NCI Protocol #:

DF/HCC Protocol #: 17-044

TITLE: A Phase 2 Study of lamivudine in patients with p53 mutant metastatic colorectal cancer

Coordinating Center: DF-HCC

***Principal Investigator (PI):** Aparna R. Parikh, M.D.
Massachusetts General Hospital
Aparna.Parikh@mgh.harvard.edu

Other Investigators: David T. Ting, M.D.
Massachusetts General Hospital
dting1@mgh.harvard.edu

David P. Ryan, M.D.
Massachusetts General Hospital
dpryan@mgh.harvard.edu

Statistician:
Hui Zheng
Massachusetts General Hospital
HZHENG1@PARTNERS.ORG

Study Coordinator/Data Manager:
Ashley O'Meara
Massachusetts General Hospital
55 Fruit St.
617-643-5411
617-643-8399
alomeara@mgh.harvard.edu

Responsible Research Nurse:
Patricia Lynch, RN
Massachusetts General Hospital
32 Fruit St.
617-643-0816
Lynch.Patricia2@mgh.harvard.edu

Agent(s): Lamivudine commercially obtained

Study Exempt from IND Requirements per 21 CFR 312.2(b).

Protocol Type / Version # / Version Date: Original/March 30, 2017
Version 2, December 01, 2017
Version 3, January 23, 2018
Version 4, May 2, 2019
Version 5, November 14, 2019

ClinicalTrials.gov Identifier: NCT03144804

SCHEMA

Eligible patients with p53mutant/deficient metastatic colorectal cancer

28 day treatment cycles of lamivudine until progression

TABLE OF CONTENTS

1. Berretta, J. and A. Morillon, Pervasive transcription constitutes a new level of eukaryotic genome regulation. EMBO Rep, 2009. 10(9): p. 973-82.....42

2. Jacquier, A., The complex eukaryotic transcriptome: unexpected pervasive transcription and novel small RNAs. Nat Rev Genet, 2009. 10(12): p. 833-44. 42

3. Jurka, J., et al., Repbase Update, a database of eukaryotic repetitive elements. Cytogenet Genome Res, 2005. 110(1-4): p. 462-7.....42

4. Ting, D.T., et al., Aberrant overexpression of satellite repeats in pancreatic and other epithelial cancers. Science, 2011. 331(6017): p. 593-6. 42

5. Bersani, F., et al., Pericentromeric satellite repeat expansions through RNA-derived DNA intermediates in cancer. Proc Natl Acad Sci U S A, 2015. 112(49): p. 15148-53.....42

6. Wylie, A., et al., p53 genes function to restrain mobile elements. Genes Dev, 2016. 30(1): p. 64-77. 42

7. Liu, M.C., et al., Circulating tumor cells: a useful predictor of treatment efficacy in metastatic breast cancer. J Clin Oncol, 2009. 27(31): p. 5153-9. 42

8. Tol, J., et al., Circulating tumour cells early predict progression-free and overall survival in advanced colorectal cancer patients treated with chemotherapy and targeted agents. Ann Oncol, 2010. 21(5): p. 1006-12.....42

9. Reid, A.H., et al., Significant and Sustained Antitumor Activity in Post-Docetaxel, Castration-Resistant Prostate Cancer With the CYP17 Inhibitor Abiraterone Acetate. J Clin Oncol, 2010. 28(9): p. 1489-95.....42

10. Danila, D.C., et al., Phase II multicenter study of abiraterone acetate plus prednisone therapy in patients with docetaxel-treated castration-resistant prostate cancer. J Clin Oncol, 2010. 28(9): p. 1496-501.....42

11. Ozkumur, E., et al., Inertial focusing for tumor antigen-dependent and -independent sorting of rare circulating tumor cells. Sci Transl Med, 2013. 5(179): p. 179ra47. 42

12. Kishikawa, T., et al., Quantitation of circulating satellite RNAs in pancreatic cancer patients. JCI Insight. 1(8).....42

13. Romanus, D., et al., *Does health-related quality of life improve for advanced pancreatic cancer patients who respond to gemcitabine? Analysis of a randomized phase III trial of the cancer and leukemia group B (CALGB 80303).* J Pain Symptom Manage, 2012. **43**(2): p. 205-17.

14. Gourgou-Bourgade, S., et al., *Impact of FOLFIRINOX compared with gemcitabine on quality of life in patients with metastatic pancreatic cancer: results from the PRODIGE 4/ACCORD 11 randomized trial.* J Clin Oncol, 2013. **31**(1): p. 23-9.
15. Crippa, S., et al., *Quality of life in pancreatic cancer: analysis by stage and treatment.* J Gastrointest Surg, 2008. **12**(5): p. 783-93; discussion 793-4.
16. Kelsen, D.P., et al., *Pain and depression in patients with newly diagnosed pancreas cancer.* J Clin Oncol, 1995. **13**(3): p. 748-55.
17. Boyd, A.D. and M. Riba, *Depression and pancreatic cancer.* J Natl Compr Canc Netw, 2007. **5**(1): p. 113-6.
18. Edgerly, M. and T. Fojo, *Is there room for improvement in adverse event reporting in the era of targeted therapies?* J Natl Cancer Inst, 2008. **100**(4): p. 240-2.
19. Berry, D.L., *Patient-reported symptoms and quality of life integrated into clinical cancer care.* Semin Oncol Nurs, 2011. **27**(3): p. 203-10.
20. Oberguggenberger, A., et al., *Is the toxicity of adjuvant aromatase inhibitor therapy underestimated? Complementary information from patient-reported outcomes (PROs).* Breast Cancer Res Treat, 2011. **128**(2): p. 553-61.
21. Meyers, F.J., et al., *Simultaneous care: a model approach to the perceived conflict between investigational therapy and palliative care.* J Pain Symptom Manage, 2004. **28**(6): p. 548-56.
23. Aaronson, N.K., et al., *The European Organization for Research and Treatment of Cancer QLQ-C30: a quality-of-life instrument for use in international clinical trials in oncology.* J Natl Cancer Inst, 1993. **85**(5): p. 365-76.
24. Watanabe, S.M., et al., *A multicenter study comparing two numerical versions of the Edmonton Symptom Assessment System in palliative care patients.* J Pain Symptom Manage, 2011. **41**(2): p. 456-68.
25. Zigmond, A.S. and R.P. Snaith, *The hospital anxiety and depression scale.* Acta Psychiatr Scand, 1983. **67**(6): p. 361-70
26. *The Journal of Infectious Diseases*, Volume 171, Issue 6, 1 June 1995, Pages 1438–1447
27. Aguirre, A.J., et al., *Activated Kras and Ink4a/Arf deficiency cooperate to produce metastatic pancreatic ductal adenocarcinoma.* Genes Dev, 2003. **17**(24): p. 3112-26
28. Bardeesy, N., et al., *Both p16(Ink4a) and the p19(Arf)-p53 pathway constrain progression of pancreatic adenocarcinoma in the mouse.* Proc Natl Acad Sci U S A, 2006. **103**(15): p. 5947-52.
29. Hezel, A.F., et al., *Genetics and biology of pancreatic ductal adenocarcinoma.* Genes Dev, 2006. **20**(10): p. 1218-49.
30. leeuwen et al. evaluation of safety and efficacy of 3tc (lamivudine) in patients with asymptomatic or mildly symptomatic human immunodeficiency virus infection: a phase i/ii study j infect dis. 1995 may;171(5):1166-71.)
31. Bersani f et al. Pericentromeric satellite repeat expansions through rna-derived dna intermediates in cancer. PNAS 2015 December, 112 (49) 15148-15153.

1. OBJECTIVES

1.1 Study Design

This is an open-label, single-arm Phase II study of lamivudine in patients who have progressed on systemic therapy for advanced colorectal cancer with TP53 mutations. The phase II study would have a two-stage design, with a target accrual of 20 evaluable patients for the first stage and a total of 32 patients for the whole study. If no responses seen in the first 20 patients, the trial will be terminated. If at least 1 patient in the first stage has response, the trial would proceed to the second stage. Patients will be treated with lamivudine at 600 mg po bid continuously for 28-day cycles. Patients enrolled at a dose level of 150 mg bid will have the dose increased to 600 mg bid, the rationale for which is explained in section 1.2. Tumor assessments will be performed every 8 weeks until documented disease progression by RECIST criteria or drug intolerance. The estimated time for accrual of the patients is 6 months.

1.2 Rationale for dose increase from 150 mg BID to 600 mg BID

In a phase I/II trial assessing the safety, pharmacokinetics, and activity of lamivudine, 97 patients with AIDS or HIV were given lamivudine at a dose of 0.5-20.0 mg/kg/day. Median CD4 count was 128/mm3. The MTD was not reached. AEs at the higher doses includes mild headaches, insomnia, and abdominal symptoms, and the study showed a trend towards lower down neutrophil counts at the highest doses [26].

Furthermore, evaluation of lamivudine (3TC) in vivo with a p53 mutant colorectal cancer cell line (SW620) was done at a dose of 50 mg/kg given by intraperitoneal (IP) injection 3 times a week. When divided per day this is 21 mg/kg/day, which is the highest dose used in the human Phase I trial. As can be seen in the Figure below, this dose was effective in causing a significant difference in xenograft tumor growth and indication of tumor reduction when compared to control SW620 xenografts. Prior evaluation of a lower dose of the NRTI ddC at 25 mg/kg in SW620 xenografts that has been published by our group [31] showed decrease in tumor growth, but not as robust as the 3TC at this higher dose.

All mice tolerated therapy and at necropsy did not have any indication of end organ damage from this higher dose. This indicates that the higher dose proposed would be efficacious to cause tumor reduction in patients without appreciable increase in adverse side effects.

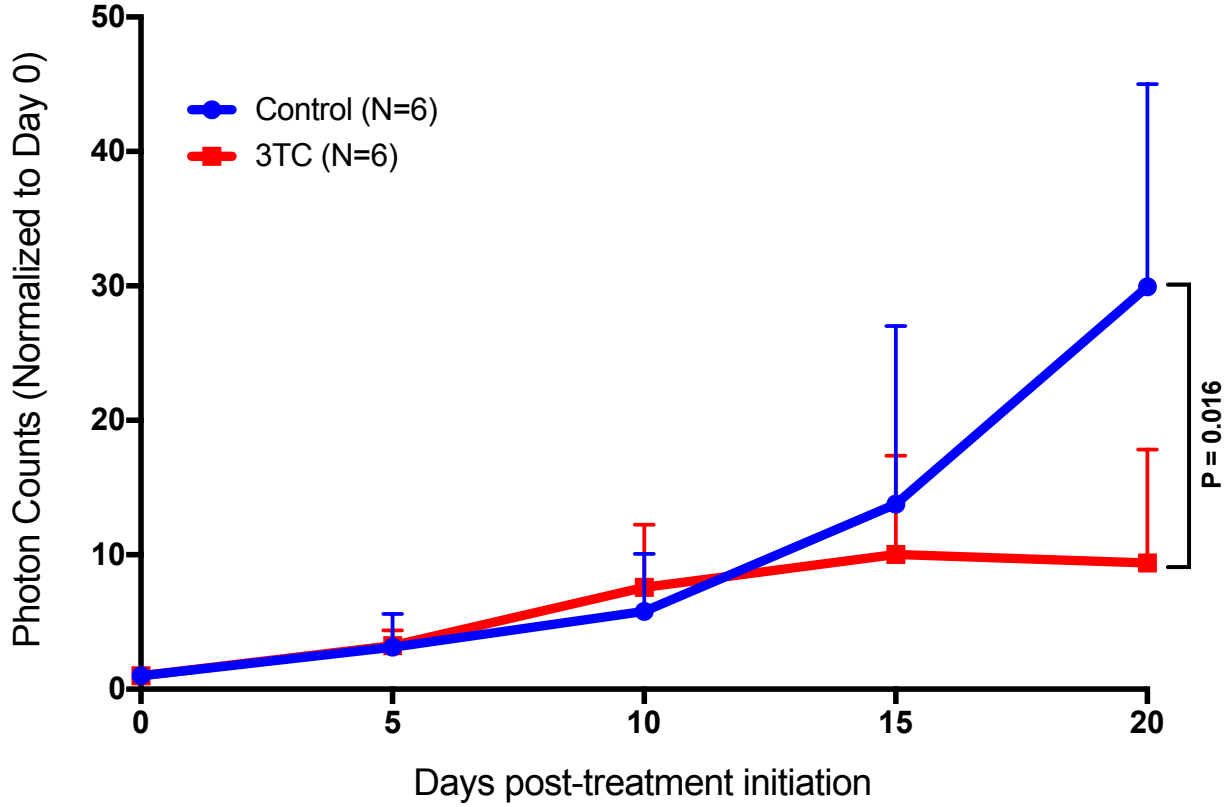

Photon Counts (Normalized to Day 0)
50
40
30
20
10
0
Control (N=6)
3TC (N=6)
P = 0.016
0
5
10
15
20
Days post-treatment initiation

Figure 1A

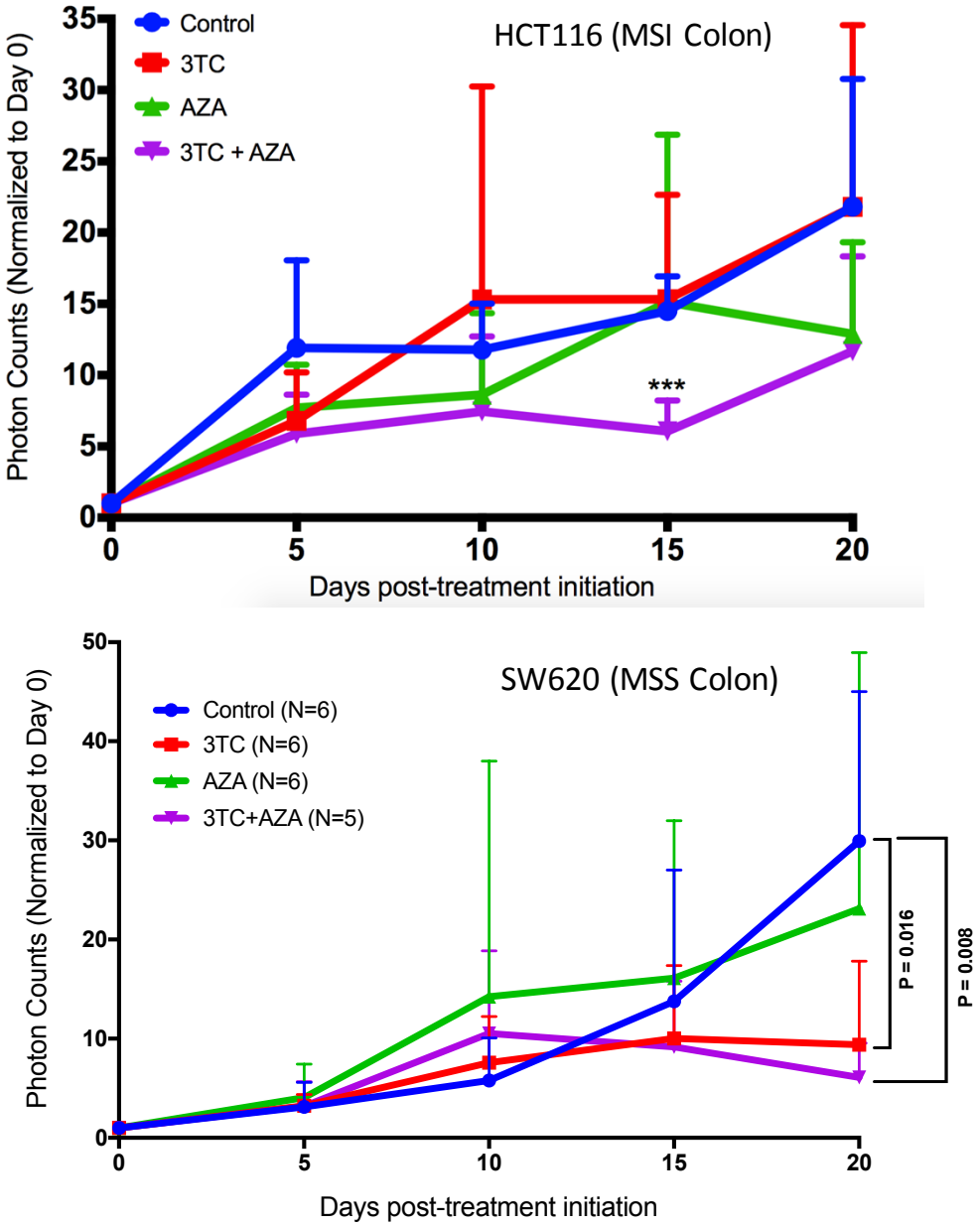
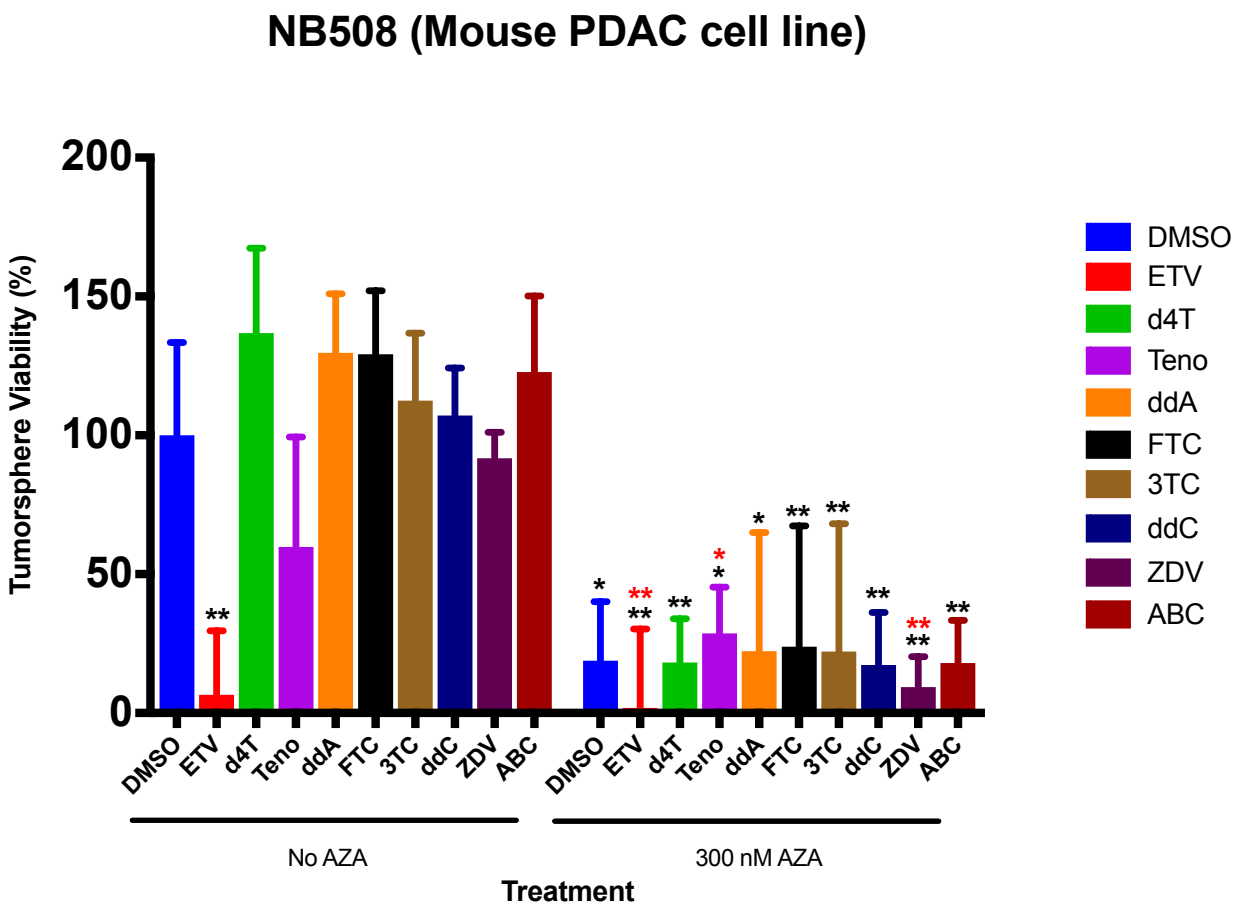


Fig. 1B: Lamivudine (3TC) with or without Azacitidine (AZA) given to colon cancer cell line mouse xenografts monitored by in vivo luciferase imaging. Note the more significant effect of combined 3TC+AZA in the HCT116 MSI colon cancer compared to SW620 MSS colon cancer, which is concordant with in vitro data.

Pancreatic cancer cell line data

In addition to colon cancer, we have additional data in mouse pancreatic cancer cell lines, which have been shown to be models of human disease with an activated KRASG12D mutation and loss of the tumor suppressor P53 [27-29]. A total of 4 of 8 mouse pancreatic cancer cell lines

have shown synergy with the combination. NB508 mouse pancreatic cancer (PDAC) cell line shown in Fig. 1C with demonstration of the highest potency of combination NRTI and AZA.



Black * indicates statistical significance when compared to DMSO No AZA (T-test)
Red * indicates statistical significance when compared to DMSO with AZA (T-test)
Black + Red star is indicative of synergy between AZA and NRTI

Fig. 1C: All NRTIs without or with Azacitidine (AZA) given to mouse PDAC cell line NB508 ($Kras^{G12D}$, Trp53 loss) showing significant sensitivity to combination of AZA to NRTI in pancreatic cancer.

With the preclinical data suggesting that a higher dose of lamivudine is more cytotoxic and clinical data that suggests no significant toxicity, we have no reason to believe there will be additional toxicity patients in these colorectal cancer patients. We propose to increase the dosing of lamivudine to 600 mg bid.

We will plan to treat 6 patients at the new dose level and not enroll any more patients until the end of a 28-day DLT period. If there is less than or equal to 1 DLTs, we will plan to continue enrollment for all patients at the 600 mg bid dose level. If 2/6 patients have a DLT, we will resume dosing at the 300 mg bid dose.

Dose limiting toxicity will be defined as any of the following AEs considered possibly related to lamivudine that occur any time from the initial dose of study treatment with severity graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE), Version 4.0

Hematologic Toxicity:

-Grade 4 neutropenia lasting ≥ 7 days
-Grade 3 or 4 neutropenia complicated by fever ≥ 38.0°C or infection
-Grade 4 thrombocytopenia
-Grade 3 thrombocytopenia complicated by hemorrhage
-Grade 4 anemia

Non-Hematologic Toxicity: Any treatment related Grade 3 or higher non-hematologic toxicity except elevations in GGT that is deemed clinically relevant and unmanageable by the investigator.

-Grade ≥ 3 AST/ALT elevation [exceptions may be made for transient (e.g. lasting < 7 Days) Grade 3 elevations of ALT/AST in the presence of known liver metastases and without evidence of other hepatic injury, if agreed by the Principal Investigator
- Grade ≥ 2 AST/ALT elevation and Grade ≥ 2 bilirubin elevation [exceptions may be made for transient (e.g. lasting < 7 days) elevations of ALT/AST and bilirubin in the presence of known liver metastases without evidence of other hepatic injury, if agreed upon by the treating physician and the Principal Investigator
-Grade 3 or 4 non-hematologic toxicity (excluding fatigue or anorexia lasting < 7 days, or Grade 3 nausea and/or vomiting that persists for < 2 days following appropriate supportive care).

As mentioned above, in a phase i/ii study, 7 levels of lamivudine therapy (from 0.5 to 20.0 mg/kg/day) were studied in 104 human immunodeficiency virus-infected patients with cd4 cell counts < or = 400 x 10(6)/l. mild and transient episodes of diarrhea, headache, fatigue, nausea, and abdominal pain were the most frequent events reported. there were no dose-limiting toxicities [30].

Nine patients have been treated at 150 mg BID with no DLTs.

1.3 Primary Objectives

To determine the response rate of lamivudine in patients with stage 4 colorectal cancer who are p53 mutated.

1.4 Secondary Objectives

1.1.1 To determine the progression free survival
1.1.2 To determine the overall survival
1.1.3 To document changes in HSATII expression in circulating tumor cells of patients on treatment
1.1.4 To document changes in HSATII expression in tumors of patients on lamivudine

2. BACKGROUND

2.1 Study Disease(s)

The initial management of stage 4 colorectal cancer that is not amenable to curative surgical resection consists of combinations of oxaliplatin, irinotecan, 5-FU, anti-VEGF antibodies, and anti-EGFR antibodies (in kras wt disease). After progression of disease on combinations of these agents, patients have limited therapeutic options. Recently, it has been demonstrated that the <5% of patients with microsatellite unstable disease may have marked responses to anti-PD1 therapy. For the >95% of patients with microsatellite stable disease, treatment options are limited to the disease stabilizing compounds of regorafenib and lonsurf. With these agents, responses are rarely seen and the average progression free survival is 2-4 months.

2.2 IND Agent(s)

Lamivudine, a synthetic nucleoside analogue, is phosphorylated intracellularly to its active 5'-triphosphate metabolite, lamivudine triphosphate. The nucleoside analogue is incorporated into viral DNA by HIV reverse transcriptase and HBV polymerase, resulting in DNA chain termination. Lamivudine triphosphate is a weak inhibitor of mammalian alpha-, beta-, and gamma-DNA polymerases. This study is using doses already FDA approved.

2.3 *Other Agent(s)*

Not applicable

2.4 Rationale

HSATII Expression

Next generation sequencing technology has recently provided unprecedented resolution of the cancer transcriptome and has revealed significant expression of non-coding sequences (ncRNAs) in normal and cancer tissues. These ncRNAs provide a previously unexplored source of novel cancer biomarkers and therapeutic targets [1, 2]. We performed RNA sequencing of primary tumors and a variety of normal tissues and identified significant transcription emanating from pericentromeric heterochromatic regions of the genome previously thought to be inactive due to heavy epigenetic silencing. Pericentromeric heterochromatin is comprised of large tandem arrays of repetitive elements called satellites, and these regions are known to be differentially

methylated in a variety of malignancies [3]. Our analysis of all human satellites identified the ncRNA HSATII satellite as being exquisitely specific for a wide variety of cancers including pancreatic, prostate, lung, ovarian, and renal cell carcinomas compared to normal tissues [4]. We confirmed HSATII expression in pancreatic cancer by using a branched chain nucleic acid RNA in situ hybridization (RNA-ISH) platform applicable to standard formalin fixed paraffin embedded (FFPE) tissue. HSATII RNA-ISH demonstrated high expression across approximately 600 epithelial cancers including carcinomas of the pancreas, lung, prostate, breast, colon, thyroid, ovarian, and kidney. Moreover, RNA-ISH in both mouse models and human specimens indicate that HSATII derepression occurs in early preneoplastic lesions of the pancreas (PanIN and IPMN) and breast (DCIS). Together, this indicates that therapeutically targeting HSATII expression would be applicable across a broad range of epithelial cancers and has the opportunity to prevent the progression of preneoplastic lesions to invasive cancer.

Sensitivity to Reverse Transcriptase Inhibitors
More recently, we have discovered that these satellite sequences are actively reverse transcribed (RT) in cancers and lead to progressive expansion of satellite sequences in tumor genomes [5]. Analysis of colon cancer TCGA whole genome sequencing data demonstrated that 50% of primary samples had significant copy number gain of HSATII in tumor genomes. Notably, HSATII expression and RT was completely absent in cancer cell lines grown in standard adherent 2D conditions, while is massively induced in the setting of tumorigenesis as a xenograft or growth as 3D tumorspheres in vitro. Using the nucleoside reverse transcriptase inhibitors (NRTI) ddC and d4T and HSATII specific locked nucleic acids (LNA), we have found significant reduction of tumor cell growth in colon cancer 3D tumorspheres and xenografts, but not in standard 2D growth conditions. Together, this indicates that HSATII RT inhibition is a novel therapeutic anti-cancer target that is seen in vivo, but would be missed in standard 2D cell line screens.

Sensitivity to Reverse Transcriptase Inhibitors in TP54 mutant colon cancer
Initial screening of active NRTIs currently used in HIV and HBV indicate lamivudine (3TC), zalcitabine (ddC), emtricitabine (FTC), and entecavir have similar effects across a panel of colon cancer cell lines in 3D tumorspheres. In colon cancer 3D tumorspheres (Fig.1, we screened a panel of FDA approved NRTIs) and have identified lamivudine (3TC), zalcitabine (ddC), emtricitabine (FTC), and entecavir as active compounds against HSATII RT with associated cytotoxicity. Preliminary analysis indicates that TP53 mutant colon cancers (SW620, DLD-1, HCT-15) are preferentially susceptible to NRTI cytotoxicity compared to wildtype (LOVO, HT-28, HCT-116, GP5D, HCT-8) colon cancer cell lines which is correlated to higher basal HSATII expression levels. This linkage between repeat RNA expression with TP53 loss has recently been described in model organisms. TP53 loss of function was linked to derepression of repetitive elements such as HSATII [6].

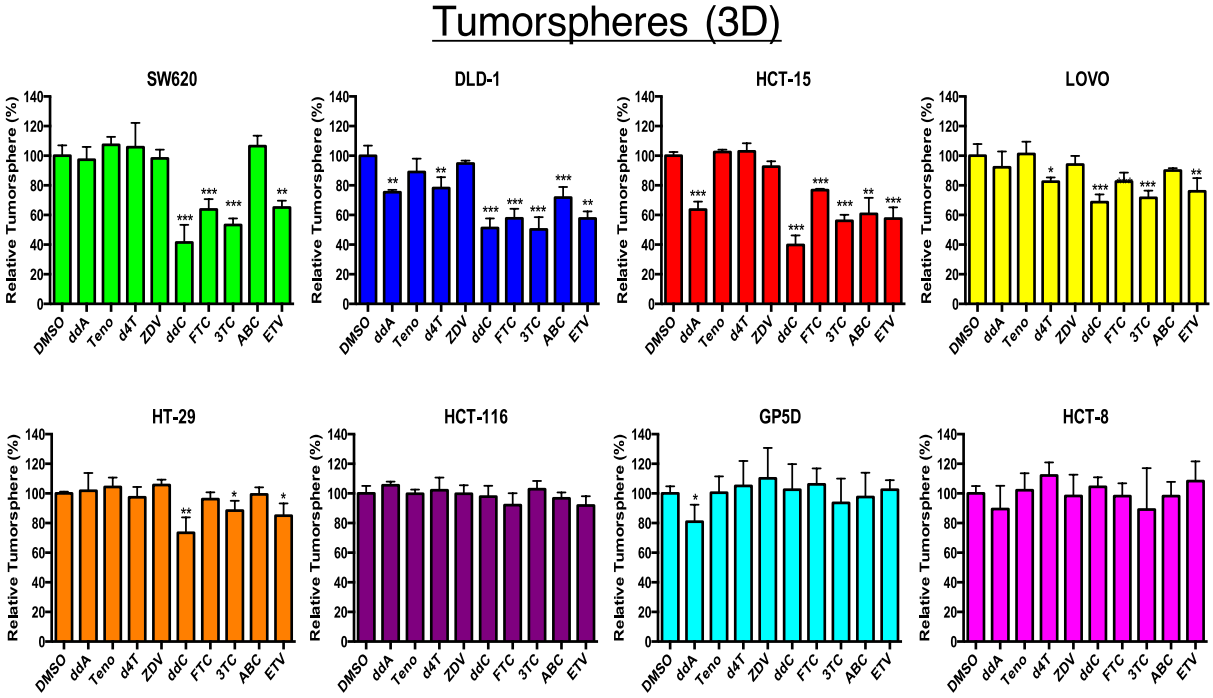


Fig. 1: A panel of 9 NRTIs and DMSO control were tested in 3D tumor spheres of 8 colon cancer cell lines. Notably, the C and G analogues consistently had more significant effects across 5 of 8 cell lines. There are clear differences in sensitivity to NRTIs between cancer cell lines.

Given the lack of therapeutic modalities for patients who progress on initial combination therapy, we propose to evaluate lamivudine in this study in colon cancers with TP53 mutation given the relationship between TP53 mutation, high HSATII repeat expression, and sensitivity to NRTIs. HSATII RNA-ISH will be used on archived tissue specimens in formalin fixed paraffin embedded (FFPE) tissue to be evaluated as a correlative biomarker that may improve prediction of sensitivity to lamivudine. Lamivudine (2′,3′-dideoxy-3′-thiacytidine) is a nucleoside analog reverse transcriptase inhibitor that is approved for the management of patients with hepatitis B and HIV.

2.5 Correlative Studies Background

A) RNA and DNA analysis of Pretreatment Diagnostic Sample

Archived formalin fixed paraffin embedded (FFPE) material from the patient's diagnostic biopsy and/or resection specimen will be analyzed for RNA and DNA markers to predict response to NRTI (lamivudine) treatment. A pre-study biopsy will be required if a biopsy was not done after the most recent treatment received. The tumor biopsy should be collected, if medically feasible, in the opinion of the investigator. We have developed a RNA-ISH assay applicable to FFPE sectioned slides for repeat non-coding RNAs including HSATII (Fig. 1). The assay has been automated, but is not CLIA certified at this time. Given this early trial, we will assess this HSATII RNA-ISH and other repeats for exploratory biomarker development.

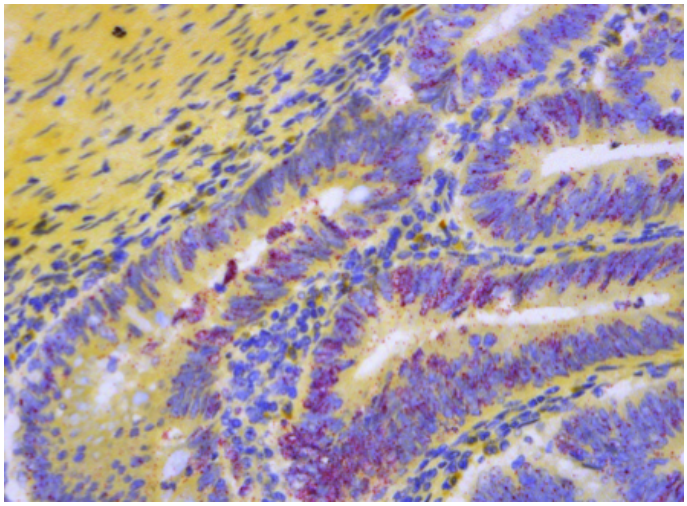


Fig. 2: RNA-ISH for HSATII (red) in primary colon cancer FFPE specimen. HSATII detected in cancer cells, but not adjacent stroma. Counter stain Hematoxylin (blue)

Preliminary data has already indicated that TP53 mutation/deletion may predict sensitivity to NRTIs. However, the spectrum of other DNA aberrations in cancers as they relate to sensitivity to NRTIs has not been explored. In addition, other RNA transcripts may prove to be useful in conjunction with mutations and HSATII RNA expression status as predictive biomarkers of response. We will perform RNA-seq and whole exome/whole genome DNA sequencing on total nucleic acids extracted from FFPE archived excess tissue slides. This analysis will also shed mechanistic insight into the relationship of HSATII expression with other RNAs and genomic features.

B) Blood Based Biomarkers for Disease Response Monitoring

Monitoring of on target effects of NRTI on repetitive elements will be done through exploratory blood based biomarker assays.

CTC numbers have been shown to correlate with response to therapy in breast [7], colon [8], and prostate cancer [9, 10]. CTC analysis will be done using the CTC-iChip, a microfluidic device that relies on a combination of immunomagnetic labeling, hydrodynamic sorting, inertial focusing, and magnetic driven separation to isolate CTCs [11]. This platform has isolated CTCs from a wide variety of malignancies. We have demonstrated the ability to detect HSATII in CTCs in metastatic cancer patients (Fig. 2) and will evaluate CTCs in patients on enrollment and every 2 months on trial.

Exosomes have also been shown to be a promising biomarker that can be purified from plasma or serum. We

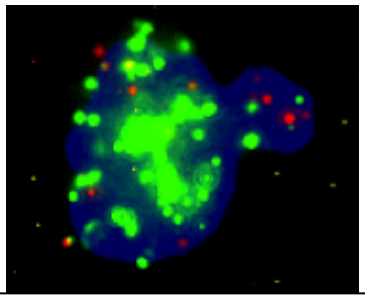


Fig. 3: CTC captured from metastatic PDAC patient stained by RNA-FISH for HSATII (green) and CK (red). DAPI nuclear stain (blue)

have recently developed a microfluidic exosome capture chip ^{Exo}Hb-chip that can purify exosomes. Notably, HSATII has been detected in the serum and exosomes of cancer patients [12].

Blood will be separated for analysis for circulating tumor cells (CTCs) and exosomes. CTCs will be enumerated with standard immunofluorescent markers, HSATII RNA-ISH, and RNA-sequencing. Exosomes will be analyzed by RNA-sequencing. Additional plasma and leukocytes (buffy coat) will be archived for potential future analysis.

RNA and DNA sequencing data will be shared with collaborating investigators for correlative science research. Original sequencing data will be shared with collaborators, but all samples will be de-identified and labeled with anonymized coding. In addition, all original data will be uploaded to the appropriate NCBI archive (i.e. GEO, dbGAP, SRA) as directed by grant funding and publication rules.

2.6 Patient-reported outcomes (PRO) background

There are few published studies measuring patient-reported symptoms and quality of life (QOL) outcomes in patients with pancreatic cancer. Most of the existing studies assessing QOL in patients with pancreatic cancer are focused on individuals with advanced disease.[13, 14] The few studies that have included patients with operable pancreatic cancer included small sample sizes in heterogeneous patients.[15, 16] Despite the fact that depression is a frequently reported symptom in patients with pancreatic cancer, the prevalence among different stages of disease has not been thoroughly investigated.[17] A more detailed and comprehensive understanding of the burdens faced by patients with locally advanced pancreatic cancer will identify areas for clinicians to enhance their supportive care efforts.

Studying patients' symptom burden and QOL while they are participating in a clinical trial provides an opportunity to better understand their disease- and treatment-related outcomes. Patients' symptom burden and QOL are better indicators of their treatment tolerability than clinician-reported toxicity monitoring. Combining objective endpoints, such as response rate, with subjective patient-reported outcomes has become increasingly important in determining efficacy, toxicity, and safety and for allowing comparisons across treatment arms.[18] Additionally, evaluating patient-reported measures may help highlight patients' difficulties with treatment adherence by demonstrating additional side effects and toxicities of therapy.[19] Increased attention to patients' symptom burden and QOL while they are participating in a clinical trial provides an opportunity to improve their quality of care.[20, 21] Thus, we aim to describe QOL, symptom burden and mood in this study population to help us better identify the side effects and challenges faced by patients with locally advanced pancreatic cancer.

We will use the EORTC QLQ-C30, a validated instrument designed for prospective clinical trials that evaluates five functions (physical, role, cognitive, emotional, and social), and nine symptoms (fatigue, pain, nausea and vomiting, dyspnea, loss of appetite, insomnia, constipation, diarrhea, and financial difficulties) to measure QOL.[22] We will use the Edmonton Symptom

Assessment System-revised (ESAS-r) to measure symptoms, which has been previously validated in patients with advanced cancer.[23] The ESAS-r consists of ten items assessing pain, fatigue, drowsiness, nausea, anorexia, dyspnea, depression, anxiety, well-being, and a free-response item. We will include diarrhea as the free-response item. The ten items are scored on a scale of 0-10 (0 reflecting no reported presence of the symptom and 10 reflecting the worst possible severity of the symptom). We will instruct patients that items are to be rated based on the previous 24-hour period. We will use the Hospital Anxiety and Depression Scale (HADS) to assess symptoms of depression and anxiety.[24] The HADS is a 14-item questionnaire that contains two 7-item subscales assessing depression and anxiety symptoms during the past week. The questionnaire consists of a four-point item response format that quantifies the degree to which participants experience a particular emotion. Scores on each subscale range from 0 to 21, with a cutoff of 8 or greater denoting clinically significant depression or anxiety symptoms.

3. PARTICIPANT SELECTION

3.1 Eligibility Criteria

Eligibility, particularly regarding past treatments, will be reviewed on a case by case basis and deviations or exceptions will be submitted to the IRB as needed.

3.1.1 Patients must have histologically confirmed adenocarcinoma of the colon that has metastasized (stage 4) and is TP53 mutant/deleted by a CLIA approved genetic test. Only known loss of function TP53 mutation/deletion will be eligible for this study.

3.1.2 Participants must have measurable disease, defined as at least on lesion that can be accurately measured in at least one dimension (longest diameter to be recorded for non-nodal lesions and short axis for nodal lesions) as > 20mm with conventional techniques or > 10 mm with spiral CT scan, MRI or calipers by clinical exam. See section 11 for evaluation of measurable disease

3.1.3 Patients must be resistant to or intolerant of 5FU, oxaliplatin, irinotecan, bevacizumab and cetuximab/panitumumab (if RAS wild type)

3.1.4 Age 18 or older.

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

3.1.6 Life expectancy of greater than 8 weeks.

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

- absolute neutrophil count ≥1,200/mcL
- platelets ≥75,000/mcL
- total bilirubin ≤1.5 × institutional upper limit of normal within normal
- AST(SGOT)/ALT(SGPT) ≤5 × institutional upper limit of normal
- creatinine within normal institutional limits OR
- eGFR ≥60 mL/min/1.73 m^2 for participants with creatinine levels not within institutional normal OR
- Creatinine Clearance (calculated per Cockcroft-Gault equation) ≥60 mL/min for participants with creatinine levels not within

institutional normal

3.1.8 The effects of *lamivudine* on the developing human fetus are known to be teratogenic. For this reason, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately. Men treated or enrolled on this protocol must also agree to use adequate contraception prior to the study, for the duration of study participation, and 4 months after completion of *lamivudine* administration.

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

3.2 Exclusion Criteria

3.2.1 Participants who have had chemotherapy or radiotherapy within 3 weeks (6 weeks for nitrosourea or mitomycin C) prior to entering the study or those who have not recovered from adverse events due to agents administered more than 3 weeks earlier. Radiotherapy maybe administered within 3 weeks of study entry at the discretion of the investigator and treating physician.

3.2.2 Participants who are receiving any other investigational agents.

3.2.3 Participants with known brain metastases should be excluded from this clinical trial because of their poor prognosis and because they often develop progressive neurologic dysfunction that would confound the evaluation of neurologic and other adverse events.

3.2.4 History of allergic reactions attributed to compounds of similar chemical or biologic composition to *lamivudine*.

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

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

3.2.7 HIV-positive participants on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with *lamivudine*

3.2.8 HBV positive participants will be excluded given the known effects of lamivudine on HBV.

3.3 Inclusion of Women and Minorities

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

4. REGISTRATION PROCEDURES

4.1 General Guidelines for DF/HCC Institutions

Institutions will register eligible participants in the Clinical Trials Management System (CTMS) OnCore. Registrations must occur prior to the initiation of protocol therapy. Any participant not registered to the protocol before protocol therapy begins will be considered ineligible and registration will be denied.

An investigator will confirm eligibility criteria and a member of the study team will complete the protocol-specific eligibility checklist.

Following registration, participants may begin protocol therapy. Issues that would cause treatment delays should be discussed with the Overall Principal Investigator (PI). If a participant does not receive protocol therapy following registration, the participant's registration on the study must be canceled. Registration cancellations must be made in OnCore as soon as possible.

4.2 Registration Process for DF/HCC Institutions

DF/HCC Standard Operating Procedure for Human Subject Research Titled *Subject Protocol Registration* (SOP #: REGIST-101) must be followed.

4.3 General Guidelines for Other Investigative Sites

Not applicable

4.4 Registration Process for Other Investigative Sites

Not applicable

5. TREATMENT AND/OR IMAGING PLAN

5.1 Treatment Regimen

Lamivudine will be administered every 4 weeks, with 28 consecutive days defined as a treatment cycle. Treatment will be administered on an *outpatient* basis. Reported adverse events and potential risks are described in Section 7. Appropriate dose modifications are described in Section 6. No investigational or commercial agents or therapies other than those described

Regimen Description					
Agent	***Premedications; Precautions***	***Dose***	***Route****	***Schedule***	***Cycle Length***
Lamivudine	Take with food.	600 mg tablet	PO BID.	Daily,	28 days

Patients enrolled onto the study as of DFCI amendment # 5 received 150 mg BID dose. The participant will be requested to maintain a medication diary of each dose of medication. The medication diary will be returned to clinic staff at the end of each cycle

5.2 Pre-Treatment Criteria

5.2.1 Cycle 1, Day 1: Patients are required to meet eligibility criteria within 14 days of starting therapy. Day 1 labs must be reviewed prior to treatment.

5.2.2 Subsequent Cycles. Toxicity related to therapy should return to baseline function or grade 2 or less prior to initiating subsequent cycles of therapy.

5.3 Agent Administration

Lamivudine, a synthetic nucleoside analogue, is phosphorylated intracellularly to its active 5'-triphosphate metabolite, lamivudine triphosphate. The nucleoside analogue is incorporated into viral DNA by HIV reverse transcriptase and HBV polymerase, resulting in DNA chain termination. Lamivudine triphosphate is a weak inhibitor of mammalian alpha-, beta-, and gamma-DNA polymerase

Toxicities

Frequent
- Gastrointestinal: Diarrhea (adults, 18%;), Nausea (33%)
- Neurologic: Headache (35%)
- Respiratory: Cough (adults, 18%;), Nasal symptom (rhinorrhea) (adults, 20%;
- Other: Fever (adults, 10%;), Malaise and fatigue (27%)

Occasional
- Pancreatitis

Rare
- Endocrine metabolic: Fat maldistribution, Lactic acidosis
- Hepatic: Hepatomegaly, Relapsing type B viral hepatitis
- Black Box Warning

Lactic acidosis and severe hepatomegaly with steatosis, including fatal cases, have been reported. Severe acute exacerbations of hepatitis B have been reported in patients who have discontinued antihepatitis B therapy (including lamivudine) or who are coinfected with hepatitis B virus (HBV) and HIV-1 and have discontinued lamivudine; monitor hepatic function upon discontinuation of therapy. Epivir(R) tablets and oral solution (used to treat HIV-1 infection) contain a higher dose of the active ingredient (lamivudine) than Epivir-HBV(R) tablets and oral solution (used to treat chronic hepatitis B infection). Patients with HIV-1 infection should receive only dosage forms appropriate for treatment of HIV-1. Epivir-HBV(R) is not approved for the treatment of HIV-1 infection. HIV-1 resistance may emerge in chronic hepatitis B-infected patients with unrecognized or untreated HIV-1 infection; offer HIV counseling and testing to all patients prior to beginning treatment with Epivir-HBV(R) and periodically during therapy

How Supplied
150 mg tablet
Store Lamivudine tablets at 25 degrees Celsius (77 degrees Fahrenheit) in a tightly closed container. Store lamivudine oral solution and tablets between 20 to 25 degrees Celsius (68 to 77 degrees Fahrenheit) in a tightly closed bottle

Dosing Information
The starting dose of lamivudine is 600 mg po BID. Drug can not be crushed, chewed or dissolved in water. Should the drug be vomiting within 1 hour of administration, drug can be re dosed. If a patient completely misses their study drug, they should take their next scheduled dose. Drug may be administered with meals. There are no prohibited foods and can be taken with or without water.

Dose Adjustments
Dose delays and modifications will be made using the following recommendations. Toxicity assessments will be done using the CTEP Active Version of the NCI Common Terminology Criteria for Adverse Events (CTCAE) which is identified and located on the CTEP website at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

If possible, symptoms should be managed symptomatically. In the case of toxicity, appropriate medical treatment should be used (including anti-emetics, anti-diarrheals, etc.).

All adverse events experienced by participants will be collected from the time of the first dose of study treatment, through the study and until the final study visit. Participants continuing to experience toxicity at the off study visit may be contacted for additional assessments until the toxicity has resolved or is deemed irreversible.

Lamivudine will be held at the discretion of the provider for any >/= to grade 3 hematologic or non-hematologic toxicities such as headache, fatigue, cytopenias, nausea or vomitting, diarrhea, pancreatitis, neutropenia, infection, myalgias or respiratory symptoms When patients recover to baseline or grade 2 toxicity (or toxicity at the discretion of provider) , patients will be restarted at 600 mg po BID, unless the provider feels otherwise.

For grade 1 or 2 toxicity deemed to be unacceptable by the patient or the treating physician, doses can be lowered to 150 mg bid or 300 mg po daily. Dose may be increased up to 600 mg bid at the discretion of the provider.

If toxicity doesn't resolve within 12 weeks of last dose, permanently discontinue.

Patients will be removed from study if they develop grade 3 or higher toxicities either at the 150 mg bid dosing or 300 mg daily dose, unless otherwise discussed with the PI and treating physician.

Dose can be lowered to as low as 150 mg bid OR 300 mg qd depending on the type of toxicity.

Of note, no dose adjustments are required for patients with impaired hepatic function but should patients develop clinical or laboratory findings suggestive of lactic acidosis and/or hepatomegaly, lamivudine should be stopped.

For renal function, should renal function become impaired, patients may continue at 600 mg twice daily dosing for Creatinine Clearance >/= 50. Should Creatinine clearance fall to 30 - <50, patients will be restarted at 300 mg once daily. For patients with a Creatinine Clearance, less than 30, patients should be taken off study unless deriving clinical benefit per treating physician. If deriving clinical benefit, patients with a Creatinine Clearance of 15-29 may remain on study at 100 mg po once daily and for a Creatinine Clearance of 5-14, 50 mg po once daily.If >/= 3 grade toxicity hasn't resolved to baseline or grade 2 within 4 weeks, treatment should be permanently discontinued. If patient was previously deriving clinical benefit per the treating physician and >/= 3 grade toxicity hasn't resolved to grade 2 or baseline by 4 weeks, treatment may be held for a maximum of 8 weeks.

5.3.1 *CTEP and/or CIP IND Agent(s), or other IND agent*

Not applicable

5.3.2 *Other Agent(s)*

Palliative radiation will be allowed if clinically indicated on the study

5.3.3 *Other Modality(ies) or Procedures*

Not applicable

5.3.4 *Investigational Imaging Agent Administration*

Not applicable

5.4 *For phase 1 protocols only:* **Definition of Dose-Limiting Toxicity (DLT)**

Not applicable

5.5 General Concomitant Medication and Supportive Care Guidelines

Because there is a potential for interaction of *lamivudine* with other concomitantly administered drugs, the case report form must capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies.

5.6 Criteria for Taking a Participant Off Protocol Therapy

Duration of therapy will depend on individual response, evidence of disease progression and tolerance. In the absence of treatment delays due to adverse event(s), treatment may continue for indefinitely or until one of the following criteria applies:

- Disease progression
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Participant demonstrates an inability or unwillingness to comply with the oral medication regimen and/or documentation requirements
- Participant decides to withdraw from the protocol therapy
- General or specific changes in the participant's condition render the participant unacceptable for further treatment in the judgment of the treating investigator

Participants will be removed from the protocol therapy when any of these criteria apply. The reason for removal from protocol therapy, and the date the participant was removed, must be documented in the case report form (CRF). Alternative care options will be discussed with the participant.

A QACT Treatment Ended/Off Study Form will be filled out when a participant is removed from protocol therapy. This form can be found on the QACT website or obtained from the QACT registration staff.

In the event of unusual or life-threatening complications, treating investigators must immediately notify the Overall PI, *David P. Ryan, M.D. 617-724-0245.*

5.7 Duration of Follow Up

Participants will be followed for *4 weeks* after removal from protocol therapy or until death, whichever occurs first. Participants removed from protocol therapy for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event.

5.8 Criteria for Taking a Participant Off Study

Participants will be removed from study when any of the following criteria apply:

- Lost to follow-up
- Withdrawal of consent for data submission
- Death

The reason for taking a participant off study, and the date the participant was removed, must be documented in the case report form (CRF).

For Centralized Subject Registrations, the research team submits a completed Off Treatment/Off Study form to ODQ when a participant comes off study. This form can be found on the ODQ website or obtained from the ODQ registration staff.

For Decentralized Subject Registrations, the research team updates the relevant Off Treatment/Off Study information in OnCore.

6. DOSING DELAYS/DOSE MODIFICATIONS

Dose delays and modifications will be made as indicated in the following table(s). The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for dose delays and dose modifications. A copy of the CTCAE version 4.0 can be downloaded from the CTEP website http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

Dose Level	Lamivudine Dose
Starting dose	600 mg po q 12 hours +/- 2 hours
-1	300 mg po q 12 hours +/- 2 hours
-2 ***	150 mg po q 12 hours +/- 2 hours

*** For Creatinine Clearance 30 - <50 mL/min, 300 mg qd is okay.

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of reported and/or potential AEs (Section 7.1) and the characteristics of an observed AE (Section 7.2) will determine whether the event requires expedited reporting **in addition** to routine reporting.

Expedited Reporting Guidelines

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

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.

Death due to progressive disease should be reported as **Grade 5 "Neoplasms benign, malignant and unspecified (incl cysts and polyps) - Other (Progressive Disease)"** under the system organ class (SOC) of the same name. Evidence that the death was a manifestation of underlying disease (*e.g.*, radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted.

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

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)
NOTE: Investigators **MUST** immediately report to the sponsor (NCI) **ANY** 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 AdEERS 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 AdEERS 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

Late Phase 2 and Phase 3 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 AdEERS within the timeframes detailed in the table below.

<table>
<tr><th>Hospitalization</th><th>Grade 1 Timeframes</th><th>Grade 2 Timeframes</th><th>Grade 3 Timeframes</th><th>Grade 4 & 5 Timeframes</th></tr>
<tr><td>Resulting in Hospitalization ≥ 24 hrs</td><td colspan="3">10 Calendar Days</td><td rowspan="2">24-Hour 5 Calendar Days</td></tr>
<tr><td>Not resulting in Hospitalization ≥ 24 hrs</td><td colspan="2">Not required</td><td>10 Calendar Days</td></tr>
</table>

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 AdEERS 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 4, and Grade 5 AEs

Expedited 10 calendar day reports for:

- Grade 2 adverse events resulting in hospitalization or prolongation of hospitalization
- Grade 3 adverse events

[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

7.1 *For CTEP protocols only:* **Comprehensive Adverse Events and Potential Risks List(s) (CAEPRs)**

Not applicable

7.1.1 CAEPRs for CTEP IND Agent(s)

Not applicable

7.1.1.1 CAEPR for *[CTEP IND Agent #1]*

Not applicable

7.1.1.2 CAEPR for *[CTEP IND Agent #2]*

Not applicable

7.1.2 Adverse Event List(s) for *[Other Investigational Agent(s)]*

Not applicable

7.1.3 Adverse Event List(s) for Commercial Agent(s)

Not applicable

7.1.4 CAEPR for *[CIP IND Agent #1)]*

Not applicable

7.1.5 Adverse Event List(s) for CIP (*e.g.* Study-Specific) Commercial Imaging Agents

Not applicable

7.2 *For CTEP protocols only:* **Adverse Event Characteristics**

Not applicable

7.3 *For CTEP protocols only:* **Expedited Adverse Event Reporting**

Not applicable

7.4 *For CTEP protocols only:* **Routine Adverse Event Reporting**

Not applicable

7.5 *For CTEP protocols only:* **Secondary Malignancy**

Not applicable

7.6 *For CTEP protocols only:* **Second Malignancy**

Not applicable

7.7 *For non-CTEP protocols only:* **Expected Toxicities**

Toxicities
Common

- Gastrointestinal: Diarrhea (adults, 18%; pediatrics, 8%), Nausea (33%)
- Neurologic: Headache (35%)
- Respiratory: Cough (adults, 18%; pediatrics, 15%), Nasal symptom (adults, 20%; pediatrics, 8%)
- Other: Fever (adults, 10%; pediatrics, 25%), Malaise and fatigue (27%)

Serious

- Endocrine metabolic: Fat maldistribution, Lactic acidosis
- Gastrointestinal: Pancreatitis, Nausea and Abdominal Pain
- Hepatic: Hepatomegaly (pediatrics, 11%), Relapsing type B viral hepatitis
- Black Box Warning

Lactic acidosis and severe hepatomegaly with steatosis, including fatal cases, have been reported. Severe acute exacerbations of hepatitis B have been reported in patients who have discontinued antihepatitis B therapy (including lamivudine) or who are coinfected with hepatitis B virus (HBV) and HIV-1 and have discontinued lamivudine; monitor hepatic function upon discontinuation of therapy. Epivir(R) tablets and oral solution (used to treat HIV-1 infection) contain a higher dose of the active ingredient (lamivudine) than Epivir-HBV(R) tablets and oral solution (used to treat chronic hepatitis B infection). Patients with HIV-1 infection should receive only dosage forms appropriate for treatment of HIV-1. Epivir-HBV(R) is not approved for the treatment of HIV-1 infection. HIV-1 resistance may emerge in chronic hepatitis B-infected patients with unrecognized or untreated HIV-1 infection; offer HIV counseling and testing to all patients prior to beginning treatment with Epivir-HBV(R) and periodically during therapy

Patients will be given a higher dose of lamivudine that what is currently approved but the dose was studied and deemed to be safe in HIV patients. As described above, we will also plan to do a

safety run in ensuring safety on the higher dose before accruing the rest of the study.

7.8 *For non-CTEP protocols only:* Adverse Event Characteristics

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

- **For expedited reporting purposes only:**
  - AEs for the agent(s) that are listed above should be reported only if the adverse event varies in nature, intensity or frequency from the expected toxicity information which is provided.
  - Other AEs for the protocol that do not require expedited reporting are outlined in the next section (Expedited Adverse Event Reporting) under the sub-heading of Protocol-Specific Expedited Adverse Event Reporting Exclusions.

- **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.

7.9 *For non-CTEP protocols only:* Expedited Adverse Event Reporting

7.9.1 Investigators **must** report to the Overall PI any serious adverse event (SAE) that occurs after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment on the local institutional SAE form.

7.9.2 DF/HCC Expedited Reporting Guidelines

Investigative sites within DF/HCC will report AEs directly to the DFCI Office for Human Research Studies (OHRS) per the DFCI IRB reporting policy.

Attribution	DF/HCC Reportable AEs				
	Gr. 2 & 3 AE Expected	**Gr. 2 & 3 AE Unexpected**	**Gr. 4 AE Expected**	**Gr. 4 AE Unexpected**	**Gr. 5 AE Expected or Unexpected**
Unrelated Unlikely	Not required	Not required	5 calendar days#	5 calendar days	24 hours*

Possible Probable Definite	Not required	5 calendar days	5 calendar days#	5 calendar days	24 hours*
# If listed in protocol as expected and not requiring expedited reporting, event does not need to be reported.					
* For participants enrolled and actively participating in the study ***or*** for AEs occurring within 30 days of the last intervention, the AE should be reported within 1 business day of learning of the event.					

7.10 Expedited Reporting to the Food and Drug Administration (FDA)

The Overall PI, as study sponsor, will be responsible for all communications with the FDA. The Overall PI will report to the FDA, regardless of the site of occurrence, any serious adverse event that meets the FDA's criteria for expedited reporting following the reporting requirements and timelines set by the FDA.

7.11 Expedited Reporting to the NIH Office of Biotechnology Activities (OBA)

Not applicable

7.12 Expedited Reporting to the Institutional Biosafety Committee (IBC)

Not applicable

7.13 Expedited Reporting to Hospital Risk Management

Participating investigators will report to their local Risk Management office any participant safety reports or sentinel events that require reporting according to institutional policy.

7.14 *For non-CTEP protocols only:* Routine Adverse Event Reporting

All Adverse Events **must** be reported in routine study data submissions to the Overall PI on the toxicity case report forms. **AEs reported through expedited processes (e.g., reported to the IRB, FDA, etc.) must also be reported in routine study data submissions.**

8. PHARMACEUTICAL AND/OR IMAGING AGENT INFORMATION

A list of the adverse events and potential risks associated with the investigational or other agents administered in this study can be found in Section 7.1.

8.1 Lamivudine

8.1.1 Description

Lamivudine, a synthetic nucleoside analogue, is phosphorylated intracellularly to its active 5'-

triphosphate metabolite, lamivudine triphosphate. The nucleoside analogue is incorporated into viral DNA by HIV reverse transcriptase and HBV polymerase, resulting in DNA chain termination. Lamivudine triphosphate is a weak inhibitor of mammalian alpha-, beta-, and gamma-DNA polymerase

8.1.2 **Form**

150mg tablet

8.1.3 **Storage and Stability**

Store Lamivudine tablets at 25 degrees Celsius (77 degrees Fahrenheit) in a tightly closed container. Store lamivudine oral solution and tablets between 20 to 25 degrees Celsius (68 to 77 degrees Fahrenheit) in a tightly closed bottle

8.1.4 **Compatibility**

Not applicable

8.1.5 **Handling**

Qualified personnel, familiar with procedures that minimize undue exposure to themselves and the environment, should undertake the preparation, handling, and safe disposal of the chemotherapeutic agent in a self-contained and protective environment.

8.1.6 **Availability**

Lamivudine will be obtained commercially

8.1.7 **Preparation**

Not applicable

8.1.8 **Administration**

Lamivudine will be administered at a starting dose of 600 mg po BID. For the purposes of this protocol the term BID is equivalent to every 12 hours +/- 2 hours.

8.1.9 **Ordering**

Lamivudine will be stored by the MGH Research Pharmacy. Please check with the MGH Research Pharmacy for each new patient enrolled.

8.1.10 **Accountability**

The investigator, or a responsible party designated by the investigator, should maintain a

careful record of the inventory and disposition of the agent using the NCI Drug Accountability Record Form (DARF) or another comparable drug accountability form. (See the NCI Investigator's Handbook for Procedures for Drug Accountability and Storage.)

8.1.11 **Destruction and Return**

Unused drug will be returned to study staff for proper handling and destruction.

8.2 ***IND Agent #2***

Not applicable

8.3 ***Other Agent #1***

Not applicable

8.4 ***Other Agent #2***

Not applicable

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

9.1 Biomarker Studies

No paired biomarker for Ph II but biomarker work planned as detailed in 9.4 as correlative work

9.2 ***For IDE protocols only:*** **Investigational Device Information**

Not applicable

9.3 ***For IDE protocols only:*** **Investigational Device Information**

Not applicable

9.4 Laboratory Correlative Studies

9.4.1 **RNA in situ Hybridization of Repeats as Novel Biomarker of Response**

Preliminary data in colon cancer cell lines indicate that HSATII high cell lines are much more sensitive to NRTI treatment [5]. The LINE-1, TERRA, and HERV-H repeats are known to also be affected by NRTI treatment and will be analyzed in parallel with HSATII.

We will utilize is RNA in situ hybridization (RNA-ISH) for targeted elements in standard archived formalin fixed paraffin embedded (FFPE) slides using the Affymetrix ViewRNA platform [4]. This assay has been optimized on the automated Leica Bond RX platform and

sequential slides will be stained for HSATII, LINE-1, TERRA, HERV-H, and GAPDH (RNA control). Only archived excess tissue will be utilized for this work. A pre-study biopsy will be required if a biopsy was not done after the most recent treatment received. The tumor biopsy should be collected, if medically feasible, in the opinion of the investigator.

9.4.1.1 Collection of Specimen(s)

Archived FFPE blocks will be obtained from the Department of Pathology

9.4.1.2 Handling of Specimens(s)

FFPE blocks will be sectioned on a microtome according to standard procedure in the MGH histopathology core facility.

9.4.1.3 Shipping of Specimen(s)

FFPE blocks will be sent to the MGH core through standard internal transport mechanisms.

9.4.1.4 Site(s) Performing Correlative Study

MGH Cancer Center – Charlestown Navy Yard Building 149

9.4.2 **DNA and RNA sequencing of tumor for exploratory science**

Preliminary analysis indicates TP53 mutation/deletion status may predict sensitivity to NRTIs. However, the full spectrum of other genomic aberrations has not been evaluated. If a CLIA certified molecular pathology targeted sequencing assay is requested for clinical care on these specimens, we will utilize that mutation data for correlative analysis. If the assay is not requested, total nucleic acids will be extracted from 1-5 10 micron thick slides in archived excess FFPE tissue per protocol (RecoverAll ThermoFisher #AM1975). DNA will be prepped for whole exome sequencing using the Illumina whole exome sequencing kit and sequenced on the Illumina NextSeq platform. Given repetitive elements are not represented in whole exome sequencing, additional whole genome sequencing analysis to assess repeat genomic copy number changes may be performed.

RNA markers including innate immune response genes (i.e. interferon response genes) have also shown to be linked to repeat expression and we will perform RNA-seq on FFPE total nucleic acid extracted material. RNA-seq data will be analyzed with unsupervised clustering, principal component analysis, differential expression, and machine learning algorithms to determine if there is a transcriptional signature associated with response to NRTIs.

RNA and DNA sequencing data will be shared with collaborating investigators for correlative science research. Original sequencing data will be shared with collaborators, but all samples will be de-identified and labeled with anonymized coding. In addition, all original data will be uploaded to the appropriate NCBI archive (i.e. GEO, dbGAP, SRA) as directed by grant funding and publication rules.

9.4.2.1 Collection of Specimen(s)

Archived FFPE blocks will be obtained from the Department of Pathology

9.4.2.2 Handling of Specimens(s)

FFPE blocks will be sectioned on a microtome according to standard procedure in the MGH histopathology core facility.

9.4.2.3 Shipping of Specimen(s)

FFPE blocks will be sent to the MGH core through standard internal transport mechanisms.

9.4.2.4 Site(s) Performing Correlative Study

MGH Cancer Center – Charlestown Navy Yard Building 149

9.4.3 **Blood Based Biomarkers to Monitor Disease Response**

Blood will be processed for CTC purification, plasma collection, and mononuclear cell isolation (Fig. 3). Approximately 10 mL of blood will be processed through the CTC-iChip platform for CTC isolation. We will divide the CTC product into three different analyses including 1) Standard IF staining 2) RNA-ISH staining and 3) bulk frozen CTC product for future qRT-PCR or sequencing analysis.

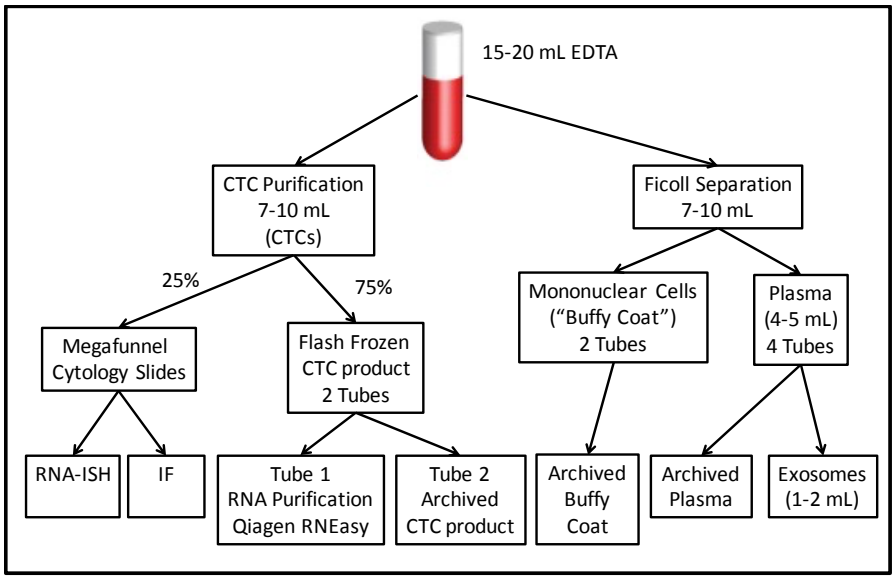


Fig. 3: Schematic of blood processing from patients on protocol.

The bulk frozen CTC product will be preserved using RNAlater stabilization solution (Qiagen Cat No. 76106). RNAlater has been shown to significantly increase RNA recovery from CTC products and still permits the isolation and analysis of DNA and proteins from CTC product. One tube of RNAlater stabilized CTC product will be purified for RNA using the RNeasy Micro Kit (Qiagen Cat No. 74004). The other tube of RNAlater stabilized CTC product will be flash frozen and stored at -80°C for future exploratory research.

The other 10 mL of blood will have plasma and mononuclear cells separated by Ficoll based

density centrifugation followed by immediate flash freezing and storage at -80°C. A 1-2 mL aliquot of plasma will be thawed and processed for exosomes capture on the HB-ExoChip and/or ultracentrifugation based methods. RNA will be isolated from exosomes using TRIzol (Thermo Fisher Cat No. 10296028) based purification. This RNA will be either used immediately for analysis or stored at -80°C.

9.4.3.1 Collection of Specimen(s)

All patients will have blood drawn into two 10 mL EDTA purple top tubes for analysis before treatment and then every two months on treatment for the duration of the study.

9.4.3.2 Handling of Specimens(s)
Specimens will be stored at room temperature and sent within 1 hour to the CTC laboratory for processing at the MGH Cancer Center, Building 149, Charlestown, MA.

9.4.3.3 Shipping of Specimen(s)

Blood will be transported to MGH Cancer Center, Building 149, Charlestown, MA via Partners Healthcare approved vendor US Ground.

9.4.3.4 Site(s) Performing Correlative Study:

MGH Cancer Center – Charlestown Navy Yard Building 149

9.4.4 **Post Treatment Biopsies**

9.4.4.1 Collection of Specimen(s)

Mandatory fresh post-treatment biopsies will be collected and divided from 32 patients in 2 for 1) formalin for standard formalin fixed paraffin embedding (FFPE) and 2) fresh frozen in liquid nitrogen or dry ice bath. FFPE slides will be stained using the Affymetrix ViewRNA platform on the automated Leica Bond RX platform. Sequential slides will be stained for HSATII, LINE-1, TERRA, HERV-H, and GAPDH (RNA control). Fresh frozen tumors will be processed for RNA and DNA extraction with the AllPrep spin column kit (Qiagen 80204). RNA will be analyzed by RNA-sequencing using the Illumina Total RNA-seq library construction and sequencing on the NextSeq 2500 platform. Excess RNA and DNA will be archived and stored at -80 degrees C. DNA may be analyzed by whole genome, whole exome, or targeted gene sequencing. These mandatory post treatment biopsies will be done around cycle 2 day 1 of treatment. This biopsy should be +/- 1 week of treatment and of same lesion as pre-study biopsy if safe to do.

9.4.4.2 Handling of Specimens(s)

Fresh post-treatment biopsies will be collected and divided in 2 for 1) formalin for standard formalin fixed paraffin embedding (ffpe) and 2) fresh frozen in liquid nitrogen or dry ice bath.

9.4.4.3 Shipping of Specimen(s)

Fresh post-treatment biopsies will be transported to MGH Cancer Center, Building 149, Charlestown, MA via Partners Healthcare approved vendor US Ground.

9.4.4.4 Site(s) Performing Correlative Study:

MGH Cancer Center – Charlestown Navy Yard Building 149

9.5 Special Studies

Not applicable

10. STUDY CALENDAR

Baseline evaluations are to be conducted within 2 weeks prior to start of protocol therapy. Scans and x-rays must be done ≤4 weeks prior to the start of therapy. In the event that the participant's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy.

Assessments must be performed prior to administration of any study agent. Study assessments and agents should be administered within ± 3 days of the protocol-specified date, unless otherwise noted.

	Pre-Study	Cycle 1*				Cycle 2+		2 months	Off Study[G]
		Day1	Day 8	Day 15	Day 22	Day1	Cycle 2 Day15[I]		
LAMIVUDINE[A]		X	X	X	X	X	X		
Informed consent	X								
Demographics	X								
Medical history	X								
Concurrent meds	X	X	X	X	X	X	X		
Physical exam	X	X	X	X	X	X	X		X
Vital signs	X	X	X	X	X	X	X		X
Height	X								
Weight	X	X	X	X	X	X	X		X
Performance status	X	X	X	X	X	X	X		X
CBC w/diff, plts	X	X	X	X	X	X	X		X
Serum chemistry[B]	X	X	X	X	X	X	X		X
EKG (as indicated)	X								
Adverse event evaluation		X	X	X	X		X		X
Tumor measurements	X	Tumor measurements are repeated every **8** weeks. Documentation (radiologic) must be provided for participants removed from study for progressive disease.							X
Radiologic	X	Radiologic measurements should be performed every **8** weeks.							X

evaluation									
B-HCG[C]	X								
Tumor biopsy (archival)[D***]	X								
Post treatment biopsy						X**			
Correlative studies[F]	X								
Surveys[H]	X							X	
HIV test	X								
HBV test	X								
CEA		X				X			

*Patient can remain on treatment until progression
**Patient will have biopsy at cycle 2 day 1 or prior should treatment be stopped prior. This biopsy should be +/- 1 week of treatment and of same lesion as pre-study biopsy if safe to do.
***A pre-study biopsy will be required if a biopsy was not done after the most recent treatment received. The tumor biopsy should be collected, if medically feasible, in the opinion of the investigator

A. Lamivudine is to be taken twice daily for all 28 days of the cycle
B. Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, phosphorus, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.
C. Serum pregnancy test (women of childbearing potential.)
D. See section 9.4.1
E. See section 9.4.4
F. Two 10ml EDTA tubes will be collected prior to the start of therapy and every 8 weeks while on therapy
G. Off-study evaluation. Note: for IND/IDE trials, follow up visits or other contact are required in order to identify SAEs during the 30 days following the end of study treatment.
H. Surveys will be administered at baseline on the date of enrollment or prior to start and then at every 2 months at the time of scans.
I. Cycle2 Day15 Visit will only be done at the discretion of the provider.

11. MEASUREMENT OF EFFECT

11.1 Antitumor Effect – Solid Tumors

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

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

11.1.1 Definitions

Evaluable for Target Disease response. Only those participants 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 target disease response. These participants will have their response classified according to the definitions stated below.

(Note: Participants who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

Evaluable Non-Target Disease Response. Participants who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

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

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

Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, abdominal masses (not followed by CT or MRI), and cystic lesions are all considered non-measurable.

Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same participant, these are preferred for selection as target lesions.

Target lesions. All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself

to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target lesions. All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow up.

11.1.3 Methods for Evaluation of Disease

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

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions. Clinical lesions will only be considered measurable when they are superficial (*e.g.*, skin nodules and palpable lymph nodes) and ≥10 mm in diameter as assessed using calipers (*e.g.*, skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Conventional CT and MRI. This guideline has defined measurability of lesions on CT scan based on the assumption that CT thickness is 5mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size of a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (*e.g.* for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image

acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

11.1.4 Response Criteria

11.1.4.1 Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

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

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

11.1.4.2 Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis).

Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD): Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

11.1.4.3 Evaluation of New Lesions

The finding of a new lesion should be unequivocal (i.e. not due to difference in scanning technique, imaging modality, or findings thought to represent something other than tumor (for example, some 'new' bone lesions may be simply healing or flare of pre-existing lesions). However, a lesion identified on a follow-up scan in an anatomical location that was not scanned at baseline is considered new and will indicate PD. If a new lesion is equivocal (because of small size etc.), follow-up evaluation will clarify if it truly represents new disease and if PD is confirmed, progression should be declared using the date of the initial scan on which the lesion was discovered.

11.1.4.4 Evaluation of Best Overall Response

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

For Participants with Measurable Disease (*i.e.*, Target Disease)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
CR	CR	No	CR	≥4 wks Confirmation**
CR	Non-CR/Non-PD	No	PR	≥4 wks Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	Documented at least once ≥4 wks from baseline**
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.
** Only for non-randomized trials with response as primary endpoint.
*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Note: Participants with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration.*" Every effort should be made to document the

objective progression even after discontinuation of treatment.

For Participants with Non-Measurable Disease (*i.e.*, Non-Target Disease)

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD
* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised		

11.1.5 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started, or death due to any cause. Participants without events reported are censored at the last disease evaluation).

Duration of overall complete response: The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented, or death due to any cause. Participants without events reported are censored at the last disease evaluation.

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

11.1.6 Progression-Free Survival

Overall Survival: Overall Survival (OS) is defined as the time from randomization (or registration) to death due to any cause, or censored at date last known alive.

Progression-Free Survival: Progression-Free Survival (PFS) is defined as the time from randomization (or registration) to the earlier of progression or death due to any cause. Participants alive without disease progression are censored at date of last disease evaluation.

Time to Progression: Time to Progression (TTP) is defined as the time from randomization (or registration) to progression, or censored at date of last disease evaluation for those without progression reported.

11.2 Antitumor Effect – Hematologic Tumors

Not applicable

11.3 Other Response Parameters

Not applicable

12. DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7.0 (Adverse Events: List and Reporting Requirements).

12.1 Data Reporting

12.1.1 Method

The QACT will collect, manage, and perform quality checks on the data for this study.

12.1.2 Responsibility for Data Submission

Investigative sites within DF/HCC or DF/PCC are responsible for submitting data and/or data forms to the QACT according to the schedule set by the QACT.

12.2 Data Safety Monitoring

The DF/HCC Data and Safety Monitoring Committee (DSMC) will review and monitor toxicity and accrual data from this study. The committee is composed of clinical specialists with experience in oncology and who have no direct relationship with the study. Information that raises any questions about participant safety will be addressed with the Overall PI and study team.

The DSMC will review each protocol up to four times a year or more often if required to review toxicity and accrual data. Information to be provided to the committee may include: up-to-date participant accrual; current dose level information; DLT information; all grade 2 or higher unexpected adverse events that have been reported; summary of all deaths occurring with 30 days of intervention for Phase I or II protocols; for gene therapy protocols, summary of all deaths while being treated and during active follow-up; any response information; audit results, and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

12.3 Multicenter Guidelines

Not applicable

12.4 Collaborative Agreements Language

Not applicable

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Endpoints

The primary endpoint for this study is to describe the ORR. All patients with measurable disease who receive at least one dose of lamivudine will be considered evaluable. The two-stage design to test the null hypothesis that the response rate is <=1% versus the alternative that response rate is >=10%. After testing the drug on 20 patients in the first stage, the trial will be terminated if 0 respond. If the trial goes on to the second stage, a total of 32 patients will be studied. If the total number responding is less than or equal to 1, the treatment is rejected. The type I error is 3.6% and the power of the test is 80%.

Secondary endpoint includes median progression-free survival (PFS), overall disease control rate, and median overall survival (OS). We will apply Kaplan-Meier method to estimate the distribution of overall patient survival and progression free survival. Kaplan-Meier curves will be plotted to illustrate survival probabilities in OS and PFS. Estimated median survival time with 95 % confidence intervals will be estimated and reported. Cox proportional hazards model will be applied to estimate the effect of confounding factors such as demographic and clinical factors

13.2 Sample Size, Accrual Rate and Study Duration

After testing the drug on 20 patients in the first stage, the trial will be terminated if 0 respond. If the trial goes on to the second stage, a total of 32 patients will be studied. We anticipate being able to accrue 32 patients in one year of study duration for an average of 3-4 patients a month, which we do not anticipate being a problem given our mCRC volume and the prevalence of p53 mutations in this segment. A total of 12 evaluable participants will be accrued in 3 months. Up to an additional 2 months of follow-up will be required after the last participant to observe the participant's response. Should there be 1 response; the study will continue to enroll the additional patients over an expected time course of 3 months with an additional 2 months of follow-up for an expected study duration of approximately 1 year.

Accrual Targets					
Ethnic Category	**Sex/Gender**				
	Females		**Males**		**Total**
Hispanic or Latino	1	+	2	=	3
Not Hispanic or Latino	11	+	18	=	29

Ethnic Category: Total of all subjects	12	(A1)	+	20	(B1)	=	32 (C1)
Racial Category							
American Indian or Alaskan Native	0		+	0		=	0
Asian	1		+	3		=	4
Black or African American	2		+	3		=	5
Native Hawaiian or other Pacific Islander	0		+	0		=	0
White	9		+	14		=	23
Racial Category: Total of all subjects	12	(A2)	+	20	(B2)	=	32 (C2)
	(A1 = A2)			(B1 = B2)			(C1 = C2)

13.3 Stratification Factors

Not applicable

13.4 Interim Monitoring Plan

See 13.1 for two-staged design

13.5 Analysis of Primary Endpoints

See 13.1 for two-staged design

13.6 Analysis of Secondary Endpoints

Secondary correlative science endpoints will describe HSATTII expression in circulating tumor cells, archived resected/biopsied tumors as well as mandatory post treatment biopsies. This will be evaluated in correlation with outcome parameters as described in detail in the biomarker and correlative science section 9.4. These correlative endpoints are exploratory in nature.

13.7 Reporting and Exclusions

13.7.1 Evaluation of Toxicity

All patients who receive study drug will be considered evaluable for toxicity.

13.7.2 Evaluation of the Primary Efficacy Endpoint

Analysis will be by intent to treat.

Total accrual may be adjusted to account for patients inevaluable for response, ie those patients not completing 1 cycle of therapy.

14. PUBLICATION PLAN

The results should be made public within 24 months of reaching the end of the study. The end of the study is the time point at which the last data items are to be reported, or after the outcome data are sufficiently mature for analysis, as defined in the section on Sample Size, Accrual Rate and Study Duration. If a report is planned to be published in a peer-reviewed journal, then that initial release may be an abstract that meets the requirements of the International Committee of Medical Journal Editors. A full report of the outcomes should be made public no later than 3 (3) years after the end of the study.

REFERENCES

1. BERRETTA, J. AND A. MORILLON, *PERVASIVE TRANSCRIPTION CONSTITUTES A NEW LEVEL OF EUKARYOTIC GENOME REGULATION.* EMBO REP, 2009. 10(9): P. 973-82.
2. JACQUIER, A., *THE COMPLEX EUKARYOTIC TRANSCRIPTOME: UNEXPECTED PERVASIVE TRANSCRIPTION AND NOVEL SMALL RNAS.* NAT REV GENET, 2009. 10(12): P. 833-44.
3. JURKA, J., ET AL., *REPBASE UPDATE, A DATABASE OF EUKARYOTIC REPETITIVE ELEMENTS.* CYTOGENET GENOME RES, 2005. 110(1-4): P. 462-7.
4. TING, D.T., ET AL., *ABERRANT OVEREXPRESSION OF SATELLITE REPEATS IN PANCREATIC AND OTHER EPITHELIAL CANCERS.* SCIENCE, 2011. 331(6017): P. 593-6.
5. BERSANI, F., ET AL., *PERICENTROMERIC SATELLITE REPEAT EXPANSIONS THROUGH RNA-DERIVED DNA INTERMEDIATES IN CANCER.* PROC NATL ACAD SCI U S A, 2015. 112(49): P. 15148-53.
6. WYLIE, A., ET AL., *P53 GENES FUNCTION TO RESTRAIN MOBILE ELEMENTS.* GENES DEV, 2016. 30(1): P. 64-77.
7. LIU, M.C., ET AL., *CIRCULATING TUMOR CELLS: A USEFUL PREDICTOR OF TREATMENT EFFICACY IN METASTATIC BREAST CANCER.* J CLIN ONCOL, 2009. 27(31): P. 5153-9.
8. TOL, J., ET AL., *CIRCULATING TUMOUR CELLS EARLY PREDICT PROGRESSION-FREE AND OVERALL SURVIVAL IN ADVANCED COLORECTAL CANCER PATIENTS TREATED WITH CHEMOTHERAPY AND TARGETED AGENTS.* ANN ONCOL, 2010. 21(5): P. 1006-12.
9. REID, A.H., ET AL., *SIGNIFICANT AND SUSTAINED ANTITUMOR ACTIVITY IN POST-DOCETAXEL, CASTRATION-RESISTANT PROSTATE CANCER WITH THE CYP17 INHIBITOR ABIRATERONE ACETATE.* J CLIN ONCOL, 2010. 28(9): P. 1489-95.
10. DANILA, D.C., ET AL., *PHASE II MULTICENTER STUDY OF ABIRATERONE ACETATE PLUS PREDNISONE THERAPY IN PATIENTS WITH DOCETAXEL-TREATED CASTRATION-RESISTANT PROSTATE CANCER.* J CLIN ONCOL, 2010. 28(9): P. 1496-501.
11. OZKUMUR, E., ET AL., *INERTIAL FOCUSING FOR TUMOR ANTIGEN-DEPENDENT AND -INDEPENDENT SORTING OF RARE CIRCULATING TUMOR CELLS.* SCI TRANSL MED, 2013. 5(179): P. 179RA47.
12. KISHIKAWA, T., ET AL., *QUANTITATION OF CIRCULATING SATELLITE RNAS IN PANCREATIC CANCER PATIENTS.* JCI INSIGHT. 1(8).
13. Romanus, D., et al., *Does health-related quality of life improve for advanced pancreatic cancer patients who respond to gemcitabine? Analysis of a randomized phase III trial of the cancer and leukemia group B (CALGB 80303).* J Pain Symptom Manage, 2012. **43**(2): p. 205-17.
14. Gourgou-Bourgade, S., et al., *Impact of FOLFIRINOX compared with gemcitabine on quality of life in patients with metastatic pancreatic cancer: results from the PRODIGE 4/ACCORD 11 randomized trial.* J Clin Oncol, 2013. **31**(1): p. 23-9.
15. Crippa, S., et al., *Quality of life in pancreatic cancer: analysis by stage and treatment.* J Gastrointest Surg, 2008. **12**(5): p. 783-93; discussion 793-4.

16. Kelsen, D.P., et al., *Pain and depression in patients with newly diagnosed pancreas cancer.* J Clin Oncol, 1995. **13**(3): p. 748-55.
17. Boyd, A.D. and M. Riba, *Depression and pancreatic cancer.* J Natl Compr Canc Netw, 2007. **5**(1): p. 113-6.
18. Edgerly, M. and T. Fojo, *Is there room for improvement in adverse event reporting in the era of targeted therapies?* J Natl Cancer Inst, 2008. **100**(4): p. 240-2.
19. Berry, D.L., *Patient-reported symptoms and quality of life integrated into clinical cancer care.* Semin Oncol Nurs, 2011. **27**(3): p. 203-10.
20. Oberguggenberger, A., et al., *Is the toxicity of adjuvant aromatase inhibitor therapy underestimated? Complementary information from patient-reported outcomes (PROs).* Breast Cancer Res Treat, 2011. **128**(2): p. 553-61.
21. Meyers, F.J., et al., *Simultaneous care: a model approach to the perceived conflict between investigational therapy and palliative care.* J Pain Symptom Manage, 2004. **28**(6): p. 548-56.
22. Aaronson, N.K., et al., *The European Organization for Research and Treatment of Cancer QLQ-C30: a quality-of-life instrument for use in international clinical trials in oncology.* J Natl Cancer Inst, 1993. **85**(5): p. 365-76.
23. Watanabe, S.M., et al., *A multicenter study comparing two numerical versions of the Edmonton Symptom Assessment System in palliative care patients.* J Pain Symptom Manage, 2011. **41**(2): p. 456-68.
24. Zigmond, A.S. and R.P. Snaith, *The hospital anxiety and depression scale.* Acta Psychiatr Scand, 1983. **67**(6): p. 361-70
25. Zigmond, A.S. and R.P. Snaith, *The hospital anxiety and depression scale.* Acta Psychiatr Scand, 1983. **67**(6): p. 361-70
26. *The Journal of Infectious Diseases*, Volume 171, Issue 6, 1 June 1995, Pages 1438–1447
27. Aguirre, A.J., et al., *Activated Kras and Ink4a/Arf deficiency cooperate to produce metastatic pancreatic ductal adenocarcinoma.* Genes Dev, 2003. **17**(24): p. 3112-26
28. Bardeesy, N., et al., *Both p16(Ink4a) and the p19(Arf)-p53 pathway constrain progression of pancreatic adenocarcinoma in the mouse.* Proc Natl Acad Sci U S A, 2006. **103**(15): p. 5947-52.
29. Hezel, A.F., et al., *Genetics and biology of pancreatic ductal adenocarcinoma.* Genes Dev, 2006. **20**(10): p. 1218-49.
30. leeuwen et al. evaluation of safety and efficacy of 3tc (lamivudine) in patients with asymptomatic or mildly symptomatic human immunodeficiency virus infection: a phase i/ii study j infect dis. 1995 may;171(5):1166-71.)
31. Bersani f et al. Pericentromeric satellite repeat expansions through rna-derived dna intermediates in cancer. PNAS 2015 December, 112 (49) 15148-15153

APPENDIX A PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (*e.g.*, light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

APPENDIX B MULTICENTER GUIDELINES

Not applicable

APPENDIX C INFORMATION ON POSSIBLE DRUG INTERACTIONS

Not applicable

APPENDIX D BIOASSAY TEMPLATES

Not applicable