurements <sup>4</sup>	√																							√ <sup>4</sup>	√		
Pharmacodynamic Samples <sup>5 &amp; 5a</sup>		√ <sup>11</sup>					√	√	√							√									√		
Serum for microRNA <sup>12</sup>		√						√	√							√											
Tumor Needle Biospy <sup>6</sup>	√																										
PET/CT <sup>7</sup>	√																										
Concomitant Meds	√	√ <sup>11</sup>																						√	√		
Survival follow-up																										√	

1. Comprehensive metabolic panel (CMP) to include measurement of sodium potassium, chloride, bicarbonate/CO<sup>2</sup>, BUN, creatinine, glucose, total bilirubin, corrected calcium (CC= Serum calcium+0.8(4-Serum albumin)), total proteins, albumin, AST, ALT, alkaline phosphatase, LDH, magnesium, and phosphate.
2. Pregnancy test required within 14 days of starting study drug for women of child bearing potential only.
3. Patients who are over 40 or have had previous myocardial infarction greater than 6 months prior to entry will be required to have a negative or low probability cardiac stress test for cardiac ischemia, as well as EKG and MUGA. Subsequent cardiac tests may be performed at the discretion of the Principal Investigator as clinically indicated.
4. Include CT scan, bone scan, MRI or ultrasound (additional tests may be performed as clinically indicated) Perform on week 11 of each cycle (+/- 7days) and every 8-12 weeks (+/- 2 weeks) on entinostat. Bone scan is required at baseline for patients with bone pain or evidence of bone lesions on CT scan.
5. Obtain pre-dose on day -14; day -7; pre-dose day 1 and at 8 hrs and 24 hrs post dose of entinostat; and pre-dose day 15 during the first cycle and at the time of progression.
  - 5a. Obtain PD sample on cycle 2 pre-dose day 1 and day 15 pre-dose; Repeat for cycle 3 pre-dose day 1 and day 15 pre-dose.
6. Optional Core biopsy and fine needle aspirate obtained within 21 days prior to start of cycle 1 and on day 30 of cycle 1 +/- 2 days.
7. To be performed prior to cycle 1 and on week 5 to assess changes in tumor blood metabolism.
- 8.** Maintenance Period, Local labs results acceptable.
  - 8a. May be performed every 8 – 12 weeks between cycles.
9. Performed 30 days (+/- 4 days) following last dose of study drug.
  - 9a. every three months afterward assessment may be obtained via telephone.
10. Beginning of course 2 (day 15) may be delayed up to 7 days.
11. Must be performed within 3 days prior to day -14.
12. Obtain Serum for microRNA for cycles 2 and 3 on day 1 pre-dose and at time of progression

## 9. MEASUREMENT OF EFFECT

Patients will undergo CT scans at week 11 (+/- 7 days) of each cycle during aldesleukin administration and then every 8-12 weeks (+/- 2 weeks).

### **Response Evaluation Criteria in Solid Tumors (RECIST V.1.0) Quick Reference**

Only patients with measurable disease at baseline should be included in protocols where objective tumor response is the primary endpoint.

**Measurable disease** - the presence of at least one measurable lesion. If the measurable disease is restricted to a solitary lesion, its neoplastic nature should be confirmed by cytology/histology.

**Measurable lesions** - lesions that can be accurately measured in at least one dimension with longest diameter  $\geq 20$  mm using conventional techniques or  $\geq 10$  mm with spiral CT scan.

**Non-measurable lesions** - all other lesions, including small lesions (longest diameter  $< 20$  mm with conventional techniques or  $< 10$  mm with spiral CT scan), i.e., bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusion, inflammatory breast disease, lymphangitis cutis/pulmonis, cystic lesions, and also abdominal masses that are not confirmed and followed by imaging techniques; and.

- 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.
- Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes). For the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

### 9.1 Methods of Measurement

- CT and MRI are the best currently available and reproducible methods to measure target lesions selected for response assessment. Conventional CT and MRI should be performed with cuts of 10 mm or less in slice thickness contiguously. Spiral CT should be performed using a 5 mm contiguous reconstruction algorithm. This applies to tumors of the chest, abdomen and pelvis. Head and neck tumors and those of extremities usually require specific protocols.
- Lesions on chest X-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.
- When the primary endpoint of the study is objective response evaluation, ultrasound (US) should not be used to measure tumor lesions. It is, however, a possible alternative to clinical

measurements of superficial palpable lymph nodes, subcutaneous lesions and thyroid nodules. US might also be useful to confirm the complete disappearance of superficial lesions usually assessed by clinical examination.

- The utilization of endoscopy and laparoscopy for objective tumor evaluation has not yet been fully and widely validated. Their uses in this specific context require sophisticated equipment and a high level of expertise that may only be available in some centers. Therefore, the utilization of such techniques for objective tumor response should be restricted to validation purposes in specialized centers. However, such techniques can be useful in confirming complete pathological response when biopsies are obtained.
- 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 when all lesions have disappeared.
- Cytology and histology can be used to differentiate between PR and CR in rare cases (e.g., after treatment to differentiate between residual benign lesions and residual malignant lesions in tumor types such as germ cell tumors).

### **Baseline documentation of “Target” and “Non-Target” lesions**

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

## **9.2 Response Criteria**

### **9.2.1 Evaluation of target lesions**

- \* Complete Response (CR): Disappearance of all target lesions
- \* Partial Response (PR): At least a 30% decrease in the sum of the LD of target lesions, taking as reference the baseline sum LD

- \* Progressive Disease (PD): At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions
- \* Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started

### 9.2.2 Evaluation of non-target lesions

- \* Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level
- \* Incomplete Response/ Stable Disease (SD): Persistence of one or more non-target lesion(s) or/and 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 (1)

(1) Although a clear progression of “non-target” lesions only is exceptional, in such circumstances, the opinion of the treating physician should prevail and the progression status should be confirmed later on by the review panel (or study chair).

### 9.2.3 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 PD the smallest measurements recorded since the treatment started). In general, the patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

Target lesions	Non-Target lesions	New Lesions	Overall response
CR	CR	No	CR
CR	Incomplete response/SD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD



PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

- Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be classified as having “symptomatic deterioration”. Every effort should be made to document the objective progression even after discontinuation of treatment.
- In some circumstances it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends on this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) to confirm the complete response status.

#### 9.2.4 Confirmation

- The main goal of confirmation of objective response is to avoid overestimating the response rate observed. In cases where confirmation of response is not feasible, it should be made clear when reporting the outcome of such studies that the responses are not confirmed.
- To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat assessments that should be performed no less than 4 weeks after the criteria for response are first met. Longer intervals as determined by the study protocol may also be appropriate.
- In the case of SD, follow-up measurements must have met the SD criteria at least once after study entry at a minimum interval (in general, not less than 6-8 weeks) that is defined in the study protocol

#### 9.2.5 Duration of overall response

- The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever status is recorded first) until the first date that recurrence or PD is objectively documented, taking as reference for PD the smallest measurements recorded since the treatment started.

#### 9.2.6 Duration of stable disease

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

Therefore, it is highly recommended that the protocol specify the minimal time interval required between two measurements for determination of SD. This time interval should take into account the expected clinical benefit that such a status may bring to the population under study.

### **9.2.7 Reporting of results**

- 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 8) unknown (not assessable, insufficient data).
- All of the patients who met the eligibility criteria should be included in the main analysis of the response rate. Patients in response categories 4-8 should be considered as failing to respond to treatment (disease progression). Thus, an incorrect treatment schedule or drug administration does not result in exclusion from the analysis of the response rate. Precise definitions for categories 4-8 will be protocol specific.
- All conclusions should be based on all eligible patients.
- Sub-analyses may then be performed on the basis of a subset of patients, excluding those for whom major protocol deviations have been identified (e.g., early death due to other reasons, early discontinuation of treatment, major protocol violations, etc.). However, these sub-analyses may not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding patients from the analysis should be clearly reported.
- The 95% confidence intervals should be provided

## **10 REGULATORY AND REPORTING REQUIREMENTS**

### **10.1 Protocol Amendments**

Any changes to the protocol will be made in the form of an amendment and must be approved by CTEP and the IRB before implementation.

### **10.2 Informed Consent**

An investigator will explain to each subject (or legally authorized representative) the nature of the study, its purpose, procedures involved, expected duration, potential risks and benefits. Each subject will be informed that participation in the study is voluntary and that he may withdraw from the study at any time, and that withdrawal of consent will not affect her subsequent medical treatment. This informed consent will be given by means of a standard written statement and will be submitted for IRB approval prior to use. No patient will enter the study before her informed

consent has been obtained.

### 10.3 Adverse Events

Expedited adverse event (AE) reporting for this study is via CTEP-AERS (CTEP Adverse Event Reporting System), accessed via the secure CTEP website: (<https://eapps-ctep.nci.nih.gov/ctepaers/pages/task?rand>). The reporting procedures are presented in the “CTEP, NCI Guidelines: Adverse Event Reporting Requirements” and can be downloaded from: (<http://ctep.cancer.gov/protocolDevelopment/electronicapplications/docs/aeguidelines.pdf>).

The descriptions and grading scales found in the CTEP Active Version of the NCI Common Terminology Criteria for Adverse Events (CTCAE) will be utilized for adverse event reporting. The CTEP Active Version of the CTCAE is identified and located on the CTEP website at: [http://ctep.cancer.gov/protocolDevelopment/electronic\\_applications/ctc.htm](http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm). All appropriate treatment areas should have access to a copy of the CTEP Active Version of CTCAE. A list of adverse events that have occurred or might occur (Comprehensive Adverse Events and Potential Risks list) can be found in Section 6 (Pharmaceutical Information). A copy of the current Agent-Specific Adverse Event List (ASAEL) is sent with LOI approval letters. The ASAEL is updated periodically; copies of the updated list are available on request via e-mail from [aemd@tech-res.com](mailto:aemd@tech-res.com).

Information about all adverse events, whether volunteered by the subject, discovered by investigator questioning, or detected through physical examination, laboratory test or other means, will be collected, recorded, and followed as appropriate.

All adverse events experienced by subjects will be collected from the time of consent, throughout the study, and until the final study visit. Subjects may be contacted for additional assessments, as necessary.

#### Definitions

Adverse Event (AE): Any undesirable sign, symptom or medical condition occurring after starting study drug (or therapy) even if the event is not considered to be related to the study.

Medical conditions/diseases present before starting study treatment are only considered adverse events if they worsen after starting study treatment (any procedures specified in the protocol). Adverse events occurring before starting study treatment but after signing the informed consent form will be recorded. Abnormal laboratory values or test results constitute adverse events only if they induce clinical signs or symptoms, require therapy or as required by SPEER.

Serious adverse event or reaction: Any untoward medical occurrence that at any dose: results in death, is life-threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability / incapacity, or is a congenital anomaly / birth defect.

The definition of serious adverse event (experience) also includes *important medical event*.

**NCI Protocol #: 7870**

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

Unexpected adverse event: An adverse event, which varies in nature, intensity or frequency from information on the investigational drug/agent provided in the Investigator's Brochure, package insert or safety reports. Any adverse event that is not included in the informed consent is considered "unexpected".

Expected (known) adverse event: An adverse event, which has been reported in the Investigator's Brochure. An adverse event is considered "expected", only if it is included in the informed consent document as a risk.

## **Relationship**

The relationship of all adverse events and serious adverse events to study medication will be assigned as follows:

Definitely: An adverse event which has a timely relationship to the administration of the investigational drug/agent, follows a known pattern of response, for which no alternative cause is present

Probably: An adverse event, which has a timely relationship to the administration of the investigational drug/agent, follows a known pattern of response, but for which a potential alternative cause may be present

Possibly: An adverse event, which has a timely relationship to the administration of the investigational drug/agent, follows no known pattern of response, but a potential alternative cause does not exist.

Unrelated: An adverse event, for which there is evidence that it is definitely related to a cause other than the investigational drug/agent. In general, there is no timely relationship to the administration of the investigational drug/agent, or if there is a timely relationship, the event does not follow a known pattern of response, and there is an alternative cause.

### **10.3.1 Reporting Procedures**

#### General

All adverse events will be captured on the appropriate study-specific case report forms (CRFs). All serious adverse events, regardless of causality to study drug, will be reported promptly to the Principal Investigator and/or the Study Coordinator. Participating network sites refer to appendix D for reporting instructions.

Institutional Review Board (IRB)

All adverse events/serious adverse events will be reported to the IRB per current institutional standards. If an adverse event requires modification of the study protocol and informed consent, these modifications will be provided to the IRB with the report of the adverse event.

External AgenciesExpedited Adverse Event Reporting – NCI/CTEP

Expedited reports are submitted to CTEP via the secure CTEP-AERS application accessed via the CTEP web site

(<https://eapps-ctep.nci.nih.gov/ctepaers/pages/task?rand>). Those AEs that do not require expedited reporting must be reported in routine (CDUS) study data submissions. AEs reported through CTEP-AERS must **also** be reported in routine study data submissions.

In the rare event when Internet connectivity is disrupted a 24-hour notification is to be made to NCI by telephone at: 301-897-7497 or 301-897-7402 for CIP studies or fax to 301-230-0159. An electronic report must be submitted immediately upon re-establishment of the internet connection.

Expedited Reporting Guidelines: Phase 1 Trials Utilizing an Agent under a CTEP IND: CTEP-AERS Reporting Requirements for Adverse Events That Occur Within 30 Days<sup>1</sup> of the Last Dose of the Investigational Agent.

	Grade 1	Grade 2	Grade 2	Grade 3		Grade 3		Grades 4 & 5 <sup>2</sup>
	Unexpected and Expected	Unexpected	Expected	Unexpected with Hospitalization	Unexpected without Hospitalization	Expected with Hospitalization	Expected without Hospitalization	Unexpected and Expected
<b>Unrelated Unlikely</b>	Not Required	Not Required	Not Required	10 Calendar Days	Not Required	10 Calendar Days	Not Required	24-Hour; 5 Calendar Days
<b>Possible Probable Definite</b>	Not Required	10 Calendar Days	Not Required	24-Hour; 5 Calendar Days	24-Hour; 5 Calendar Days	10 Calendar Days	Not Required	24-Hour; 5 Calendar Days
<p><sup>1</sup> Adverse events with attribution of possible, probable, or definite that occur <u>greater</u> than 30 days after the last dose of treatment with an agent under a CTEP IND require reporting as follows:</p> <p>CTEP-AERS 24-hour notification followed by complete report within 5 calendar days for:</p> <ul style="list-style-type: none"> <li>Grade 3 unexpected events with hospitalization or prolongation of hospitalization</li> <li>Grade 4 unexpected events</li> </ul>								

- Grade 5 expected events and unexpected events

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

December 15, 2004

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

- Expedited AE reporting timelines defined:
  - “24 hours; 5 calendar days” – The investigator must initially report the AE via CTEP-AERS within 24 hours of learning of the event followed by a complete CTEP-AERS report within 5 calendar days of the initial 24-hour report.
  - “10 calendar days” - A complete CTEP-AERS report on the AE must be submitted within 10 calendar days of the investigator learning of the event.
- Any medical event equivalent to CTCAE grade 3, 4, or 5 that precipitates hospitalization (or prolongation of existing hospitalization) must be reported regardless of attribution and designation as expected or unexpected with the exception of any events identified as protocol-specific expedited adverse event reporting exclusions.
- Any event that results in persistent or significant disabilities/incapacities, congenital anomalies, or birth defects must be reported via CTEP-AERS if the event occurs following treatment with an agent under a CTEP IND.
- Use the NCI protocol number and the protocol-specific patient ID provided during trial registration on all reports.

Phase 2 Trials Utilizing an Agent under a CTEP IND: CTEP-AERS Reporting Requirements for Adverse Events That Occur Within 30 Days<sup>1</sup> of the Last Dose of the Investigational Agent

	Grade 1	Grade 2	Grade 2	Grade 3		Grade 3		Grades 4 & 5 <sup>2</sup>	Grades 4 & 5 <sup>2</sup>
	Unexpected and Expected	Unexpected	Expected	Unexpected with Hospitalization	Without Hospitalization	Expected with Hospitalization	without Hospitalization	Unexpected	Expected
<b>Unrelated Unlikely</b>	Not Required	Not Required	Not Required	10 Calendar Days	Not Required	10 Calendar Days	Not Required	10 Calendar Days	10 Calendar Days
<b>Possible Probable Definite</b>	Not Required	10 Calendar Days	Not Required	10 Calendar Days	10 Calendar Days	10 Calendar Days	Not Required	24-Hour; 5 Calendar Days	10 Calendar Days

<sup>1</sup> Adverse events with attribution of possible, probable, or definite that occur greater than 30 days after the last dose of treatment with an agent under a CTEP IND require reporting as follows:

CTEP-AERS 24-hour notification followed by complete report within 5 calendar days for:

- Grade 4 and Grade 5 unexpected events

CTEP-AERS 10 calendar day report:

- Grade 3 unexpected events with hospitalization or prolongation of hospitalization
- Grade 5 expected events

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

December 15, 2004

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

- Expedited AE reporting timelines defined:
  - “24 hours; 5 calendar days” – The investigator must initially report the AE via CTEP-AERS within 24 hours of learning of the event followed by a complete CTEP-AERS report within 5 calendar days of the initial 24-hour report.
  - “10 calendar days” - A complete CTEP-AERS report on the AE must be submitted within 10 calendar days of the investigator learning of the event.
- Any medical event equivalent to CTCAE grade 3, 4, or 5 that precipitates hospitalization (or prolongation of existing hospitalization) must be reported regardless of

attribution and designation as expected or unexpected with the exception of any events identified as protocol-specific expedited adverse event reporting exclusions.

- Any event that results in persistent or significant disabilities/incapacities, congenital anomalies, or birth defects must be reported via CTEP-AERS if the event occurs following treatment with an agent under a CTEP IND.
- Use the NCI protocol number and the protocol-specific patient ID provided during trial registration on all reports.

Expedited Adverse Event Reporting Exclusions – Not Applicable

### 10.3.2 Unanticipated Problems

#### Definition

An Unanticipated Problem is any unexpected event that affects the rights, safety or welfare of subjects or others that result from the research. This may involve physical, social, psychological, legal or other risks.

An event is an Unanticipated Problem when it fulfills the definition above; it must be related or possibly related to the research and it must be Serious (see section 8.2). Unanticipated is defined as the nature, severity, or frequency of the event is not consistent with the current Investigator's Brochure, protocol or alternative risk information and is not due to the progression of a disease process.

An Unanticipated Problem (UAP) is any incident, experience, or outcome that meets all of the following criteria:

1. Unexpected (in terms of nature, severity, or frequency) given:
  - (a) the research procedures that are described in the study- related documents, including study deviations, as well as issues related to compromise of patient privacy or confidentiality of data;
  - (b) the characteristics of the subject population being studied
2. Related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research)
3. Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized and deemed **Serious**.

#### 10.3.2.1 Reporting Unanticipated Problems

The principal investigator or delegated research staff will submit an Unanticipated Problem Form to the CRS Compliance Office via Fax: 716-845-8815 or email:

**NCI Protocol #: 7870**



CRSCompliance for all unanticipated problems and adverse events that are serious and deemed related or possibly related to the research drug, biologic or intervention.

#### 10.3.2.2 Secondary AML/MDS

All secondary malignancies that occur following treatment with an agent under an NCI/IDE must be reported via CTEP-AERS. CTCAE v4.0 has three options available to describe treatment-related events:

- Leukemia secondary to on oncology chemotherapy.
- Myelodysplastic syndrome
- Treatment related secondary malignancy

If you are reporting in CTCAE v3.0, the event can be reported as: “Secondary malignancy-Other (specify)”.

### 10.4 Data and Safety Monitoring

Scheduled meetings will take place monthly and include the protocol principal investigator, study coordinators, research nurses, and, when appropriate, the collaborators, sub-investigators, and biostatistician involved with the conduct of the protocol. During these meetings the investigators will discuss matters related to: safety of protocol participants, validity and integrity of the data, enrollment rate relative to expectation, characteristics of participants, retention of participants, adherence to protocol (potential or real protocol violations), data completeness, and progress of data for secondary objectives such as surrogate markers. A review of all grade 3/4 toxicities will take place in each of the meetings as well as any delays in surgical procedure or in wound healing that may be contributed to study drug.

Quarterly reports will be sent to CTEP according to specified CTEP guidelines.

### 10.5 CTEP Multicenter Guidelines

#### 10.5.1 Responsibilities of the Principal Investigator.

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

- The Principal Investigator is responsible for the timely review of Adverse Events (AE) to assure safety of the patients.
- The Principal Investigator is responsible for the review of and timely submission of data for study analysis.

### **10.5.2 Responsibilities of the Coordinating Center**

- Each participating institution will have an appropriate assurance on file with the Office for Human Research Protection (OHRP), NIH. The Coordinating Center is responsible for assuring that each participating institution has an OHRP assurance and must maintain copies of IRB approvals from each participating site.
- Prior to the activation of the protocol at each participating institution, an OHRP form 310 (documentation of IRB approval) must be submitted to the CTEP PIO.
- The Coordinating Center assures that IRB approval has been obtained at the participating site prior to the first patient registration from that site.
- The Coordinating Center is responsible for the preparation of all submitted data for review by the Protocol Chair.
- The Coordinating Center maintains documentation of AE reports. The participating institution reports directly to CTEP with a copy to the Coordinating Center. The Coordinating Center will submit AE reports to the Protocol Chair for timely review.
- Audits may be accomplished in one of two ways: (1) source documents and research records for selected patients are brought from participating sites to the Coordinating Center for audit, or (2) selected patient records may be audited on-site at participating sites. If the NCI chooses to have an audit at the Coordinating Center, then the Coordinating Center is responsible for having all source documents, research records, all IRB approval documents, NCI Drug Accountability Record forms, patient registration lists, response assessments scans, x-rays, etc. available for the audit.

### **10.5.3 Patient Registration**

The coordinating Center serves as the central location for registering all patients enrolled in a multi-center trial. Refer to network appendix D for patient registering instructions.

### **10.6 Cooperative Research and Development Agreement (CRADA)/Clinical Trials Agreement (CTA)**

The agent(s), supplied by CTEP, DCTD, NCI, used in this protocol is/are provided to the NCI under a Collaborative Agreement (CRADA, CTA) between the Pharmaceutical Company(ies) [hereinafter referred to as "Collaborator(s)"] and the NCI Division of Cancer Treatment and Diagnosis. Therefore, the following obligations/guidelines, in addition to the provisions in the

**NCI Protocol #: 7870**

Intellectual Property Option to Collaborator@ contained within the terms of award, apply to the use of Agent(s) in this study:

1. Agent(s) may not be used for any purpose outside the scope of this protocol, nor can Agent(s) be transferred or licensed to any party not participating in the clinical study. Collaborator(s) data for Agent(s) are confidential and proprietary to Collaborator(s) and shall be maintained as such by the investigators. The protocol documents for studies utilizing investigational agents contain confidential information and should not be shared or distributed without the permission of the NCI. If a copy of this protocol is requested by a patient participating on the study or patient's family member, the individual should sign a confidentiality agreement. A suitable model agreement can be downloaded from <http://ctep.cancer.gov>.
2. For a clinical protocol where there is an investigational Agent used in combination with (an)other investigational Agent(s), each the subject of different collaborative agreements, the access to and use of data by each Collaborator shall be as follows (data pertaining to such combination use shall hereinafter be referred to as "Multi-Party Data".):
  - a. NCI must provide all Collaborators with prior written notice regarding the existence and nature of any agreements governing their collaboration with NIH, the design of the proposed combination protocol, and the existence of any obligations that would tend to restrict NCI's participation in the proposed combination protocol.
  - b. Each Collaborator shall agree to permit use of the Multi-Party Data from the clinical trial by any other Collaborator solely to the extent necessary to allow said other Collaborator to develop, obtain regulatory approval, or commercialize its own investigational agent.
  - c. Any Collaborator having the right to use the Multi-Party Data from these trials must agree in writing prior to the commencement of the trials that it will use the Multi-Party Data solely for development, regulatory approval, and commercialization of its own investigational agent.
3. Clinical Trial Data and Results and Raw Data developed under a Collaborative Agreement will be made available exclusively to Collaborator(s), the NCI, and the FDA, as appropriate and unless additional disclosure is required by law or court order. Additionally, all Clinical Data and Results and Raw Data will be collected, used and disclosed consistent with all applicable federal statutes and regulations for the protection of human subjects, including, if applicable, the *Standards for Privacy of Individually Identifiable Health Information* set forth in 45 C.F.R. Part 164.
4. When a Collaborator wishes to initiate a data request, the request should first be sent to the NCI, who will then notify the appropriate investigators (Group Chair for Cooperative Group studies, or PI for other studies) of Collaborator's wish to contact them.
5. Any data provided to Collaborator(s) for phase 3 studies must be in accordance with the guidelines and policies of the responsible Data Monitoring Committee (DMC), if there is a DMC for this clinical trial.

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

Regulatory Affairs Branch, CTEP, DCTD, NCI  
Executive Plaza North, Suite 7111  
Bethesda, Maryland 20892  
FAX 301-402-1584  
Email: anshers@mail.nih.gov

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

## **11. STATISTICAL CONSIDERATIONS**

### **11.1 Study Design-**

This is a phase I/II study of high dose interleukin 2 in combination with the histone deacetylase inhibitor entinostat in patients with metastatic renal cell carcinoma. The Phase I portion of the study will be used to evaluate the safety and tolerability of high dose interleukin 2 in combination with entinostat. High dose interleukin 2 (IL-2) is the standard of care for this patient population. It is hoped that a minimum effective dose of entinostat would enhance immune response with little additional toxicity. Because response rate may not fully reflect the additional immune response attributable to entinostat and because IL-2 is the standard of care, a single-stage design will be used in the Phase II portion of the study to estimate the efficacy of the drug combination. Secondary endpoints will be used to understand the effect of entinostat and IL-2 on progression free survival (PFS) and parameters measuring immune response. A sequential probability ratio test (SPRT) will be used to provide a statistical threshold for stopping the study in case there is evidence of a larger proportion of extended grade 4 toxicities than allowed.

### **11.2 Phase I**

Primary Objective:

High-dose interleukin (IL-2) is the current standard therapy for metastatic renal cell carcinoma and its toxicity in a phase III study has been reported<sup>5</sup>. The primary objective of this portion of the study is to determine a recommended dose of the biologic agent, entinostat, when combined

**NCI Protocol #: 7870**

with IL-2. The IL-2 dose level is 600,000 IU/kg, every 8 hours, IV. The current recommended dose of entinostat when administered as a single agent every second week continuously is 10 mg/m<sup>2</sup>. The entinostat study dose levels are 1, 3 and 5 mg, every 2 weeks, PO with 3 mg chosen as the starting dose for this study. A DLT will be defined as extended grade 4 toxicity (duration of one week or more) during the first 45 days of treatment with the recommended dose being the highest dose at which 0 or 1 DLTs are observed. A DLT rate of  $\leq 33\%$  will be permitted for further evaluation in the Phase II portion of the study.

The first dose level will have a minimum of 3 patients treated (unless the first 2 patients experience dose-limiting toxicity(s) (DLT) before 3<sup>rd</sup> patient is enrolled. If a DLT occurs in 1 patient treated at the starting dose level, another 3 patients will be treated at this dose level. If DLTs occur in 2 patients in the first 6 patients, patients will be treated at a lower dose level (entinostat 1 mg PO every 2 weeks, dose level 0) and DLT will be evaluated similarly. If DLT occurs in 1/6 patients, dose level 1 will be considered the recommended Phase II dose.

If no DLTs occur at the starting dose level 1, 3 or 4 patients will be treated at the next dose level (level 2) with 5 mg PO every 2 weeks (wait 45 days for DLT assessment on first 2 patients before 3<sup>rd</sup> patient is enrolled). If no DLTs occur at dose level 2, this dose level will be recommended for the phase II portion of the study. If a DLT occurs in 1 patient at dose level 2, another 3 or 4 patients will be treated at this dose level. If DLT occurs in 2/6 patients at dose level 2, dose level 1 will be the recommended for the Phase II. If a DLT occurs in 1/6 patients, dose level 2 will be considered the recommended Phase II dose.

Patients who experience prolonged grade 4 toxicity (more than one week) and recover to  $\leq$  Grade 1 (or to pretreatment baseline level toxicity) may continue treatment at the next entinostat lower level. Once the entinostat has been dose reduced, patients must remain at the reduced dose for the remainder of the study. Patients may have their dose de-escalated twice before being removed from the trial. Patients will be allowed to remain on the therapy provided that they are tolerating the treatment and do not develop progressive disease. No dose de-escalation for IL-2 will be performed.

#### Secondary Objective:

The secondary objective of this portion of the study will describe the toxicity and tolerability of the combination of IL-2 and entinostat. An exact binomial proportion with a 95% confidence interval will be given for prolonged grade 4 toxicity. The frequency and grade of toxicities will be tabulated for each dose level. Summary statistics (mean, standard deviation, frequency) will be used to record the number of doses of IL-2 administered during the first cycle of therapy and the toxicity after the scheduled 9<sup>th</sup> dose IL-2.

### 11.3 Phase II

#### Primary Objective:

Once a recommended dose has been determined in the Phase I portion of the study, the cohort will be expanded so that 36 new patients will be treated at this dose level. The primary objective of this phase is to evaluate the safety and efficacy of the combination of entinostat and IL-2. The primary endpoint is overall response rate (complete plus partial). Enrollment is expected to be

between 1 and 2 patients per month. The combination treatment will be considered unsuccessful if the response rate is 20% or less, and it will be considered active enough to pursue further if the response rate was 40% or greater. To test this hypothesis, the fixed sample size for a single-stage study with a type I error of 10% and a type II error of 10%, based on an exact binomial test, is 36. If 11 or more of the patients have a response, the hypothesis that the response rate is  $\leq 20\%$  is rejected with a target error rate of 0.10. This design has type I and type II errors of 0.09 and 0.09, respectively.

#### Secondary Objectives:

Secondary endpoints will be used to determine the effect of entinostat on progression free survival (PFS), survival, time-to tumor progression and parameters measuring immune response.

Failure rates will be used to compare the PFS, overall survival and time-to-tumor progression of metastatic RCC patients treated with high dose aldesleukin combined with entinostat to the historical failure rates of patients treated with high dose aldesleukin alone. The failure rate is estimated by dividing the number of events ( $d$ ) by the sum of the exposure time in the study

cohort:  $\lambda = d / \sum t_i$ . The 95% confidence interval is calculated as:  $\lambda \pm Z_{1-\alpha/2} \frac{\lambda}{\sqrt{d}}$ .

The historical PFS failure rate observed in the randomized trial of IL-2 reported by the Cytokine Working Group<sup>5</sup> was 2.77 per person year (median PFS of 3 months). Patients eligible for this study are expected to be homogenous group with comparable prognostic characteristics. Enrollment is expected to be between 1 and 2 patients per month with an accrual period of 6 years and 12 months of follow-up. With a total of 34 events in 36 patients, the study would yield 79% power to detect a decrease in the PFS failure rate to 1.85 per person year of follow-up (an increase of 1.5 months in the median PFS compared to the historical median) at an alpha level of 0.1 (one-sided).

We do not expect that different doses of entinostat during the period off aldesleukin (maintenance) will affect the primary endpoint since objective responses have been rarely observed with single agent entinostat. We expect that the vast majority of patients will be able to tolerate the 10 mg maintenance dose if the phase II recommended dose in combination with aldesleukin is 5 mg. Should we observe discrepancies in the maintenance dose level of entinostat we will consider subgroup analysis to determine the secondary objective PFS in the different group of patients.

The toxicity of IL-2 when combined with entinostat will be further characterized following the phase II portion of this study. The exact binomial proportion for prolonged grade 4 toxicities with the 95% confidence interval will be given. Additional toxicity frequencies, proportions, and 95% CIs will be given by type and grade of toxicity.

The association between responses (e.g. response, progression free and overall survival) and baseline laboratory parameters (e.g. CD4+, CD4+/Foxp3, CD8+, Ki 67, Tumor) will be summarized graphically (boxplots, scatterplots, Kaplan-Meier curves) and numerically (means, medians). For binary outcomes such as response, logistic regression will be used to assess the significance of associations individually (univariate) and while adjusting for other variables (multivariate). The Cox proportional hazards model will be used to model the effect of these

**NCI Protocol #: 7870**

parameters on time-to-event outcomes.

Logistic regression will be used to explore the relationship between entinostat exposure (plasma concentrations) with pharmacodynamic (PD) endpoints (e.g., toxicity and histone acetylation in peripheral blood mononuclear cells and change in T cell subset population).

We will determine if initial levels of specific T lymphocytes (Tregs) or changes in the level of specific T lymphocytes from baseline might predict for response to this combination therapy. In this study Tregs are defined as CD4+CD25<sup>hi</sup> T cells. The hypotheses are that low baseline levels of Treg would be associated with an increased probability of response and that decreasing Tregs from baseline would be associated with an increased probability of response. Continuous baseline values of Treg (expressed as a frequency) and the change in Treg from baseline (4 weeks post treatment – pretreatment) as well as binary versions of these variables will be explored for an association with response. A 30% decrease from baseline will be used as the binary cutpoint for Treg change.

Two PET-derived indices of glucose metabolism: Standard Uptake Value (SUV) and FDG influx rate constant (Ki) will be used to determine whether early changes in tumor metabolisms by FDG PET/CT scan anticipate anatomic changes in patients receiving the combination of entinostat and IL-2. These two parameters will be measured at baseline and at week 5 using identical PET/CT acquisition protocols. Continuous change from baseline and binary (30% decrease from baseline) measures of these predictors will also be explored for an association with response. The hypothesis for these predictors is that treatment would decrease glucose metabolism, increasing response.

Data analysis for Treg and PET correlative studies:

This study will provide estimates of means and standard deviations for baseline Treg, change in Treg at week four and changes in the PET glucose metabolism indices at week five. Transformations of these predictors will be considered before analyses. For each endpoint, we will determine how well the responders and non-responders are distinguished. For binary predictors, the sensitivity and specificity with 95% confidence intervals will be reported. T tests will be used to compare the mean change between responders and non-responders. If there are sufficient numbers of responders, partial responders and non-responders an ANOVA will be used to compare changes in these three groups. If complete data are obtained for CD4+CD25<sup>hi</sup> T cells at multiple time points post treatment, repeated measures ANOVA will be performed to evaluate data for trends over time.

#### 11.4 Toxicity monitoring

A sequential probability ratio test (SPRT) will be used to provide a monitoring boundary for stopping the study in case there is moderate evidence that the proportion of extended grade 4 toxicities is 20% as opposed to the permitted rate of 5%. This monitoring boundary will be used to evaluate the toxicity data after every patient. The SPRT is based on testing two simple hypotheses on the basis of the likelihood ratio. The likelihood function for a binary toxicity outcome is binomial and the log likelihood ratio will be used to determine the relative evidence in favor of two hypothesized prolonged grade 4 toxicity rates,  $p_1=0.2$  and  $p_2=0.05$ . The

likelihood ratio is:

$$\text{Likelihood ratio (For } p_1 \text{ vs } p_2 \mid \text{Data)} = \frac{\Pr(\text{data} \mid \theta = 0.2)}{\Pr(\text{data} \mid \theta = 0.05)} = \frac{\theta^x (1 - \theta)^{N-x}}{\theta^x (1 - \theta)^{N-x}}$$

where  $N=36$  and  $x$  is the number of toxicities observed. We will stop the study if there is moderate evidence,  $LR \geq 8.0$ , that the proportion of prolonged grade 4 toxicities is 20% versus 5%. The stopping boundary corresponding to this LR is given below. If the number of toxicities falls above the line any point along the study, the study will be stopped. Table 1 gives the observed number of prolonged toxicities for which this boundary would be crossed after each patient accrued ( $N$ ). The rate of erroneously stopping the study using this boundary is approximately  $1/8$  or  $0.125$ .

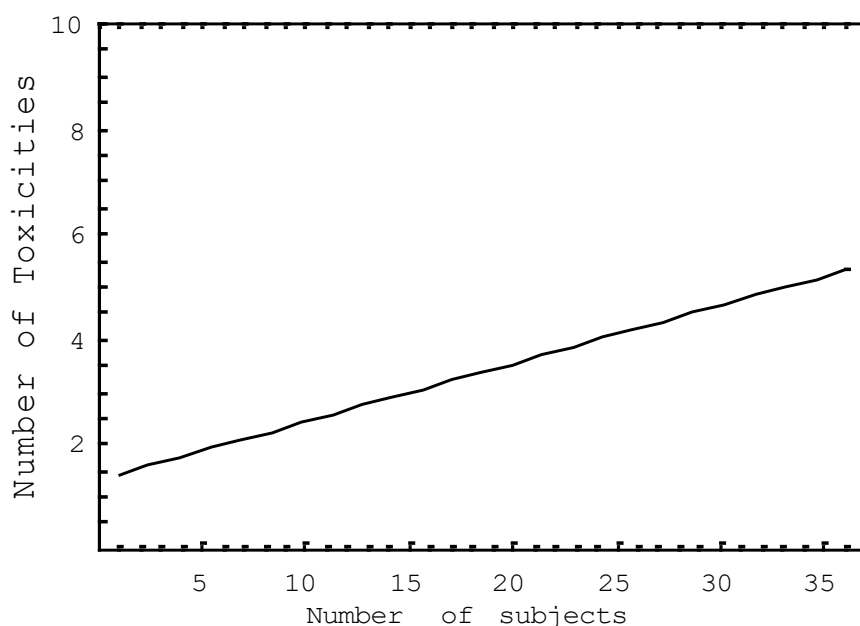


Table 1. Observed number of prolonged toxicities (Tox) where SPRT boundary would be crossed after each patient accrued ( $N$ ).

N	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Tox	2	2	2	2	2	2	3	3	3	3	3	3	3	3	3	4	4	4

N	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
Tox	4	4	4	4	4	4	5	5	5	5	5	5	5	5	5	6	6	6

### 11.5 Patient Compliance

All patients will receive study drug under direct observation by the site personnel. Dosing date

NCI Protocol #: 7870

Protocol Version Date 5/29/19

page 55 of 79



and time will be recorded by the site staff on the CRF. For all other doses, the patient will self-administer his/her dose of oral medications per schedule and record the date and time in the patient diary. If a patient regurgitates any tablets, the patient should not repeat that dose, but should resume his/her scheduled subsequent doses.

Pill counts will be utilized to monitor compliance as well as the patient diary. Patients will be supplied with a diary in which to record the date and time of each self-administered dose of study drugs. Patients will also be asked to record any partial doses. Study site staff will review the diary with the patient at each scheduled clinic visit and transcribe all pertinent information onto the CRF. The diaries will be retained at the study site as part of the source documentation and new diary pages issued to the patient following each visit. Patients will be asked to return all study medication bottles (empty, partial or full) to the study staff.

### **11.6 Data quality assurance**

This study will be monitored regularly. Monitoring procedures include one or more visits designed to clarify all prerequisites before the study commences. Interim monitoring visits will take place on a regular basis according to a schedule fixed by mutual agreement. During these visits, the CRA will check for completion of the entries on the CRFs, their compliance with the study protocol and with GCP, and will compare the CRF entries with the source data.

For source data verification, at least the following information must be included in the patient note/file:

- Date of patient's written informed consent,
- Patient participation in a clinical study and the study number,
- Patient visit dates,
- Serious adverse events,
- Adverse events,
- Confirmation of the diagnosis of the indication being treated,
- Study drug administration (e.g., start of study treatment, dose),
- Concomitant disease medications and dose (including parenteral nutrition and corticosteroid dose), and
- Primary endpoint.

The CRA will collect the appropriate copy or copies of the completed forms. He/she will also verify the correct use of the investigational product. Entinostat will not be supplied to the investigator site prior to approval from the IRB/IEC and regulatory authority (if applicable). At a final visit, the CRA will check all remaining material including the remaining quantities of the investigational product and will organize its return.

In addition, the CRA will determine whether all AEs and SAEs have been appropriately documented and whether the SAEs were reported within the time periods required.

A member of the Quality Assurance Unit may arrange to visit the investigator in order to audit the performance of the study at the study site and the study documents originating there. A sponsor designee will usually accompany the auditors. The investigator will be informed about the outcome of the audit.

## **11.7 Reporting and Exclusion**

### **11.7.1 Evaluation of toxicity**

All patients will be evaluable for toxicity from the time of their first dose of entinostat

### **11.7.2 Evaluation of response**

All conclusions should be based on all eligible patients who initiate entinostat treatment. Sub-analyses may then be performed on the basis of a subset of patients, excluding those for whom major protocol deviations have been identified (e.g., early death due to other reasons, early discontinuation of treatment, major protocol violations, etc.).

However, these sub-analyses may not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding patients from the analysis should be clearly reported.

Prolonged lymphocyte decrease is not considered a DLT since it is a known side effect of high dose interleukin 2 and it is not considered clinically relevant.

Enrolled patients who are deemed to be not eligible/not evaluable will be replaced.

### **11.7.3 Discontinuation and Withdrawal of Subjects**

**11.7.3.1** All patients who initiate protocol treatment will be included in the overall evaluation of response (intent-to-treat analysis). All reasons for discontinuation of treatment should be documented clearly in the medical record. Note: Patients who sign a consent form, but do not initiate study drug will be replaced.

**11.7.3.2** Non-compliance with the study protocol; including, but not limited to not attending the majority of scheduled visits. The PI will determine when non-compliance should lead to removal from study. Note: The patients will still be included in the overall evaluation of response (intent-to-treat analysis)

**11.7.3.3** Unacceptable major toxicity. Note: The patients will still be included in the overall evaluation of response (intent-to-treat analysis).

**11.7.3.4** At patient's own request. Note: The reason for discontinuation from the study must be documented. The patients will be included in the overall evaluation of response (intent-to-treat analysis) if any protocol therapy was administered prior to withdrawal.

## **12. ETHICS**

### **12.1 Institutional Review Board**

The study protocol and any amendment that is not solely of an administrative nature must be approved by an Independent Ethics Committee (IEC) or Institutional Review Board (IRB).

### **12.2 Evaluation of benefits and risks/discomforts**

Potential **benefits**: Patients will receive evaluation and treatment of their malignancy as a result of participating in this trial. The trial will provide information on how the combination of entinostat and aldesleukin should be administered to patients, but may or may not help a specific patient personally. This treatment may offer temporary control of the disease, but is not curative by this protocol. Because of the study design, patients may be treated with a drug dose that is too low to be effective. Therefore, neither benefit can be promised nor can the chance of benefit be predicted. Alternative approaches to entering this trial, including supportive care only, will also be discussed before the verbal and written consent is obtained regarding the risks, benefits, and the treatment requirements of this trial.

**Measures for minimizing risk**: Administering entinostat and aldesleukin to patients may involve risks that are currently unforeseeable. Side effects can be unpredictable in nature and severity, although all care will be taken to minimize them. If patients suffer any physical injury as a result of participating in this study, immediate medical treatment is available at the treatment center. Frequent blood work will be taken to monitor side effects. Although no compensation is available, any injury will be evaluated and treated in keeping with the benefits or care to which patients are entitled under applicable regulations. Malignancies with no further standard treatment options generally have a poor prognosis. Therefore, patients may experience significant treatment-related morbidity, and/or complications from progression of their disease.

### **12.3 Risks/benefits analysis**

Data gathered from both clinical and laboratory evaluations in this trial will be analyzed frequently to ensure safety of patients. Any new or significant finding(s) found during the course of the research will be shared and explained to each participant since that may affect a patient's willingness to participate further. Patient's anonymity will be protected to the maximum extent in all publications and presentations that result from this research.

### **12.4 Patient information and consent**

The investigator will explain the nature of the study, its purpose and associated procedures, the

expected duration, and the potential benefits and risks of participation to each patient prior to his/her entry into the study (i.e., before examinations and procedures associated with selection for the study are performed). Each patient will have ample opportunity to ask questions and will be informed about the right to withdraw from the study at any time without any disadvantage and without having to provide reasons for this decision. Following this informative discussion, a patient will be asked if he/she is willing to sign and personally date a statement of informed consent. Only if the patient voluntarily agrees to sign the informed consent statement and has done so, may he/she enter the study. The patient will receive a copy of the signed and dated informed consent form.

The signed informed consent statement is to remain in the investigator's files. The investigator will document on each CRF that he/she has informed the patient and that the patient has signed the informed consent statement.

The informed consent form and any other written information provided to patients will be revised whenever important new information becomes available that may be relevant to the patient's consent, or there is an amendment to the protocol which necessitates a change to the content of the written informed consent form. The investigator will inform the patient of changes in a timely manner and will ask the patient to confirm continuation of his/her participation in the study by his/her signature on the revised informed consent form. Any revised written informed consent form must receive the IRB's approval/favorable opinion in advance of use.

## **12.5 Data handling and record keeping**

### **12.5.1 Case report forms**

This study has been assigned to be monitored by the Clinical Trials Monitoring Service (CTMS). Theradex is the pharmaceutical company that administers the Clinical Trials Monitoring Service. CTMS Data is required to be submitted to CTMS at least every two weeks, via the NCI/DCTD case report forms or the electronic case report form (ACES). Details on the protocol specific requirements will be communicated to the principal investigator by Theradex. Adverse event data will also be collected in the electronic data entry system used by Roswell Park Clinical Research Services. Furthermore, the investigator will document in the patient files (hospital files). If corrections are necessary, they will be entered by an authorized member of the investigator's staff in the following manner: the wrong entry will be crossed out with a single line so that it remains legible, and the correct entry will be placed next to it. All corrections will be initialed and dated by the site staff making the correction. For corrections concerning adverse events or the primary variable, a reason for any alteration must be provided.

**NCI Protocol #: 7870**

Protocol Version Date 5/29/19

page 59 of 79

Any documents related to the study must be archived at the study site or in a central archive. This includes the careful listing of the identities of the patients involved in the study. This list and the signed informed consent statements are key documents in the files to be stored by the investigator.

Patient (hospital) files will be archived according to local regulations. All documents related to the study must be retained until at least 15 years after the end of the study. At the end of this period, CTEP will inform the investigator as to when these documents no longer need to be retained.

### **12.5.2 Laboratory data and reference laboratories**

For laboratory services provided by the investigator, the investigator must supply the sponsor with a complete list of all normal laboratory values for the laboratory utilized, as well as proof of laboratory certification by the Clinical Laboratory Improvement Amendments. Shipping information for the pharmacodynamic samples is contained in section 7.6.

### **12.5.3 Patient registry**

The investigator should maintain a patient registry of all patients entered into the study in the event a safety issue arises after study completion.

### **12.5.4 Drug accountability**

The designated personnel will confirm receipt of the drugs investigational product in writing and will use the investigational product only within the framework of this clinical study and in accordance with this study protocol. He/she will keep a record of the investigational product dispensed, preferably on the CRF. The investigational product must be stored in a locked storage facility and protected from unauthorized access. Any unused, partially used, or empty containers of the investigational product will be returned to the sponsor at the termination of the study at the latest. Receipt, distribution, and return of the investigational product must be properly documented on the forms provided by the sponsor giving the following information: Study number, sender, receiver, date, mode of transport, quantity, batch number, and expire or retest date, if applicable.

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## APPENDIX A: Protocol for quantitative analysis of tumor metabolism and tumor blood flow using $^{18}\text{F}$ FDG–PET/CT imaging.

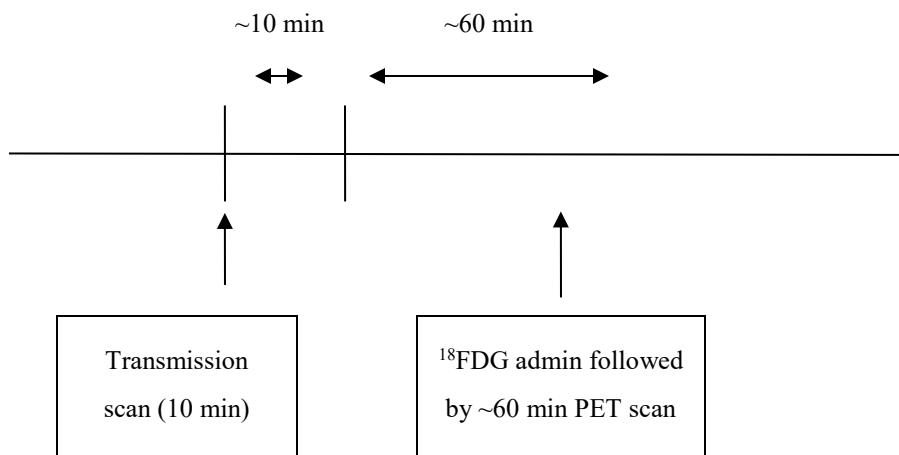
### 1. Synopsis

In order to assess the effect of entinostat and aldesleukin on tumor metabolism, measurements with PET/CT utilizing  $^{18}\text{F}$ FDG will be performed in a standardized fashion. Studies will be undertaken in eligible patients participating in the clinical study.

Patients will receive 7 mCi of  $^{18}\text{F}$ FDG. Dynamic acquisition of 23 frames will be performed from 0-60 minutes post injection. Full kinetic analysis will be undertaken using standard modeling techniques.

PET/CT studies will be undertaken before initiating entinostat administration and 5 weeks after initiation of aldesleukin treatment.

These studies will be conducted on patients enrolled by the Sydney Kimmel Comprehensive Cancer Center at Johns Hopkins University (JHU). Additional institutions may also participate as the study progresses.





## 2. Glossary

PET/CT	Positron Emission Tomography/Computerized Tomography
ROI	Region of Interest
SUV	Standardized Uptake Value
JHU	Johns Hopkins University
GE	General Electric
FDG	Fluorodeoxyglucose
T <sub>1/2</sub>	Half Life
K1	Model parameter representing influx of tracer in the ROI.

## 3. Background and Rationale

### 3.1. Tumor Metabolism

Positron Emission Tomography (PET) is uniquely suited to evaluate metabolic activity in human neoplasms for diagnostic imaging purposes. The glucose analogue fluorine-18-fluorodeoxyglucose (<sup>18</sup>FDG) has been proven successful as a PET imaging agent for detection and localization of many forms of cancer. The elevated rate of glycolysis in many types of tumor cells enhances the uptake of <sup>18</sup>FDG in neoplasms relative to normal tissues. The most commonly used techniques for quantitative analysis are standardized uptake values (SUV) and simplified tracer kinetic modeling using Patlak-Gjedde analysis. Standardized uptake values are confined to the measurement of radioactivity concentrations at a fixed time point.

<sup>18</sup>FDG PET/CT imaging may provide a useful pharmacodynamic tool in the assessment of tumor response upon immunotherapy in combination with HDAC inhibitors. <sup>18</sup>FDG PET/CT imaging may also predict tumor response before anatomic assessment and help selecting patients who may benefit for further treatment with high dose aldesleukin.

### 4. Patient Selection

PET/CT scans will be performed in all eligible patients.

Prior to any PET/CT study specific investigations being undertaken, freely given informed consent must be obtained from the patient.

Only patients with suitable lesions ( $\geq$  approximately 1.0 cm) as measured by CT will be included in this sub study. Intrathoracic lesions are preferred, since the arterial input function is derived noninvasively from a large blood vessel (thoracic aorta).

## 5. Procedures

NCI Protocol #: 7870

Protocol Version Date 5/29/19

page 64 of 79

One scan per patient will be performed at each session. Each patient will undertake 2 separate sessions: before initiating entinostat administration and 5 weeks after initiation of aldesleukin treatment.

At each session, one  $^{18}\text{F}$ FDG PET/CT scan acquisition will be performed. Changes in metabolism from the baseline study will be computed.

## **6. Tracer Preparation**

On the scheduled day of PET/CT assessments  $^{18}\text{F}$ FDG will be produced at our cyclotron facility

## **7. Scanning Protocol**

An eight-ring GE 4096 tomograph (GE Corp, Milwaukee, WI) will be used for scanning. The scanner has an axial resolution of 6.7 mm and in-plane resolution of 6.5 mm for studies conducted on patients enrolled by the Sydney Kimmel Comprehensive Cancer Center at Johns Hopkins. If additional centers are opened to undertake the sub study, every reasonable attempt will be made to standardize acquisition procedures to allowing pooling of data.

The subject will be positioned in the tomograph in a supine position with the tumor region within the gantry and fixed on the bed by loose Velcro bands. Before emission scanning, a transmission scan for correction of photon attenuation will be performed for 10 minutes with a removable ring source containing 68 Ge.

$^{18}\text{F}$ FDG will be undertaken immediately follow with the patient not moving from the PET/CT scanner. In this manner, the same transmission data can be used for analysis of the emission  $^{18}\text{F}$ FDG. Subjects will receive 10 ml of  $^{18}\text{F}$ FDG e i. v. (7 mCi) over 30 seconds using an automated injector. Dynamic acquisition of 23 frames will be performed as previously described; from 0-60 minutes post injection. Full kinetic analysis will be available using standard modelling techniques.

## **8. Safety**

Safety assessment and recording as described in the main protocol should be adhered to for patients enrolled in the sub study.

## **9. Analysis**

### **9.1 General**

Analysis of PET/CT scan data will be undertaken at JHU using standard modelling techniques. Time-activity curves for each image slice are averaged over all image planes containing tumor to reduce noise in the resulting individual slice time-activity curves.

Kinetic modelling will be performed in the  $^{18}\text{F}$ FDG (three compartment) studies. SUV corrected for lean body mass is preferred. Region of interest (ROI) will be placed using comparator anatomic data (CT).

**NCI Protocol #: 7870**

## **9.2 Metabolism**

Metabolism studies are performed after the intravenous injection of  $^{18}\text{F}$ FDG.

Relative changes in tumor metabolism (mg/gm of tissue/min) and SUV will be estimated. SUV for tumor may be regarded as a surrogate for metabolism.

## **9.3 Interpretation of Estimated Parameters**

It is anticipated that with effective treatment there will be declines in the estimated tumor metabolic parameter. The precision of tumor SUV (semi quantitative metabolic rate) is reproducible within 10%. Further, SUV can be compared between institutions by comparing the SUV determined at JHU at varying times post  $^{18}\text{F}$ FDG injection with the SUV determined at the same time point post injection.

**Reference**

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**APPENDIX B: 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 C: HDIL-2 (HIGH DOSE ALDESLEUKIN) ADMINISTRATION GUIDELINES**

The toxicities of IL-2 therapy are thought to result primarily from a capillary leak syndrome (CLS) as well as lymphoid infiltration which have been observed histologically in many organs. The earliest clinical manifestations of CLS are hypotension and tachycardia which can be seen two hours after the first dose of high-dose of IL-2. Decreased systemic vascular resistance has been measured at this time point and contributes to the early drop in blood pressure. Because of these changes, if patients are receiving antihypertensive medications they should be discontinued prior to initiating high-dose IL-2.

Intravenous fluids are the initial therapy for hypotension and the goal is to maintain systolic blood pressure greater than 80-90 mmHg. It is common to require additional fluid in the first 24 hours of therapy in response to hypotension and oliguria. After initial resuscitation, most patients reach a steady state of relative hypotension and tachycardia that will be maintained until the final doses of IL-2. No intervention is generally needed for these parameters unless they exceed predetermined guidelines or oliguria ensues. Fluid replacement of 1-1.5 liters/day above maintenance requirements should not be exceeded because of extravasation and compounding of generalized edema and pulmonary congestion. The use of additional fluids late in the course of treatment should be restricted because the transient benefit they achieve may be overshadowed by the unwanted consequences of fluid leak (special caution must be exercised if body weight is >10% above baseline). Crystalloid is favored over colloid for the treatment of hypotension based on a randomized study that showed both were equally effective. If hypotension persists despite judicious fluid resuscitation, vasopressor support with an agonist such as phenylephrine is indicated. The hypotensive effects of each IL-2 dose generally peak 4-6 hours after infusion and vasopressors are titrated accordingly. When phenylephrine can be weaned to <0.5 µg/kg/min it is generally safe to proceed with additional IL-2 dosing. As immunotherapy nears completion, phenylephrine requirements increase; doses approaching 1.5-2.0 µg/kg/min and inability to wean by 8 hours suggest that IL-2 infusions should be discontinued. Blood pressure measurements generally return to baseline within 24-48 hours of discontinuation of IL-2.

Within the first 8 hours of starting IL-2, decreased urine output is frequent and is a consequence of hypotension and decreased intravascular volume. Renal dysfunction during IL-2 has been described as prerenal in nature, transient and without evidence of intrinsic renal damage. Oliguria is first treated with fluid boluses; if 1-1.5 Liters of crystalloid does not restore urine

flow, an indwelling urinary catheter is inserted and dopamine at renal perfusion doses (2 µg /kg/ min) is initiated. Urine output greater than 10-20 cc hour must be established before additional IL-2 dosing can be considered. Serum creatinine levels are measured at least daily and are considered when making decisions about IL-2 dosing.

Patients receiving high-dose IL-2 may experience progressive shortness of breath during therapy which very infrequently requires endotracheal intubation or drainage of a pleural effusion. The mechanism of pulmonary congestion has been attributed to increased vascular permeability. Careful pretherapy screening of patients who are smokers or have large tumor burdens in the lung (FEV1 and forced vital capacity should be greater than 65% of predicted and PaO<sub>2</sub> >75 on room air), advising smokers to quit 2 weeks before therapy as well as judicious fluid replacement during therapy have resulted in a low incidence of severe toxicity. The selective monitoring of transcutaneous O<sub>2</sub> saturation has been very helpful in patients experiencing pulmonary symptoms. O<sub>2</sub> saturation should be maintained above 95% and if this level cannot be met by 4L O<sub>2</sub> by nasal cannula or 40% O<sub>2</sub> by mask, IL-2 dosing should be discontinued. The auscultation of rales in the lung bases is not infrequent during therapy but progression to the mid lung fields coupled with marginal O<sub>2</sub> saturation are reasons to discontinue IL-2 dosing. Chest radiographs revealing interstitial edema and pleural effusions provide complementary information to the clinical assessment and are performed selectively. After discontinuation of IL-2, symptoms of pulmonary congestion resolve promptly and recovery is aided by rapid diuresis.

One of the more visible sequelae of CLS is the generalized total body edema and consequent weight gain during high-dose IL-2. Neurovascular compression from edema is rare and is best treated with elevation of the involved extremity and use of compression garments. Cautious use of fluids to correct hypotension and oliguria is important in minimizing edema. Diuresis is not always possible during therapy because patients may be hypotensive and are frequently unresponsive to diuretics. After stopping IL-2 and when the blood pressure is returning to baseline (and not requiring vasopressors), diuresis should be vigorously initiated by pharmacologic means. Fluid balance is rapidly restored and the majority of patients are discharged at or below admission weight about 3 days after stopping IL-2.

### **Pre-Therapy Assessment and Interventions**

- Confirm no recent (< 7 days) history of infections (respiratory, renal, IV site, other). If present, delay IL-2 by 1 week.
- Check labs (i.e. normalized since last IL-2), EKG (i.e. no changes since IL-2), CXR (i.e. no effusion since IL-2).
- Draw research labs if appropriate.

- Start acetaminophen, indomethacin and H2 blocker 8-12 hours before IL-2.
- Premedicate patient for nausea (before IL-2 dose)

### During Therapy Assessment and Interventions

- Start maintenance IV at 100 cc hr before IL-2 is started.

### PATIENT MONITORING GUIDELINES

	<u>Ward</u>	<u>ICU</u>
Vitals signs	Every 4hr	Every 1hr
Intake and output	Every 4hr	Every 1hr
Weight	Daily	Daily
Mental Status	Every 8hr	Every 8 hr
IV site	Every 8hr (change arm IV every 3 days)	Every 8 hr
<u>Labs</u>		
CBC	Daily	BID
Lytes, BUN, Crt, Glu	Daily	BID
ALT, AST, total bili	Daily	Daily
Ca <sup>++</sup> , Mg <sup>++</sup> , phosphorus	Daily	Daily
PT, PTT	Daily after day 2.	Daily
CK, total	Daily	Daily
TSH, T3, T4	Each cycle	Each cycle
Urinalysis	Each cycle	Each cycle
EKG	Each cycle	Each cycle
CXR	Each cycle	Each cycle

### Target blood pressure during therapy

<u>Normal BP for patient</u>	<u>Target BP on therapy</u>
<100 mm Hg	>80 mm Hg
100-120 mm Hg	>85 mm Hg
>120 mm Hg	>90 mm Hg

### IL-2 dosing

NCI Protocol #: 7870

Protocol Version Date 5/29/19

page 70 of 79

No dose reductions of IL-2 are performed. IL-2 doses are delayed according to symptomatic recovery from the previous dose. Delay greater than 24 hours should result in discontinuation of a course of IL-2.

Guidelines for delay or discontinuation of IL-2 are given below. The presence of relative criteria implies that a patient is nearing the completion of a course of therapy but that with appropriate corrective measures or with a time delay to allow for recovery, it may be safe to proceed. Several relative criteria are usually an indication to discontinue dosing. The presence of absolute criteria is generally considered an indication to stop dosing IL-2.

NO steroids, systemic or topical, should be used at any time while patients are receiving immunotherapy.

### DOSE MODIFICATION FOR HDIL-2

(Delay or discontinuation only; no dosage adjustment)

<b>System</b>	<b>Relative Criteria</b>	<b>Absolute Criteria</b>
Cardiac	Sinus Tach 120-130	Sinus Tach >130 (persists after correcting hypotension, fever, stopping dopamine) EKG changes of ischemia Atrial fibrillation Supraventricular tachycardia Ventricular arrhythmias (frequent PVCs, bigeminy, v-tach) Elevated CPK-MB
Dermatologic		Moist desquamation
Gastrointestinal	Diarrhea, 1000 cc/shift  Ileus/abdominal distention	Diarrhea, 1000 cc/shift x 2 Vomiting not responsive to medication Severe abdominal distention affecting breathing Severe abdominal pain, unrelenting
Hemodynamic	Max Neo-Syneprine	Max Neo-Syneprine

NCI Protocol #: 7870

Protocol Version Date 5/29/19

page 71 of 79



	1-1.5 ug/kg/min	1.5-2.0 ug/kg/min
	Min Neo-Syneprine	Min Neo-Syneprine
	>0.5 ug/ kg/ min	> 0.8 ug/ kg/ min
Hemorrhagic	Guaiac+ sputum, emesis, Platelets 30-50,000/mm <sup>3</sup>	Frank blood in sputum, emesis, stool Platelets <30,000/mm <sup>3</sup>
Infectious		Strong clinical suspicion or documented
Neurologic	Vivid dreams Emotional lability	Mental status not reversible in 2 hrs. Disorientation Hallucinations
Pulmonary	Resting shortness of breath 3-4 L O <sub>2</sub> NC for sat >95%	>4 L O <sub>2</sub> by NC for sat >95% 40% O <sub>2</sub> mask for sat > 95% Endotracheal Intubation
	Rales 1/3 up chest	Rales 1/2 up chest Pleural effusion requiring tap or chest tube while on therapy
Renal	Urine 80-160 cc/8 hour Urine 10-20 cc/hr Creatinine 2.5-2.9 mg/dl	Urine <80 cc/8 hour Urine <10 cc/ hr Creatinine >3.0 mg/dl
Weight Gain	15% weight gain over baseline	

## INTERVENTION

### Observation category   Action

≤ 3 Relative criteria	Initiate corrective measures
>3 Relative criteria	Stop IL-2 for current course
1 Absolute criterion	Stop IL-2 for current course

**CORRECTIVE MEASURES**

<b>Symptom/sign</b>	<b>Corrective measure</b>
Arrhythmia (other than sinus tach)	Stop IL-2 (most arrhythmias). Correct electrolytes, minerals, anemia, hypoxia; use medications as indicated
Anemia	Transfuse PRBCs to achieve Hct > 28 % during IL-2 dosing.
Acidosis	Total CO <sub>2</sub> (bicarbonate) < 20 mmol/ L, give 50 meq bicarb IV If CO <sub>2</sub> <18 mmol/L, give 100 meq bicarb IV
Chills (generally after first or second dose)	Warm blankets as first measure. IV Demerol if persist.
CK elevation	Measure isoenzymes, EKG. If have evidence of myocarditis, must stop IL-2. Will need a MUGA stress test before next cycle of IL-2 to rule out Myocardial dysfunction. Future IL-2 may be considered if the MUGA is normal.
Dermatitis	Oatmeal baths, lotions (NO <u>steroid</u> or alcohol containing lotions).
Diarrhea	Antidiarrheals (alternate medications). Avoid overuse because of complicating ileus and distention.
Edema	Elevate symptomatic extremity. Use fluids judiciously.
Epigastric pain	Evaluate cause, give indomethacin rectally, and consider antacids.
Fever breakthrough	Increase frequency of indomethacin to every 6 hr. Consider septic work-up if happens after first 24 hrs. of therapy i.e. high spike, 38.5 or above, during therapy.
Hypoalbuminemia	Observe
Hypocalcemia	Maintain above lowest normal at institution (compensate for low albumin).
Hypokalemia	Maintain potassium above 3.6 mmol/L.
Hypomagnesemia	Maintain above lowest normal at institution (compensate for low

	albumin)
Hypotension	Use crystalloids; maintain systolic BP > 80-90 mm Hg. If hypotension persisting in spite of optimal fluid therapy consider vasopressors. See under dose modification for hemodynamic criteria
Infection	Stop IL-2 and treat infection as indicated
Mucositis/stomatitis	Frequent oral care, mouthwashes, topical anesthetics, nystatin.
Oliguria	Fluid boluses at first. If 1-1.5 L of crystalloid does not restore U/O, insert Foley catheter and initiate renal dose dopamine (2 ug/kg/min). U/O should be 20-30 cc hour at least.
Nausea vomiting	Antiemetics (alternate medications and routes if not effective).
Nasal congestion	Room humidifier, decongestant (NO topical steroids).
Poor venous access	Insert PIC or central line. Antibiotic  Prophylaxis started with PIC or central line inserted and continued until line out (remove line as soon as not needed for fluids, vasopressors, replacements, diuretics, etc).  Prophylaxis started with PIC or prophylaxis started with PIC or central line inserted and continued until line out (remove line as soon as not needed for fluids, vasopressors, replacements, diuretics, etc). Prophylaxis started with PIC or central line inserted and continued until line out (remove line as soon as not needed for fluids, vasopressors, replacement, diuretics)
Pruritus	Oatmeal baths, lotions, antipruritics
Shortness of breath	Check transcutaneous O <sub>2</sub> sat. If <95% use O <sub>2</sub> . Use fluids judiciously. Do not use inhalational steroids
Tachycardia (sinus)	Correct fever, hypotension, hypoxia, anemia, consider discontinuation of dopamine if used.

Thrombocytopenia      Consider transfusion if count < 20,000/mm<sup>3</sup>

### Concomitant Medications Used During IL-2 Therapy\*

Medication	Dose/Frequency/Route	Side Effect Treated
Acetaminophen	650 mg q4h, PO/PR	Fever, myalgia
Indomethacin	50-75 mg q8h, PO/PR	Fever, myalgia
Meperidine HCL	25-50 mg q1h, IV prn	Chills
Ranitidine HCL	50 mg q8h, IV	Gastritis
Aluminum hydroxide (200 mg)	30 ml q3h, PO prn	Gastric upset
Magnesium hydroxide 200 mg		
Simethicone 20 mg		
Droperidol	1 mg q4-6h, IV prn	Nausea
Prochlorperazine	25 mg q4h, PR prn or 10 mg q6, IV prn	Nausea
Ondansetron HCL	10 mg q8h, IV prn	Nausea
Granisetron HCL	0.01 mg/kg daily, prn	Nausea
Lorazepam	0.5 – 1.0 mg q6h, PO/IV prn	Nausea, anxiety
Haloperidol	1-5 mg q1h, IV/IM prn	Agitation
Loperamide	2 mg q3h, PO prn	Diarrhea
Diphenoxylate HCL	(2.5 mg) q3h, PO prn	Diarrhea
Atropine sulfate (25 µg)		
Codeine Sulfate	30 – 60 mg q4H, PO prn	Diarrhea
Tucks	Apply locally, prn	Perianal discomfort
Hydroxyzine HCL	10 – 20 mg q6h, PO prn	Pruritis
Diphenhydramine HCL	25 – 50 mg q4h, PO/IV prn	Pruritis
Oatmeal powder/baths	Apply locally prn	Pruritis
Lubriderm 8 oz. with 0.25% Camphor and 0.25% menthol	Apply locally prn	Pruritis
Sodium bicarbonate	6 tsp/1500 ml Swish & swallow prn	Mucositis
Lidobenalox oral	5 ml q3h, PO prn	Mucositis
Pseudoephedrine HCL	30 mg q6h, PO prn	Sinus congestion
Temazepan	15-30 mg qhs, PO prn	Insomnia
Zolpidem	5-10 mg qhs, PO prn	Insomnia

NCI Protocol #: 7870

Protocol Version Date 5/29/19

page 75 of 79

Normal saline	250-500 ml IV prn	Oliguria, hypotension
Dopamine HCL	400 mg/250 ml, IV prn (2 µg/kg/min)	Oliguria
Phenylephrine	40 mg/100 ml, titrate IV prn (0.1 – 2.0 µg/kg/min)	Hypotension
Calcium gluconate 10%	1 gm (over 1h), IV prn	Hypocalcemia
Magnesium sulfate	1 gm (over 1h), IV prn	Hypomagnesemia
Potassium phosphate	10 – 15 mmole (over 6h), IV prn	Hypophosphatemia
Potassium chloride	10 meq (over 1h), IV prn	Hypokalemia
Cefazolin	1-2 gm q6h, IV	Prevent line sepsis
Clindamycin	900 mg q8h, IV	Prevent line sepsis

Abbreviations: q: every; PO: orally; PR: rectally; IV: intravenously; PRN: as needed.

\* Douglas Schwartzenruber, Biologic Therapy of Cancer, editors: VT De Vita, Jr, S Hellman and SA Rosenberg, 2nd edition, JB Lippincott, 1995, pg. 235-249.

## Appendix D: Instructions for Network Sites

### 1. CONTACT INFORMATION

All questions related to the protocol or study implementation should be directed to:

Roswell Park Cancer Institute  
CRS Quality Assurance Office  
GBSB 1930  
Buffalo, New York 14263

**Telephone:**

716-845-8084 or 716-845-1205 - M-F; 8:00 AM to 5:30 PM

716-845-2300 - After hours, Weekends and Holidays: request the RPCI Principal Investigator

**Fax:** 716-845-8743

### 2. INFORMED CONSENT

- Informed Consent must be obtained by the **Investigator** from any patients wishing to participate, **prior to any procedures or change in their treatment**
- An informed consent template is provided by RPCI and can be amended to reflect institutional requirements
- All consent changes **must** be reviewed by Roswell Park Cancer Institute Quality Assurance Office prior to submission to the site IRB.
- The informed consent must be IRB approved
- Always check that you are using the correct date and version of the IRB approved consent.

### 3. PATIENT REGISTRATION

The **Subject Enrollment Log** must be faxed to the CRS Quality Assurance Office within 24 hours of the date the patient is consented. Once the Principal Investigator has determined that the eligibility criteria have been met, complete the **Patient Registration Form and fax it** to the RPCI Quality Assurance Coordinator at (716) 845-8743.

Note: The patient completes the **Gender, Race, and Ethnicity form** and this is placed in the study binder.

**Roswell Park Cancer Institute does not grant exceptions to eligibility criteria.**

### 4. STUDY DEVIATIONS

- If a deviation has occurred to eliminate hazard, this should be reported to the RPCI Quality Assurance Office, site IRB and any other regulatory authority involved in the trial.
- ANY study deviation will be recorded on the Study Deviation Log
- Patients who are inadvertently enrolled, with significant deviation(s) from the study-specified criteria, will be removed from the study
- Notify RPCI of any early patient withdrawal and appropriately document the discontinuation and the reason why.

## 5. STUDY DOCUMENTATION

- Study documents must be filled out completely and correctly. Ditto marks are not allowed.
- If an entry has been documented in error put a single line through the entry and initial and date the change. The auditor must be able to read what has been deleted.
  - Do NOT use white-out, magic marker, scratch-outs
  - Do NOT erase entries
- Use only black ink for documentation on the accountability form and any other study forms.

## 6. DRUG ACCOUNTABILITY

**Drug accountability will be strictly maintained, recording quantities of study drug received, dispensed to patients and wasted, lot number, date dispensed, patient ID number and initials, quantity returned, balance remaining, manufacturer, expiration date, and the initials of the person dispensing the medication.**

- Responsibility rests solely with the Principal Investigator but can be delegated as appropriate (e.g. to pharmacy personnel)
- Records must be maintained regarding receipt, dispensing, return, waste and disposition of all investigational agents
- Study drug supply should only be used in accordance with the IRB approved study
- Drug accountability forms are protocol and agent specific, they are study source documents and will be used to verify compliance with the study
- Any discrepancies shall be documented and explained
- An inventory count should be performed with each transaction
- Drug accountability forms shall be stored with study related documents
- Each medication provided for this study and each dosage form and strength must have its own Drug accountability.
- Do **NOT** “transfer”, “borrow” or “replace” supplies between studies
- Dispensing the wrong study supply is considered a **medication error**
- **Never** replace investigational agents with commercial product

## 7. SERIOUS ADVERSE EVENT REPORTING:

The site Investigator or designated research personnel will report all serious adverse events, whether related or unrelated to the study drug(s) to the **IRB in accordance with their local institutional guidelines**. Expedited adverse event (AE) reporting for this study is via CTEP-AERS (CTEP Adverse Event Reporting System), accessed via the secure CTEP web site (<https://eapps-ctep.nci.nih.gov/ctepaers/pages/task?rand>). The reporting procedures to be followed are presented in the “CTEP, NCI Guidelines: Expedited Adverse Event Reporting Requirements” which can be downloaded from the *CTEP web site* (<http://ctep.cancer.gov/protocolDevelopment/elcetricapplications/docs/aeguidelines.pdf>). The site will notify the CRS Quality Assurance Office within one business day of being made aware of the SAE by faxing a copy of the expedited reports submitted to CTEP via CTEP-AERS. A preliminary written report must follow within 24 hours (1 business day) of the oral notification. A complete follow-up report must be filed within 10 working days.

## 8. UNANTICIPATED PROBLEM REPORTING:

An Unanticipated Problem (UAP) is any incident, experience, or outcome that meets **all** of the following criteria:

1. Unexpected (in terms of nature, severity, or frequency) given:
  - (a) the research procedures that are described in the study- related documents, including study deviations, as well as issues related to compromise of patient privacy or confidentiality of data;
  - (b) the characteristics of the subject population being studied;
2. Related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research);
3. Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized;

For all adverse events occurring that are unanticipated and related or possibly related to the research drug, biologic or intervention the participating physician or delegated research staff from each site will notify **their local IRB in accordance with their local institutional guidelines**. The site must also notify the CRS Quality Assurance Office within 24 hours of being made aware of the Unanticipated Problem by completing the **RPCI Unanticipated Problem Report Form** and faxing it to the CRS Quality Assurance office.