# F-aza-T-dCyd (NSC801845), a Novel Cytidine Analog, in Comparative Cell Culture and Xenograft Studies with the Clinical Candidates T-dCyd, F-T-dCyd, and Aza-T-dCyd 🅰🅲

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## ABSTRACT

In this article, 5-aza-4′-thio-2′-β-fluoro-2′-deoxycytidine (F-aza-T-dCyd, NSC801845), a novel cytidine analog, is first disclosed and compared with T-dCyd, F-T-dCyd, and aza-T-dCyd in cell culture and mouse xenograft studies in HCT-116 human colon carcinoma, OVCAR3 human ovarian carcinoma, NCI-H23 human NSCLC carcinoma, HL-60 human leukemia, and the PDX BL0382 bladder carcinoma. In three of five xenograft lines (HCT-116, HL-60, and BL-0382), F-aza-T-dCyd was more efficacious than aza-T-dCyd. Comparable activity was observed for these two agents against the NCI-H23 and OVCAR3 xenografts. In the HCT-116 study, F-aza-T-dCyd [10 mg/kg intraperitoneal (i.p.), QDx5 for four cycles], produced complete regression of the tumors in all mice with a response that proved durable beyond postimplant day 150 (129 days after the last dose). Similarly, complete tumor regression was observed in the HL-60 leukemia xenograft when mice were dosed with F-aza-T-dCyd (10 mg/kg i.p., QDx5 for three cycles). In the PDX BL-0382 bladder study, both oral and i.p. dosing of F-aza-T-dCyd (8 mg/kg QDx5 for three cycles) produced regressions that showed tumor regrowth beginning 13 days after dosing. These findings indicate that further development of F-aza-T-dCyd (NSC801845) is warranted.

**Graphical Abstract:** http://mct.aacrjournals.org/content/molcanther/20/4/625/F1.large.jpg.
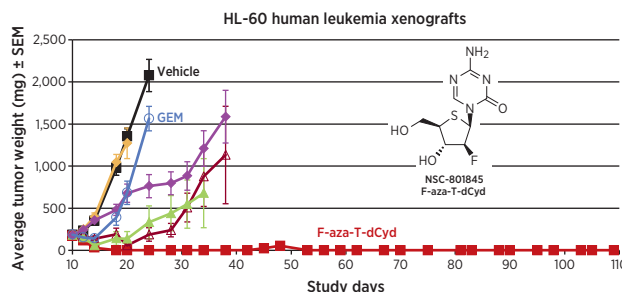
## Introduction

Cytidine analogs remain an area of active drug discovery and development with five FDA-approved drugs, including cytarabine, which was approved in 1969 for the treatment of acute myeloid leukemia (AML; ref. 1). DNMT1, a maintenance methyltransferase that contributes to the hypermethylation and silencing of tumor suppressor genes, is a major molecular target of two of these drugs, azacytidine and decitabine, which were approved for myelodysplastic syndromes in 2004 and 2006, respectively (2). The latter two drugs have also been tested in leukemia and solid tumor clinical trials as single agents and in combination therapies (3). When DNMT1 is depleted by drug treatment, the existing methyl pattern on genes is no longer maintained in replicated cells resulting in reactivation of tumor suppressor genes (4). At least two cell division cycles are required after drug exposure to maximize reexpression of silenced genes (2). In addition, DNMT1 has roles independent of its methyltransferase activity and a DNMT1 knockout results in decreased cell viability preceded by events consistent with activation of a DNA damage response. Azacytidine and decitabine contain aza-cytosine bases connected to a ribose ring that is notable by the presence or absence of a hydroxy group at the 2-position. 4′-thio-2′-deoxycytidine (T-dCyd) and 5-aza-4′-thio-2′-deoxycytidine (aza-T-dCyd) are two related sulfur-containing deoxy-cytidine analogs that deplete DNMT1 both *in vitro* and *in vivo* in tumor cells (5). Both agents were effective (i.p. dosing) in slowing the growth of tumors in NCI-H23 human NSCLC xenografts in athymic nude mice (nu/nu NCr). T-dCyd and aza-T-dCyd are currently in phase I clinical trials at the NCI (Bethesda, MD; NCT02423057 and NCT03366116, respectively). The representative examples of cytidine agents are shown in **Fig. 1**.

The incorporation of a fluorine atom into the chemical structure of cytidine derivatives has been an effective strategy for modulating the pharmacokinetic and pharmacodynamic parameters of the nucleoside (6). Through the ability of fluorine to increase lipophilicity and affect electronic and steric factors, fluorine atoms can be used to block metabolism and produce changes in target potency, selectivity, and

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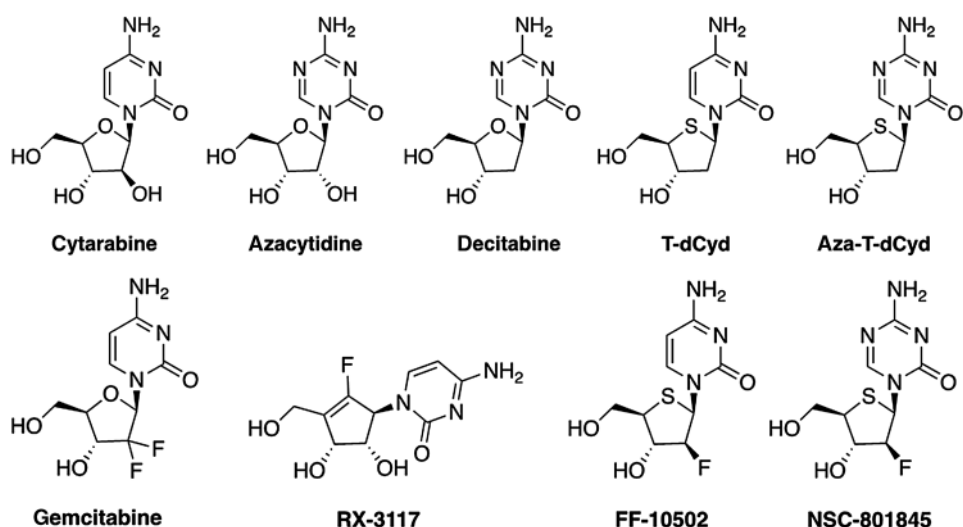
**Figure 1.**
Structures of cytidine FDA-approved drugs and investigational agents, including fluorine-containing cytidine compounds.

overall toxicity associated with the modified derivative. Gemcitabine is a widely clinically used fluorine containing cytidine drug, which is approved for use in pancreatic, ovarian, breast, and non–small cell lung cancers. Gemcitabine is a prodrug, which is phosphorylated intracellularly and incorporated into DNA during DNA synthesis, thus, terminating further DNA chain elongation (7–9). DNA repair processes are unable to remove gemcitabine resulting in cell death. RX-3117 is another fluorine containing agent that can downregulate DNMT-1 and can be incorporated into RNA and DNA (10). RX-3117 has shown significant efficacy in several colon, lung, and pancreatic human xenograft models including against tumor lines that are resistant to gemcitabine (11). RX-3117 has completed phase I trial and has undergone phase II trial in metastatic bladder cancer as a single agent and phase II trial in metastatic pancreatic cancer in combination with abraxane (NCT02030067 and NCT03189914). FF-10502 (F-T-dCyd) is a fluorine-containing thio-nucleoside that inhibits DNA-polymerase and is superior to gemcitabine in targeting pancreatic cancer cells (12). FF-10502 is currently in phase I/II clinical trial in solid tumors and lymphomas (NCT02661542; ref. 13).

Herein, the synthesis and first disclosure of a novel fluorine containing cytidine analog, 5-aza-4′-thio-2′-β-fluoro-2′-deoxycytidine (F-aza-T-dCyd, NSC801845), is described (14). F-aza-T-dCyd is compared with several related cytidine analogs [including T-dCyd, aza-T-dCyd, and F-T-dCyd (FF-10502)], in the NCI-60 cell assay. In addition, the results of a comparative *in vivo* efficacy study are presented with F-aza-T-dCyd, gemcitabine, T-dCyd, aza-T-dCyd, and FF-10502 in several human tumor xenograft studies, including HCT-116 human colon carcinoma, OVCAR3 human ovarian carcinoma, NCI-H23 human NSCLC carcinoma, and HL-60 human leukemia as well as a patient-derived xenograft, BL0382 bladder carcinoma.

## Materials and Methods

### Compound synthesis

The FDA-approved drugs, 5-azacytidine, decitabine, and gemcitabine, were obtained from the DTP chemical repository (available from NCI at: https://dtp.cancer.gov/organization/dscb/obtaining/default.htm). The investigational agent RX-3117 was purchased from ChemScene. T-dCyd and aza-TdCyd were synthesized as described previously (15). FF-10502 (F-T-dCyd) was synthesized at NCI according to a modification (14) of methods described previously (16). Briefly, NSC-801845 was synthesized in 13 steps as described in **Fig. 2** (14). A detailed description and experimental details for the synthesis of F-aza-T-dCyd (NSC-801845) and FF-10502 are found in the Supplementary Material.
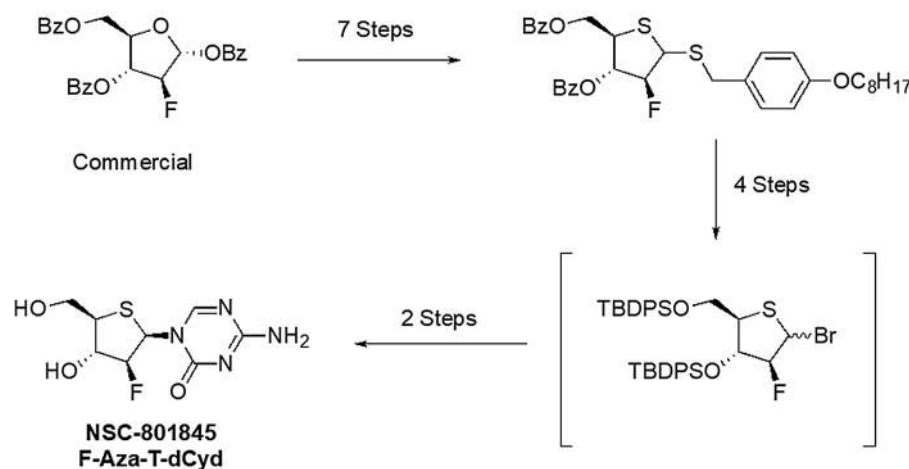


**Figure 2.**
Synthesis of NSC-801845.

### Cell culture

NCI-60 cell lines were obtained from the NCI Developmental Therapeutics Program Tumor Repository. For each lot of cells, the Repository performed Applied Biosystems AmpFLSTR Identifiler testing with PCR amplification to confirm consistency with the published Identifiler STR profile for the given cell line (17–19). Each cell line was tested for *Mycoplasma* when it was accepted into the repository; routine *Mycoplasma* testing of lots was not performed. Cells were kept in continuous culture for no more than 20 passages. The optimal seeding densities for each of the cell lines at each time point assessed were determined prior to performing the concentration response studies (20–22). The NCI-60 screen was performed as described at: https://dtp.cancer.gov/discovery_development/nci-60/default.htm. Briefly, the NCI-60 human tumor lines were grown in RPMI1640 medium supplemented with 5% FBS and 2 mmol/L L-glutamine. For experiments, cells were inoculated into 96-well plates in 100 µL of complete medium at plating densities ranging from 5,000 to 40,000 cells per well depending on the doubling time of individual lines. The plates were incubated at 37°C in humidified 5% $CO_2$/95% air for 24 hours. Compounds were formulated in DMSO. The plates were incubated for 48 hours. For staining, sulforhodamine B (SRB) solution (100 µL) at 0.4% (w/v) in 1% acetic acid was added to each well, and plates were incubated for 10 minutes at room temperature. The SRB was solubilized, and the absorbance at 515 nm was read. Using the absorbance measurements time zero (Tz), control growth (C), and test growth (Ti), the percent cell growth was calculated. Growth inhibition of 50% ($GI_{50}$) is calculated from $[(Ti-Tz)/(C-Tz)] \times 100 = 50$, which is the compound concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells.

### *In vivo* studies

Human tumor xenografts were generated in 4- to 6-week-old female athymic nude mice (nu/nu NCr) or NSG mice by subcutaneous injection of tumor cells (HL-60, NCI-H23, OVCAR-3, HCT-116) grown *in vitro* using RPMI1640 with 10% FBS and 2 mmol/L L-glutamine (23).

The BL0382F1232 patient-derived xenograft (PDX) model, was originally developed by Jackson Laboratories and received from JAX as cryopreserved fragments (available as JAX # TM00020; ref. 24). Upon receipt, we serially passaged the tumor to create a cryopreserved bank of tumor fragments. For drugs studies, vials of cryopreserved tumor were thawed, implanted into NSG mice, and the resulting tumors passaged into cohorts of mice to establish the study mice as described for other xenograft models (23).

The mice were housed in an AAALAC-accredited facility with food and water provided *ad libitum*. When tumors reached the predetermined starting weight (staging weight), the animals were randomized into experimental groups and treatment was initiated. Groups included a vehicle control group as well as the drug-treated groups. Drug doses were selected on the basis of prior experience or newly conducted mouse tolerability studies as described elsewhere (23). Tumors were monitored by bidirectional caliper measurements, and the tumor weights were calculated as tumor weight (mg) = (tumor length in mm × tumor width in mm2)/2. Data collection was performed using the StudyLog software program StudyDirector (Studylog Systems, Inc.). Data were calculated and plotted using Microsoft EXCEL. Significant differences in response between controls and each treatment group were calculated using Student *t* test.

## Results

### Synthesis of F-aza-T-dCyd (NSC801845)

The incorporation of a fluorine atom into the cytidine aza-T-dCyd was accomplished starting from the commercially available (2R, 3S,4R, 5R)-5-((benzoyloxy)methyl)-3-fluorotetrahydrofuran-2,4-diyl dibenzoate. Primarily utilizing chemistry applied previously to the synthesis of the des-fluoro-thio sugar, the intermediate 2-bromo-3-β-fluoro thio sugar was prepared in 11 steps and immediately coupled with silylated aza-cytosine to produce upon deprotection F-aza-T-dCyd (NSC801845; ref. 14). A comparison of calculated LogP values (cLogP values obtained from ChemDraw v.18) for aza-T-dCyd (−4.37) and F-aza-T-dCyd (-3.93) suggests a lipophilicity increase of a half-log value for the novel fluorinated agent (**Fig. 3**).
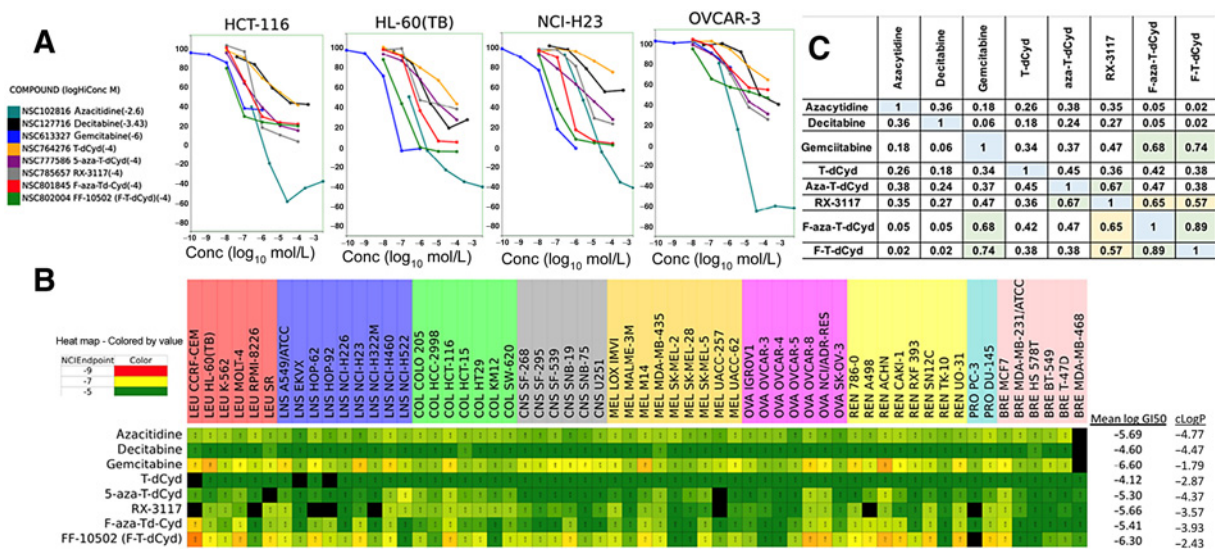
**Figure 3.**
NCI-60 concentration response, heatmap view, and matrix COMPARE.

### Cell culture studies

The eight cytidine agents were evaluated in the 5-concentration NCI60 cell line assay (**Fig. 3**). Representative concentration response curves from four of the NCI60 cell lines showed a 10-fold to 100-fold difference in sensitivity of the cells to the eight compounds (**Fig. 3**). The most cytotoxic compounds were gemcitabine, FF-10502 (F-T-dCyd), F-aza-T-dCyd (NSC801845), followed by RX-3117. The least cytotoxic compounds were decitabine and T-dCyd. The NCI60 heatmap based upon the $GI_{50}$ values showed the full range of activity in the assay and indicated some similarities in the patterns of cell line sensitivities for some of the eight compounds. The hematologic malignancy cell lines were generally sensitive to the compounds except TdCyd. Among the NSCLC lines, NCI-H460 was sensitive, whereas EKVX and NCI-H226 were less responsive. With the exception of gemcitabine and FF-10502 (F-T-dCyd), the CNS malignancy cell lines were generally nonresponsive. MDA-MB-435 was most sensitive among the melanoma lines, and SK-Mel-2 and SK-Mel-5 were the least responsive. The ovarian cancer line OVCAR8 was sensitive to the cytidine analogs while the ovarian cancer cell line OVCAR-4 was generally nonresponsive to the cytidine analogs. Among the renal cell carcinoma cell line panel, the ACHN cell line was very responsive and the TK-10 cell line was the least responsive. The breast and prostate cancer panel cell lines had mixed responses to the eight cytidine analogs with only MCF-7 showing relative sensitivity to the group.

A $GI_{50}$ matrix grid-COMPARE analysis, employing a standard Pearsons correlation, run with the eight cytidine analogs revealed a strong COMPARE correlation between F-aza-T-dCyd (NSC801845) and FF-10502 (F-T-dCyd; 0.89) as well as between gemcitabine and F-aza-T-dCyd (NSC-801845; 0.68) and between gemcitabine and FF-10502 (F-T-dCyd; 0.74; **Fig. 3C**). Examination of the corresponding mean graphs of F-aza-T-dCyd, F-T-dCyd, and gemcitabine further demonstrates the similarities between the NCI-60 patterns between these agents (Supplementary Fig. S1). The carbocyclic sugar analog,

RX-3117 also showed interesting COMPARE correlations with strong correlation with aza-T-dCyd (0.67) and F-aza-T-dCyd (NSC801845; 0.65) with somewhat lower correlations to FF-10502 (F-T-dCyd; 0.57) and gemcitabine (0.47). Interestingly, the correlation between F-aza-T-dCyd (NSC801845) and aza-T-dCyd was relatively low (0.45). TGI and $LC_{50}$ values were not examined in a COMPARE analysis because neither parameter was reached at 100 μmol/L for any of the cytidine agents except gemcitabine.

### *In vivo* studies

*In vivo* studies were carried out with five of the eight cytidine agents, T-dCyd, aza-T-dCyd, gemcitabine, FF-10502 (F-T-dCyd), and F-aza-T-dCyd (NSC801845) in mouse xenograft studies with five tumor types, including HCT-116 human colon carcinoma, OVCAR3 human ovarian carcinoma, NCI-H23 human NSCLC carcinoma, HL-60 human leukemia, and the patient-derived xenograft BL0382 human bladder carcinoma. (**Figs. 4–6**). Doses and schedules for the known cytidine agents [T-dCyd and aza-T-dCyd (5), gemcitabine (25), F-T-dCyd (12)] were chosen at or near the MTD previously observed in these and other tumor-bearing models. For F-aza-T-dCyd, an MTD was determined for single and multiple daily i.p. dosing and these doses and schedules were used in the five xenograft studies, which were carried out in a sequential and iterative fashion (26). For example, with the observation of noteworthy activity for F-aza-T-dCyd in the HL-60 and HCT-116 xenografts, an oral-dosed arm for this agent was added to the OVCAR-3 and BL0382 studies. In xenograft studies, T-dCyd was the least effective of the cytidine analogs in four of five xenografts. In three of five xenograft lines (HCT-116, HL-60, and the PDX BL-0382), F-aza-T-dCyd (NSC801845) was more efficacious than aza-T-dCyd (administered at the MTD of 1.5 mg/kg intraperitoneal). Comparable activity was observed for these two agents against the NCI-H23 and OVCAR3 xenografts.
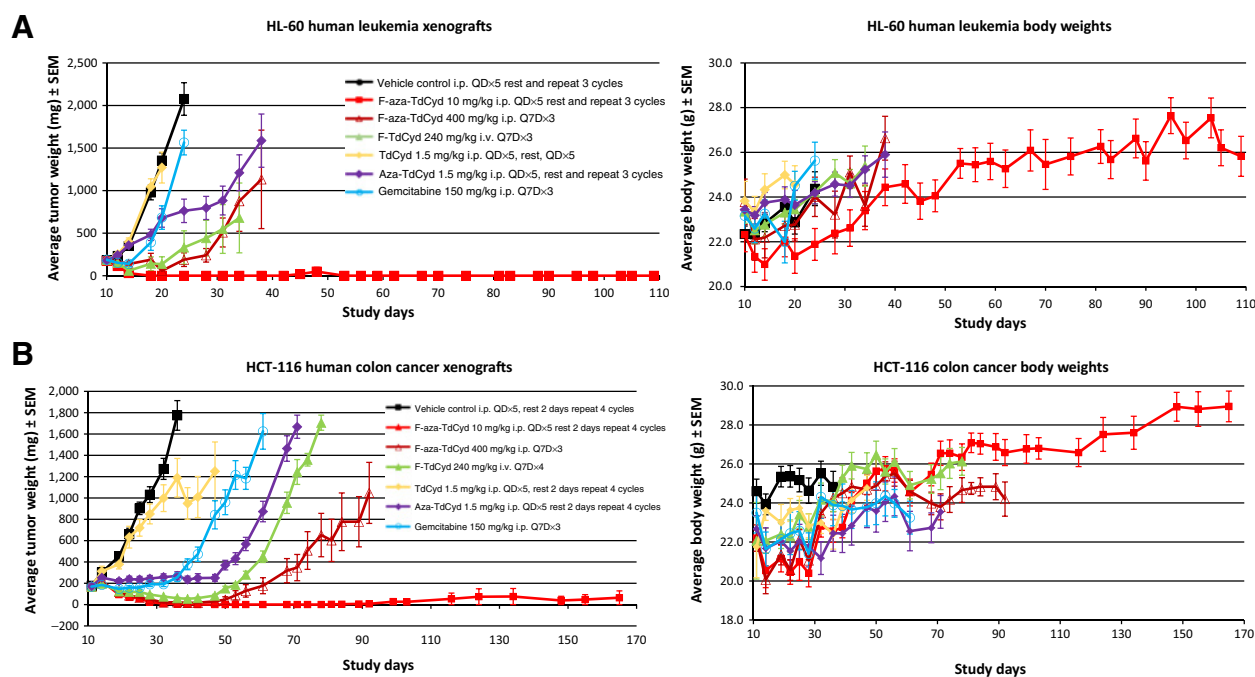
**Figure 4.**
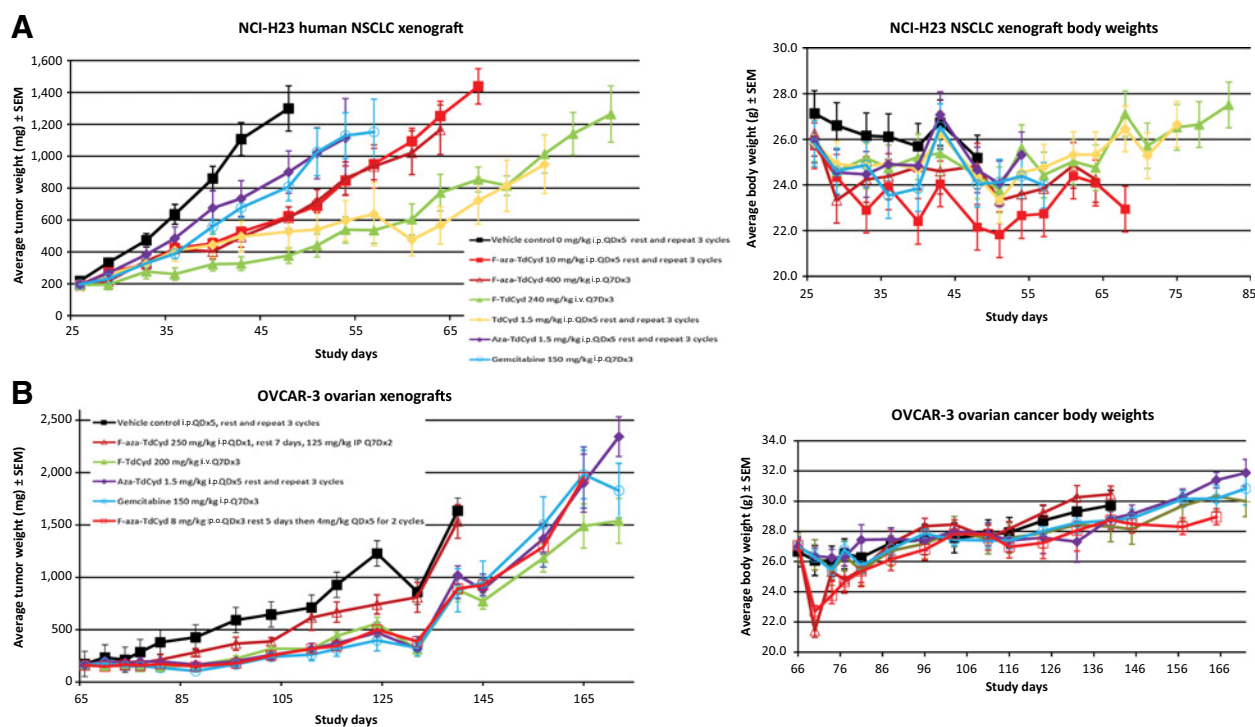HCT-116 colon carcinoma, HL-60 leukemia xenografts.

**Figure 5.**
OVCAR3 ovarian and NCI-H23 NSCLC lung carcinoma xenografts.

In the HCT-116 study, F-aza-T-dCyd (NSC801845; 10 mg/kg i.p., QDx5 for four cycles), produced complete regression of the tumors in all mice with a response that proved durable out to 150 days (129 days after the last dose; $P = 8 \times 10^{-9}$; **Fig. 4**). In the HCT-116 model, regression was also observed with FF-10502 [F-T-dCyd; 240 mg/kg intravenously (i.v.), every 7 days for four cycles], however, tumor regrowth was observed upon cessation of treatment ($P = 1 \times 10^{-8}$). Both aza-T-dCyd and gemcitabine provided modest suppression of tumor growth as well in the HCT116 tumor ($P = 1 \times 10^{-7}$ and $P = 1 \times 10^{-7}$, respectively). A once weekly dose of F-aza-TdCyd (400 mg i.p. for three cycles) in this study was also effective in initially causing regression in the HCT116 tumor ($P = 1 \times 10^{-8}$), but this treatment proved to be less durable over time compared with five daily doses for four cycles. Mean body weights in the HCT-116–bearing animals were decreased initially with F-aza-T-dCyd treatment but recovered to normal levels of growth throughout the remainder of the study. Similar body weight effects were observed with the dosing of other cytidine agents in this study.

A similar complete tumor regression was observed in the HL-60 leukemia xenografts when mice were dosed with F-aza-T-dCyd (10 mg/kg i.p., QDx5 for three cycles) with a response that proved durable out to 45 days ($P = 3 \times 10^{-8}$; **Fig. 4**). Tumor regression was also observed with F-aza-T-dCyd (400 mg/kg i.p., Q7Dx3; $P = 3 \times 10^{-7}$) or FF-10502 (240 mg/kg i.v., Q7Dx4; $P = 2 \times 10^{-5}$), but tumor growth in this model resumed after cessation of either treatment. The antitumor effects for treatment with aza-T-dCyd and gemcitabine were minimal, and T-dCyd was ineffective in the HL-60 leukemia model. Mean body weights were generally unaffected by any of the cytidine treatment protocols in the HL-60 xenografts.

In the OVCAR3 ovarian tumor xenograft model, similar levels of tumor growth suppression were observed with F-aza-TdCyd [8 to 4 mg/kg orally (p.o.) QDx5], FF-10502 (200 mg/kg i.v., Q7Dx3), aza-T-dCyd (1.5 mg/kg i.p., QDx5), or gemcitabine (150 mg/kg i.p., Q7Dx3; **Fig. 5**). Treatment with F-aza-T-dCyd (250 mg/kg i.p.) administered weekly was minimally effective in this model. Mouse body weights initially dropped slightly more than 10% upon initial treatment with F-aza-T-dCyd but recovered normally throughout the remainder of the xenograft study.

In the NCI H-23 NSCLC lung carcinoma xenograft model, none of the cytidine agents showed significant efficacy, with only F-T-dCyd (240 mg/kg i.v., Q7Dx3) and T-dCyd (1.5 mg/kg i.p., QDx5) having minimal effects on tumor growth suppression (**Fig. 5**). No difference was observed between the effects of weekly and daily administered doses of F-aza-T-dCyd. Mouse body weights were generally unaffected by any of the cytidine treatment protocols in the NCI-H-23 NSCLC study.

In the PDX BL0382 bladder carcinoma, both oral and intraperitoneal dosing of F-aza-T-dCyd (8 mg/kg p.o., QDx5 for three cycles; 8 mg/kg QDx5 IP, QDx5 for three cycles) produced regressions that showed tumor regrowth 13 days after dosing though at a growth rate below that of the control group (**Fig. 6**). Although drug levels in the blood were not determined, the similar efficacy observed at the same doses with the oral and i.p. routes of administration in this model suggests that F-aza-T-dCyd (NSC801845) has significant oral activity in mice. This level of efficacy compared quite well with that observed after treatment with gemcitabine (150 mg/kg i.p., Q7Dx3). A weekly dose of F-aza-T-dCyd (250 mg/kg i.p., Q7Dx3) proved somewhat less effective although there was good tumor growth control throughout the dosing period. Treatments with F-T-dCyd, aza-T-dCyd, and T-dCyd were less effective in the BL-0382 patient-derived xenograft model. Mouse body weights were generally unaffected by any of the cytidine treatment protocols in this study.
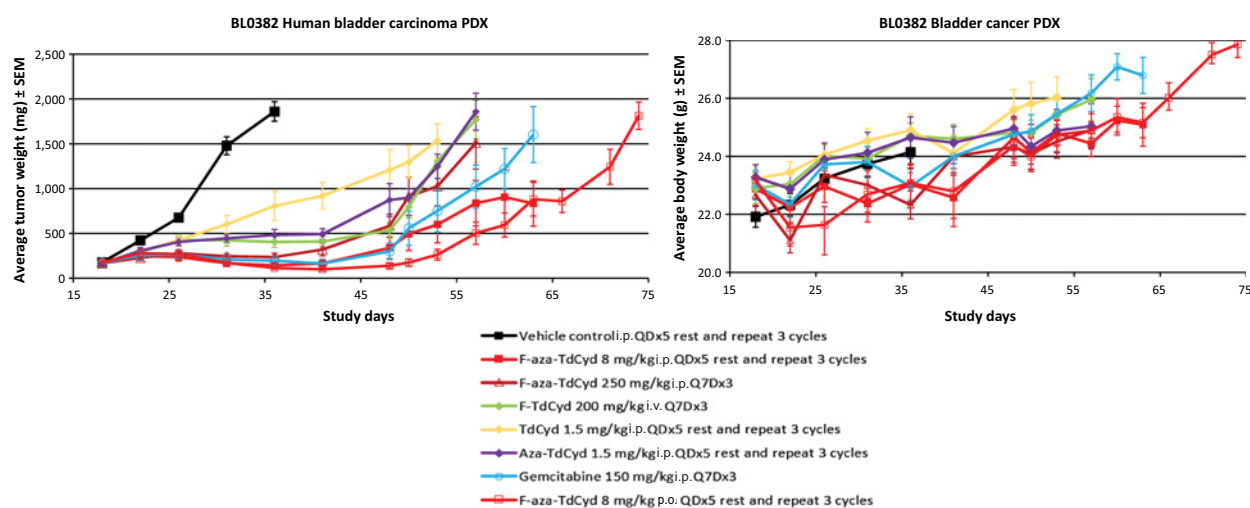
**BL0382 Human bladder carcinoma PDX**

**BL0382 Bladder cancer PDX**

- ■ Vehicle control i.p. QDx5 rest and repeat 3 cycles
- ■ F-aza-TdCyd 8 mg/kg i.p. QDx5 rest and repeat 3 cycles
- ▲ F-aza-TdCyd 250 mg/kg i.p. Q7Dx3
- ✦ F-TdCyd 200 mg/kg i.v. Q7Dx3
- ◆ TdCyd 1.5 mg/kg i.p. QDx5 rest and repeat 3 cycles
- ◆ Aza-TdCyd 1.5 mg/kg i.p. QDx5 rest and repeat 3 cycles
- ● Gemcitabine 150 mg/kg i.p. Q7Dx3
- ■ F-aza-TdCyd 8 mg/kg p.o. QDx5 rest and repeat 3 cycles

**Figure 6.**
BL0382 bladder carcinoma.

## Discussion

The NCI-60 human tumor cell line panel, consisting of cell lines from nine tumor types, has been used to profile potential oncology chemotherapeutic agents for the past 25 years (20). In addition, the NCI-60 screen has proven to be a useful tool for the oncology research community to further its understanding of the biology of cancer and the molecular targets and mechanisms of action of new oncology agents. In this regard, the COMPARE algorithm has been a useful tool for the direct comparison of sensitivity patterns resulting from the effects of compounds on cell growth in the 2-day NCI-60 assay (27). The qualitative nature of these sensitivity patterns (regardless of potency) can often be correlative to the target mechanisms associated with the test compounds (28). Independent of whether a specific molecular target has been identified, high COMPARE (Pearson's) correlation between two test compounds is often indicative of a shared molecular mechanism of action.

Of the eight cytidine agents evaluated in the NCI-60 cell line panel, FF-10502 (F-T-dCyd) and gemcitabine were generally more potent based on the their respective mean $GI_{50}$ (growth inhibition) values across the entire panel. These two agents were followed by F-aza-T-dCyd (NSC801845), azacytidine, and RX-3117 with aza-T-dCyd and T-dCyd being less cytotoxic based on their respective mean $GI_{50}$ potencies in the 2-day assay. However, by COMPARE analysis, F-aza-T-dCyd (NSC801845) correlated highly with FF-10502 (F-T-dCyd) and gemcitabine (correlations 0.89 and 0.68, respectively), suggesting a possible shared DNA-damaging mechanism of action among these three agents. Interestingly, the $GI_{50}$ sensitivity patterns between F-aza-T-dCyd and aza-T-dCyd have a low correlation (0.45), even though these two agents differ in structure by only the presence or absence of a single fluorine atom.

Although correlation of the sensitivity patterns from the NCI-60 screen can often be associated with mechanistic information, the link between potency in the cell-line assay and *in vivo* efficacy is not universally realized. In this set of compounds, the two most potent cell culture agents, FF-10502 (mean log $GI_{50}$ −6.30) and gemcitabine (mean log $GI_{50}$ −6.60), have readily detected antitumor activity in four of the five xenograft models. However, the overall impressive efficacy associated with F-aza-T-dCyd (NSC801845), which produced regres-

sion of tumors in three of five of the xenografts models is not predicted by its $GI_{50}$ potencies (mean log $GI_{50}$ −5.41) in the NCI-60 assay. Several parameters could be factors in accounting for the *in vitro/ in vivo* disconnect surrounding F-aza-T-dCyd (NSC801845), including the nature of the 2-day cell assay, mechanism of action, pharmacokinetics of the agent in mice, and compound residence time in cells. The data indicate the tumor regression is durable upon cessation of compound dosing and that oral delivery of F-aza-T-dCyd (NSC801845) produced efficacy on par with or greater than intraperitoneal delivery, further highlights the attractive nature of this new cytidine agent. These xenograft data clearly demonstrate that F-aza-T-dCyd (NSC801845) has remarkable activity relative to the comparator set against multiple tumor lines. Thus, further characterization of this novel cytidine derivative as a potential antitumor agent is warranted.

### Authors' Disclosures

The Editor-in-Chief of *Molecular Cancer Therapeutics* is an author on this article. In keeping with AACR editorial policy, a senior member of the *Molecular Cancer Therapeutics* editorial team managed the consideration process for this submission and independently rendered the final decision concerning acceptability. J. Morris reported employment from NIH during the conduct of the study; in addition, J. Morris had a patent for WO2020/068657 pending. D.G. Wishka reported personal fees from the United States Government during the conduct of the study; in addition, D.G. Wishka had a patent for WO 2020/068657 A1 pending. O.D. Lopez reported personal fees from Kelly Government Solutions at NIH during the conduct of the study; personal fees from Kelly Government Solutions at NIH outside the submitted work; in addition, O.D. Lopez had a patent for WO 2020/068657 pending. M.W. Kunkel reported other from NIH during the conduct of the study and other from NIH outside the submitted work. No potential conflicts of interest were disclosed by the other authors.

### Disclaimer

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### Authors' Contributions

**J. Morris:** Conceptualization, data curation, formal analysis, supervision, investigation, visualization, methodology, writing–original draft, project administration, writing–review and editing. **D.G. Wishka:** Conceptualization, resources, investigation, methodology, writing–review and editing. **O.D. Lopez:** Conceptualization,

resources, investigation, methodology, writing–review and editing. **V. Rudchenko:** Resources, methodology. **G. Huang:** Resources. **S.N. Hoffman:** Resources. **S. Borgel:** Resources**. K. Georgius:** Resources. **J. Carter:** Resources. **H. Stotler:** Resources. **M.W. Kunkel:** Data curation, formal analysis, visualization, writing–review and editing. **J.M. Collins:** Supervision, writing–review and editing. **M.G. Hollingshead:** Resources, data curation, formal analysis, investigation, visualization, methodology, writing–review and editing. **B.A. Teicher:** Resources, data curation, formal analysis, investigation, visualization, methodology, writing–review and editing.

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