Multi-Histology Phase II Study of 5-Fluoro-2'-Deoxycytidine with Tetrahydrouridine (FdCyd + THU)

Abbreviated Title: Ph II FdCyd+THU

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Précis

Background:

- 5-Fluoro-2'-deoxycytidine (FdCyd), a fluoropyrimidine nucleoside analog, has a short (10-minute) half-life and is rapidly degraded *in vivo* by cytidine deaminase. However, co-administration with tetrahydrouridine (THU), an inhibitor of cytidine/deoxycytidine deaminase, has been shown to increase the AUC of the parent compound more than 4-fold. Increased FdCyd exposure allows it to be taken up intracellularly and converted to its triphosphate, which is incorporated into DNA and inhibits the action of the enzyme DNA methyltransferase (DNMT). Inhibition of DNMT, and in turn DNA methylation, can result in the re-expression of tumor suppressor genes.

Primary objective:

- Determine progression-free survival (PFS) and/or the response rate (CR + PR) of FdCyd administered 5 days per week for 2 weeks, in 28-day cycles, by intravenous infusion over 3 hours along with THU in patients with breast cancer, head and neck cancer, non-small cell lung cancer, and urothelial transitional cell carcinoma.

Exploratory objectives:

- Evaluate whether treatment with FdCyd and THU alters DNA methylation patterns in tumor biopsy samples before and during treatment by LINE-1 analysis.
- Evaluate the safety and tolerability of FdCyd (100 mg/m^2) + THU (350 mg/m^2) administered 5 days per week for 2 weeks, in 28-day cycles, by intravenous infusion over 3 hours.
- Measure changes in the number of CTCs following treatment with FdCyd plus THU.

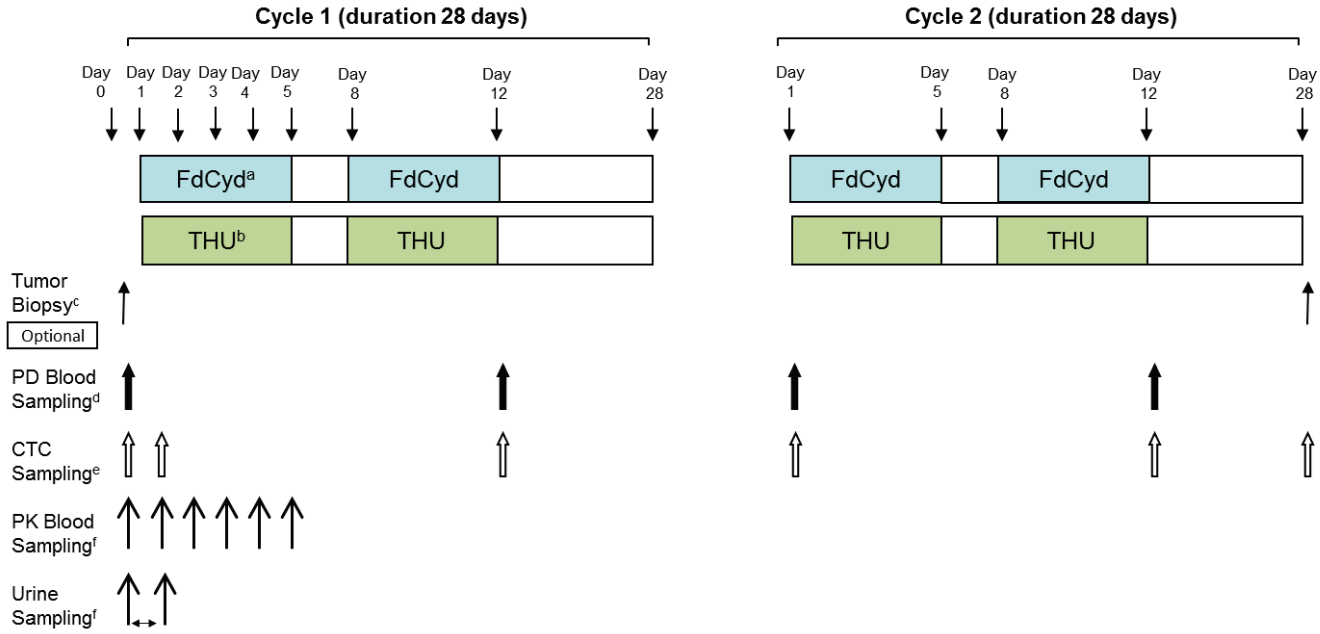
Eligibility:

- Patients with histologically documented non-small cell lung cancer, head and neck cancer, urothelial transitional cell carcinoma, and breast carcinoma.

Design:

- This is a multicenter trial with NCI as the coordinating center and the California Cancer Consortium and UPMC as participating sites.
- FdCyd will be administered as an IV infusion over 3 hours with 20% of the daily dose of THU administered as an IV push and the remaining 80% co-administered with FdCyd by 3-hour infusion daily for 5 consecutive days of treatment per week for 2 consecutive weeks, followed by 2 weeks of no treatment, in a 28-day cycle.
- Blood and optional tumor biopsies for pharmacodynamic and pharmacokinetic studies will be obtained.
- The study will accrue a maximum of 165 patients including all centers.

Schema



a. FdCyd: 3-hour infusion in 5% dextrose injection, USP.

b. THU: 20% of the daily dose will be given by IV push, then 80% of the daily dose by 3-hour infusion in 5% dextrose injection, USP at the same time as FdCyd.

c. Optional tumor biopsies will be collected prior to treatment in Cycle 1 and after completion of Cycle 2 (i.e., following the restaging imaging scans).

d. Blood samples for PD analysis will be collected prior to drug administration on Day 1 in Cycles 1, 2, and 4, and after the completion of treatment on Cycles 1, 2 and 4 (Day 12 +/- 1 day).

e. Blood samples for circulating tumor cells will be collected prior to drug administration on Day 1, on Cycle 1 Day 2 following treatment (up to 24 hours after end of infusion), on Cycle 1 Day 12 (+/- 1 day), on Day 1 (+/- 1 day) of Cycle 2 and all subsequent cycles, on Day 12 (+/- 1 day) of Cycles 2, 4, and 6, and at time of disease progression.

f. Blood and urine samples for PK analysis will be collected from 3 patients in each cohort (6 of the first 12 patients with urothelial transitional cell carcinoma) during Cycle 1 only. Blood will be collected pre-drug infusion and at 15 min, 30 min, 1 hr, 2 hr, and 2.5 hr during the infusion and then 15 min, 30 min, 1 hr, 2 hr, 4 hr, and 6 hr post completion of infusion on Cycle 1 Day 1, and pre-drug infusion on Days 2, 3, 4, and 5. Urine will be collected prior to treatment and at every void from 0 to 24 hours post drug infusion on Cycle 1 Day 1.

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1 Study Objectives

1.1 Primary Objective

- Determine progression-free survival (PFS) and/or the response rate (CR + PR) of FdCyd administered 5 days per week for 2 weeks, in 28-day cycles, by intravenous infusion over 3 hours along with THU in patients with breast cancer, head and neck cancer, non-small cell lung cancer, and urothelial transitional cell carcinoma.

1.2 Exploratory Objectives

- Evaluate whether treatment with FdCyd and THU alters DNA methylation patterns in tumor biopsy samples before and during treatment by LINE-1 analysis.
- Evaluate the safety and tolerability of FdCyd (100 mg/m^2) + THU (350 mg/m^2) administered 5 days per week for 2 weeks, in 28-day cycles, by intravenous infusion over 3 hours.
- Measure changes in the number of CTCs following treatment with FdCyd plus THU.

2 Background and Rationale

2.1 Background

2.1.1 Methylation in Cancer

The observation that changes in DNA methylation potentially play a role in neoplastic transformation has existed for nearly 30 years [1]. Over the past decade, the importance of DNA methylation in the development of cancer has become more evident.

DNA methylation involves the addition of a methyl group to any of the four bases which make up the coding sequence of DNA. In humans, methylation typically occurs after DNA synthesis and is catalyzed by one of the DNA methyltransferases (DNMT). DNA methylation that occurs in regulatory (i.e., promoter) sequences can promote or suppress the expression of particular genes. In mammals, DNA methylation occurs almost exclusively in the 5' position of cytosine residues within the dinucleotide CpG. CpG-rich regions, also termed CpG islands, span the promoters of many genes. In many normal cells, these CpG islands are not methylated and the nearby gene is active. Methyl-binding proteins recognize methylated CpG islands and are thought to be responsible for the initiation of the events resulting in chromatin condensation and gene silencing. The derangement of methylation patterns in human tumors results in a global hypomethylation of genes with hypermethylation of certain promoters of key genes such as retinoblastoma 1 (*RB1*), *p16*, von Hippel-Lindau (*VHL*) and MutL protein homologue 1 (*MLH1*), among others [2]. A more detailed description of the methylation changes thought to play integral roles in the cancers to be studied in trial is included below.

2.1.2 Breast Cancer

In the United States, over 180,000 women are diagnosed with breast cancer annually, making it the most common cancer amongst females and the second most common cause of cancer death among women [3]. While great strides have been made over the past

30 years in the treatment of patients with localized breast cancer, up to 5% of patients present with metastatic disease at diagnosis and long-term survival for these patients with therapy is less than 5% [4]. The median PFS for patients who have failed multiple lines of therapy is measured in months. A recent review of published clinical trials in patients with metastatic breast cancer concluded that "There is little evidence from trials reported from 2000 to 2007 that major survival differences exist between many commonly employed chemotherapy regimens" [5]. Taxane-containing regimens offer a slight survival advantage, but for patients with taxane-resistant or refractory cancers, no new agents substantially improve outcome. Median PFS rates of 3.8 months and 4.2 months were obtained in a Phase II study of heavily and minimally pretreated patients with metastatic breast cancer, respectively, on a Phase II study of gemcitabine and cisplatin [6]. Addition of the epothilone B analog ixabepilone to capecitabine in a Phase III trial in patients with locally advanced or metastatic cancer prolonged progression-free survival in the combination group from 4.2 to 5.8 months over capecitabine alone; pretreatment or resistance to anthracyclines and resistance to taxanes were eligibility criteria [7]. Response rates and PFS after failure of 2 lines of therapy are disappointing. A retrospective review of 578 patients with metastatic breast cancer, analyzing for overall survival related to each line of chemotherapy, reported PFS of 2-3 months and overall survival of 8 months after failing 2 lines of therapy [8].

Number of lines of chemotherapy	No. of patients	Median OS (months)	Median TTF (months)
1 line and more	487	22.5	3.97
2 lines and more	331	17	3.45
3 lines and more	225	12.3	2.79
4 lines and more	141	9.3	2.93
5 lines and more	77	8.7	2.99
6 lines and more	40	8.2	2.07
7 lines and more	25	7.5	3.68
8 lines and more	11	Not evaluable	Not applicable

Table 1: Overall survival (OS) related to lines of chemotherapy in patients with metastatic breast cancer. OS was evaluated between Day 1 of the first chemotherapy line and the date of last report. Time to treatment failure (TTF) was evaluated between Day 1 of the chemotherapy line and the day of progression of disease. From Tacca et al. 2009 [8]

The integral roles of the estrogen and progesterone receptors as well as Her2neu in the pathogenesis of some breast cancer have led to the development of multiple therapies. However, with the knowledge that silencing of key tumor suppressor genes may play a part in breast carcinogenesis comes the opportunity to improve on current treatment approaches. Decreased expression of the tumor suppressor gene *WWOX* has been detected in over 60% of breast cancer tumor samples in one series [9]. When the demethylating agent decitabine was used in a breast xenograft model, tumor growth regressed and *WWOX* expression was

restored [10]. Other genes found to be methylated in breast tumor samples include *BRCA1*, *ER*, *PR*, *RASSF1A*, and *E-cadherin* [11-14].

2.1.3 Head and Neck Cancer

Patients with locally recurrent or metastatic head and neck cancer have a poor prognosis. Investigations into multiple cisplatin-based chemotherapy regimens and more recently, taxane-containing regimens have provided slight improvement in survival compared to supportive care alone, but the median survival for patients with recurrent and metastatic head and neck squamous cell carcinoma (HNSCC) is 4 to 6 months [15, 16]. New treatment modalities are needed. A median PFS of 9.6 weeks was obtained in a Phase II study of erlotinib in heavily pretreated patients with locally recurrent or metastatic HNSCC [17]. A retrospective review of 151 patients with HNSCC refractory to platinum-based chemotherapy treated between 1990 and 2000 at seven different centers with second line therapies showed an overall survival of 103 days (95% confidence interval [CI]: 77-126 days) and an overall response rate of < 3% [18].
Administering cetuximab with platinum-based chemotherapy as first-line treatment in patients with recurrent or metastatic HNSCC increased median progression-free survival (PFD) from 3.3 to 5.6 months [15]. However, the difference in median PFS from an ECOG Phase III study of patients with recurrent/metastatic HNSCC randomly assigned to receive cisplatin with cetuximab or with placebo were statistically insignificant: 2.7 months for patients on placebo and 4.2 months for patients on cisplatin and cetuximab [19].

Analysis of tumor samples has revealed that several tumor suppressor genes thought to play a role in head and neck cancer development are methylated. Using Restriction Landmark Genomic Scanning (RLGS), 5 candidate genes were identified. These included septin 9 (*SEPT9*), sodium-coupled monocarboxylate transporter (*SLC5A8*), functional smad-suppressing element on chromosome 18 (*FUSSEL18*), early B-cell factor 3 (*EBF3*), and iroquois homeobox 1 (*IRX1*) [20]. In the 42 tumors studied, 27% to 67% demonstrated methylation of at least one of the 5 genes listed. Over 50% demonstrated methylation of 2 or more genes. Treatment of head and neck cell lines with decitabine restored mRNA expression of these genes. The role of these genes in tumor suppression was corroborated by colony forming assays demonstrating growth restriction associated with gene expression [21]. Additional genes thought to be methylated in squamous cell carcinoma of the head and neck that may contribute to tumor growth include *MGMT*, *p16*, and *DAPK* [20].

2.1.4 Non-small Cell Lung Cancer

Lung cancer remains the most common cause of cancer mortality in the United States with over 160,000 deaths attributed to the disease in 2007. Non-small cell lung cancer (NSCLC) comprises approximately 85% of all primary lung malignancies and includes predominantly adenocarcinoma, squamous cell, and large cell carcinomas, in decreasing order of incidence. For patients with unresectable disease, cure rates remain dismal and combination chemotherapy provides palliative benefit as well as modest prolongation of survival. Median survival for treated patients with a poor performance status has been reported as 2.9 to 5.8 months [22, 23]. Treatment options for patients who have progressed after a one prior regimen include docetaxel, pemetrexed, and erlotinib [24]. In the recent Phase II study comparing chemotherapy (docetaxel or pemetrexed) alone, bevacizumab

plus chemotherapy, and bevacizumab plus erlotinib, median PFS times were 3.0, 4.8, and 4.4 months, respectively [25].

Response rates and improvement in PFS are modest in metastatic NSCLC after failure of first-line therapy. In the initial Phase II study with gefitinib, patients were randomized to receive 250 mg or 500 mg daily of gefitinib (IDEAL trial). The objective tumor response rates were 18.4% and 19.0% among evaluable patients, symptom improvement rates were 40.3% and 37.0%, median progression free survival times were 2.7 and 2.8 months, and median overall survival times were 7.6 and 8.0 months, for 250 mg and 500 mg of gefitinib respectively [26]. The BR.21 placebo controlled, double-blinded study randomized patients who had progressed on chemotherapy in a 2:1 manner to receive erlotinib or placebo. The response rate was 8.9% in the erlotinib group and <1% in the placebo group ($p<0.001$). Time to tumor progression was 2.2 months and 1.8 months, respectively ($p<0.001$) with an overall survival of 6.7 months and 4.7 months, ($p<0.001$) in favor of erlotinib [27].

Given the low response rates and poor PFS from agents that are currently FDA approved for the treatment of patients with relapsed NSCLC, we feel that in this initial trial of FdCyd +THU in NSCLC targeting a RR of 20% and a PFS of 4 months is appropriate in this disease setting.

A number of genes are hypermethylated in non-small cell lung cancers, and hypermethylation of certain genes has been associated with a decrease in overall survival [28]. In the largest series to date, tumor samples from 150 patients with various stages of NSCLC of either squamous or adenocarcinoma histology were analyzed [29]. Those with methylated *p16* had a statistically significant decrease in median survival compared to those in whom the *p16* promoter was not hypermethylated (21.7 months vs. 62.5 months, $p = 0.0001$). Methylation of the *p16* promoter is thought to be induced by tobacco smoke exposure; a direct correlation between *p16* promoter methylation and number of pack-years smoked, duration of smoking history, and indirect correlation with time elapsed since smoking cessation has been demonstrated in a large series of patients with NSCLC [30]. When studied in combination with other genes in a series of tumor samples from patients with NSCLC, healthy volunteers and smokers without evidence of lung cancer, the hypermethylation of *p16* appeared to be a late event during lung cancer progression [31]. In a NSCLC xenograft study, the use of decitabine resulted in tumor growth suppression and was associated with restoration of *FHIT* and *WWOX* gene expression [32].

2.1.5 Urothelial Transitional Cell Carcinoma

Urothelial transitional cell carcinoma (TCC) originates in the urothelium and accounts for about 90% of bladder cancers [33]. It is estimated that in 2008 there will be 69,000 new diagnoses of urinary bladder cancer in the United States with approximately 14,000 deaths [3]. Epidemiological studies have identified several chemical exposures associated with urinary bladder cancer, including cigarette smoking [34, 35]. Relative survival decreases as the carcinoma spreads to more distant sites. For patients whose disease is not beyond the muscle wall, 75% 5-year progression-free survival can be achieved. However, this survival rate diminishes markedly with more invasive disease such that almost no patients

with lymph node involvement survive 5 years [36, 37]. The need for improved therapies especially in heavily pretreated patients is great (Table 2). Median progression-free survival in a Phase II trial of ixabepilone in patients with advanced transitional cell carcinoma was 2.7 months [38]. A median PFS of 3.0 months (95% CI: 2.4–3.8) was achieved in a Phase II study of the microtubule inhibitor vinflunine in patients with advanced TCC progressing after first-line platinum therapy [39]; the subsequent Phase III study also conferred a 2-month overall survival advantage (6.9 vs. 4.6 months) [40].

Drug	No. of pts	Evaluable pts	CR	PR	%	Duration (months)	TTP (months)	OS (months)
Paclitaxel	31	31	—	3	10		2.2	7.2
	14	14		1	7	7.4		
	45	37	—	2	5	3.0		
Docetaxel	30	30	—	4	13	4.0		9.0
Oxaliplatin	20	18	—	1	6			
Lapatinib	59	59	—	2	3			
Bortezomib	18	11	—	—	0			
Pemetrexed	47	47	3	10	28	3.0		9.8

Table 2: Results from recently published Phase II trials in previously treated patients with advanced TCC. TTP: time to progression; OS: overall survival. From Culine et al. 2006 [39].

As summarized in Table 3, salvage chemotherapy in patients with urothelial malignancies has low response rates and low PFS and overall survival [41].

Drug	n	Eligibility	RR (%)	Median PFS (months)	Median OS (months)
Ifosfamide	56	1 prior regimen	20	2.4	5.5
Ifosfamide– docetaxel	22	1 prior platinum-based regimen	25		
Ifosfamide– paclitaxel	13	1 prior regimen	15		
Paclitaxel (24 h)	9	1 prior regimen	56		
Paclitaxel (weekly)	31	1 prior regimen for advanced disease, prior adjuvant chemotherapy and taxanes allowed	10	2.2	7.2
Docetaxel (every 3 weeks)	30	1 prior cisplatin regimen, prior taxanes not allowed	13		9.0
Carboplatin–paclitaxel	44	1 prior platinum regimen within 1 year, prior taxanes not allowed	16	4	6
Gemcitabine	35	1 prior platinum regimen	22.5	5.0	
Gemcitabine	30	1 prior cisplatin regimen	11	4.9	8.7
Gemcitabine–paclitaxel	41	1 prior cisplatin regimen including perioperative therapy	60	14.4	
Pemetrexed	47	1 prior regimen including perioperative therapy within 12 months	27.7	2.9	9.6
Pemetrexed	12	1 prior regimen	8		
Vinflunine	51	1 prior platinum regimen	18	3.0	6.6
Ixabepilone	42	1 prior platinum regimen, prior taxane allowed	11.9	2.7	8.0

Drug	n	Eligibility	RR (%)	Median PFS (months)	Median OS (months)
Oxaliplatin	18	1 prior regimen for advanced disease, prior adjuvant chemotherapy >6 months earlier not counted	6		

Table 3: Reported Phase II trials of salvage chemotherapy for patients with advanced urothelial cancer. OS: Overall survival; PFS: Progression-free survival; RR: Response rate. From Sonpavde et al. 2008 [41]

In a series of over 340 bladder cancer samples, investigation of 16 tumor suppressor genes determined a nonrandom methylation pattern ($p < 0.0001$) and the suggestion of a CpG island methylator phenotype [42]. Methylation of death-associated protein (DAP) kinase in patients with superficial bladder cancer is associated with an increased risk of recurrence ($p < 0.001$) [43]. Methylation of *RASSF1A* in a series of 55 bladder tumors was detected in over 60% of the samples. In bladder cancer cell lines that do not express *RASSF1A*, treatment with decitabine resulted in gene re-expression [44]. However, a Phase II study of the histone deacetylase inhibitor SAHA in 14 patients with advanced TCC had limited efficacy with median disease-free survival and overall survival times of 1.1 and 4.3 months, respectively [45].

2.2 FdCyd

FdCyd is a fluoropyrimidine analogue which has been shown to inhibit the enzyme DNMT, thus inhibiting DNA methylation. FdCyd administered by itself to mice or humans suffers much the same fate as other fluoropyrimidines including 5-fluoro-2'-deoxyuridine (FdUrd), *in vivo*, such that the ubiquitous presence of thymidine and uridine phosphorylases causes rapid cleavage of FdUrd to FUra in most animals and in humans (Figure 1) [46].

However, a critical difference in the degradation of FdCyd, a cytosine nucleoside, is that it is not subject to cleavage of the glycosidic bond in mammals [47, 48]. An obligatory first step in its degradation is deamination by cytidine/deoxycytidine (Cyd/dCyd) deaminase. Although it was originally proposed that deamination of FdCyd to FdUrd was responsible for its activity (5), later work has clearly demonstrated that FdCyd can be phosphorylated to 5-fluoro-2'-deoxycytidylate (FdCMP) by deoxycytidine kinase and the nucleotide deaminated to 5-fluoro-2'-deoxyuridine 5'-monophosphate (FdUMP) by deoxycytidylate (dCMP) deaminase [49, 50]. The activity of dCMP deaminase is reported to be higher in common human malignancies than in most normal tissues, which suggests the possibility of selective cytotoxicity toward neoplastic cells [49].

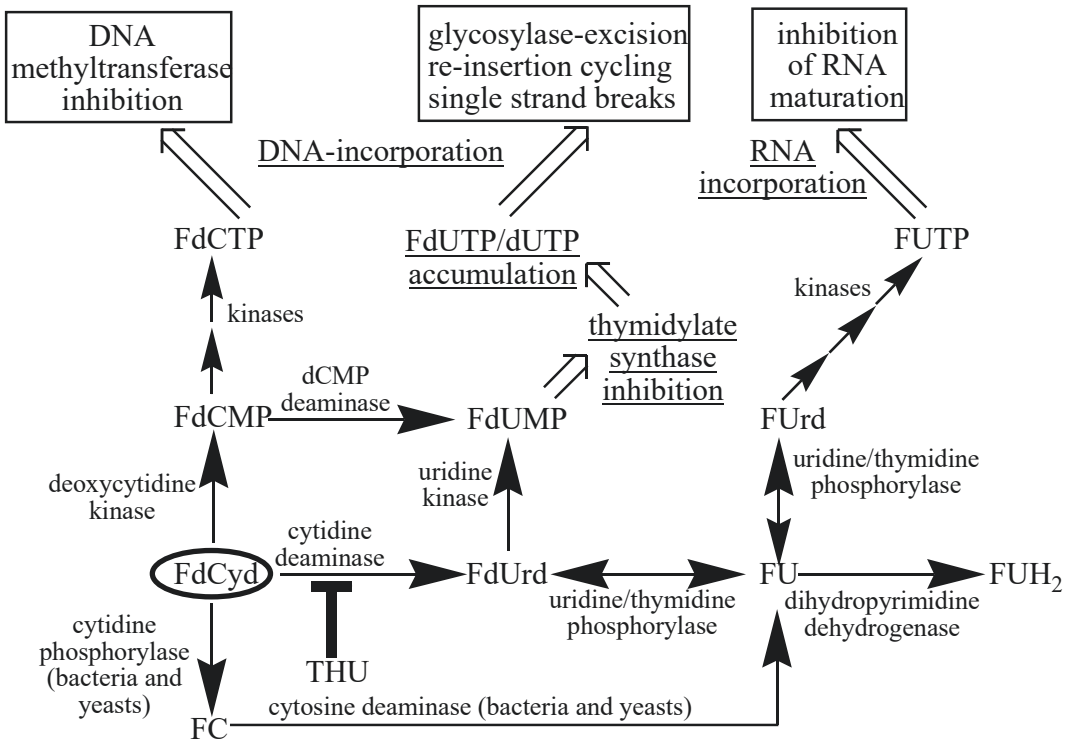

Figure 1: The metabolic pathway of FdCyd [51] (Courtesy: Drs. J. Beumer and M. Egorin; used with permission)

In addition to inhibition of thymidylate synthase, the incorporation of FdCyd into DNA may contribute to its antineoplastic activity. Incorporation correlated with cytotoxicity in the human breast carcinoma line MCF-7. It was suggested that inhibition of DNA methylation distinguishes the effects of FdCyd from the effects of FdUrd and fluorouracil [52]. The expression of genes for metabolic enzymes silenced by DNA methylation, including deoxycytidine kinase, may also be reactivated by inhibitors of DNA (cytosine-5)-methyltransferase [53]. It has been shown that oligonucleotides containing FdCyd form tight-binding ternary complexes with the methyl group from S-adenosylmethionine and purified DNA (cytosine-5)-methyltransferase *in vitro*, adding support to the hypothesis that this enzyme is an additional site of action for FdCyd [54].

2.3 THU

Because FdCyd is phosphorylated by deoxycytidine kinase, the deamination of the nucleoside FdCyd may be inhibited without limiting its metabolism to FdUMP or its incorporation into DNA. THU is an effective non-toxic inhibitor of Cyd/dCyd deaminase, both *in vitro* and *in vivo* [55]. THU has been shown to decrease deamination of the deoxycytidine analog 1-ß-D-arabinofuranosylcytosine (araC) in humans [56] and thereby to increase the potency of araC [57]. Either with or without THU, araC is only activated by deoxycytidine kinase. 1-ß-D-Arabinofuranosyluridine (araU), the deamination product of araC, may have some modulatory activity but is relatively non-toxic. Decreasing the concentration of araU, *per se*, is of little benefit to the patient. Consequently, the combination of araC and THU was qualitatively the

same as araC alone. A lower dose of araC was required for activity when combined with THU, but the spectrum of activity and toxicity was the same as for araC alone [57]. In contrast to araU, FUra is not an innocuous compound and reducing patient exposure to FUra may well reduce undesired side effects of treatment with FdCyd. Improvement in therapeutic indices have been shown during *in vivo* treatment of two murine neoplasms, Lewis lung carcinoma and adenocarcinoma-755 (both the solid and ascites forms); FdCyd with THU was more effective than equitoxic doses of FUra, FdUrd, or FdCyd alone [49]. The toxicities of FdCyd have been compared with those of FUra, FdUrd, 5-fluoroorotic acid, and 5-fluorouridine in mice, rats, cats, dogs, and *Rhesus* monkeys.

Co-administration of FdCyd with THU increases the AUC of FdCyd. Using a liquid chromatography/tandem mass spectrometry (LC-MS/MS) assay developed for the quantitation of THU in plasma, co-administration of THU (100 mg/kg) with FdCyd (25 mg/kg) decreased FdCyd clearance in mice by a factor of five relative to administration of FdCyd alone (from 2.1 to 0.4 mL/min); co-administration increased the percentage of parent FdCyd recovered in 16-hour (1.61% increasing to 35.6%) and 24-hour (1.94% increasing to 21.96%) urine collections [58, 59]. FdCyd in combination with THU has demonstrated *in vitro* and *in vivo* efficacy in breast, lung, and lymphoma models [49, 54].

2.4 Preclinical Studies

2.4.1 Animal Toxicology Studies

From the results of the animal studies, FdCyd is expected to produce in patients the same kinds of toxic effects caused by FdUrd and FUra. However, the LD50 of FdCyd relative to that of FdUrd differed depending on animal species. In mice, cats, and *Rhesus* monkeys, the two deoxynucleosides were approximately equitoxic. In dogs, FdCyd was 5 times more toxic than FdUrd; in rats, 14-times. The level of Cyd/dCyd deaminase activity is reported to be low in dogs and undetectable in rats [60].

We determined the toxicity and activity of 5 days of intravenous FdCyd, administered as a daily bolus with 100 mg/kg/day of THU, in CDF1 mice. The LD50 was estimated to be 31 mg/kg/day with a 95% confidence interval of 27 to 38 mg/kg/day. The highest non-lethal dose (25 mg/kg/day x 5) produced reversible toxicity, an 18% weight loss during treatment, and depletion of myeloid bone marrow elements. In a pilot toxicology study, animals were treated with 35 mg/kg/day for 5 days and necropsied 3 days after the last dose. Bone marrow hyperplasia was present, consistent with a compensatory response secondary to prior marrow destruction. Small and large intestinal epithelial atrophy with concomitant crypt epithelial regeneration was also consistent with compensatory response to prior intestinal epithelial destruction. Gastric glandular atrophy and lymphoid depletion of spleen and thymus glands was clearly evident. FdCyd was highly effective in the L5178Y/TK- tumor model often used to assess the activity of thymidylate synthase inhibitors in mice. Doses between 10 and 25 mg/kg/day resulted in 100% long-term survivors (LTS). Three of 4 mice treated with 5 mg/kg/day were LTS. Three of 12 mice treated with 2.5 mg/kg/day were LTS, and the dying animals had an increase in life span of 74%, compared with saline-treated, tumor-bearing controls (p=0.0001 by log rank test).

2.4.2 Human cell lines

In vitro time-dependence of the effect of FdCyd: *In vitro* experiments exposing human tumor cell lines to various concentrations of FdCyd for various periods of time indicated that exposure to FdCyd for periods of <1 week resulted in no discernable effect on DNA methylation by methylation-specific PCR, even at concentrations above 1 µM. However, changes in DNA methylation occurred with exposure times ≥2 weeks at concentrations of 1 µM and above (Table 4). In the human breast cancer cell line MCF-7, the 5'-promotor region of the glutathione S-transferase P1 gene (*GSTP1*) was heavily methylated and the mRNA for this gene was not detected by RT-PCR prior to treatment with FdCyd. There was a time-dependent decrease in DNA methylation and increase in mRNA expression of this gene during treatment of the MCF-7 cells with 1 µM FdCyd (E.M. Newman, unpublished observations; Figure 2).

Gene	SFN	RASSFIA	RARB	APC	HIN-1	PNRC1	TWIST1	P14	P16
Location	1p36.11	3p21.3	3p24	5q21-22	5q35-qter	6q15	7q21.2	9q21	9q21
RT-PCR	+	-	-	+	-	+	-	-	-
+DAC	+	+	+	+	+	+	+	-	-
+FdCyd	+	+	+	+	+	+	+	-	-

Gene	SYK	SNCG	GSTP1	CCND2	PYCARD	CDH1	HIC-1	BRCA1	TIMP3
Location	9q22	10q23	11q13	12p13	16q11.2	16q22.1	17p13.3	17q21	22q12.3
RT-PCR	-	+	+	+	+	-	+	+	+
+DAC	+	+	+	+	+	+	+	+	+
+FdCyd	+	+	+	+	+	+	+	+	+

Table 4: Hypermethylated-silenced genes become re-expressed following treatment with FdCyd. RT-PCR analysis (+, expressed; -, not expressed) of the human breast cancer cell line MDA-MB 231 following treatment with 1 µM FdCyd x 4 weeks [61].

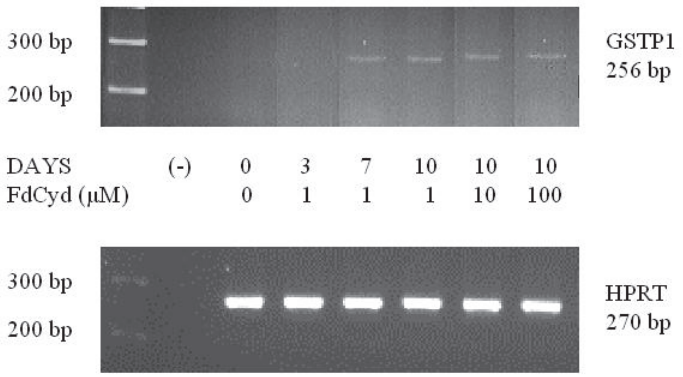

Figure 2: DNA methyltransferase inhibition by FdCyd. Exposure-dependent activation of the methylcytosine-silenced gene, *GSTP1*, at 1 µM FdCyd in MCF-7 cells; enhanced gene expression requires 7-10 days. The constitutively active HPRT gene was used as a control (Newman and Sowers; personal communication).

These *in vitro* findings are consistent with the ability of decitabine (DAC), another DNA (cytosine-5)-methyltransferase inhibitor, to induce fetal hemoglobin (HbF) in patients with sickle cell anemia in whom treatment with hydroxyurea did not increase HbF (28). In that clinical trial, patients were treated with DAC 5 days a week for 2 weeks, followed by 2 weeks off therapy and a second 2 weeks on DAC treatment. The percentage of HbF increased during the 2 weeks of treatment, remained stable during the 2 weeks off treatment, and rose again during the second 2 weeks of treatment, reaching a peak of 13% that persisted for 2 weeks before falling below 90% of the peak percentage. In the ongoing Phase I trial of FdCyd, preliminary pharmacokinetic (PK) analysis performed on plasma samples obtained near the end of the infusion from patients treated with 20, 40, and 80 mg/m^2/day indicated that all patients treated with 40 mg/m^2/day and above obtained plasma concentrations of FdCyd ≥ 1 μM. Increases in the percentage of HbF expression have been measured in patient samples from the ongoing Phase I trial (see Figure 5 below; Section 2.7.3).

2.5 Clinical studies

The NCI is a participating site on the current Phase I study of FdCyd and THU (06-C-0221; A Phase I Trial of 5-Fluoro-2'-Deoxycytidine with Tetrahydrouridine in Advanced Malignancies, PI Dr. James Doroshow). The trial was initiated in 1999, and the first 20 patients were treated with FdCyd + THU administered as an IV infusion over 3 hours along with the infusion of THU daily for 5 consecutive days of treatment in 21-day cycles. The daily dose of FdCyd was escalated geometrically in cohorts of 3 patients from 2.5 to 80 mg/m^2/day. The dose of THU was fixed at 350 mg/m^2/day. Eighteen patients completed at least one cycle and were evaluable for toxicity. There were no toxicities ≥ Grade 3 attributable to the treatment other than anemia and lymphopenia. Seven patients had stable disease after 2 cycles of treatment; 3 received additional cycles of FdCyd/THU (2 CRC; 1 NSCLC) [62].

Review of the PK data (see Section 2.5.1) and the available experimental data supported longer exposure times; thus, the schedule was changed from 1 week (daily x5) to 2 consecutive weeks (5 days per week for two weeks) of dosing, which is the regimen that will be explored in the current trial. The dose of 80 mg/m^2/day for 5 days q 21days was well tolerated. Therefore, to ensure patient safety, the dose was modified to 40 mg/m^2/day for 5 consecutive days of treatment per week for 2 consecutive weeks, q28 day cycles. Through the NCI RAID program, additional drug was made available, and patients enrolled on the revised schedule. Six patients were treated at this dose level followed by 3-6 patients at each subsequent dose level. To date, 29 patients have been treated on this regimen as part of the ongoing Phase I trial. Six patients have completed at least one cycle of therapy at the 134 mg/m^2/day dose level. Patients are currently being accrued to the 180 mg/m^2/day dose level. Toxicities have been generally mild, and have included Grade 1/2 nausea, vomiting, diarrhea, headache, fatigue; and grade 1 leucopenia and thrombocytopenia (see Section 7.1). There has been one DLT, a case of Grade 3 colitis at the 134 mg/m^2/day dose level that responded to conservative management.

The proposed starting dose for this trial, 100 mg/m^2/day, has been safely administered to 6 patients to date (ALT elevation observed in one patient was reversible and considered unlikely related to the study agents, see Section 2.5.3).

2.5.1 Pharmacokinetics

A hydrophilic-interaction liquid chromatographic-MS/MS method developed by Dr. Beumer and colleagues to simultaneously quantify FdCyd and downstream metabolites (FdUrd, 5-FU, FUrd, 5-FC) in mouse and human plasma [30] was employed to evaluate the PK of FdCyd in the first 20 patients on the Phase I study of FdCyd and THU. The end-of-infusion FU and FdUrd concentrations in plasma are <10% of those observed after therapeutic continuous infusions of FU or FdUrd. No FdCyd or metabolite was detected in day 5 pretreatment plasma. FdCyd concentrations increased with the dose of FdCyd (Figure 3). Peak plasma concentrations of FdCyd (mean ± SD) increased from 110 ± 50 nM in patients receiving 5 mg/m^2/day (n = 3) to 1.54 ± 0.17 µM in patients receiving 40 mg/m^2/day to 5.5 ± 0.9 µM in patients receiving 80 mg/m^2/day (n = 3) [63].

These results demonstrate that, at doses as low as 20 mg/m^2, the observed FdCyd concentrations exceeded that which has been shown to inhibit *in vitro* DNA methylation in MCF-7 cells (24-hour incubation) [52]. This suggests that the intended biological effect may occur at an FdCyd dose level that is below the MTD, which was not reached in this cohort. A more complete evaluation of the clinical pharmacokinetics of FdCyd + THU will be performed once accrual to this trial is completed and all data are available.

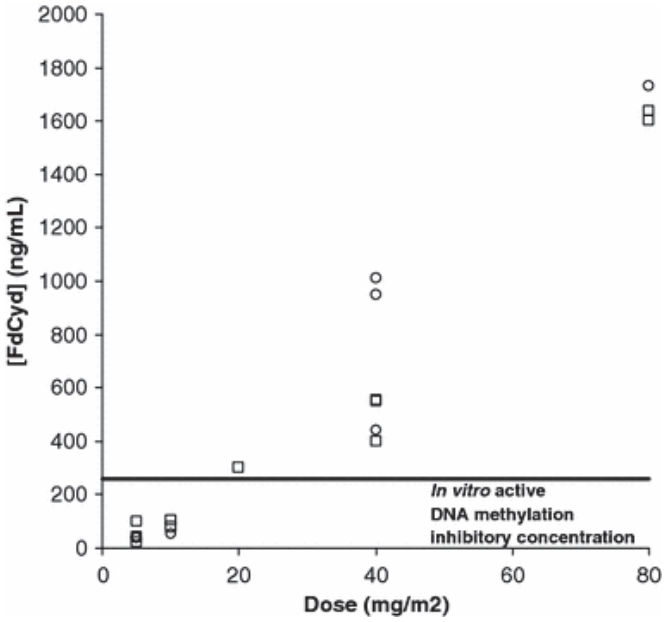

Figure 3: End-of-infusion plasma concentrations of FdCyd achieved after i.v. administration of various doses of FdCyd co-administered with 350 mg/m^2 THU on day 1 (*open square*) or day 5 (*open circle*) in patients participating in the ongoing Phase I trial of FdCyd and THU.

2.5.2 Clinical Efficacy

One heavily pretreated patient with metastatic breast cancer had a confirmed partial response (PR) documented by CT scan (> 90 % reduction in the sum of the target lesions) while receiving treatment on the Phase I protocol at the FdCyd dose level of 67 mg/m^2/day (Figure 4). Although significant clinical improvement was noted after 2 cycles of

treatment, the PR was obtained after 4 cycles, and continued shrinkage of the tumor was noted after 6 cycles. The patient maintained a PR for 16 months before progression. Figure 4 shows the pre- and post-treatment CT scans demonstrating the 90% reduction in tumor size as well as the observed improvement in skin involvement.

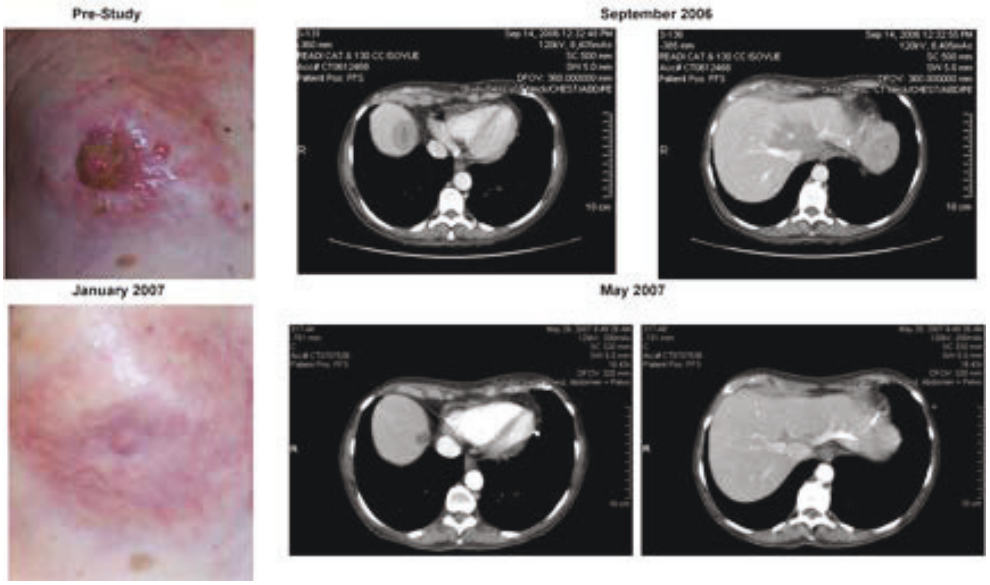

Figure 4: Pre- and post-treatment CT scans of a 61-year-old female with metastatic breast cancer who had received multiple chemotherapy and hormonal regimens including high-dose chemotherapy and autotransplant prior to enrolling on the Phase I trial of FdCyd +THU.

Data are available on 82 patients accrued to date (February 2016). The NSCLC cohort closed after 25 patients were accrued as the criteria for continuation after the first 20 patients (at least 7 instances of 4-month PFS) were not met. The head and neck cohort is also closed after enrolling 21 patients; clinical benefit was observed in six patients including one who achieved a partial response before coming off study after the 4th cycle for disease progression. Five other patients had stable disease, four for at least 4 cycles and one for 2 cycles.

To date, 22 evaluable patients have been accrued to the breast cancer cohort. Clinical benefit has been observed in 9 patients, including one with stage IV breast cancer who had stable disease for 5 cycles with progression after 7 cycles. Six other patients had stable disease for at least 2 cycles, and two have had confirmed partial responses, one of whom remained on study for 8 cycles before disease progression.

Of the 14 evaluable patients accrued to the urothelial transitional cell carcinoma cohort, two have had partial responses and two met the criteria for continuation (4-month PFS).

2.5.3 Toxicity Observed on Protocol 06-C-0221

Notable toxicities observed on the ongoing Phase I protocol, including the current dose level of 180 mg/m^2/day of FdCyd, include Grade 3 colitis at the 134 mg/m^2 dose level (DLT), which resolved with conservative management.

One patient developed an electrolyte imbalance that did not resolve immediately upon supplementation, but did resolve within 96 hours, at the FdCyd dose level of 40 mg/m^2/day.

Another patient developed an elevation of ALT to >5 times the upper limit of normal on the second cycle of treatment at the FdCyd dose level of 100 mg/m^2/day. The elevation in ALT was more likely due to concurrent medication with ondansetron, and it resolved without sequelae when both the ondansetron and the FdCyd/THU were stopped, but a contribution to the toxicity by FdCyd/THU cannot be ruled out entirely. Of note, this patient did not develop significantly elevated transaminases during a subsequent course of FdCyd/THU without ondansetron and did develop elevation of transaminases again when he received ondansetron as part of another chemotherapy regimen.

2.6 Rationale

The proposed trial is a Phase II study of FdCyd plus THU in patients with advanced non-small cell lung cancer, head and neck cancer, urothelial transitional cell carcinoma, or breast cancer. FdCyd is being evaluated as an antitumor agent due to its ability to inhibit DNA methylation, resulting in epigenetic remodeling. Results of the PK studies from the ongoing Phase I clinical trial of FdCyd and THU indicate that all patients treated with ≥ 40 mg/m^2/day achieved a plasma concentration of at least 1 μM FdCyd. Initial clinical results from the Phase I trial include a partial response (>90% reduction in target lesions) that lasted 16 months in one patient with extensively pre-treated breast cancer enrolled at the NIH. This patient was treated at the 67 mg/m^2/day dose level. The response seen in a patient with breast cancer as well as the activity of demethylating agents in a breast cancer xenograft model provide a rationale for testing the agent in this disease. Most of the patients expected to be accrued to this protocol will have failed multiple lines of treatment; their median PFS without therapy is not expected to be more than 2 months (3 months for patients with breast cancer). The study design will simultaneously discriminate, for patients with advanced non-small cell lung cancer, head and neck cancer, urothelial transitional cell carcinoma, or breast cancer, between tumor response rates of 20% vs. 5% or 4-month (6-month for the breast cancer stratum) PFS rates of 50% vs. 25% (corresponding to median PFS of 4 vs. 2 months; 6 vs. 3 months for the breast cancer stratum).

The growing evidence for the role of methylated genes in the pathogenesis of bladder, head and neck, and non-small cell lung cancer provide the basis for a trial of FdCyd/THU in patients with these diseases. Along with clinical response, effects of study treatment on methylation of CpG islands and gene re-expression will be evaluated.

2.7 Correlative Studies

2.7.1 LINE1 CpG Island Methylation by Pyrosequencing

Hypermethylation of CpG islands as a method of gene silencing is well-established, and it is clear that genes throughout the cancer genome can be hypermethylated. Dr. Allen Yang's laboratory at the University of Southern California (USC)/Norris Comprehensive Cancer Center has demonstrated that measurement of hypermethylation of CpG islands in Long Interspersed Nuclear Elements (LINE1 Sequences) is a reasonable method of determining overall levels of genome methylation [64]. The method employs primer sets capable of hybridizing to 12,000 + copies of LINE1 sequences in the human genome. These sequences harbor significant numbers of CpG islands. The state of methylation of these CpG islands is established by PCR of the genomic DNA with and without bisulfite pretreatment of the DNA, which allows discrimination of methylated from non-methylated sequences [65]. Information is obtained from the DNA by sequencing the PCR product on a pyrosequencing instrument, the Pyromark MD (Biotage AB, Uppsala, Sweden). The information consists of a short run of average sequence of the LINE1 CpG islands of 12,000 + genes, and not the sequence of individual genes; information collected actually reflects the inhibition of DNA methyltransferase activity on the bulk DNA in the presence or absence (pre-treatment measurements) of the drug.

The DCTD Pharmacodynamic Assay Development and Implementation Section (PADIS) has tested and confirmed the effect of the demethylating agent decitabine in altering DNA methylation on three CpG islands found in the LINE1 sequence in several nude mouse xenograft models (Table 5). Xenografts were implanted and staged to 150-200 mg before treatment. Decitabine or vehicle was administered IV for 10 consecutive days, with sampling of the subcutaneous tumors by 18-gauge needle biopsy under anesthesia on days 1 or 3, 5 or 7, and 9 or 10. Currently, the PADIS laboratory is setting up the pyrosequencing method at NCI-Frederick, and assay validation will be accomplished by transferring the assay into NCI from Dr. Yang's laboratory using a common set of specimens.

Xenograft Sensitivity to Decitabine: Percent Methylation Inhibition of 3 CpG Line1 Islands

		Days Post-Dose			
		Day 5	Day 7	Day 9	Day 10
Xenografts	Decitabine				
HL60	2 mg/kg	18.5*			32.4*
	1 mg/kg	18.9*			19*
	0.5 mg/kg	6.9			12.8*
IGROV-1	2 mg/kg		12.8*	18.2*	
	1 mg/kg		14.2*	17*	
	0.5 mg/kg		4.8	10.8*	
HCT-116	2 mg/kg	15.8*			6.66
	1 mg/kg	13.4*			17.8*
	0.5 mg/kg	6.3			14.3*
MDA-MB231	2 mg/kg	9.1*			0
	1 mg/kg	8.4*			4.9
	0.5 mg/kg	0			6.2

Table 5: Effect of the demethylating agent decitabine on LINE1 CpG Island methylation in 4 xenograft models. Xenografts are stack-ranked by demethylation response, but this ranking was concordant with decitabine effect on xenograft growth inhibition in all four models, which was dose-dependent. Asterisks (*) indicate statistically significant inhibition. Percent CV of the LINE1 pyrosequencing assay results was 5% or less in the vehicle control groups used to set the baseline for establishing statistical significance. No drug effect on methylation was observed at any dose level on day 1 or day 3 of decitabine treatment, consistent with the proposed mechanism of action of DNA methyltransferase inhibitors.

2.7.2 Measurement of Methylation-Silenced Gene Products by mRNA Transcript Assays

In addition to documented genome-wide alterations in CpG island methylation caused by inhibition of DNA methyltransferase activity, a number of specific genes have been reported to be silenced in cancer and re-expressed after treatment with DNMT1 inhibitors. One of these is E-cadherin; other targets of interest include alpha- and beta-catenin.

We have examined alterations in E-cadherin and catenin expression levels in the same HL-60 xenograft model (treated with decitabine) described above. Expression of E-cadherin was analyzed by RT-qPCR employing an assay validated for performance on the Taqman platform. Controls included actin and alpha- and beta-catenin, neither of which is silenced by CpG island methylation. Results are shown in Table 6, and indicate statistically significant upregulation of E-cadherin mRNA levels at Day 5, consistent with literature reports [66, 67].

Group	Day	Catenin A1	Catenin B1	E-cadherin-10	E-cadherin-15	huACTB
Vehicle	1	33.33	27.04	38.90	38.89	23.86
	5	33.83	26.97	38.34	38.18	23.64
	10	34.72	26.91	37.68	38.02	23.78
Decitabine:						
2 mg/kg	1	33.41	27.21	38.53	38.65	23.83
	5	34.03	28.39	36.55#	37.13	25.04*
	10	32.97	28.48	37.45	38.26	26.35*
1 mg/kg	1	32.98	26.81	38.20	38.56	23.33
	5	33.17	26.90	36.64#	36.92#	23.79
	10	33.09	28.07	37.87	38.63	25.29*
0.5 mg/kg	1	33.89	27.66	38.45	38.85	24.23
	5	34.57	28.60	37.39	37.58	25.01*
	10	33.55	27.76	38.38	38.73	24.99*

Table 6: Analysis of mRNA expression levels for specific genes in response to decitabine treatment. Results are the means for 4 mouse xenografts per treatment group at each time point in the HL60 model. Control vehicle (human actin; huACTB) Ct values by RT-qPCR were 23.76 +/- 0.436; control vehicle E-cadherin values were 38.35 +/- 0.685. Asterisks (*) indicate decreases in mRNA expression levels >2 SD from the mean vehicle. These decreases were observed in biopsy sections with evidence of substantial cell killing by immunohistochemistry, and further correlated with inhibition of tumor growth. Hash marks (#) indicate an increase in mRNA expression >2 SD from the mean vehicle.

Other mRNA assays for measuring re-expression of specific genes silenced by methylation are being explored in PADIS, and may be employed to analyze patient specimens if they have been analytically validated and demonstrated to correlate appropriately with LINE1 and xenograft growth curves in the preclinical modeling experiments described above. As of Amendment V (9/18/17), such additional mRNA assays validated include those for DAPK1, p16, MGMT, RASSF1a, and TIMP3. Furthermore, if sufficient tumor tissue is available, p16, DNMT1, and other relevant proteins may be assessed using validated immunofluorescence microscopy assays.

In addition, the Illumina RNA expression system has been optimized to search for alterations in expression profile of over 25,000 genes. These hybridization array chips typically require 100 ng RNA per chip, quantities which can be readily extracted from needle biopsies. As this technology measures RNA expression with pre-designed hybridization probes, sequence analysis is not performed. Instead, differences in the quantity of an mRNA are detected. The benefit of this approach is that it could enable investigators to sort out the difference between clinical responders and non-responders, which will help understand the pathways responsible for effective mechanism of action of FdCyd, and may also shed some light on pathways involved in toxicity of the compound. Expression analysis by this method will be performed in Dr. Paul Meltzer's laboratory (NCI/CCR).

2.7.3 Measurement of Fetal Hemoglobin Gene Re-expression in Blood

Fetal hemoglobin gene expression is silenced perinatally by CpG island methylation, and has been reported to be re-expressed in response to decitabine treatment [68]. A quantitative real-time RT-PCR method of analysis of γ- and β-globin mRNAs in primary human erythroid cells has been developed [69], and we have preliminary data indicating that this quantitative method can be applied to RNA extracted from peripheral RBC. We obtained RBC samples diluted in RNAzol for RNA isolation and analysis. Preliminary results from the ongoing Phase I study of FdCyd plus THU are presented in Figure 5.

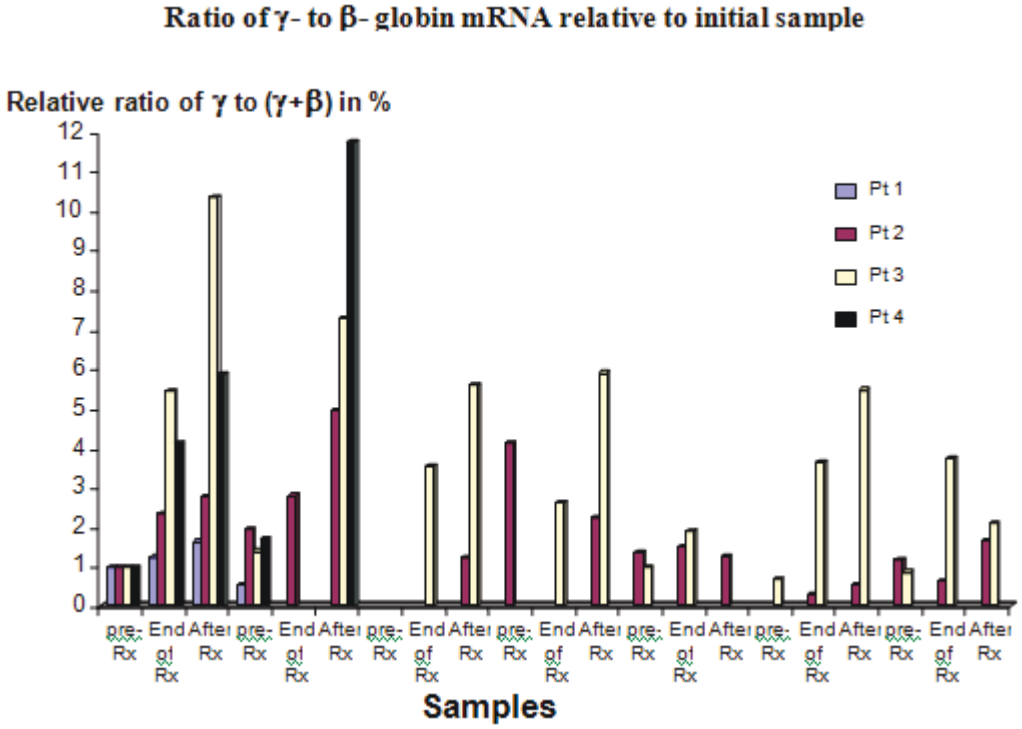

Figure 5: RT-PCR data showing changes in fetal (γ) hemoglobin expression in 4 patients on the ongoing Phase I trial of FdCyd +THU. Results are presented relative to pretreatment (baseline) values and confirm that the silenced fetal hemoglobin gene is reactivated following FdCyd treatment. *Note:* isolation of RBCs as an exploratory analysis of fetal hemoglobin expression levels was suspended with Amendment E (3/16/11).

2.7.4 Measurement of Circulating Tumor Cells (CTC) in Peripheral Blood

CTCs may provide information on disease progression and response to therapy in patients with advanced cancer [70, 71]. In Figure 6, the number of CTCs in blood from 2 patients treated with topotecan is superimposed on the percentage of CTCs expressing γ-H2AX—one of the earliest markers of DNA double-strand breaks. Levels of γ-H2AX can be used as a dosimeter and biomarker for drug-induced DNA damage.

We propose to use the Veridex CellSearch instrument system, which can detect a single CTC in 7.5 mL of patient blood, to measure changes in the number of CTCs in patients on this study. This device has been approved by the FDA for monitoring response to therapy in patients with breast, prostate, and colon cancer; other cancer indications are being evaluated. The instrument is already supporting prostate vaccine trials in the CCR's Laboratory of Tumor Immunology and Biology with the goal of developing a large enough patient database within NCI to understand the best use of the device in monitoring patient response during clinical trials. CTCs are being collected from patients in the Developmental Therapeutics Section Clinic to obtain data on CTC count variability.

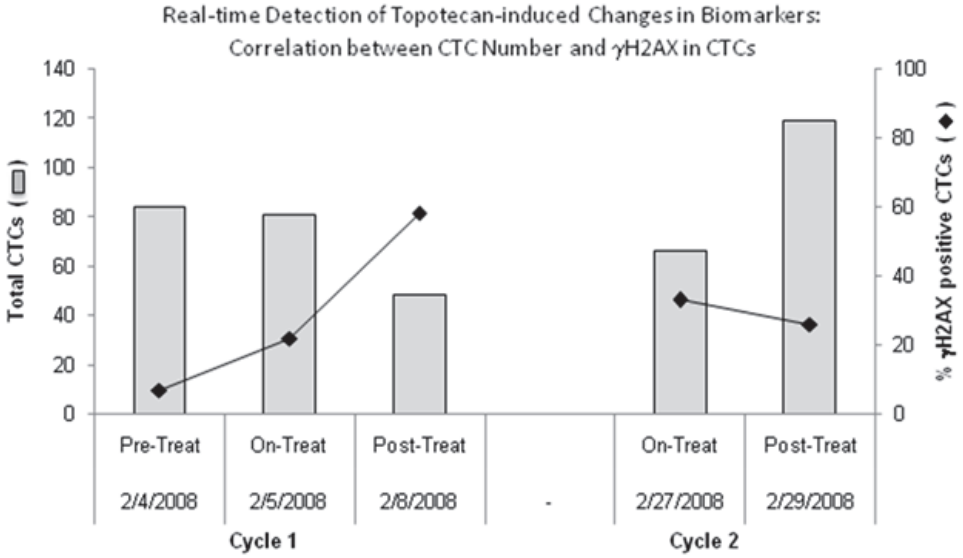

Figure 6: The number of CTCs in blood from a patient treated with topotecan (bars) is superimposed with the percentage of CTCs expressing γ-H2AX (diamond symbols).

A further important application of collecting CTCs in trials is their potential as tumor biopsy surrogates to measure drug effects on molecular targets. The number of CTCs at the various timepoints, if measurable, will be correlated in a pilot fashion with clinical information on patient response to drug therapy (Section 9.0).

Preliminary PD data from this trial indicated that 17 (33%) of 52 patients had more than one CTC at baseline, 15 (29%) patients had detectable CTCs after treatment (but not baseline), and 20 (39%) had no measurable CTCs at any time point.

3 Patient Selection

3.1 Eligibility Criteria

- Patients must have histologically documented metastatic or unresectable non-small cell lung cancer, head and neck cancer, urothelial transitional cell carcinoma, or breast cancer whose disease has progressed after at least one line of standard therapy.

- Patients with solid tumors (non-small cell lung cancer, head and neck cancer, urothelial transitional cell carcinoma, and breast cancer) must have measurable disease, defined as at least one lesion that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥ 20 mm with conventional techniques or as ≥ 10 mm with spiral CT scan. Patients with the above tumor types whose disease is limited to the skin are eligible at the discretion of the PI and must have a physical exam with documentation of skin lesion(s) by color photography, including a ruler to estimate the size of the lesion(s).
- Diagnosis of malignancy must be confirmed by the department of pathology at the institution where the patient is enrolled prior to patient enrollment.
- Any prior therapy must have been completed ≥ 4 weeks prior to enrollment on protocol and the participant must have recovered to eligibility levels from prior toxicity. Patients should be at least six weeks out from nitrosoureas and mitomycin C. Prior radiation should have been completed ≥ 4 weeks prior to study enrollment and all associated toxicities resolved to eligibility levels. Patients must be ≥ 2 weeks since any investigational agent administered as part of a Phase 0 study (also referred to as an "early Phase I study" or "pre-Phase I study" where a sub-therapeutic dose of drug is administered) at the PI's discretion, and should have recovered to eligibility levels from any toxicities.
- Age ≥ 18 years. Because no dosing or adverse event data are currently available on the use of FdCyd and THU in patients < 18 years of age, children are excluded from this study, but may be eligible for future pediatric Phase I combination trials.
- Karnofsky performance status ≥ 60%, see Appendix A.
- Life expectancy of greater than 3 months.
- Patients must have normal organ and marrow function as defined below:

– absolute neutrophil count	≥1,500/mcL3.4.1
– platelets	≥100,000/mcL
– total bilirubin	<1.5 X institutional upper limit of normal
– AST(SGOT)/ALT(SGPT)	≤ 3 X institutional upper limit of normal; ≤ 5 X ULN for patients with liver metastases
– creatinine **OR**	<1.5 X institutional upper limit of normal
– creatinine clearance	≥60 mL/min for patients with creatinine levels above 1.5 X institutional upper limit of normal.

- Because FdCyd has been shown to be teratogenic in animals, pregnant women will be excluded from this trial. Nursing women are also excluded, as there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with FdCyd. Women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) for the duration of study participation, and for 3 months after completion of study. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she or her partner should inform the treating physician immediately.

- Ability to understand and the willingness to sign a written informed consent document.
- Patients should not be receiving any other investigational agents.

3.2 Exclusion Criteria

- Patients with clinically significant illnesses which would compromise participation in the study, including, but not limited to: active or uncontrolled infection, immune deficiencies or confirmed diagnosis of HIV infection, active infection with Hepatitis B or Hepatitis C, uncontrolled diabetes, uncontrolled hypertension, symptomatic congestive heart failure, unstable angina pectoris, myocardial infarction within the past 6 months, uncontrolled cardiac arrhythmia; or psychiatric illness/social situations that would limit compliance with study requirements.
- History of allergic reactions attributed to fluoropyrimidines (e.g., capecitabine, fluorouracil, fluorodeoxyuridine) or tetrahydrouridine.

3.3 Research Eligibility Evaluation

3.3.1 Clinical Evaluations

- A complete history and physical examination will be completed within 8 days prior to subject enrollment. This will include evaluation of measurable disease and determination of performance status.
- Diagnostic imaging studies must be performed within 28 days prior to enrollment.

3.3.2 Laboratory Evaluations

Eligibility evaluation laboratory tests should be performed within 8 days prior to subject enrollment unless stated otherwise.

- Hematological profile: CBC with differential and platelet count
- Biochemical profile: electrolytes, BUN, creatinine, glucose, AST, ALT, bilirubin, calcium, phosphorous, albumin, magnesium, alkaline phosphatase, LDH
- Urine pregnancy test in women of childbearing potential

3.3.3 Pathology Review

A block or stained slides of primary tissue from the time of diagnosis will be required from each study subject to confirm diagnosis. Tissue blocks from a known recurrence will be accepted if the original tumor samples are unavailable. Diagnosis of malignancy must be confirmed by the department of pathology at the site where the patient is enrolled.

3.4 Patient Registration

Eligible participants will be entered on study by a member of the Coordinating Center study team.

3.4.1 Coordinating Site Registration Process

On Study: Authorized staff must register an eligible candidate with NCI Central Registration Office (CRO) within 24 hours of signing consent. A registration Eligibility

Checklist from the Web site (http:// home.ccr.cancer.gov/intra/eligibility/welcome.htm) must be completed and sent via encrypted email to: NCI Central Registration Office (HOIS) <ncicentralregistration-l@mail.nih.gov>. After confirmation of eligibility at the Central Registration Office, CRO staff will call pharmacy to advise them of the acceptance of the patient on the protocol prior to the release of any investigational agents. Verification of Registration will be forwarded electronically via e-mail. Please note: it is very important for all registrars to acquire encrypted e-mail from NIH Help Desk, since the verification of registration includes patient's information. A recorder is available during non-working hours.

3.4.2 Participating Site Registration

All patients must be registered through the NCI Central Registration Office (CRO) via the Coordinating Center. A protocol registration form will be completed by the Coordinating Center, NCI CCR. To register a subject after the participant has signed consent, complete Appendix H (Eligibility/Pre-Registration Worksheet) and fax or email the completed Appendix H along with all supporting records to the Coordinating Center's Research Nurse: Jennifer Zlott, Fax: (301) 480-7281, zlottjh@mail.nih.gov. The Coordinating Center will notify you either by e-mail or fax that the protocol registration form has been received. The Coordinating Center will register the patient and provide the participating site with the patient's unique patient/subject ID number. This unique ID number is to be used on all research samples and data entry for this subject. Questions about eligibility should be directed to the Coordinating Center's Research Nurse.

3.5 Off Protocol Therapy and Off-Study Procedure

NCI Clinical Center: Authorized staff must notify the Central Registration Office when a patient is taken off protocol therapy and when a patient is taken off-study. A Participant Status Updates Form from http://home.ccr.cancer.gov/intra/eligibility/welcome.htm must be completed and sent via encrypted email to: NCI Central Registration Office (HOIS) ncicentralregistration-l@mail.nih.gov.

Participating sites: Authorized staff must notify the Coordinating Center Central Registration Office (CRO) when a patient is taken off protocol therapy and when a patient is taken off-study. The Participant Status Updates Form included in Appendix H must be completed and sent via encrypted email to: NCI Central Registration Office (HOIS) (ncicentralregistration-l@mail.nih.gov) **and** faxed or emailed to the Coordinating Center's Research Nurse, Jennifer Zlott, Fax: (301) 480-7281, zlottjh@mail.nih.gov.

4 Treatment Plan

This is a multicenter Phase II trial of FdCyd and THU in patients with breast cancer, head and neck cancer, non-small cell lung cancer, and urothelial transitional cell carcinoma who have failed at least one prior line of therapy (Section 3.1).

Patient evaluations will be performed throughout the study as described below. Baseline history, physical examination, and laboratory evaluations must be conducted within 8 days prior to start of protocol therapy. If protocol therapy is started within 8 days of the eligibility screening evaluations (see Section 3.3.1 and Section 3.3.2), the results from these screening evaluations may be used as baseline measurements. If >8 days have passed since the screening evaluations, the medical history, physical examination, and laboratory evaluations must be repeated prior to starting protocol therapy. Baseline tumor imaging must be performed within 28 days prior to start of protocol therapy. If protocol therapy is started within 28 days of the eligibility screening tumor imaging, the screening evaluation imaging results may be used as baseline measurements; if >28 days have passed since the screening evaluation tumor imaging, the imaging must be repeated prior to starting protocol therapy.

History and physical exam will be performed at baseline (within 8 days prior to the start of protocol therapy), and physical exams will be performed again during weeks 1 and 2 of cycle 1, and at the start of every cycle thereafter (within 3 days prior to treatment). The patient will be contacted by phone during weeks 3 and 4 of cycle 1. Labs (CBC with differential; serum chemistries, including glucose) will be performed at baseline (within 8 days prior to the start of protocol therapy), weekly during cycle 1, and at the start (within 3 days prior to treatment) and during weeks 1 and 2 of every cycle thereafter. Samples for correlative studies will be collected as described in Section 9. Appropriate imaging studies will be performed at baseline (within 28 days prior to the start of protocol therapy), and repeat imaging scans will be performed every 2 cycles for disease restaging (every 3 cycles for patients on study for more than one year, every 4 cycles for patients on study more than 3 years).

4.1 FdCyd and THU Administration

FdCyd will be administered as an IV infusion over 3 hours with 20% of the daily dose of THU administered as an IV push and the remaining 80% of the daily dose co-administered with FdCyd over a 3-hour infusion, daily for 5 consecutive days of treatment per week, for 2 consecutive weeks, followed by 2 weeks of no treatment. This means treatment will be administered on days 1-5 and 8-12 of each cycle, +/- 1 day for scheduling reasons (e.g., weekends, holidays, or patient convenience). Additionally, 1 or 2 doses may be missed if they fall on a holiday, and treatment may be delayed up to 2 weeks for holidays or patient convenience after discussion with the coordinating center. The dose of THU is fixed at 350 mg/m^2/day. The dose of FdCyd is 100 mg/m^2/day and will not be escalated. The cycle length will be 28 days. Dose modifications are outlined in Section 5.

Patients will be treated at 100 mg/m^2 FdCyd, a dose shown to be well tolerated at the same schedule in 6 patients in the ongoing Phase I study.

Blood and urine samples for PK analysis will be obtained from a total of 15 patients accrued during Cycle 1 only. Samples will be collected from 3 patients with each of the indications listed below except urothelial transitional cell carcinoma; samples will be collected from 6 of the first 12 patients with urothelial transitional cell carcinoma as the presence of ileal loops or neobladders in this population may have an impact on drug pharmacokinetics (Section 9.0).

Blood and optional tumor biopsies will be obtained from all patients to evaluate drug effect (Section 9.0).

Patients with the indications listed below whose disease has progressed after at least one line of therapy are eligible to participate per Section 3.1 (Eligibility Criteria).

A Non-Small Cell Lung Cancer
B Urothelial Transitional Cell Carcinoma
C Head and Neck Cancer
D Breast Cancer

Patients will also be required to keep a study diary (Appendix B) to record any side effects, and any concurrent medications taken.

5 Dosing Delays/Modifications

5.1 Dose Modifications

Toxicities should have resolved to ≤ Grade 2 (except lymphopenia) prior to starting the next cycle. Treatment may be delayed for a maximum of 2 weeks beyond the actual cycle length of 28 days; in case toxicities do not resolve to Grade 2 or less, the patient will not receive further therapy on this protocol and will be followed for resolution of toxicities.

Treatment will be held for all Grade 3 toxicities (hematological and non-hematologic, with the exception of electrolyte abnormalities and lymphopenia) until resolved to Grade 2 (except lymphopenia), regardless of when the toxicity occurs during the cycle.

5.1.1 Grade 2 drug-related toxicity: No changes will be made to the doses of FdCyd and THU.

5.1.2 Grade 3-4 drug-related non-hematologic toxicities: Doses of both FdCyd and THU will be held until toxicities recover to ≤ Grade 2 (with the exception of electrolyte abnormalities). For the first occurrence of Grade 3 drug-related toxicity, study drugs can be re-initiated at the same dose level. For second occurrence, the dose of FdCyd will be reduced by one dose level while THU will remain at 350 mg/m2/day. For Grade 4 drug-related toxicities, FdCyd will be re-initiated at the next lower dose level while THU will remain at 350 mg/m2/day. Electrolyte abnormalities will not require dose reduction if resolution to Grade 2 or less is documented within 24 hours. A maximum of 2 dose reductions of FdCyd will be allowed before a patient is taken off study. Dose modifications for nausea, vomiting and diarrhea will be made only if it is refractory to treatment (refer to Section 5.3).

5.1.3 Grade 3 and 4 drug-related hematologic toxicities: FdCyd will be re-initiated at the next lower dose level while THU will remain at 350 mg/m2/day (except leucopenia with neutropenia <4 days or any degree of anemia or lymphopenia). In case of leucopenia with neutropenia <4 days or any degree of anemia or lymphopenia, study drugs will be re-initiated at the same dose level.

5.2 Dose Reduction

For the purposes of dose modification, the dose will be reduced to the next lower dose level as indicated in the table below. Patients who have had their dose de-escalated should have correlative studies performed at the new dose, if feasible. No more than 2 dose reductions will be allowed.

Dose Modification Table

Dose Level	FdCyd Dose	THU dose
-2	67 mg/m^2/day	350 mg/m^2/day
-1	80 mg/m^2/day	350 mg/m^2/day
1	100 mg/m^2/day	350 mg/m^2/day

5.3 General Concomitant Medication and Supportive Care Guidelines

All patients will be provided with the best available supportive care.

5.3.1 Vomiting

Nausea and vomiting will be considered refractory if it does not resolve to ≤ Grade 1 with treatment with a combination of at least 2 antiemetics.

5.3.2 Diarrhea

If diarrhea develops and does not have an identifiable cause other than study drug administration, anti-diarrheals such as Lomotil (diphenoxylate HCl 2.5 mg + atropine sulfate 0.025 mg/tablet) dosed according to package insert or loperamide 4 mg po after the first unformed stool with 2 mg po every 2 hours as long as unformed stools continue (4 mg every 4 hours while asleep). No more than 16 mg of loperamide should be taken in during a 24-hour period. This regimen can be repeated for each diarrheal episode. Diarrhea will be considered refractory if it does not resolve within 24 hours ≤ to Grade 2 with the above regimen (16 mg, or less if there is resolution of the symptoms, of loperamide in a 24-hour period).

5.3.3 Electrolyte Abnormalities

If hypokalemia, hypophosphatemia, or hypomagnesemia occur, the patient may receive oral or IV supplementation to correct the abnormality. If hyponatremia occurs, the patient may receive 0.9% Sodium Chloride intravenously to correct the abnormality.

5.3.4 Neutropenia

Febrile neutropenia is a life-threatening complication requiring hospitalization and urgent broad-spectrum antibiotics, as well as an aggressive search for the source and microbial cause of the episode. If clinically indicated, filgastrim will be administered per accepted guidelines (ASCO or Clinical Center). Study medications will not be reinitiated until at least 24 hours after filgastrim administration.

5.3.5 Anemia

Symptomatic anemia should be treated with red blood cell infusion and is recommended if the hemoglobin falls below 8 mg/dL. Use of erythropoeitin is allowed per accepted guidelines.

5.3.6 Thrombocytopenia

Thrombocytopenia will be treated conservatively. In the absence of bleeding, fever, or a necessary invasive procedure, platelet transfusions should be given for a platelet count $\leq 10{,}000/mm^3$. In case the patient is febrile or has other evidence of infection, platelet transfusions should be given for a platelet count $\leq 20{,}000/mm^3$. If invasive procedure(s) are planned or the subject develops bleeding, platelet transfusions should be administered in accordance with standard of practice, usually maintaining a platelet count above $50{,}000/mm^3$.

5.4 Duration of Therapy

In the absence of treatment delays due to adverse event(s), subjects may continue on the study as long as they are tolerating the drugs and responding to the treatment, or until one of the following criteria are met:

- Disease progression,
- Intercurrent illness that prevents further administration,
- More than 2 dose reductions required for toxicity (as described in Section 5.1)
- Patient decides to withdraw from the study, or
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the opinion of the principal investigator.

5.5 Duration of Follow-up

Patients will be followed for 30 days after the last dose is administered or until one of the following occurs: patient enrolls on another protocol, patient receives standard of care, or death, whichever comes first. The follow-up will consist of a phone call between Days 27-30 after the last dose to evaluate adverse events that were ongoing and any new events that might be deemed related to the therapy. Unacceptable toxicities (i.e., AEs related to the intervention) that have not resolved by Day 30 post-treatment will be followed via biweekly phone calls until stabilization or resolution.

5.6 Criteria for Removal From Study

Patients will be removed from study for one of the following reasons: completed 30-day follow-up period, toxicities are unresolved but stabilized, patient enrolls on another protocol, or patient receives standard of care. The reason for study removal and the date the patient was removed must be documented in the medical record.

6 Human Subjects Protection

6.1 Rationale for Subject Selection

This study will be open to all individuals regardless of gender, ethnicity, or race provided that the aforementioned inclusion and exclusion criteria are met. For safety reasons, pregnant women and children are excluded from this study. The NCI is the coordinating center for this multi-institutional study. This study will be recruited through internal referral, our physician referral base, and through various cancer information hotlines (i.e., Clinical Studies Support Center, 1-800-4Cancer.) Participants should realize that there is no guarantee of benefit to them from participation in this trial. The results of this trial may benefit future cancer patients. To date, there is no information that suggests that differences in drug metabolism or effect on tumor would be expected in one ethnic group compared to another. Efforts will be made to extend accrual to each representative population, but a balance must be struck between participant safety considerations and limitations on the number of individuals exposed to potentially ineffective treatments on the one hand and the need to explore racial/ethnic aspects of clinical research on the other hand. If differences in outcome that correlate to ethnic identity are noted, a follow-up study may be written to investigate those differences more fully.

Inclusion of Women and Minorities:
This study will be open to all individuals regardless of gender, ethnicity, or race provided that the aforementioned inclusion and exclusion criteria are met.

6.2 Justification for Exclusions

Because FdCyd has been shown to be teratogenic in animals, pregnant women will be excluded from this trial. Nursing women are also excluded, as there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with FdCyd. Participants with unstable or serious medical conditions (active or uncontrolled infection, immune deficiencies or confirmed diagnosis of HIV infection, Hepatitis B, Hepatitis C, or uncontrolled diabetes, uncontrolled hypertension, symptomatic congestive heart failure, unstable angina pectoris, myocardial infarction within the past 6 months, uncontrolled cardiac arrhythmia; or psychiatric illness/social situations that would limit compliance with study requirements) are excluded due to the possibility that the underlying condition may obscure the attribution of effect and adverse events with respect to the combination of FdCyd with THU and may limit study compliance.

6.3 Participation of Children

Participants under the age of 18 will be excluded from study because no dosing or adverse event data are currently available for the use of FdCyd and THU in participants <18 years of age.

6.4 Evaluation of Benefits and Risks/Discomforts

There may or may not be any clinical benefit to a patient from participation in this trial. Their participation will benefit future cancer patients. Potential risks include the possible occurrence of any of a range of side effects that are listed in the consent document. The procedure for

protecting against or minimizing risks will be to medically evaluate patients as described in protocol Section 6.6. Although no compensation is available, any injury will be fully evaluated and treated in keeping with the benefits or care to which participants are entitled under applicable regulations.

6.5 Consent and Assent Process and Documentation

An associate or principal investigator on the trial will inform patients of the purpose, alternatives, drug administration plan, research objectives and follow-up of this trial. The patient will be provided an IRB-approved consent for review and signature and his/her questions will be answered. After a decision is made to enroll into the study, a signature will be obtained from the patient. The original signed consent goes to Medical Records; a copy will be placed in the research record.

All patients must have a signed informed consent form and an on-study (confirmation of eligibility) form filled out and signed by a participating investigator before entering on study.

For NCI only: adults unable to give consent are excluded from enrolling in the protocol. However, re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation, all subjects ≥ age 18 will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. Note: The PI or AI will contact the NIH Ability to Consent Assessment Team for evaluation. For those subjects that become incapacitated and do not have pre-determined substitute decision maker, the procedures described in MEC Policy 87-4 for appointing a surrogate decision maker for adult subjects who are (a) decisionally impaired, and (b) who do not have a legal guardian or durable power of attorney, will be followed.

6.6 Procedure for Protecting Against or Minimizing Any Potential Risks

All care will be taken to minimize side effects, but they can be unpredictable in nature and severity. This study may involve risks to patients, which are currently unforeseeable. All patients will be monitored for side effects from taking study medication and from undergoing blood sampling procedures. The research component of this study required to obtain 2 CT tumor biopsies confers radiation exposure at an effective dose of 1.6 rem. This dose is below NIH RSC guidelines for adults of 5.0 rem per year and represents a slightly greater than minimal risk to patients.

6.6.1 Patient Advocate

The patients' rights representative is available to patients receiving treatment on this protocol at the NIH Clinical Center at (301) 496-2626 in Building 10 of the Clinical Research Center, Room 1-3521, on the Bethesda NIH campus. Patients enrolled at other sites will be given information regarding their local patient advocate. Patients will be informed that they can contact the study PI or RN at any time with questions about their

medical care, and that the patients' rights representative is also available to answer non-medical questions about the study.

7 Adverse Events: List And Reporting Requirements

7.1 Comprehensive Adverse Events and Potential Risks Lists (CAEPRs) for 5-Fluoro-2'-deoxycytidine (FdCyd, NSC 48006) with Tetrahydrouridine (THU, NSC 112907)

7.1.1 CAEPRs for CTEP-Supplied Investigational Agent(s)

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 (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification. *Frequency is provided based on 166 patients.* Below is the CAEPR for 5-Fluoro-2'-deoxycytidine with tetrahydrouridine (FdCyd/THU).

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

Version 2.2, January 17, 2014[1]

Adverse Events with Possible Relationship to 5-Fluoro-2'-deoxycytidine with tetrahydrouridine (FdCyd/THU) (CTCAE 4.0 Term) [n= 166]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
Anemia			***Anemia (Gr 2)***
	Febrile neutropenia		
CARDIAC DISORDERS			
	Sinus tachycardia		***Sinus tachycardia (Gr 2)***
GASTROINTESTINAL DISORDERS			
	Abdominal distension		
	Abdominal pain		***Abdominal pain (Gr 2)***
	Constipation		***Constipation (Gr 2)***
Diarrhea			***Diarrhea (Gr 2)***
	Dry mouth		
	Dyspepsia		
	Flatulence		
		Ileus	
	Mucositis oral		***Mucositis oral (Gr 2)***
Nausea			***Nausea (Gr 2)***
Vomiting			***Vomiting (Gr 2)***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
	Chills		***Chills (Gr 2)***

	Edema limbs		
Fatigue			***Fatigue (Gr 2)***
	Fever		***Fever (Gr 2)***
	Non-cardiac chest pain		
	Pain		
INVESTIGATIONS			
	Activated partial thromboplastin time prolonged		***Activated partial thromboplastin time prolonged (Gr 2)***
Alanine aminotransferase increased			***Alanine aminotransferase increased (Gr 2)***
Alkaline phosphatase increased			***Alkaline phosphatase increased (Gr 2)***
Aspartate aminotransferase increased			***Aspartate aminotransferase increased (Gr 2)***
	Blood bilirubin increased		***Blood bilirubin increased (Gr 2)***
	Cholesterol high		
	Creatinine increased		***Creatinine increased (Gr 2)***
Lymphocyte count decreased			***Lymphocyte count decreased (Gr 2)***
Neutrophil count decreased			***Neutrophil count decreased (Gr 2)***
Platelet count decreased			***Platelet count decreased (Gr 2)***
	Weight loss		***Weight loss (Gr 2)***
White blood cell decreased			***White blood cell decreased (Gr 2)***
METABOLISM AND NUTRITION DISORDERS			
Anorexia			***Anorexia (Gr 2)***
	Dehydration		***Dehydration (Gr 2)***
	Hypercalcemia		
	Hyperglycemia		***Hyperglycemia (Gr 2)***
	Hyperkalemia		
	Hypermagnesemia		
Hypoalbuminemia			***Hypoalbuminemia (Gr 2)***
	Hypocalcemia		***Hypocalcemia (Gr 2)***
	Hypokalemia		***Hypokalemia (Gr 2)***
	Hypomagnesemia		***Hypomagnesemia (Gr 2)***
Hyponatremia			***Hyponatremia (Gr 2)***
	Hypophosphatemia		***Hypophosphatemia (Gr 2)***
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Arthralgia		
	Generalized muscle weakness		
	Myalgia		
NERVOUS SYSTEM DISORDERS			
	Dizziness		***Dizziness (Gr 2)***
	Dysgeusia		
	Headache		***Headache (Gr 2)***
	Peripheral motor neuropathy		***Peripheral motor neuropathy (Gr 2)***
	Peripheral sensory		

	neuropathy		
RENAL AND URINARY DISORDERS			
	Proteinuria		
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Cough		
	Dyspnea		***Dyspnea (Gr 2)***
	Epistaxis		
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
	Alopecia		
	Dry skin		
	Hyperhidrosis		
	Palmar-plantar erythrodysesthesia syndrome		
	Pruritus		
	Rash maculo-papular		
VASCULAR DISORDERS			
	Hypotension		***Hypotension (Gr 2)***

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

Also reported on FdCyd/THU trials but with the relationship to FdCyd/THU still undetermined:

CARDIAC DISORDERS - Aortic valve disease; Atrial fibrillation; Cardiac arrest; Mitral valve disease; Myocardial infarction; Palpitations; Pericardial effusion; Pericarditis; Tricuspid valve disease; Ventricular tachycardia
EAR AND LABYRINTH DISORDERS - Ear pain; Middle ear inflammation; Tinnitus
EYE DISORDERS - Blurred vision; Conjunctivitis; Dry eye; Eye disorders - Other (right eye blindness [90-95% visual acuity loss]); Eye disorders - Other (subconjuctival hemorrhage); Eye disorders - Other (visual changes); Eye pain; Floaters; Photophobia; Watering eyes
GASTROINTESTINAL DISORDERS - Bloating; Colitis; Colonic obstruction; Duodenal ulcer; Dysphagia; Esophagitis; Gastrointestinal disorders - Other (pyloric ulcer); Gastrointestinal pain; Hemorrhoids; Lip pain; Oral pain; Rectal hemorrhage; Typhlitis; Upper gastrointestinal hemorrhage
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Edema face; Flu like symptoms; Infusion site extravasation; Injection site reaction; Localized edema; Malaise
INFECTIONS AND INFESTATIONS - Enterocolitis infectious; Eye infection; Lung infection; Mucosal infection; Sepsis; Skin infection; Upper respiratory infection
INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Bruising; Vascular access complication
INVESTIGATIONS - CPK increased; Hemoglobin increased; INR increased; Investigations - Other (bicarbonate)
METABOLISM AND NUTRITION DISORDERS - Alkalosis; Hypernatremia; Hypertriglyceridemia; Hyperuricemia; Hypoglycemia

MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Arthritis; Back pain; Bone pain; Chest wall pain; Joint range of motion decreased; Neck pain; Pain in extremity
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS) - Tumor pain
NERVOUS SYSTEM DISORDERS - Cognitive disturbance; Nervous system disorders - Other (neuropathy - cranial); Neuralgia; Syncope; Tremor
PSYCHIATRIC DISORDERS - Agitation; Anxiety; Confusion; Depression; Insomnia
RENAL AND URINARY DISORDERS - Cystitis noninfective; Hematuria; Hemoglobinuria; Renal and urinary disorders - Other (bladder pain); Renal and urinary disorders - Other (urethra pain); Urinary frequency; Urinary incontinence; Urinary tract pain
REPRODUCTIVE SYSTEM AND BREAST DISORDERS - Breast pain; Gynecomastia; Pelvic pain
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Allergic rhinitis; Bronchopulmonary hemorrhage; Hiccups; Hypoxia; Pleural effusion; Sore throat
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Bullous dermatitis; Nail discoloration; Periorbital edema; Purpura; Rash acneiform; Skin and subcutaneous tissue disorders - Other (folliculitis); Skin hyperpigmentation; Skin hypopigmentation; Skin ulceration
VASCULAR DISORDERS - Flushing; Hot flashes; Hypertension

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

7.2 Adverse Event Characteristics

CTCAE term (AE description) and grade: The descriptions and grading scales found in the revised NCI Common Terminology Criteria for AE (CTCAE) version 3.0 will be utilized until December 31, 2010 for AE reporting. CTCAE version 4.0 will be utilized beginning January 1, 2011 and until March 31, 2018. CTCAE version 5.0 will be utilized for AE reporting beginning April 1, 2018. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov).

'Expectedness': AEs can be 'Unexpected' or 'Expected' (see Section 7.1 above) for expedited reporting purposes only. 'Expected' AEs (the ASAEL) are ***bold and italicized*** in the CAEPR (Section 7.1.1).

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

7.3 Expedited Adverse Event Reporting

7.3.1 Expedited AE reporting for this study must use CTEP-AERS (CTEP Adverse Event Expedited Reporting System), accessed via the CTEP home page (HUhttp://ctep.cancer.govUH). The reporting procedures to be followed are presented in the "CTEP, NCI Guidelines: Adverse Event Reporting Requirements" which can be downloaded from the CTEP home page (HUhttp://ctep.cancer.govUH). These requirements are briefly outlined in the table below (Section 7.3.2).

A 24-hour notification is to be made to CTEP by telephone at 301-897-7497 only when internet connectivity is disrupted. Once internet connectivity is restored, an AE report submitted by phone must be entered electronically into CTEP-AERS by the original submitter at the site.

CTEP-AERS is programmed for automatic electronic distribution of reports to the following individuals: Study Coordinator of the Lead Organization, Principal Investigator, and the local treating physician. CTEP-AERS provides a copy feature for other e-mail recipients.

7.3.2 Expedited Reporting Guidelines

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

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)
NOTE: Investigators **MUST** immediately report to the sponsor (NCI) **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)
An adverse event is considered serious if it results in **ANY** of the following outcomes:

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

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

Hospitalization	Grade 1 and Grade 2 Timeframes	Grade 3-5 Timeframes
Resulting in Hospitalization ≥ 24 hrs	10 Calendar Days	24-Hour 5 Calendar Days
Not resulting in Hospitalization ≥ 24 hrs	Not required	

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

Expedited AE reporting timelines are defined as:

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

[1]Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:

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

- All Grade 3, 4, and Grade 5 AEs

Expedited 10 calendar day reports for:

- Grade 2 AEs resulting in hospitalization or prolongation of hospitalization

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

Effective Date: May 5, 2011

Note: A death on study requires both routine and expedited reporting regardless of causality, unless as noted below. Attribution to treatment or other cause must be provided.

IMPORTANT: Deaths clearly due to progressive disease should be reported as Grade 5 "Disease Progression" in the system organ class (SOC) "General disorders and administration site conditions." Evidence that the death was a manifestation of underlying disease (e.g., radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted. Deaths clearly due to progressive disease should NOT be reported via CTEP-AERS but rather should be reported via routine reporting methods (*e.g.*, CDUS and/or CTMS).

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

7.3.3 Protocol-Specific Expedited Adverse Event Reporting Exclusions

Lymphopenia (any grade), alopecia (any grade), anemia (grade 2), electrolyes (grade 2; sodium, potassium, phosphorous, and magnesium), albumin (grade 2), hyperuricemia (grade 3), INR (grade 2), and PTT (grade 2) will not be reported via CTEP-AERS but will be included in the routine data submissions.

7.3.4 Pregnancy, Fetal Death, and Death Neonatal

NOTE: When submitting CTEP-AERS reports for "Pregnancy", "Pregnancy loss", or "Neonatal loss", the Pregnancy Information Form should be completed for patients who became pregnant on study, and faxed along with any additional

medical information to **301-230-0159.** The potential risk of exposure of the fetus to the investigational agent(s) or chemotherapy agent(s) should be documented in the "Description of Event" section of the CTEP-AERS report.

7.3.4.1 Pregnancy

- Because patients who become pregnant on study risk intrauterine exposure of the fetus to agents which may be teratogenic, DCTD/DCP is requesting that pregnancy should be reported in an expedited manner via CTEP-AERS as Grade 3 "*Pregnancy, puerperium and perinatal conditions - Other (pregnancy)*" under the *Pregnancy, puerperium and perinatal conditions* SOC.
- The pregnancy outcome for patients on study should be reported via CTEP-AERS at the time the outcome becomes known, accompanied by the same Pregnancy Report Form used for the initial report.

7.3.4.2 Pregnancy loss

- Pregnancy loss is defined in CTCAE as "Death in utero."
- Any pregnancy loss should be reported expeditiously, as Grade 4 "Pregnancy loss" under the Pregnancy, puerperium and perinatal conditions SOC.
- A pregnancy loss should NOT be reported as a Grade 5 event under the Pregnancy, puerperium and perinatal conditions SOC, as currently CTEP-AERS recognizes this event as a patient death.

7.3.4.3 Death Neonatal

- Neonatal death, defined in CTCAE as "A disorder characterized by cessation of life occurring during the first 28 days of life" that is felt by the investigator to be at least possibly due to the investigational agent/intervention, should be reported expeditiously.
- A neonatal death should be reported expeditiously as Grade 4 "Death neonatal" under the General disorders and administration SOC.
- Neonatal death should NOT be reported as Grade 5 "Death neonatal" under the General disorders and administration SOC. If reported as such, the CTEP-AERS interprets this as a death of the patient being treated.

7.3.5 NCI-IRB Expedited Reporting of Adverse Events, Unanticipated Problems, and Deaths

7.3.5.1 Definitions

Adverse event
An adverse event is defined as any reaction, side effect, or untoward event that occurs during the course of the clinical trial associated with the use of a drug in humans,

whether or not the event is considered related to the treatment or clinically significant. For this study, AEs will include events reported by the patient, as well as clinically significant abnormal findings on physical examination or laboratory evaluation. A new illness, symptom, sign or clinically significant laboratory abnormality or worsening of a pre-existing condition or abnormality is considered an AE. All AEs must be recorded on the AE case report form unless otherwise noted in Section 7.3.3.

All AEs, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until satisfactory resolution. AEs should be reported up to 30 days following the last dose of study drug.

An abnormal laboratory value will be considered an AE if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

Suspected adverse reaction
Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

Unexpected adverse reaction
An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application. "Unexpected", also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

Serious

An Unanticipated Problem or Protocol Deviation is serious if it meets the definition of a Serious Adverse Event or if it compromises the safety, welfare, or rights of subjects or others.

Serious Adverse Event

An adverse event or suspected adverse reaction is considered serious if in the view of the investigator or the sponsor, it results in any of the following:

- Death,
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant disability/incapacity
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Disability

A substantial disruption of a person's ability to conduct normal life functions.

Life-threatening adverse drug experience

Any adverse event or suspected adverse reaction that places the patient or subject, in the view of the investigator or sponsor, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.

Protocol Deviation (NIH Definitions)

Any change, divergence, or departure from the IRB approved research protocol.

Non-compliance (NIH Definition)

The failure to comply with applicable NIH Human Research Protections Program (HRPP) policies, IRB requirements, or regulatory requirements for the protection of human research subjects.

Unanticipated Problem

Any incident, experience, or outcome that:

- Is unexpected in terms of nature, severity, or frequency in relation to

  (a) the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents, and (b) the characteristics of the subject population being studied; **AND**

- Is related or possibly related to participation in the research; **AND**

- 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.

7.3.5.2 NCI IRB and Clinical Center Director Reporting Requirements

The Coordinating Center PI will report in the NIH Problem Form to the NCI-IRB and NCI Clinical Director:

- All deaths, except deaths due to progressive disease
- All Protocol Deviations
- All Unanticipated Problems
- All serious non-compliance

Reports must be received within 7 days of Coordinating Center PI awareness via iRIS.

7.3.5.3 NCI-IRB Reporting of IND Safety Reports

Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported to the NCI IRB.

7.4 Multicenter Guidelines for Expedited Adverse Event Reporting

7.4.1 Expedited Adverse Event Reporting for Participating Sites

7.4.1.1 Adverse Event Reporting via CTEP-AERS:

Follow sponsor expedited AE reporting requirements in Section 7.3.2. Copy Jennifer Zlott (zlottjh@mail.nih.gov) and Alice Chen, MD (chenali@@mail.nih.gov) on all CTEP-AERS reports.

7.4.1.2 Adverse Event Reporting to NCI IRB:

The site PI must immediately report to the Coordinating Center PI any serious adverse event, whether or not considered drug related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the drug caused the event within 24 hours of PI awareness of the event. The site PI must also report any protocol deviations or violations to the Coordinating Center PI within 7 days of PI awareness. Participating centers must also submit the report to their IRB in accordance with their institutional policies.

Follow NCI IRB expedited AE reporting requirements in Section 7.3.4. Complete the NCI IRB Expedited AE form supplied by the Coordinating Center. Send the completed form to Jennifer Zlott, RN either by e-mail to (zlottjh@mail.nih.gov) or by fax to (301) 480-4612.

7.5 Routine Adverse Event Reporting

All Adverse Events must be reported in routine study data submissions. AEs reported through CTEP-AERS must also be reported in routine study data submissions.

AML/MDS events will be reported via CTEP-AERS (in addition to routine AE reporting mechanisms). In CTCAE v5, the event(s) may be reported as either: 1) Leukemia secondary to oncology chemotherapy, 2) Myelodysplastic syndrome, or, 3) Treatment related secondary malignancy.

7.5.1 NCI IRB Requirements for Coordinating Site PI Reporting at Continuing Review

System Organ Class	CTCAE Term	Grade	# of Events since last CR	Total # of Events	Attribution to Research	Serious?	Unexpected?

The protocol PI will report to the NCI-IRB:

1. A summary of all protocol deviations in a tabular format to include the date the deviation occurred, a brief description of the deviation and any corrective action.
2. A summary of any instances of non-compliance
3. A tabular summary of the following adverse events:

   - All Grade 2 **unexpected** events that are possibly, probably or definitely related to the research;
   - All Grade 3 and 4 events that are possibly, probably or definitely related to the research;
   - All Grade 5 events regardless of attribution;
   - All Serious Events regardless of attribution.

NOTE: Grade 1 events are not required to be reported.

8 Pharmaceutical Information

8.1 Compound Name: 5-Fluoro-2'-deoxycytidine (NSC 48006)

Formula: $C_9H_{12}FN_3O_4$
Molecular Weight: 245.21
Other Names: FdCyd

Description: Fluoropyrimidine antimetabolite.

Dosage and Formulation: 5-Fluoro-2'-deoxycytidine (FdCyd) is supplied by the DCTD and distributed by the PMB/NCI as a sterile, single use vial. Each vial contains 100 mg (20 mg/mL; 5 mL) of a clear, colorless solution.

Preparation: The prescribed daily dose should be further diluted in 5% dextrose injection, USP prior to administration.

Storage: Intact vials should be stored at controlled room temperature.

Stability: Shelf life stability testing of the intact vials is on-going. CAUTION: The single-use dosage form contains no antibacterial preservatives. Therefore, it is advised that the product be discarded 8 hours after initial entry.

Method of Administration: FdCyd may be mixed with tetrahydrouridine (THU, NSC 112907) in 5% dextrose injection, USP and is stable for at least 24 hours at room temperature.

Toxicities: A list of the adverse events and potential risks associated with FdCyd can be found in Section 7.1.

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

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

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

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 Drug Accountability Record Form (DARF). (See the NCI Investigator's Handbook for Procedures for Drug Accountability and Storage.)

8.2 Compound Name: Tetrahydrouridine (NSC 112907)

Formula: $C_9H_{16}N_2O_6$
Molecular Weight: 248.2
Other Names: THU

Description: Cytidine deaminase inhibitor.

Dosage and Formulation: Tetrahydrouridine Injection is supplied by the DCTD and distributed by the PMB/NCI as a 500 mg vial (10 mg/mL; 50 mL). The clear, colorless solution contains 10 mg tetrahydrouridine per mL with dibasic phosphate, USP, monobasic phosphate, USP used as buffer to maintain pH at 7.4.

The total daily dose of THU will be 350 mg/m^2. Each day, 20% of the dose of THU will be injected as an IV push; 80% of the dose of THU diluted in 5% dextrose injection, USP will be infused over 3 hours.

Using retention of 90% of the initial potency as the criterion for stability, FdCyd and THU were stable when mixed together in 5% dextrose injection, USP for at least 24 hours, both at controlled room temperature and at 40°C (IND #54,223).

Preparation: Tetrahydrouridine may be administered without further dilution or may be further diluted in 0.9% Sodium Chloride Injection, USP or 5% dextrose injection, USP to a concentration as low as 2.5 mg/mL.

Storage: Intact vials should be stored in the refrigerator (2° – 8°C).

Stability: Shelf life stability testing of the intact vials is ongoing. THU as diluted above is stable for at least 24 hours at room temperature. CAUTION: The single-use dosage form contains no antibacterial preservatives. Therefore, it is advised that the product be discarded 8 hours after initial entry.

Toxicities: A list of the adverse events and potential risks associated with THU can be found in Section 7.1.

Availability: This is an investigational agent supplied to investigators by the DCTD, NCI.

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

agents for the study should be ordered under the name of one lead investigator at that institution.

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

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 Drug Accountability Record Form (DARF). (See the NCI Investigator's Handbook for Procedures for Drug Accountability and Storage.)

9 Correlative Studies

Tumor biopsies and blood samples will be obtained to characterize the effects of FdCyd and THU on the biology of tumor cells and to determine the pharmacokinetics of both the FdCyd and THU. Blood samples will be collected to measure DNA methylation and DNA methyltransferase activity. Blood will also be collected to measure changes in the number of CTCs before and during treatment.

9.1 Tissue Sample Acquisition and Processing

9.1.1 Timing of Biopsies

Tumor biopsies are optional in this trial.

Biopsies will be performed at the following times:

- After consent, prior to treatment
- After the completion of Cycle 2 (i.e., following the restaging imaging scans)

9.1.2 Biopsy Procedure

Serial tumor biopsies will be obtained through Interventional Radiology by a percutaneous approach. A maximum of 2 core biopsies 18-gauge in diameter will be obtained at each time point. Only percutaneous biopsies will be performed on patients with solid tumors. It is estimated that there will be between 2 to 5 million cells from each biopsy. If a site is deemed appropriate for biopsy with minimal risk to the participant by agreement between the investigators and Interventional Radiology, an attempt at biopsy will be made. The use of imaging to facilitate biopsies will be decided by members of the Interventional Radiology team and may include ultrasound, CT scan, or MRI. Should CT scan be needed for biopsy, the number of scans for each procedure will be limited to the minimum number needed to safely obtain a biopsy.

Tumor biopsies and local anesthesia will be administered only if they are considered to be of low risk to the participant as determined by the investigators and Interventional Radiology.

All cases will be carefully reviewed with the interventional radiologists who have extensive experience in performing such procedures. Only if the procedure is considered to be low risk then we will proceed with tumor biopsy in a given participant. If the participant refuses a tumor biopsy, he/she will still remain on study and receive study medication and all other correlative studies will be performed.

If an initial attempt at percutaneous biopsy is unsuccessful, the patient will be given an option to proceed with a repeated attempt at percutaneous biopsy. In case biopsy samples are unable to be obtained for a given patient, the patient will still remain on study, receive study medication, and all other correlative studies will be performed.

9.1.3 Laboratory Contact, NIH Clinical Center

For patients enrolled at the NCI, at least 24 hours prior to taking the biopsy, the research nurse will contact the NCI Phase I/II PK/PD Support Group in NIH Building 10: E-mail: NCIPK-PDsupportgroup@mail.nih.gov; Pager: 102-12798; Phone: (301) 451-1169; Fax: (301) 480-5871. Tubes pre-labeled with the participant ID, biopsy date, protocol #, and site of tissue biopsy will be provided.

Refer to Appendix F for biopsy processing and shipping information.

9.1.4 Measurement of Methylation-Silenced Gene Products by mRNA Transcript Assays in Tumor Biopsies

Biopsies will be utilized to measure methylation-silenced gene products by mRNA transcript assays prior and post treatment. Expression of E-Cadherin, DAPK1, p16, MGMT, RASSF1a, and TIMP3 will be analyzed by RT-qPCR employing an assay validated for performance on the Taqman platform. The kit is purchased from ABI. Tumor specimens will be extracted with RNA-Easy, and the RNA purified, DNAse-treated, and quantified by absorbance. cDNA generated will be measured on a Nanodrop spectrophotometer; 20 ng of cDNA will be loaded into each of triplicate wells in 96-well plates for PCR amplification. Controls include actin and beta-catenin, neither of which is silenced by CpG island methylation. All samples will be initially stored and processed in Dr. Kinders' laboratory (PADIS/LHTP/NCI-Frederick). If sufficient tumor tissue is available, immunofluorescence microscopy–based assessments of p16, DNMT1, and other relevant proteins may also be performed.

9.2 Pharmacodynamic Studies

9.2.1 Blood Collection for PD Studies

PD samples will be collected for the following: plasma for DNA methylation status and mononuclear cells for assessing DNA methyltransferase activity. Two 4-mL EDTA (lavender-top) tubes of blood will be collected from the patient at each time point. See Appendix F for reagents, shipping instructions, and procedures for PD studies. Current

SOPs can be found on the DCTD Web site:
http://dctd.cancer.gov/ResearchResources/ResearchResources-biomarkers.htm

Blood will be collected at the following times for PD analysis:

- Prior to drug administration on Day 1 for Cycles 1, 2, and 4
- At the completion of treatment on Cycles 1, 2, and 4 (Day 12 +/- 1 day)

9.2.2 Blood Collection for CTC Studies

Blood for CTCs will be collected to determine the dose response to FdCyd/THU treatment in terms of the fraction of CTCs expressing p16.
Prior to CTC collection, each participating site should e-mail a request for specimen collection and shipping materials from NCI_PD_Support_CellSearch@mail.nih.gov.

Approximately 8 mL of blood will be collected from the patient into a 10-mL CellSave preservative tube at each time point.

Blood will be collected at the following times:

- Prior to treatment on Cycle 1, Day 1
- Cycle 1, Day 2 following treatment (up to 24 hours after end of infusion)
- Cycle 1, Day 12 (+/- 1 day)
- Day 1 (+/- 1 day) of Cycle 2 and all subsequent cycles
- Day 12 (+/- 1 day) of Cycles 2, 4, and 6
- At time of disease progression

9.2.3 CTC collection

The blood will be collected (~8 mL) at the time points mentioned above. Whole blood will be collected aseptically by venipuncture or from a venous port into a CellSave preservative tube. Invert tube 8 times following collection to distribute anticoagulant and preservative through blood. The collected blood samples are stable for up to 96 hours at room temperature (15˚C to 30˚C) prior to processing.

9.2.4 Laboratory Contact, NIH Clinical Center

For patients enrolled at the NCI, at least 24 hours prior to taking the blood samples, the research nurse will contact the NCI Phase I/II PK/PD Support Group in NIH Building 10: E-mail: NCIPK-PDsupportgroup@mail.nih.gov; Pager: 102-12798; Phone: (301) 451-1169; Fax: (301) 480-5871.

Testing and data analysis will be performed by Dr. Kinders (PADIS/LHTP/Frederick National Laboratory for Cancer Research). Refer to Appendix F for Participating Site sample shipping material request procedures and shipping information.

9.3 Pharmacokinetics Studies (Cycle 1 Only)

FdCyd will be assayed in plasma and urine from 15 patients. Blood will be drawn from a site other than the one used to administer chemotherapy (from a contralateral limb or a central venous line of patients receiving FdCyd and THU by peripheral vein, or from a peripheral vein of patients receiving FdCyd and THU by central venous line). Urine will be collected to determine renal excretion of the drug.

9.3.1 PK Urine and Blood Samples

Blood samples for PK analysis will be obtained from a total of 15 patients during Cycle 1 only. Samples will be collected from 3 patients from each cohort except urothelial transitional cell carcinoma; samples will be collected from 6 of the first 12 patients with urothelial transitional cell carcinoma as any ileal loops or neobladders in this patient population may have an impact on drug pharmacokinetics. One 3-mL heparinized (green-top) tube preloaded with 30 µL of 100 mg/mL of ZEB (see Appendix D for preparation of tubes) will be obtained for each of the time points. The actual time of collection will be noted for each sample, and the sample will be placed immediately on ice. See Appendix E for reagents and the procedure for PK studies.

Blood will be collected at the following times:

- Cycle 1, Day 1 prior to treatment
- Cycle 1 Day 1 at 15 min, 30 min, 1 hr, 2 hr, and 2.5 hr during the infusion
- Cycle 1 Day 1 at 15 min, 30 min, 1 hr, 2 hr, 4 hr, and 6 hr post completion of infusion
- Cycle 1 Days 2, 3, 4, and 5 prior to treatment

Based upon the results of the initial measurements, sampling times may be adjusted but neither the total number of samples nor the total amount of blood will be increased. All samples will be immediately placed on ice. Samples should be processed within 10 minutes after collection.

A baseline urine collection of 10 mL will be collected just before drug administration. Urine will then be collected at every void from 0 to 24-hr post drug infusion on Cycle 1 Day 1 for PK analysis. Samples should be placed on wet ice immediately after collection. The total volume of urine collected for the 24-hour time period will be mixed, measured, and noted. A minimum sample of 10 mL will be saved by nursing in a specimen cup. The remainder of the unused sample can be discarded. Samples will be processed according to the procedures in Appendix E.

Record the date, planned time and actual time of collection for each specimen on the pharmacokinetic form; the study number and the patient's unique patient identifier should be included on this form. In addition, the date and exact time when the FdCyd and THU doses were administered should be recorded on the appropriate pharmacokinetic form (Appendix G).

9.4 Sample Collection and Processing at the NIH Clinical Center

Biospecimens will be collected and processed using validated SOPs that will ensure both specimen quality and patient confidentiality pursuant to informed consent provisions.

Using a computerized inventory system and a backup hardcopy process, all specimen collection and processing steps will be documented and the specific location of each specimen will be tracked. Each new specimen collected will be assigned a unique barcode identifier that can be linked to the original specimen collected and other relevant information within the inventory system. To ensure patient confidentiality, only containers used for the initial specimen collections will be labeled with patient identifiers. Only the barcode identifier will be applied to all subsequent specimen containers. When specimens are processed and aliquoted, no patient information will be included on the new containers. Original specimen containers will be discarded. Only barcode-labeled specimens without patient identifiers will be shipped for analysis and/or storage. Specimen labels will indicate: protocol number, order in which the patient enrolled on the trial, type of sample, collection time, and total volume collected, as appropriate. Samples from sets of at least three patients will be grouped for scientific analysis.

The inventory process contains other security provisions sufficient to safeguard patient privacy and confidentiality. Access to the inventory system and associated documents will be restricted to appropriate individuals. Requests to use specimens stored in the repository must be approved. The only patient information available in the inventory system will be the patient sex, diagnosis, and level of informed consent given. SOPs ensure that any changes in informed consent made by a patient and relayed to the PI will be reflected in the inventory system to ensure that specimens are destroyed as appropriate. All laboratory personnel will be trained to adhere to SOPs and will be monitored for high-quality performance.

Following completion of this study, samples will remain in storage as detailed above. Access to these samples will only be granted following IRB approval of an additional protocol, granting the rights to use the material.

If, at any time, a patient withdraws from the study and does not wish for their existing samples to be utilized, the individual must provide a written request. Following receipt of this request, the samples will be destroyed (or returned to the patient, if so requested), and reported as such to the IRB. Any samples lost (in transit or by a researcher) or destroyed due to unknown sample integrity (i.e., broken freezer allows for extensive sample thawing, etc.) will be reported as such to the IRB.

Any new use of these samples will require prospective IRB review and approval; any loss or destruction of samples and the planned disposition of samples after the protocol is terminated will be reported to the IRB.

10 Study Calendar

Eligibility screening evaluations are to be conducted within 8 days prior to enrollment, with the exception of diagnostic imaging, which must be done within 28 days prior to enrollment. Baseline history, physical examination, and laboratory evaluations are to be conducted within 8 days prior to the start of protocol therapy. If protocol therapy is started within 8 days of the eligibility screening evaluations, values from the screening evaluations may be used as baseline measurements; if > 8 days have passed since the screening evaluations, the medical history, physical examination, and laboratory evaluations must be repeated prior to starting protocol therapy. Baseline imaging scans must be done within 28 days prior to the start of protocol therapy. If protocol therapy is started within 28 days of the eligibility screening tumor imaging, the screening evaluation imaging results may be used as baseline measurements; if >28 days have passed since the screening evaluation tumor imaging, the imaging must be repeated prior to starting protocol therapy. The research team may perform additional safety/monitoring tests as clinically indicated.

	Pre-Study Eligibility Screening	Baseline Clinical Evaluation	Cycle 1				Cycle 2				Cycle 3 onwards
			Wk 1	Wk 2	Wk 3	Wk 4	Wk 1	Wk 2	Wk 3	Wk 4	
FdCyd[a]			X	X			X	X			X
THU[a]			X	X			X	X			X
Informed consent	X										
Demographics	X										
Med.History	X	X[i]					X				X (prior to treatment)
Physical exam[b]	X	X[i]	X	X			X				X (prior to treatment)
Height and Weight	X	X[i]					X				X
Performance Status	X	X[i]	X	X	X	X	X				X
CBC w/diff, plts[c]	X	X[i]	X	X	X	X	X	X			X
Serum chemistry[c]	X	X[i]	X	X	X	X	X	X			X
PD blood samples[d]			X	X			X	X			X (days 1, 12)
Blood samples for CTC[f]			X	post-treatment CTC blood sample collection described in Section 9.2.2							
Optional Tumor Biopsies[j]			X								X (at first restaging)
Tumor Measurement[e]	X	X[i]									X
Radiologic Evaluation[e]	X	X[i]									X
B-HCG[g]	X										
PK blood and urine samples[h]			X								
Adverse event evaluation			X……X								

a. FdCyd and THU will be administered IV on Days 1-5 and 8-12 of each cycle +/- 1 day for scheduling reasons (e.g., weekends, holidays, or patient convenience).

b. A physical examination including vital signs will be conducted pre-study and during weeks 1 and 2 of Cycle 1, and then within 3 days prior to treatment every cycle thereafter. The patient will be contacted by phone during weeks 3 and 4 of Cycle 1.

c. Serum chemistries (sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose, AST, ALT, bilirubin, calcium, phosphorous, albumin, magnesium, alkaline phosphatase, LDH) and CBC with differential and platelets will be performed at baseline, weekly during cycle 1, then within 3 days prior to the start of every cycle and during weeks 1 and 2 of every cycle.

d. PD samples will be collected at multiple time points as specified in Section 9.

e. Radiologic examination will be performed at baseline and then every two cycles (every three cycles for patients on study more than one year and every 4 cycles for patients on study more than 3 years).

f. Blood samples for CTCs will be collected prior to study and as specified in Section 9.2.2.

g. Urine pregnancy test (women of childbearing potential).

[h] Blood and urine for PK analysis will be obtained during Cycle 1 only as specified in Section 9.
[i] Values from eligibility screening tests may be used as baseline evaluation values if the test was performed within 8 days of start of protocol therapy (or, for radiologic evaluation and tumor measurement, within 28 days of start of protocol therapy). See Section 3.3 and Section 4.
[j] Optional tumor biopsies will be performed as described in Section 9.1.1.

11 Measurement of Effect

Radiologic evaluation for tumor measurements will be performed at baseline within 28 days prior to the start of protocol therapy and then every 2 cycles.

11.1 Antitumor Effect – Solid Tumors

Response and progression will be evaluated in this study using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee [JNCI 92(3):205-216, 2000]. Changes in only the largest diameter (unidimensional measurement) of the tumor lesions are used in the RECIST criteria.

11.1.1 Definitions

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

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

11.1.2 Disease Parameters

Measurable disease. Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥20 mm with conventional techniques (CT, MRI, ultrasound) or as ≥10 mm with spiral CT scan. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters)

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter <20 mm with conventional techniques or <10 mm using spiral CT scan), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonis, inflammatory breast disease, abdominal masses (not followed by CT or MRI), and cystic lesions are all non-measurable.

Target lesions. All measurable lesions up to a maximum of 5 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 response.

Non-target lesions. All other lesions (or sites of disease) including any measurable lesions over and above the 10 target lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence or absence of each should be noted throughout follow-up.

11.1.3 Methods for Evaluation of Measurable Disease

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

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination when both methods have been used to assess the antitumor effect of a treatment.

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

Conventional CT and MRI: These techniques 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.

Ultrasound (US): When the primary endpoint of the study is objective response evaluation, 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.

Endoscopy, Laparoscopy: The utilization of these techniques 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 reference centers.

However, such techniques may be useful to confirm complete pathological response when biopsies are obtained.

Tumor markers: Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response. Progression of disease will be determined per RECIST criteria as outlined below.

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

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

11.1.4 Response Criteria

11.1.4.1 Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions

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

Progressive Disease (PD): At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum of the 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 of the LD since the treatment started.

11.1.4.2 Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

Incomplete Response/ Stable Disease (SD): Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD): Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions Although a clear progression of “non-target” lesions only is exceptional, the opinion of the treating physician

should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Response for This Category Also Requires:
CR	CR	No	CR	>4 weeks confirmation
CR	Non-CR/Non-PD	No	PR	>4 weeks confirmation
PR	Non-PD	No	PR	
SD	Non-PD	No	SD	documented at least once >4 weeks from baseline
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD*	Yes or No	PD	
Any	Any	Yes	PD	
In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression. Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration.*" Every effort should be made to document the objective progression even after discontinuation of treatment.				

11.1.4.3 Evaluation of Best Overall Response

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

11.1.4.4 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectively documented.

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

12 Data Reporting / Regulatory Requirements

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7.

12.1 Data Reporting

12.1.1 Method

This study will be monitored by the Clinical Data Update System (CDUS) version 3.0. Cumulative CDUS data will be submitted quarterly to CTEP by electronic means. Reports are due January 31, April 30, July 31, and October 31. Instructions for submitting data using the CDUS can be found on the CTEP Web site (http://ctep.cancer.gov).

Note: **All** adverse events that have occurred on the study, including those reported through CTEP-AERS, must be reported via the monitoring method identified above.

12.1.2 Responsibility for Submission

Study participants are responsible for submitting CDUS data and/or data forms to the Coordinating Center quarterly to allow time for Coordinating Center compilation, Principal Investigator review, and timely submission to CTEP (see Section 12.1.1.). The Coordinating Center is responsible for compiling and submitting CDUS data to CTEP for all participants and for providing the data to the Principal Investigator for review.

12.2 Data Safety and Monitoring Plan

The investigators at each participating center will be responsible for the collection, maintenance, and quality control of the study data. Adverse events observed in patients enrolled on the trial will be monitored in real time by the Principal and Associate Investigators, and attribution of these events to the research will be determined at the end of each treatment cycle in each subject. The clinical research team (PI, adjunct PI, research nurses, data managers) will meet weekly when patients are being actively treated on the trial to discuss each patient in detail and ensure that all events are graded appropriately, and that the attribution to study drug is correct. The Coordinating Center is responsible for establishing conference calls between participating sites at least on a monthly basis to discuss the observed toxicities and protocol issues.

All SAEs will be reported through CTEP-AERS to CTEP, to the Coordinating Center PI at NCI, and forwarded to the IRB per Section 7. In all cases where the dose of the study treatment has been reduced/modified or the patient withdrawn due to unusual or unusually severe toxicity considered related to the study treatment, the investigator must contact and inform the Coordinating Center PI. All sites will be monitored by the CTEP drug monitor who will receive data from all participating sites.

Data will be monitored regularly by the principal investigator in order to identify significant toxicity trends. Any new significant finding that may affect the patient's willingness to continue in the study will be shared with patients.

Confidentiality will be maintained as much as possible, consistent with applicable regulations. Names of participants or identifying material will not be released without patient permission, except when such release is required by law. No patient's name or identifying information will be released in any publication or presentation. Records are maintained according to current legal requirements, and are made available for review according to the requirements of the FDA or other authorized user, only under guidelines established by the Federal Privacy Act.

Safety Monitoring Committee:
Because this is a multi-institutional Phase II protocol for which the Developmental Therapeutics Clinic (DTC), NCI is the Coordinating Center, it will be monitored by the DTC Safety Monitoring Committee, NCI. Reviews will occur annually concurrent with NCI IRB continuing reviews.

12.3 Multicenter Guidelines

This protocol will open initially at the NCI. The NCI IRB will be notified once the participating centers' IRBs have approved the studies to open. This protocol will follow the CCR's Clinical Research Operations' SOPs for multicenter trials.

12.3.1 IRB Approvals

As the Coordinating Center for a trial, it is the PI's responsibility to ascertain that no patients are entered on the trial at a participating institution without full IRB approval. Thus, the NCI IRB must approve the addition of each participating institution to the protocol and will require a copy of the local IRB approval from each participating institution before NCI IRB approval will be granted.

The PI will provide the NCI IRB with a copy of the participating institution's approved yearly continuing review. Registration will be halted at any participating institution in which a current continuing approval is not on file at the NCI IRB.

12.3.2 Amendments and Consents

The NCI PI will provide the NCI IRB with copies of all amendments, consents and approvals from each participating institution.

12.3.3 Data Collection

The investigators will be responsible for the collection, maintenance, and quality control of the study data. All data collected for each study subject will be entered into the Cancer Central Clinical Database (C3D), an NCI electronic case report form/database, every 2 weeks. The participating sites will be able to enter the data remotely into the web-based C3D system. Each site investigator is also responsible for maintaining all source documentation related to the study, including any films, tracings, computer discs or tapes. NCI will be responsible for data management, data analysis, and reporting. Data collection forms will be provided to the participating institutions. Required data

include, not exclusively: prior disease-related therapies with dates, disease type, stage, disease sites, with measurements, and concurrent medications.

12.3.4 Data and Center Audits

Audits will be conducted yearly to ensure data integrity and provide quality control. These audits will be conducted by the NCI research team. Selected patient charts should be audited as well as the participating institution's Standard Operating Procedures (SOP) at the time of the visit. Data from participating institutions should be available when the protocol is audited at the NCI.

12.3.5 CTEP Multicenter Guidelines

This protocol will adhere to the policies and requirements of the CTEP Multicenter Guidelines. The specific responsibilities of the Principal Investigator and the Coordinating Center (Study Coordinator) and the procedures for auditing are presented in Appendix C.

- The Principal Investigator/Coordinating Center is responsible for distributing all IND Action Letters or Safety Reports received from CTEP to all participating institutions for submission to their individual IRBs for action as required.

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

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

N/A

13 Statistical Considerations

The primary objective of this protocol is to determine if FdCyd/THU is associated with adequate response rates (RR) or progression-free survival (PFS) in patients with any of a variety of malignancies as defined below. PFS will be estimated using a Kaplan-Meier curve. The results obtained will be informally compared to those from other studies in similar patients to help determine the potential benefit of exploring use of these agents in a subsequent, more definitive study, if warranted. These patients will have been previously treated with multiple prior chemotherapeutic regimens, so that any reasonable evidence of benefit will be interpreted as being a positive indicator.

Exploratory study objectives relate to the safety of the agents in patients with various solid tumors, and an evaluation of the impact of FdCyd/THU on extent of LINE-1 methylation in tumor biopsies. Blood samples will also be collected to evaluate the number of circulating tumor cells before and during treatment. Changes from baseline will be determined in either

absolute or relative terms as appropriate, and evaluated for statistical significance, as well as to determine if the changes or the actual values at a time point are associated with clinical response. Paired comparisons with baseline will be done using a paired t-test or Wilcoxon signed rank test as appropriate, and the changes will be compared between responders and non-responders (PD) using a two-sample t-test (adequate numbers of subjects with normally distributed data in both groups) or a Wilcoxon rank sum test, if enough patients respond for this to be appropriate. In all such cases, these analyses will be considered exploratory and not formally adjusted for multiple comparisons. However, to ensure proper interpretation in the context of a potentially large number of explorations being performed, only p values <0.01 will be interpretable as being associated with statistical significance.

Patients will be enrolled into one of 4 strata as follows:

A Non-Small Cell Lung Cancer
B Breast Cancer
C Urothelial Transitional Cell Carcinoma
D Head and Neck Cancer

In each stratum, the study will be conducted with a dual-endpoint design, based on RR and 4-month (6-month for the breast cancer stratum) PFS as co-primary endpoints. In strata A, C, and D, evidence of positive results is sought in the first stage to determine if the particular stratum should be expanded into the second stage. In stratum B, a one-stage design is utilized. Also, throughout the sections below, 'response' will refer to the appearance of a positive outcome as defined for each disease.

Stratum A: Non-Small Cell Lung Cancer
Stratum A will use a trial design for patients with NSCLC that judges the agents as promising if we achieve either tumor response rates of 20% vs. 5% or 4-month PFS rates of 50% vs. 25% (corresponding to median PFS of 4 vs. 2 months). The design requires a maximum of 45 evaluable patients. For each stratum, if at least 6 objective responses (at least 13%), or at least 18 instances of 4-month PFS (at least 40%), are observed among the 45 evaluable patients, this regimen will be considered worthy of further testing in this disease. If no more than one objective response (no more than 5%), and no more than 6 instances of 4-month PFS (no more than 30%), are observed among the initial 20 patients, the stratum will be terminated early and declared negative.

This design yields at least 87% power to detect a true objective response rate of at least 20%. It yields at least 90% power to detect a true 4-month PFS rate of at least 50% (median PFS of 4 months). It yields at least .96 probability of a negative result if the true objective response rate is no more than 5% and the true 4-month PFS rate is no more than 25% (median PFS of 2 months), with approximately .58 probability, at least, of early negative stopping in this case. In addition, the over-all probability of negative results across all 4 strata, under the over-all null hypothesis, is .85. These last three probabilities are calculated assuming that tumor response rate and PFS rate are uncorrelated. If they are positively correlated, as is likely, the probabilities will be a bit higher.

Stratum B: Breast Cancer
Stratum B will use a trial design for patients with breast cancer that judges the agents as promising if we achieve either tumor response rates of 20% vs. 5% or 6-month PFS rates of 50% vs. 25% (corresponding to median PFS of 6 vs. 3 months). The design requires a maximum of 35 evaluable patients. If at least 5 objective responses (at least 14%), or at least 15 instances of 6-month PFS (at least 43%), are observed among the 35 evaluable patients, this regimen will be considered worthy of further testing in this disease. This design yields at least 85% power to detect a true objective response rate of at least 20%. It yields at least 84% power to detect a true 6-month PFS rate of at least 50% (median PFS of 6 months). It yields at least .95 probability of a negative result if the true objective response rate is no more than 5% and the true 6-month PFS rate is no more than 25% (median PFS of 3 months). In addition, the over-all probability of negative results across all 4 strata, under the over-all null hypothesis, is .85. These last three probabilities are calculated assuming that tumor response rate and PFS rate are uncorrelated. If they are positively correlated, as is likely, the probabilities will be a bit higher.

If accrual onto this stratum is slow, and the target to demonstrate promising response rate (5 responders) or PFS rate (15 patients with 6-month PFS) has already been met among the initial 30 patients, consideration will be given to stopping the trial and declaring the regimen promising. The likelihood of demonstrating such promise under the alternative hypothesis is at least .57.

Stratum C: Urothelial Transitional Cell Carcinoma
Stratum C will use a trial design for patients with urothelial transitional cell carcinoma that judges the agents as promising if we achieve either tumor response rates of 20% vs. 5% or 4-month PFS rates of 50% vs. 25% (corresponding to median PFS of 4 vs. 2 months). The design requires a maximum of 45 evaluable patients. If at least 6 objective responses (at least 13%), or at least 18 instances of 4-month PFS (at least 40%) are observed among the 45 evaluable patients, this regimen will be considered worthy of further testing in this disease. If no more than one objective response (no more than 5%), and no more than 6 instances of 4-month PFS (no more than 30%), are observed among the initial 20 patients, the stratum will be terminated early and declared negative.

This design yields at least 87% power to detect a true objective response rate of at least 20%. It yields at least 90% power to detect a true 4-month PFS rate of at least 50% (median PFS of 4 months). It yields at least .96 probability of a negative result if the true objective response rate is no more than 5% and the true 4-month PFS rate is no more than 25% (median PFS of 2 months), with approximately .58 probability, at least, of early negative stopping in this case. In addition, the over-all probability of negative results across all 4 strata, under the over-all null hypothesis, is .85. These last three probabilities are calculated assuming that tumor response rate and PFS rate are uncorrelated. If they are positively correlated, as is likely, the probabilities will be a bit higher.

Stratum D: Head and Neck Cancer

Stratum D will use a trial design for patients with head and neck cancer that judges the agents as promising if we achieve either tumor response rates of 20% vs. 5% or 4-month PFS rates of 50% vs. 25% (corresponding to median PFS of 4 vs. 2 months). The design requires a maximum of 45 evaluable patients. If at least 6 objective responses (at least 13%), or at least 18 instances of 4-month PFS (at least 40%), are observed among the 45 evaluable patients, this regimen will be considered worthy of further testing in this disease. If no more than one objective response (no more than 5%), and no more than 6 instances of 4-month PFS (no more than 30%) are observed among the initial 20 patients, the stratum will be terminated early and declared negative.

This design yields at least 87% power to detect a true objective response rate of at least 20%. It yields at least 90% power to detect a true 4-month PFS rate of at least 50% (median PFS of 4 months). It yields at least .96 probability of a negative result if the true objective response rate is no more than 5% and the true 4-month PFS rate is no more than 25% (median PFS of 2 months), with approximately .58 probability, at least, of early negative stopping in this case. In addition, the over-all probability of negative results across all 4 strata, under the over-all null hypothesis, is .85. These last three probabilities are calculated assuming that tumor response rate and PFS rate are uncorrelated. If they are positively correlated, as is likely, the probabilities will be a bit higher.

For all strata, toxicity data will be obtained, and if there are 5 or more patients in a given stratum who experience Grade 3 or greater toxicity attributable to the agent, then comparisons between cohorts across grades of toxicity may be done using a Kruskal-Wallis test for ordered columns. Otherwise, toxicities will be tabulated and described.

Comparisons between extent of LINE-1 methylation and γ- to β-globin ratio will be made by subtracting pre-treatment from post-treatment levels and determining the statistical significance of the difference using a Wilcoxon signed rank test. These measures will all be evaluated with exploratory intent, and findings will be reported without formal adjustment for multiple comparisons, but in the context of a study with multiple exploratory analyses performed.

A total of 160 evaluable patients may be required if the study enrolls patients onto the second stage of cohorts A and D. The diseases studied are quite different from one another; thus, imposing an overall rule about responses in the full spectrum of patients, prior to enrolling to the first stage is not practical.

To allow for inevaluable patients, a total of 165 patients will be set as the accrual ceiling. It is anticipated that approximately 8- 9 patients per month will be accrued such that approximately 21 months will be required to accrue up to 165 patients.

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Appendix A: Performance Status

Karnofsky Performance Scale	
Percent	Description
100	Normal, no complaints, no evidence of disease.
90	Able to carry on normal activity; minor signs or symptoms of disease.
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.
60	Requires occasional assistance, but is able to care for most of his/her needs.
50	Requires considerable assistance and frequent medical care.
40	Disabled, requires special care and assistance.
30	Severely disabled, hospitalization indicated. Death not imminent.
20	Very sick, hospitalization indicated. Death not imminent.
10	Moribund, fatal processes progressing rapidly.
0	Dead.

Appendix B: Study Diary

Patient Name: _____

	Please note any side effects you experienced on this day	**Do not list your daily medications, but note any other medication you took on this day (both prescription and non-prescription) and the reason why taken**
Monday Date:		
Tuesday Date:		
Wednesday Date:		
Thursday Date:		
Friday Date:		
Saturday Date:		
Sunday Date:		

Signature of person completing form: _____ Date: _____

Appendix C: CTEP Multicenter Guidelines

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

Responsibility of the Protocol Chair

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

Responsibilities of the Coordinating Center

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

scans, x-rays, etc. available for the audit.

Inclusion of Multicenter Guidelines in the Protocol

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

Agent Ordering

Except in very unusual circumstances, each participating institution will order DCTD-supplied investigational agents directly from CTEP (See Section 8). Investigational agents may be ordered by a participating site only after the initial IRB approval for the site has been forwarded by the Coordinating Center to the CTEP PIO.

Appendix D: Preparation of PK Blood Draw Tubes and Urine Storage Cryogenic Vials With Zebularine (ZEB)

Preparation of tubes:
Zebularine (ZEB) will be provided upon request as bulk powder. Participating center pharmacies will make up a stock solution of ZEB at 100 mg/mL (wt of zebularine/volume of water added) and will prepare 1-mL frozen aliquots.

Requests for Zebularine powder can be sent to:

Rao Vishnuvajjala, PhD
Pharmaceutical Resources Branch, NCI, NIH
9609 Medical Center Drive MSC 9734
Rockville, MD 20850-9734
Phone: (240) 276-5962
E-mail: raov@dtpepn.nci.nih.gov

**Please note: vacutainers and/or other supplies will not be provided by the NCI*

Blood Draw Tubes:
Zebularine can be added to Vacutainer tubes up to one day in advance without causing significant loss of vacuum. Using a 3/10 cc insulin syringe or other similar sized syringe with a fine needle, draw up 30 µL of the ZEB solution and transfer it to a 3-mL heparinized Vacutainer tube (cat # 366667) by piercing the stopper. Do not draw up ZEB for more than one tube at a time. You will not be able to control the amount of ZEB solution that leaves the needle, as it is sucked out by the vacuum. Because of the fine needle, you will not lose the vacuum (apart from the volume added) in the collection tube.

Urine Storage Cryogenic Vials:
Five (5) µL of the 100 mg/mL ZEB solution should be added to each cryogenic vial to accommodate the 0.5 mL urine sample. Because the cryogenic tubes are not under vacuum, you can use any appropriate device to measure the 5 µL, e.g., a micropipettor such as Pipetman. This will yield the same final concentration of ZEB in the urine samples as in the blood samples (final concentration 1 mg/mL).

Appendix E: Procedure for Blood and Urine Samples for Pharmacokinetics

Plasma:
Supplies for each sample:

- One (1) 3-mL heparinized (green-top) tube (Vacutainer®□cat #366667 or equivalent) **with zebularine** (ZEB, see Appendix D.)
- It is critical that the vials containing ZEB be used to minimize ex vivo deamination of FdCyd during processing and storage.
- Three (3) 1-mL cryogenic vials

Procedure: Obtain blood in a 3-mL heparinized (green-top) tube (Vacutainer cat #366667 or equivalent) preloaded with 30 µL of 100 mg/mL of **ZEB**. Gently invert 3 times to mix and place immediately on ice. For each time point, label 3 cryogenic vials with the coordinating center patient #, date, and time. Do not include patient identifiers on the sample labels. Centrifuge the blood at 600 x g for 10 minutes at 4□C. Transfer 0.5 mL of the plasma into each of 2 cryogenic vials and the remainder of the plasma into the third cryogenic vial, if possible without disturbing the cell pellet. Keep the plasma on ice and freeze at or below -20□C within one hour.

Urine:
Supplies for each sample:

- One (1) screw-capped centrifuge tube (15 mL)
- Three (3) 1-mL cryogenic vials with 5 µL of 100 mg/mL zebularine (ZEB, Appendix D)
- It is critical that the vials containing ZEB be used to minimize ex vivo deamination of FdCyd during storage.

Procedure: Obtain approximately 10 mL of urine from the patient and place immediately on ice. Except for the pretreatment specimen, obtain the 10 mL aliquot after the total volume of the 24-hr collection has been measured. Make sure the 24-hr urine is mixed before taking the aliquot. If the aliquot is frozen, thaw on ice or thaw at room temperature, but place on ice as soon as thawed, mix, and process as soon as possible. Pour the urine specimen into a screw-capped centrifuge tube and centrifuge at 600 g for 1 minute at 4□C to sediment debris. Transfer 0.5 mL of the urine into each of 3 cryogenic vials **with ZEB**. Label the vials with the coordinating center patient # and date (no patient identifiers). Freeze the cryogenic vials of urine at or below -20□C.

All PK samples are to be shipped to:
Jan H. Beumer, PharmD, PhD
University of Pittsburgh Cancer Institute
Room G27E Hillman Research Laboratories
5117 Centre Avenue
Pittsburgh, PA 15213

Please notify either Mr. Robert Parise or Ms. Susan Christner (412-623-3248) by telephone or fax (412-623-1212) at least 24 hours prior to shipment. Samples should be shipped to arrive Tuesday,

Wednesday, Thursday, or Friday. A name, phone number, fax number, or e-mail address should be included with samples so that receipt can be acknowledged.

The shipment of human blood samples must comply with appropriate regulations as specified by the carrier. At a minimum, all samples must be packaged within two containers with absorbent material between containers to control any spill or leakage. The outer container must be puncture-resistant (e.g., cardboard mailing tube, corrugated cardboard box). A biohazard sticker must be affixed to both the inner and outer containers.

All samples should be shipped via overnight express courier in insulated containers with enough dry ice to maintain the samples in a frozen state. A copy of each of the pharmacokinetic sample collection forms for the respective patients should be included with each shipment.

Appendix F: Procedure for Tumor Biopsy and Blood Samples for Pharmacodynamic Studies

Tumor Biopsy Samples

Biopsies (once initial processing is complete in the procedure room; Section 9.1.2) will be flash-frozen in a pre-chilled cryovial prelabeled with a Unique Identifier Code for the specimen per NCTVL HUSOP340507UH (Sample Handling Section 9.1), see the DCTD Web site for detailed instructions on biopsy collection and handling: https://dctd.cancer.gov/ResearchResources/biomarkers/docs/par/SOP340507_Biopsy_Frozen.pdf. At the NCI, frozen specimens will be transported on dry ice by Clinical Service Program courier to the PADIS lab at Frederick National Laboratory for Cancer Research, where they will be logged into the record keeping system and held in liquid nitrogen until processing.

Biopsy specimens from participating sites will be sent to:

Attention: Dan Danner
NCI-F/FNLCR
1073 Beasley Street, Building 1073
Fort Detrick
Frederick, MD 21701
Phone: 301-846-5748
NCI_PD_Support_CellSearch@mail.nih.gov

Please send an email to FNLCR PD Specimen Central Receiving (NCI_PD_Support@mail.nih.gov) to advise that biopsy samples are being prepared for shipment. State "*Protocol Name* PD Specimens Ready for Shipment" in the subject line. If needed, FNLCR PD Central Receiving can be contacted directly at 301-846-1951 (primary) or 240-344-5697 (mobile). All shipments should include a description of contents on the outside label/shipping slip and detailed packing slip included with the samples.

If the biopsy specimen is large enough (i.e., 10 mm or longer), the specimen will be cut into two parts for subsequent DNA extraction (CpG island methylation analysis) and RNA extraction. If the sample is not sufficient, it will be processed for DNA extraction only.

Processing for CpG island methylation analysis will consist of DNA extraction (DNA-Easy kit, Ambion), purification and quantitation, and aliquoting in barcoded tubes. LINE1 methylation analysis will be performed on the Biotage Pyromark in the PADIS laboratory. An additional analysis in Dr. Yang's laboratory at USC will be performed with a blind aliquot of DNA isolated from the patient biopsy specimen should this prove necessary to confirm results.

Isolation of CTCs

Prior to CTC collection, each participating site should e-mail a request for specimen collection and shipping materials from NCI_PD_Support_CellSearch@mail.nih.gov.
Allow at least six business days for receipt of the blood shipment containers; a confirmation e-mail with the expected shipping date will be sent.

Blood (~8 mL) will be collected aseptically by venipuncture or from a venous port into a CellSave preservative tube pre-labeled with a unique identifier code for the specimen per PADIS SOP LHTP003.8.1 http://dctd.cancer.gov/ResearchResources/biomarkers/docs/ctc/LHTP003.08.03_CellSave_Collection_Subm.pdf; the collected blood samples are stable for up to 96 hours at room temperature (15˚C to 30˚C) prior to processing.

At the NCI, blood will be transported by Clinical Service Program courier to the PADIS lab at Frederick National Lab. Do not ship on ice. Once at PADIS, samples will be logged into a record keeping system and processed within the 96-hour window from sample collection.

Samples from participating sites will be sent to:
Attention: Dan Danner
NCI-F/FNLCR
1073 Beasley Street, Building 1073
Fort Detrick
Frederick, MD 21701
Phone: 301 846-5748

E-mail NCI_PD_Support_CellSearch@mail.nih.gov prior to shipping with expected arrival date/time and a description of contents. All shipments should include a description of contents on the outside label/shipping slip and detailed packing slip included with the samples. Because of the 96-hour window of sample stability, **CTC samples should be shipped to arrive within a 96-hour window** as specified below:

Collection Day	Day/time samples **must** arrive at PADIS
Monday	Thursday (early morning)
Tuesday	Friday (early morning)
Wednesday	Friday (early morning)
Thursday	Friday (early morning)
Friday	Monday (early morning)
Saturday	Tuesday (early morning)
Sunday	Wednesday (early morning)

Testing and data analysis will be performed by Dr. Kinders (PADIS/LHTP/NCI-Frederick). Each ~8 mL of blood will be transferred from the CellSave preservative tube into a correspondingly labeled 15 mL AutoPrep conical tube. The sample will be processed on the CellTracks AutoPrep system, using the CellSearch CTC kit, which contains a ferrofluid-based capture reagent (monoclonal antibody to EpCam) and immunofluorescent reagents. After immunomagnetic capture and enrichment, fluorescently labeled monoclonal antibodies will be added for identification and enumeration of CTCs.

Isolation of plasma for DNA methylation analysis, and mononuclear cells for DNA methyltransferase activity

Reagents:

- Twelve mL of ficoll-hypaque (or equivalent lymphocyte separation medium with density = 1.077), at room temperature.
- Approximately 50 mL of sterile Ca^{++} and Mg^{++}-free phosphate-buffered saline (PBS) or other isotonic diluent at room temperature.
- Approximately 50 mL of sterile 0.15 M NaCl (saline).
- Seven sterile 15 mL conical centrifuge tubes.
- Eight 2-mL cryogenic vials.

Procedure: Obtain two 4-mL EDTA (lavender-top) tubes of blood from the patient.

Mononuclear cells (to assess DNA methyltransferases activity): Centrifuge one (1) 4-mL EDTA tube of blood at 1,000 g for 5 minutes. Transfer as much plasma from each tube as can be safely removed without disturbing the buffy coat to another tube. Gently resuspend the cell pellet back to the original blood volume in room-temperature PBS by inverting several times. Divide the pooled, resuspended blood into two 15-mL centrifuge tubes. Taking care to maintain sterility of the stock bottle, draw 3 mL of ficoll-hypaque for each of the four tubes into a syringe. (The ficoll-hypaque can be drawn up in advance so that it warms to room temperature in the syringe instead of warming the entire bottle.) Using a 20-ga spinal needle (long), carefully layer 3 mL of the ficoll-hypaque under the diluted blood cells in each tube. Centrifuge at 400 g for 30 minutes at room temperature.

Transfer the opaque interface from each tube with a Pasture pipette into a clean conical centrifuge tube containing 3 mL of PBS and dilute the combined cell suspension to approximately 7 mL with additional PBS. Centrifuge at 250 g for 10 minutes. Aspirate and discard the supernatant PBS. Resuspend the cell pellet in 10 mL of PBS. Centrifuge at 250 g for 10 minutes. Aspirate the supernatant PBS, leaving 0.5 to 1 mL of PBS in which to resuspend the cell pellet. Transfer the cell suspension into a cryogenic vial and centrifuge at 600 g for 10 minutes or at approximately 10,000 g in a microfuge for 1 minute. Aspirate and discard the supernatant PBS. Cap the cryogenic vial and vortex it to "smear" the pellet on the inside of the vial, then freeze at -80°C. Label the vial:

Protocol# <date>
PBMC <cycle #>
<Pt Acc #> <day of cycle>.

Plasma (to assess DNA methylation status): Using the remaining 4-mL EDTA tube, spin blood for plasma. Pool with the plasma derived from the mononuclear cell blood tube and transfer 1.5-mL aliquots into 2-mL cryogenic vials. Store at or below -20□C (-70□C preferred). Label the vials:

Protocol# <date>
Plasma <cycle #>
<Pt Acc #> <day of cycle>.

The isolated samples should be stored as directed above then shipped with the sample obtained with the first interim weekly labs. At the NCI, specimens will be transported by Clinical Service Program courier to the PADIS lab at Frederick National Laboratory for Cancer Research. At participating sites, send the samples packed in dry ice to:

Attention: Dan Danner
NCI-F/FNLCR
1073 Beasley Street, Building 1073
Fort Detrick
Frederick, MD 21701
Phone: 301-846-5748
NCI_PD_Support_CellSearch@mail.nih.gov

Please send an email to FNLCR PD Specimen Central Receiving (NCI_PD_Support@mail.nih.gov) prior to shipping with expected arrival date/time and a description of contents. State "*Protocol Name* PD Specimens Ready for Shipment" in the subject line. If needed, FNLCR PD Central Receiving can be contacted directly at 301-846-1951 (primary) or 240-344-5697 (mobile). All shipments should include a description of contents on the outside label/shipping slip and detailed packing slip included with the samples.

Note: With Amendment E (3/16/11 protocol version), isolation of RBCs from blood samples for analysis of fetal hemoglobin expression levels was suspended. RBCs collected prior to this will be stored frozen for future analysis.

Appendix G: Chemotherapy Administration Log for Pharmacokinetic Studies

(First Cycle – First week)

Patient ID_____ Institution: NCI / COH / USC / UCD / PENN
(circle one)

Height _____ cm Weight: ____ . __ kg BSA __ . __ __ m^2

Days 1-5:
THU _____mg/day rapid IV infusion (push)

THU _____mg/day continuous IV infusion over 3 hrs, with

FdCyd _____mg/day continuous IV infusion over 3 hrs.

WEEK 1:

Day 1	Exact Day/Time of THU **Push**	___/___/___, ____:____am/pm
(Hr 0)	Exact Day/Time of **Start** of THU/FdCyd Infusion	___/___/___, ____:____am/pm
	Exact Day/Time of **End** of THU/FdCyd Infusion	___/___/___, ____:____am/pm
Day 2	Exact Day/Time of THU **Push**	___/___/___, ____:____am/pm
(Hr 24)	Exact Day/Time of **Start** of THU/FdCyd Infusion	___/___/___, ____:____am/pm
	Exact Day/Time of **End** of THU/FdCyd Infusion	___/___/___, ____:____am/pm
Day 3	Exact Day/Time of THU **Push**	___/___/___, ____:____am/pm
(Hr 48)	Exact Day/Time of **Start** of THU/FdCyd Infusion	___/___/___, ____:____am/pm
	Exact Day/Time of **End** of THU/FdCyd Infusion	___/___/___, ____:____am/pm
Day 4	Exact Day/Time of THU **Push**	___/___/___, ____:____am/pm
(Hr 72)	Exact Day/Time of **Start** of THU/FdCyd Infusion	___/___/___, ____:____am/pm
	Exact Day/Time of **End** of THU/FdCyd Infusion	___/___/___, ____:____am/pm
Day 5	Exact Day/Time of THU **Push**	___/___/___, ____:____am/pm
(Hr 96)	Exact Day/Time of **Start** of THU/FdCyd Infusion	___/___/___, ____:____am/pm
	Exact Day/Time of **End** of THU/FdCyd Infusion	___/___/___, ____:____am/pm

(See separate PD flow sheet for Cycles 2 and beyond.)

Combined Specimen Collection Form (**PK/PD Flow Sheet**) for Pharmacokinetic and Pharmacodynamic Studies **for Cycle 1 – Page 1 of 4**

Patient ID _____

Cycle **1**, Day **1**:

Date	Hr:Min	Sample	Planned Time	Actual Time	RN Init.	Comments
_____ (Day 1)	:	**#1** (P1)	Pretreatment - 3 mL of blood in a green-topped tube **with ZEB**	:	_____	
	:	**#2** (B1)	Pretreatment - 2 lavender-topped tubes with 4 mL of blood each	:	_____	
	:	**#3** (B2)	Pretreatment - 7.5 mL of blood in a CellSave tube	:	_____	
	:	**#4** (U1)	Pretreatment - 10 mL of **urine** Then ask patient to void completely prior to 24-hr urine collection. **Start** 24-hr **urine collection** for FdCyd Analysis.	:	_____	

Send each sample when drawn to local lab for processing and interim storage according to Appendix E for Pharmacokinetic (green-top) samples and Appendix F for Pharmacodynamic (lavender-top) and CTC (CellSave) samples.

Urine Collection Guidelines for the First 24 Hours Post-Drug Administration
Collect and pool urine for the first 24 hours post drug (0-24 hours), and refrigerate. Please note the total volume of urine collected. At end of 24 hours, mix the urine and take a 10 mL aliquot in a sterile urine cup, refrigerate, and discard the rest.

Sample Collection for Day 1 continues on the next page.

Combined Specimen Collection Form **(PK/PD Flow Sheet)** for Pharmacokinetic and Pharmacodynamic Studies **for Cycle 1 – Page 2 of 4**

Cycle **1**, Day **1**: Patient ID _____

Date	Hr:Min	Sample	Planned Time	Actual Time	RN Init.	Comments
_____ (Day 1, **Cont**.)			**Important** – Record time infusion started	:	_____	
	:	**#5** (P2)	15 Minutes - 3 mL of blood in a green-topped tube **with ZEB**	:	_____	
	:	**#6** (P3)	30 Minutes - 3 mL of blood in a green-topped tube **with ZEB**	:	_____	
	:	**#7** (P4)	1 Hour - 3 mL of blood in a green-topped tube **with ZEB**	:	_____	
	:	**#8** (P5)	2 Hours - 3 mL of blood in a green-topped tube **with ZEB**	:	_____	
	:	**#9** (P6)	2.5 Hours - 3 mL of blood in a green-topped tube **with ZEB**	:	_____	
_____ (Day 1, **Cont**.)			**Important** – Record time infusion ended	:	_____	
	:	**#10** (P7)	15 Minutes - 3 mL of blood in a green-topped tube **with ZEB**	:	_____	
	:	**#11** (P8)	30 Minutes - 3 mL of blood in a green-topped tube **with ZEB**	:	_____	
	:	**#12** (P9)	1 Hour - 3 mL of blood in a green-topped tube **with ZEB**	:	_____	
	:	**#13** (P10)	2 Hours - 3 mL of blood in a green-topped tube **with ZEB**	:	_____	
	:	**#14** (P11)	4 Hours - 3 mL of blood in a green-topped tube **with ZEB**	:	_____	
	:	**#15** (P12)	6 Hours - 3 mL of blood in a green-topped tube **with ZEB**	:	_____	

Send each sample when drawn to local lab for processing and interim storage according to Appendix E.

Sample Collection for Days 2-5 begins on the next page.

Combined Specimen Collection Form **(PK/PD Flow Sheet)** for Pharmacokinetic and Pharmacodynamic Studies **for Cycle 1 – Page 3 of 4**

Cycle **1**, Days **2-5**: Patient ID _____

Date	Hr:Min	Sample	Planned Time	Actual Time	RN Init.	Comments
			Ask patient to void just before ending urine collection.			
_____ (Day **2**)	:	**#16** (U2)	**Before Day 2 Chemo** - End of 24-Hr **urine** collection	:	_____	
	:	**#17** (P13)	**Before Day 2 Chemo** - 3 mL of blood in a green-topped tube **with ZEB**	:	_____	
_____ (Day **2**, **Cont**.)			**Important** – Record time infusion started	:	_____	
_____ (Day **2**, **Cont**.)			**Important** – Record time infusion ended	:	_____	
	:	**#18** (B3)	**After Day 2 Chemo** (up to 24 Hours) - 7.5 mL of blood in a CellSave tube	:	_____	
_____ (Day **3**)	:	**#19** (P14)	**Before Day 3 Chemo** - 3 mL of blood in a green-topped tube **with ZEB**	:	_____	
_____ (Day **4**)	:	**#20** (P15)	**Before Day 4 Chemo** - 3 mL of blood in a green-topped tube **with ZEB**	:	_____	
_____ (Day **5**)	:	**#21** (P16)	**Before Day 5 Chemo** - 3 mL of blood in a green-topped tube **with ZEB**	:	_____	

Send each sample when drawn to local lab for processing and interim storage according to Appendix E for Pharmacokinetic (green-top) samples and Appendix F for Pharmacodynamic (lavender-top) and CTC (CellSave) samples.

Sample Collection for Day 12 begins on the next page.

Combined Specimen Collection Form **(PK/PD Flow Sheet)** for Pharmacokinetic and Pharmacodynamic Studies **for Cycle 1 – Page 4 of 4**

Cycle **1**, Continued: Patient ID _____

Date	Hr:Min	Sample	Planned Time	Actual Time	RN Init.	Comments
Cycle **1**, Day **12** +/- 1 day:						
_____ (Day 12 +/- 1 day)	:	**#22** (B4)	7.5 mL of blood in a CellSave tube	:	_____	
_____ (Day 12 +/- 1 day)	:	**#23** (B5)	2 lavender-topped tubes with 4 mL of blood each	:	_____	

Send each sample when drawn to local lab for processing and interim storage according to Appendix E for Pharmacokinetic (green-top) samples and Appendix F for Pharmacodynamic (lavender-top) and CTC (CellSave) samples.

The processed samples for Cycle 1 should be shipped according to Appendix E for Pharmacokinetic (green-top) samples and Appendix F for Pharmacodynamic (lavender-top) and CTC (CellSave) samples.

Sample Collection for Cycle 2 and beyond begins on the next page.

Flow Sheet for Cycle 2 and Beyond (See combined PK/PD flow sheet for cycle 1.)
Specimen Collection Form for Pharmacodynamic Studies

Patient ID_____ Institution: NCI / COH / USC / UCD /PENN (circle one)

Height _____ cm Weight: ____ . __ kg BSA __ . __ __ m^2

THU _____mg/day rapid IV infusion (push)

THU _____mg/day continuous IV infusion over 3 hrs, with

FdCyd _____mg/day continuous IV infusion over 3 hrs.

Cycle # _____ Start of current treatment cycle (Day 1): Date _____

Sample	Date	Time	RN Initials	Comment
#1 – Cycles 2 and 4 Pretreatment (**Day 1**) 2 lavender-topped tubes with 4 mL of blood each				
#2 – Cycles 2, 4, 6 Day 1 7.5 mL of blood in CellSave tube				
#3 – Cycles 2 and 4 Day 12 (+/- 1 day) 2 lavender-topped tubes with 4 mL of blood each				
#4 – Cycles 2, 4, 6 Day 12 (+/- 1 day) 7.5 mL of blood in CellSave tube				

Send each sample when drawn to local lab for processing and interim storage according to Appendix E for Pharmacokinetic (green-top) samples and Appendix F for Pharmacodynamic (lavender-top) and CTC (CellSave) samples.

The processed samples should be shipped according to Appendix E for Pharmacokinetic (green-top) samples and Appendix F for Pharmacodynamic (lavender-top) and CTC (CellSave) samples.

Flow Sheet for Cycle 1 and Beyond (after patients in each cohort have completed PK requirements)
Specimen Collection Form for Pharmacodynamic Studies

Patient ID_____ Institution: NCI / COH / USC / UCD / PENN (circle one)

Height _____ cm Weight: ____ . __ kg BSA __ . __ __ m^2

THU _____mg/day rapid IV infusion (bolus)

THU _____mg/day continuous IV infusion over 3 hrs, with

FdCyd _____mg/day continuous IV infusion over 3 hrs.

Cycle # _____ Start of current treatment cycle (Day 1): Date _____

Sample	Date	Time	RN Initials	Comment
#1 – Cycles 1, 2 & 4 Pretreatment **Day 1** 2 lavender-topped tubes with 4 mL of blood each				
#2 – Cycles 1, 2, 4, & 6 Pretreatment **Day 1** 7.5 mL of blood in CellSave tube				
#3 – Cycle 1, Day 2 after treatment (up to 24 hours after end of infusion) 7.5 mL of blood in CellSave tube				
#4 – Cycles 1, 2 & 4 **Day 12** (+/- 1 day) 2 lavender-topped tubes with 4 mL of blood each				
#5 – Cycles 1, 2, 4, & 6 **Day 12** (+/- 1 day) 7.5 mL of blood in CellSave tube				

Send each sample when drawn to local lab for processing and interim storage according to Appendix F for Pharmacodynamic (lavender-top) and CTC (CellSave) samples. The processed samples should be shipped according to Appendix F for Pharmacodynamic (lavender-top) and CTC (CellSave) samples.

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Appendix H: Eligibility/Pre-Registration Worksheet

A Multi-Histology Phase II Study of 5-Fluoro-2-Deoxycytidine with Tetrahydrouridine (FdCyd + THU)

Coordinating Center: NCI
Bethesda, MD 20892
Contact: Jennifer Zlott
Tel: 301-594-5664
Fax: 301-480-7281
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Protocol Chair:
James H. Doroshow, MD
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Tel: 301-496-4291
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Lead Associate Investigator:
A. P. Chen, MD
National Cancer Institute
Tel: 301-496 4291
chenali@mail.nih.gov

<table>
<tr><td colspan="2">Patient's Name: (FML)</td><td>Institution:</td></tr>
<tr><td colspan="2">Medical Record Number:</td><td>Investigator:</td></tr>
<tr><td colspan="2">Patient's Birth date:</td><td rowspan="2">Signature of Treating Physician</td></tr>
<tr><td colspan="2">Sex: _____male _____female</td></tr>
<tr><td colspan="2">IRB approval valid until (date):</td><td>Date Informed Consent was signed:</td></tr>
<tr><td>Race:
_____ Black
_____ Caucasian
_____ Asian
_____ American Indian
_____ Native Hawaiian/Pacific Islander
_____ Other _____</td><td>Ethnicity:
___Hispanic
___Non-Hispanic
___Other:_____</td><td>Projected start date of treatment:</td></tr>
</table>

INCLUSION CRITERIA: All responses must be YES. A NO response will make the subject ineligible.

	Yes	No	N/A
Histologically documented (confirmed by the department of pathology at the institution where the patient is enrolled prior to patient enrollment) metastatic or unresectable non-small cell lung cancer, head and neck cancer, urothelial transitional cell carcinoma, or breast cancer, whose disease has progressed after at least one line of standard therapy.			
Patients with solid tumors (non-small cell lung cancer, head and neck cancer, urothelial transitional cell carcinoma, and breast cancer) must have measurable disease, defined as at least one lesion that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥ 20 mm with conventional techniques or as ≥ 10 mm with spiral CT scan. Patients with head and neck cancer whose disease is limited to the skin are eligible at the discretion of the PI and must have a physical exam with documentation of skin lesion(s) by color photography, including a ruler to estimate the size of the lesion(s).			
Any prior therapy must have been completed ≥4 weeks prior to enrollment on protocol, and the participant must have recovered to eligibility levels from prior toxicity. Patients should be at least six weeks out from nitrosoureas and mitomycin C. Prior radiation should have been completed ≥4 weeks prior to study enrollment, and all associated toxicities should have resolved to eligibility levels. Patients must be ≥2 weeks since any investigational agent administered as part of a Phase 0 study, and should have recovered to eligibility levels from any toxicities.			
Age ≥ 18 years?			
Karnofsky score_____see Appendix A?			
Life expectancy > 3 months?			
Women of childbearing potential and men must agree to use adequate birth control			
Has a signed informed consent/assent been obtained by the patient or parent/legal guardian?			

EXCLUSION CRITERIA: Responses should be NO

		Yes	No	N/A
Uncontrolled intercurrent illness including, but not limited to: active or uncontrolled infection, immune deficiencies or confirmed diagnosis of HIV infection, active infection with Hepatitis B or Hepatitis C, uncontrolled diabetes, uncontrolled hypertension, symptomatic congestive heart failure, unstable angina pectoris, myocardial infarction within the past 6 months, uncontrolled cardiac arrhythmia; or psychiatric illness/social situations that would limit compliance with study requirements.				
Pregnant?	Pregnancy Test Date:			
Breastfeeding?				
History of allergic reactions attributed to compounds of similar composition to the investigational agents?				
Receiving any other investigational agents?				

Pre Study Evaluations:	**Date Done:**
History and Physical Exam (within 8 days prior to enrollment)	
Height, Weight, vital signs and performance status (within 8 days prior to enrollment)	
CBC, Diff, Plts, Blood Chemistries* (within 1 week) * Na^+, K^+, Cl^-, CO_2, Creatinine, BUN, calcium, glucose, albumin, SGPT (ALT), SGOT (AST), LDH, Alkaline Phos, Bilirubin Send actual lab results	
CT scan chest/abd/pelvis (within 28 days prior to enrollment)	
Pregnancy Test (if clinically indicated within 8 days prior to enrollment)	

DESCRIPTIVE FACTORS:

Primary: _____ Histology: _____

Other Chronic Diseases: Y / N (If yes, please explain):

PRIOR THERAPY (Please specify date, procedure, agent, dose, response. Date of last treatment required.)

YES NO

____ ____ 1. **Surgery/Biopsy:**

____ ____ 2. **Chemotherapy:**

____ ____ 3. **Radiotherapy**:

____ ____ 4. **Hormonal Therapy:**

____ ____ 5. **Immunotherapy**:

Physician's Signature: _____
Date:_____

Printed Name of Physician: _____

To be completed by participating center when registering a patient:

Date Registered with NCI _____/_____/_____
Spoke With: _____
Study ID:_____
Eligibility Checklist Completed By: _____
Assigned CRA / Data Manager: _____

A confirmation of registration will be sent to you by the NCI.

Participant Status Updates Form

Complete form and send via encrypted email to:
NCI Central Registration Office (HOIS) at ncicentralregistration-l@mail.nih.gov

Patient Information:
First name *Last name* *Middle initial*

*ID number:*_____

*Protocol Number (CC# preferred):*_____

Off Treatment Date (mm/dd/yy): _____

Off Treatment Reason: _____

Off Study Date (mm/dd/yy): _____

Choose one of the following off-study reasons:

_____ *C: Completed Study*
_____ *L: Lost to follow-up*
_____ *R: Refused Further Treatment*
_____ *T: Toxicity*
_____ *D: Death*
_____ *P: Progressive Disease*
_____ *O: Other*

Death Date: (mm/dd/yy): _____

Choose one or more of the following DOD Sources:

*Social Security Death Index SS#:*_____ *http://ssdi.rootsweb.com/*
*Obituaries Document:*_____ *http://www.legacy.com/washingtonpost/DeathNotices*
*Cause of Death*_____
*Place of Death:*_____
*Family/staff member name notifying DOD:*_____

Registrar:
*Name:*_____
*Work Phone:*_____ *Today's Date***:** _____

Comments:

Appendix I: Informed Consent Form Template for Cancer Treatment Trials

Study Title: A Multi-Histology Phase II Study of 5-Fluoro-2-Deoxycytidine with Tetrahydrouridine (FdCyd + THU)

Introduction

We invite you to take part in this research study.

First, we want you to know that:

Taking part in this research study is entirely voluntary.

You may choose not to take part, or you may withdraw from the study at any time. In either case, you will not lose any benefits to which you are otherwise entitled.

You may receive no benefit from taking part. The research may give us knowledge that may help people in the future.

Second, some people have personal, religious or ethical beliefs that may limit the kinds of medical or research treatments they would want to receive (such as blood transfusions). If you have such beliefs, please discuss them with your doctors or research team before you agree to the study.

Now we will describe this research study. Before you decide to take part, please take as much time as you need to ask any questions and discuss this study with family, friends or your personal physician or other health professional.

Why is this study being done?

This research study includes patients with advanced non-small cell lung cancer, breast cancer, urothelial transitional cell carcinoma, and head and neck cancer. You are being asked to volunteer to take part in this research study because you have advanced cancer that has progressed after receiving standard treatment or for which no effective therapy exists. We are doing this study to try to develop better treatments for cancer. On this study, two experimental drugs, FdCyd (also called 5-fluoro-2'-deoxcytidine), and THU (also called tetrahydrouridine), will be given to you. The purpose of this study is to see if the drugs work together to control your tumor growth. FdCyd is thought to work by changing how genes work in cancer cells. THU does not have any anticancer effects on its own, but it helps keep the other drug, FdCyd, from being broken down by your body. These drugs have been given to more than 170 patients as of February 2016. The two drugs have some side effects that will be reviewed with you by your medical team before you sign the consent form.

How many people will take part in this study?

Up to 165 patients will take part in this study across 6 centers in the United States.

What will happen if I take part in this study?

If you are accepted and you choose to take part, you will begin receiving FdCyd and THU. Please see the Study Chart below for more details. You will also have tests and procedures done because you are in the study to see how FdCyd and THU are affecting your body. This will include repeating some of the imaging studies (e.g., CT scans, a computerized x-ray examination) to find out if your cancer has responded. Descriptions of the tests and procedures that will be performed during the study are listed below. For some study procedures we will need you to come to _____ *[name of center]*.

FdCyd and THU will be given in cycles. Each cycle is 28 days long. You will receive FdCyd and THU on days 1-5 and 8-12 of each cycle. You may continue to receive FdCyd and THU if your cancer does not grow, if you do not have too many side effects, and if you are willing to do so.

Clinic Visits: FdCyd and THU will be given through a vein each day on days 1-5 and 8-12 of each cycle. We will ask that you come to _____ *[name of center]* on these days during each cycle. While you are at _____ *[name of center]* we will perform study tests and procedures to see how the study drugs are affecting your body. So, for each cycle you will be coming to _____ *[name of center]* for at least 12-14 days. If you develop any side effects, you may be asked to visit more often.

Standard procedures being done because you are in this study; these may be done more often because you are in the study:

- **Clinic visit:** to ask how you are feeling and to evaluate you with a physical examination every week during Cycle 1, and then at the beginning of each cycle.
- **Vital signs and physical examinations:** will be performed during the clinic visits.
- **Blood tests:** Measurement of your white blood cells, red blood cells, and platelets and measurements of your blood sugar and electrolytes and of how your liver, kidneys, and blood clotting work will be done every week during Cycle 1, and then before treatment on weeks 1 and 2 of all other cycles. Approximately 1 tablespoon (15 mL) of blood will be drawn per visit.
- **Urine test:** Depending on the results of blood tests, you may be asked to collect your urine for 24 hours for further testing.
- **CT scans** or other imaging tests such as ultrasound (an examination using sound waves) or MRI (an examination using magnetic field and radio waves) that detect your tumor will be done before the study and every 8 weeks while you are receiving study drugs (less often if you have been on study for more than one year). This is done so that any benefit of the treatment can be determined, and if your cancer is not responding to the treatment, the study team can tell you and discuss other treatment options (discussed further below).

Tests and procedures that are either being tested in this study or being done to see how the drug is affecting your body:

- **Measurement of FdCyd and THU in your blood:** We will collect blood samples from some patients to measure the amount of study drugs in the blood and to help us find out how the body handles the drugs. Blood will be collected during Cycle 1 only, on day 1 before the drugs are given, at multiple time points while the drugs are being given, at several times

up to 6 hours after the drugs are given, and then once before treatment on days 2, 3, 4, and 5. The total blood for all these tests will be about 3 tablespoons (45 mL).

- **Other research blood samples:** We will also be collecting blood samples to find out the effects of the drugs on cells in your blood. Blood samples will be collected on days 1, 2, and 12 in Cycle 1, on day 1 of Cycle 2 and every subsequent cycle, on day 12 in Cycles 2, 4, and 6, and, if your disease comes back, at the time that your disease comes back. The total blood for all these tests will be about 1/2 a cup (about 115 mL).
- **Measurement of FdCyd and THU in your urine:** We will collect urine samples from some patients to measure the amount of study drugs in the urine. Urine will be collected before the study drugs are given and for 24 hours after the drugs are given on Cycle 1, day 1.
- **Tumor biopsies:** After you are accepted to take part in the study, you will be asked to have a biopsy of your tumor (removal of a small bit of tissue for examination under a microscope) once before you receive study drugs and a second time on day 12 of Cycle 2. We are collecting biopsy samples to study how the drugs affect your tumor. Biopsies are a very important part of this trial and are done for research purposes. Tumor biopsies are optional, and no more than two biopsy procedures will be performed during the study. You may choose not to have tumor biopsies, and this will not affect your taking part in this study. If you decide not to have tumor biopsies, you will still receive study drugs and have other tests that are part of the study.

  Tumor biopsies are only collected by trained personnel. Biopsies are collected using a small bore needle under imaging guidance (CT, MRI, or ultrasound as considered appropriate by the interventional radiologist performing the biopsy). Imaging helps the specialized radiologist know that the needle has been placed into the tumor mass.

  Typical risks of biopsy collection include, but are not limited to, bleeding, infection, pain, and scarring. If you experience any complications from the biopsy, medical care will be offered to you. The amount of radiation you will receive in this study is ____ [*insert center-specific dosimetry value for 2 research biopsies*] rem (a measurement of ionizing radiation such as emitted in x-rays), which is below the guideline of 5 rem per year allowed for research subjects by the NIH Radiation Safety Committee. You will be counseled in more detail about biopsies, and you will be asked to sign a separate consent form that will describe the procedures and risks at that time. Your safety is the most important thing at all times. If upon attempting the first biopsy, no tissue can be obtained or it has caused you harm, the second biopsy procedure will not be done. After you are enrolled in this study, if for any reason the biopsies cannot be done safely, you may still receive the study drugs but the biopsies will not be done.

  I agree to allow biopsies for research purposes Yes___ No____ Initials_____

- Clinical evaluations, imaging studies, and biopsies will be done at _____ *[name of center]*.

Study Chart

The drugs are given over 28-day periods of time called cycles. FdCyd and THU are given through a vein for about 3 hours each day on days 1-5 and 8-12 of each cycle. The days you receive study drugs may change by up to 1 day for scheduling reasons (for example, weekends, holidays, or for your convenience), but FdCyd and THU will only be given for 5 days in week 1 and 5 days in week 2.

The chart below shows what will happen to you during Cycle 1 and future cycles after you sign the consent and start the study. Each cycle is numbered. The left-hand column shows the day in the cycle, and the right-hand column tells you what will happen on that day.

Day	What to do and what will happen to you
Before starting study drug	• Have a history taken of how you feel and undergo a physical examination by a Health Care Provider (HCP) • Get routine blood tests • CT scan will be done • Tumor biopsy may be performed (optional, see above)
Cycle 1 onwards, Day 1	• Have a history taken of how you feel and undergo a physical examination by a HCP • Get routine blood tests • FdCyd and THU will be given through a vein for 3 hours • Blood draws for research will be obtained • Urine samples for research will be obtained over a 24-hour period (Cycle 1 only)
Cycle 1 onwards, Day 2, 3, 4, 5	• FdCyd and THU will be given through a vein for 3 hours • Blood draws for research will be obtained (Cycle 1 only)
Cycle 1 onwards, Day 6, 7	• No treatment
Cycle 1 onwards, Day 8	• Have a history taken of how you feel and undergo a physical examination by a HCP (Cycles 1 and 2 only) • Get routine blood tests • FdCyd and THU will be given through a vein for 3 hours
Cycle 1 onwards, Day 9, 10, 11	• FdCyd and THU will be given through a vein for 3 hours
Cycle 1 onwards, Day 12	• FdCyd and THU will be given through a vein for 3 hours • Blood draws for research will be obtained (Cycles 1, 2, 4, and 6)
Cycle 1 onwards, Day 13-28	• No treatment • On approximately days 15 and 21, you will have a physical examination and blood draws for routine blood tests (Cycle 1 only)
Cycle 3 onwards	• CT scans to determine how your tumor is responding to the treatment will be done every 2 cycles (every 3 cycles if you have been on the study for more than a year or every 4 cycles if for more than 3 years) • Tumor biopsy may be performed (at the end of Cycle 2/prior to start of Cycle 3 only; optional, see above) • Blood draw for research will be obtained if your disease comes back

Are there reasons that my taking part in this study may end early?

Your study doctor will be watching you and your cancer while you are receiving FdCyd and THU. If your cancer is worsening, the study doctor will suggest that you stop taking the drugs and she or he will discuss other options with you. Also, if you are having side effects from the drugs which are dangerous to your health or too difficult for you to tolerate, you and the study team may decide that you should stop taking the drugs. The study doctor may stop you from taking part in this study at any time if he or she believes it is not in your best interest, if you do not follow the study rules, or if the study is stopped.

Stopping Therapy
Your doctor may decide to stop your therapy for the following reasons:

- if he/she believes that it is in your best interest
- if your disease comes back during treatment
- if you have side effects from the treatment that your doctor thinks are too severe
- if new information shows that another treatment would be better for you

In this case, you will be informed of the reason therapy is being stopped.
You can stop taking part in the study at any time. However, if you decide to stop taking part in the study, we would like you to talk to the study doctor and your regular doctor first.
If you decide at any time to withdraw your consent to participate in the trial, we will not collect any additional medical information about you. However, according to FDA guidelines, information collected on you up to that point may still be provided to the Cancer Therapy Evaluation Program (CTEP) at the National Cancer Institute (NCI) or designated representatives. If you withdraw your consent and leave the trial, any samples of yours that have been obtained for the study and stored at the NCI can be destroyed upon request. However, any samples and data generated from the samples that have already been distributed to other researchers or placed in the research databases **cannot** be recalled and destroyed.

What side effects or risks can I expect from being in the study?

If you choose to take part in this study, there is a risk that you may:

- Lose time at work or home and spend more time in the hospital or doctor's office than usual
- Be asked sensitive or private questions which you normally do not discuss

The agents used in this study may affect how different parts of your body work such as your liver, kidneys, heart, and blood. The study doctor will be testing your blood and will let you know if changes occur that may affect your health. There is also a risk that you could have side effects from the study drug(s)/study approach. Here are important points about side effects:

- The study doctors do not know who will or will not have side effects.
- Some side effects may go away soon, some may last a long time, or some may never go away.
- Some side effects may interfere with your ability to have children.
- Some side effects may be serious and may even result in death.

Here are important points about how you and the study doctor can make side effects less of a problem:

- Tell the study doctor if you notice or feel anything different so they can see if you are having a side effect.
- The study doctor may be able to treat some side effects.
- The study doctor may adjust the study drugs to try to reduce side effects.

The tables below show the most common and the most serious side effects that researchers know about. There might be other side effects that researchers do not yet know about. If important new side effects are found, the study doctor will discuss these with you.

Risks and side effects related to FdCyd and THU may include:

COMMON, SOME MAY BE SERIOUS In 100 people receiving FdCyd/THU, more than 20 and up to 100 may have:
• Anemia which may require blood transfusion • Diarrhea, nausea, vomiting • Tiredness • Bruising, bleeding • Infection, especially when white blood cell count is low • Loss of appetite

OCCASIONAL, SOME MAY BE SERIOUS In 100 people receiving FdCyd/THU, from 4 to 20 may have:
• Abnormal heartbeat • Bloating, constipation, heartburn, passing gas • Pain • Dry mouth, skin • Sores in mouth which may cause difficulty swallowing • Chills, fever • Swelling of arms, legs • Weight loss • Dehydration • Muscle weakness • Dizziness, headache • Changes in taste • Numbness, tingling or pain of the arms and legs • Cough, shortness of breath • Nose bleed • Hair loss, itching, rash • Increased sweating • Redness, pain or peeling of the palms and soles • Low blood pressure which may cause feeling faint

THU has no expected side effects at the dose used in this study, but THU may make the side effects of FdCyd worse.

Blood draws for routine laboratory tests may result in bruising, infection, and minor pain or discomfort comparable with a needle prick. When the medication is put through a vein in your arm, you may experience a moment of pain. In addition, there is the discomfort of having the catheter taped to your arm. Other risks include bleeding, bruising, temporary clotting of the vein, and infection.

The removal of tumor tissue for research biopsies can cause pain, bruising, and possibly infection at the site where the biopsy was taken. The biopsy procedure will be discussed in detail with you, including side effects, and we will ask you to sign a separate consent form before to the procedure.

Reproductive Risks: Because the drugs in this study can possibly affect unborn babies and infants, you should not become pregnant, father a baby, or breastfeed while you are on this study. Women of childbearing potential will be required to have a pregnancy test. If you are a woman who can become pregnant, or are the partner of a woman who can become pregnant, you will need to practice an effective form of birth control before starting study treatment and for 3 months after you finish study treatment. If you think that you or your partner is pregnant, you should tell your study doctor or nurse at once. Avoiding sexual activity is the only certain method to prevent pregnancy. But, if you choose to be sexually active, you should use an appropriate "double barrier" method of birth control (such as female use of a diaphragm, intrauterine device (IUD), or contraceptive sponge, in addition to male use of a condom) or the female should be using prescribed "birth control" pills, injections, or implants. Male participants must also use adequate contraception. If you choose to be sexually active during this study, you understand that even with use of these birth control measures pregnancy could still result. Some methods might not be approved for use in this study. Ask about counseling and more information about preventing pregnancy. For more information about risks and side effects, ask your study team.

Are there benefits to taking part in this study?

Taking part in this study may or may not make your health better. While doctors hope the combination of FdCyd and THU will be useful against your cancer, there is no proof of this yet. You should discuss other treatment options with the study team and your home doctor before deciding to take part in this study. We do know that information from this study will help doctors learn more about the combination of FdCyd and THU as a treatment for cancer. This information will also help future cancer patients.

What other choices do I have if I do not take part in this study?

Talk to your doctor about your choices before you decide if you will take part in this study. Your other choices may include:

- Getting other therapies that, although might not improve survival, may have other benefits, such as delaying disease progression.
- Taking part in another study.
- Getting no treatment.

- Getting comfort care, also called palliative care. This type of care helps reduce pain, tiredness, appetite problems, and other problems caused by cancer. It does not treat the cancer directly, but instead tries to improve how you feel. Comfort care tries to keep you as active and comfortable as possible.

Will my medical information be kept private?

We will do our best to make sure that the personal information in your medical record will be kept private. However, we cannot guarantee total privacy. Your personal information may be given out if required by law. If information from this study is published or presented at scientific meetings, your name and other personal information will not be used.

Organizations that may look at and/or copy your medical records for research, quality assurance, and data analysis include:

- The National Cancer Institute (NCI) and other government agencies, like the Food and Drug Administration (FDA), involved in keeping research safe for people.
- Designated investigators from other cancer centers participating in this study, including the City of Hope National Medical Center; the City of Hope Medical Group, Pasadena; the University of Southern California/Norris Comprehensive Cancer Center; the University of California, Davis Cancer Center, and the Penn State Cancer Institute.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

[Note to Informed Consent Authors: the above paragraph complies with the new FDA regulation found at 21 CFR 50.25(c) and must be included verbatim in all informed consent documents. The text in this paragraph cannot be revised.]

[Note to Local Investigators: The NCI has recommended that HIPAA regulations be addressed by the local institution. The regulations may or may not be included in the informed consent form depending on local institutional policy.]

What are the costs of taking part in this study?

You and/or your health plan/ insurance company will need to pay for some or all of the costs of treating your cancer in this study. Some health plans will not pay these costs for people taking part in studies. Check with your health plan or insurance company to find out what they will pay for. Taking part in this study may or may not cost your insurance company more than the cost of getting regular cancer treatment.

The study agents, FdCyd and THU, will be provided free of charge while you are participating in this study. Even though it is unlikely, there is a possibility that at some point the supply of study agent may run out necessitating taking you off-study.

[If applicable, inform the patient of any tests, procedures or agents for which there is no charge. The explanation, when applicable, should clearly state that there are charges resulting from

performance of the test or drug administration that will be billed to the patient and/or health plan. For example, "The NCI is supplying (drug) at no cost to you. However, you or your health plan may need to pay for costs of the supplies and personnel who give you the (drug)."]

You will not be paid for taking part in this study.

For more information on clinical trials and insurance coverage, you can visit the National Cancer Institute's Web site at http://cancer.gov/clinicaltrials/understanding/insurance-coverage. You can print a copy of the "Clinical Trials and Insurance Coverage" information from this Web site.

Another way to get the information is to call 1-800-4-CANCER (1-800-422-6237) and ask them to send you a free copy.

What happens if I am injured because I took part in this study?

It is important that you tell your study doctor, _____ *[investigator's name(s)],* if you feel that you have been injured because of taking part in this study. You can tell the doctor in person or call him/her at _____ *[telephone number]*.

You will get medical treatment if you are injured as a result of taking part in this study. You and/or your health plan will be charged for this treatment. The study will not pay for medical treatment.

What are my rights if I take part in this study?

Taking part in this study is your choice. You may choose either to take part or not to take part in this study. If you decide to participate, you may leave the study at any time. No matter what decision you make, there will be no penalty to you, and you will not lose any of your regular benefits. Leaving the study will not affect your medical care. You can still get your medical care from our center. We will tell you about new information or changes in the study that may affect your health or your willingness to continue in the study. In the case of injury resulting from this study, you do not lose any of your legal rights to seek payment by signing this form.

Who can answer my questions about the study?

You can talk to your study doctor about any questions or concerns you have about this study. Contact your study doctor _____ *[name(s)]* at _____ *[telephone number]*.

For questions about your rights while taking part in this study, call the _____ *[name of center]* Institutional Review Board (a group of people who review the research to protect your rights) at _____ *(telephone number). [Note to Local Investigator: Contact information for patient representatives or other individuals in a local institution who are not on the IRB or research team but take calls regarding clinical trial questions can be listed here.]*

Where can I get more information?

You may call the National Cancer Institute's Cancer Information Service at: 1-800-4-CANCER (1-800-422-6237). You may also visit the NCI Web site at http://cancer.gov/.

- For NCI's clinical trials information, go to: http://cancer.gov/clinicaltrials/
- For NCI's general information about cancer, go to http://cancer.gov/cancerinfo/

You will get a copy of this form. If you want more information about this study, ask your study doctor.

Signature

I have been given a copy of all _____ *[insert total of number of pages]* pages of this form. I have read it or it has been read to me. I understand the information and have had my questions answered. I agree to take part in this study.

Participant _____

Date _____