CUMC IRB#: AAAR6703
Version Date: 30MAR2022
NCT04476537

A phase Ib high-throughput pancreas precision oncology study investigating the feasibility, efficacy, and pharmacodynamics of cell regulatory-network analysis based therapy selection in advanced pancreatic adenocarcinoma: HIPPOCRATES Part 2.

COLUMBIA UNIVERSITY MEDICAL CENTER

Herbert Irving Comprehensive Cancer Center Protocol

NCI·CC

A Cancer Center Designated by the National Cancer Institute

Columbia University Medical Center
Herbert Irving Comprehensive Cancer Center
Version Date: 30 Mar 2022

CUMC IRB#: AAAR6703
Version Date: 30MAR2022

TITLE: A phase Ib high-throughput pancreas precision oncology study investigating the feasibility, efficacy, and pharmacodynamics of cell regulatory-network analysis based therapy selection in advanced pancreatic adenocarcinoma: HIPPOCRATES Part 2.

Coordinating Center: Columbia University Irving Medical Center

Principal Investigator: Gulam Abbas Manji, M.D./Ph.D.
161 Fort Washington Avenue
New York, NY 10032
Telephone: 212-212-304-6357
Email address: gam2140@cumc.columbia.edu

Co-Investigators: Gulam Manji, M.D., Ph.D
John Chabot, M.D.
Susan Bates, M.D.
Antonio Fojo, M.D., Ph.D
Yvonne Saenger, M.D.
Ruth White, M.D., Ph.D
Kenneth P. Olive, Ph.D.
Andrea Califano, Laurea
Armando Del Portillo, M.D., Ph.D
Lyndon Luk, M.D.
Christine Chio, Ph.D.
Serge Cremers, Ph.D.

Statistician: Jianhua Hu, Ph.D.
1130 Saint Nicholas Avenue, 1002A
New York, NY 10032
Email: jh3992@cumc.columbia.edu

Funding Source:	The Lustgarten Foundation 415 Crossways Park Drive, Suite D Woodbury, NY 11797 Phone: 516-737-1550

Initial version: 20Jul2020
Amended: 30Mar2022

Protocol Signature Page

I confirm that I have read this protocol, I understand it, and I will work according to this protocol and to the ethical principles stated in the latest version of the Declaration of Helsinki, the applicable ICH guidelines for good clinical practices, and the applicable federal, state, and local laws, rules, and regulations relating to the conduct of the protocol. I have read and understand the information in the Investigators' Brochure (or Manufacturer's Brochure) regarding the risks and potential benefits. I will promptly submit the protocol to the applicable IRB for review and approval. Once the protocol has been approved by the IRB, I understand that any modification made during the course of the study must first be approved by the IRB, prior to implementation except when such modification is made to remove an immediate hazard to the subject. I certify that I, and the study staff, have received the requisite training to conduct this research protocol. I agree to maintain adequate and accurate records in accordance with Columbia University and Herbert Irving Comprehensive Cancer Center policies, Federal, state and local laws and regulations. I agree to maintain the confidentiality of all information received or developed in connection with this protocol.

Instructions to Principal Investigator: Sign and Date this signature page and print your name. Return the original, completed and signed to the Clinical Protocol & Data Management Office. Retain a copy in the regulatory binder.

_____ _____

Signature of Principal Investigator Date

_____

Principal Investigator Name (Print)

_____

Name of Institution

Table of Contents

1. PROTOCOL SYNOPSIS

This document is a protocol for a human research study. This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Columbia University Medical Center institutional research policies and procedures.

Title	A phase Ib high-throughout pancreas precision oncology study investigating the feasibility, efficacy, and pharmacodynamics of cell regulatory-network analysis based therapy selection in advanced pancreatic adenocarcinoma: HIPPOCRATES Part 2.
Short Title	RNA precision oncology in advanced pancreatic cancer (HIPPOCRATES Part 2)
Phase	Ib
Methodology	This is a single arm, open-label phase Ib trial of FDA-approved or investigational agents in patients with locally advanced, unresectable or metastatic pancreatic cancer who have progressed on one or two prior lines of treatment to evaluate feasibility, safety, and efficacy of the OncoTreat algorithm.
Study Duration	We estimate that the trial (Parts 1 and 2) will require up to 36 months to complete--15 to 18 months for tissue acquisition on Part 1 (AAAR6703) and an additional 15-18 for treatment and follow-up to evaluate the efficacy outcomes for all subjects.
Study Center(s)	Columbia University Irving Medical Center

Objectives	1. Co-Primary objectives: • To evaluate the overall feasibility of using the OncoTreat technology to inform rational selection of off-label FDA-approved or investigational drugs in subjects with advanced pancreatic cancer (PDA) who have progressed on one or two prior lines of therapy. This will include the percentage of subjects who begin an OncoTreat-prioritized agent, defined as having received a single dose of study drug, and the "match rate," defined as the fraction of subjects who match with at least one OncoTreat-prioritized agent (p-value $< 1 \times 10^{-5}$). • To evaluate the clinical efficacy of OncoTreat by assessing the objective response rate (ORR) for subjects treated with OncoTreat-prioritized drugs according to Response Evaluation Criteria in Solid Tumors (RECIST) v1.1 criteria. 2. The secondary objectives of this study are to: • To describe the safety and tolerability, defined as the incidence of Grade 3/4 adverse events (AEs) in terms categorized and graded according to the National Cancer Institute Terminology Criteria for Adverse Events (NCI CTCAE v4.0), of using OncoTreat to inform rational treatment decisions in subjects with advanced PDA. The incidence of dose modifications, interruptions, and discontinuations will also be described. • To assess the efficacy of OncoTreat by estimating the disease control rate (DCR) at 16 and 24 weeks, the median progression-free survival (PFS), the overall survival (OS), and the CA 19-9 tumor marker response. • Assess the pharmacokinetics of each OncoTreat-prioritized agent in subjects with advanced PDA. 3. The exploratory objectives of this study are to: • Explore the relationship between drug exposure, pharmacodynamics, efficacy, and safety of each OncoTreat-prioritized agent. Pharmacodynamic response will be evaluated by comparing the master regulator signature of a given subject's tumor using

	VIPER analysis, comparing pre-treatment and on-treatment tumor samples. The changes in master regulator activity between these two samples will be compared to that predicted from cell-based and PDX-based treatment studies. • Assess whether the master regulator signature, using VIPER analysis, in a given subject's tumor changes in response to standard-of-care treatment by comparing initial and secondary biopsies collected for each subject before and after one or two lines of systemic therapy (chemotherapy or clinical trial). These data will be used to determine whether the predicted sensitivity of a patient's tumor to the first- or second-line therapy that they receive is predictive of the actual clinical outcomes that are observed during treatment. • Using the same data as in the bullet point above, assess the frequency with which chemotherapy treatments alter the predicted sensitivity of tumors to other OncoTreat-prioritized agents (p-value $< 1 \times 10^{-5}$). • Compare the standard-of-care clinical DNA mutation analysis results with matched master regulator activity profiles to correlate any clinically actionable genomic information with the top OncoTreat-prioritized drugs (p-value $< 1 \times 10^{-5}$). • Evaluate the feasibility of using the OncoTreat technology on peripheral blood circulating tumor cells (CTCs) by comparing master regulators generated from CTCs to those obtained from tumor tissue in matched pre- and on-treatment samples with an OncoTreat-informed agent. • Identify associations between master regulator profiles and additional clinical, epidemiological, physiological, imaging, or molecular features of the subject or subject's tumor. • Identify potential biomarkers, beyond the master regulator signature, using additional genomic, RNA, protein, and imaging platforms that are predictive or

	prognostic of response to each OncoTreat-prioritized agent.
Number of Subjects	We will enroll 30 subjects in Part 1 (AAAR6703) of the study to obtain detailed predicted therapy options. We anticipate accrual over 15-18 months. We estimate that 15 subjects will progress to Part 2, requiring an additional 15-18 months to complete treatment and for follow-up.
Diagnosis and Main Inclusion Criteria	Locally advanced, unresectable or metastatic pancreatic cancer who progressed on one or two prior lines of chemotherapy, for whom a precision therapy was recommended in Part 1.
Duration of administration	Treatment will continue until progressive disease, unacceptable adverse events, intercurrent illness that prevents further administration of treatment, investigator's decision to withdraw the subject, subject withdrawal of consent, pregnancy of the subject, noncompliance with trial treatment or procedure requirements, or administrative reasons requiring cessation of treatment.
Reference therapy	Not applicable – single arm study.

Statistical Methodology	This is a single arm, phase Ib study with 2 co-primary endpoints: 1. Feasibility Endpoint: We will determine the feasibility of the OncoTreat platform in guiding therapy. The primary feasibility endpoint is whether a subject begins treatment on Part 2 based on results generated from the OncoTreat analysis. Previous attempts to match subjects based on DNA profiling yielded enrollment rates of 3-5%. We hypothesize that we will validate OncoTreat matches for 50% of subjects and that at least 30% of enrolled subjects (60% of matches) will begin therapy. With 30 enrolled patients we will have 84% power to detect a treatment rate of 30% compared to a historical treatment rate of 5% with alpha of 0.05. We will assess the "match rate" – the fraction of patients who match with at least one drug. With 30 enrolled patients, we will have 90% power with alpha = 0.05 to detect a 50% match rate (15 of 30 subjects) compared to a (liberal) estimated match rate of 20% based on DNA profiling in pancreatic cancer. 2. Clinical Endpoint: The primary clinical efficacy endpoint is the objective response rate for OncoTreat-prioritized drugs according to RECIST v1.1. The phase III trial (NAPOLI-1) that resulted in the FDA-approval of nanoliposomal irinotecan and 5-fluorouracil in the second-line setting demonstrated a median OS of 6.1 months versus 4.2 months for 5-fluorouracil monotherapy (primary endpoint), mPFS of 3.1 months, and an ORR of 16% (compared to 1% in the 5-fluorouracil arm). Thus, based on historical controls, the ORR for treatment-resistant metastatic PDAC is expected to be no more than 16%. Using the above mentioned rates, a sample size of n = 9 achieves at least a 80% power to detect a difference of 25% in ORR (30% vs 5%) using a one-sided binomial test with a target alpha of 0.1. To be conservative, we use the minimum number of patients (9) expected to be treated in this calculation. If at the end of the study the total number of objective responses is greater than or equal to 2, we will be able to reject the historical rate of 5% at the significance level of 0.05 with power of 69% and OncoTreat will have demonstrated a clear signal of clinically meaningful improvement in the primary endpoint for patients with advanced, previously-treated PDA. Assuming about 30% of enrolled patients will receive an OncoTreat-informed treatment, N = 30 subjects is an appropriate sample size for this prospective study. Secondary measures include DCR, PFS, and OS as well as safety and tolerability of OncoTreat-directed therapy. The pharmacokinetics of OncoTreat-prioritized drugs in PDA subjects will also be described.

1.1 Trial Design Overview

This is a phase 1b, single-arm, single-center, open-label clinical trial for 30 evaluable patients with locally advanced or metastatic pancreatic cancer (PDA). Subjects with newly diagnosed locally advanced or metastatic PDA will be enrolled on protocol Part 1 (AAAR6703) prior to tumor biopsy. OncoTreat analysis will be conducted using a New York State CLIA-certified test. Subjects will receive physician's choice first-line cytotoxic chemotherapy (including 5-FU, gemcitabine, or capecitabine-based combinations or monotherapy) or clinical trial while preclinical validations of OncoTreat candidate treatments are performed. Preclinical results will be evaluated by the Precision Medicine Tumor Board (PMTB), which will review the data and prioritize a treatment based on the following criteria: 1) patient safety; 2) preclinical efficacy; 3) pharmacological properties; and 4) drug availability. If an agent is not identified or available at first progression, patients will continue on to second-line therapy (chemotherapy or clinical trial) and will be again assessed at the time of second progression. If an agent is identified and available, subjects will be enrolled on this protocol (Part 2), undergo a repeat biopsy and be treated on a single-arm, single-agent phase Ib clinical trial evaluating pharmacokinetics, safety, and efficacy in recurrent PDA patients.

This study will be conducted in conformance with Good Clinical Practices.

Specific procedures to be performed during the trial, as well as their prescribed times nad associated visit windows, are outlined in the Trial Flow Chart – Section 9.

1.2 Trial Schema

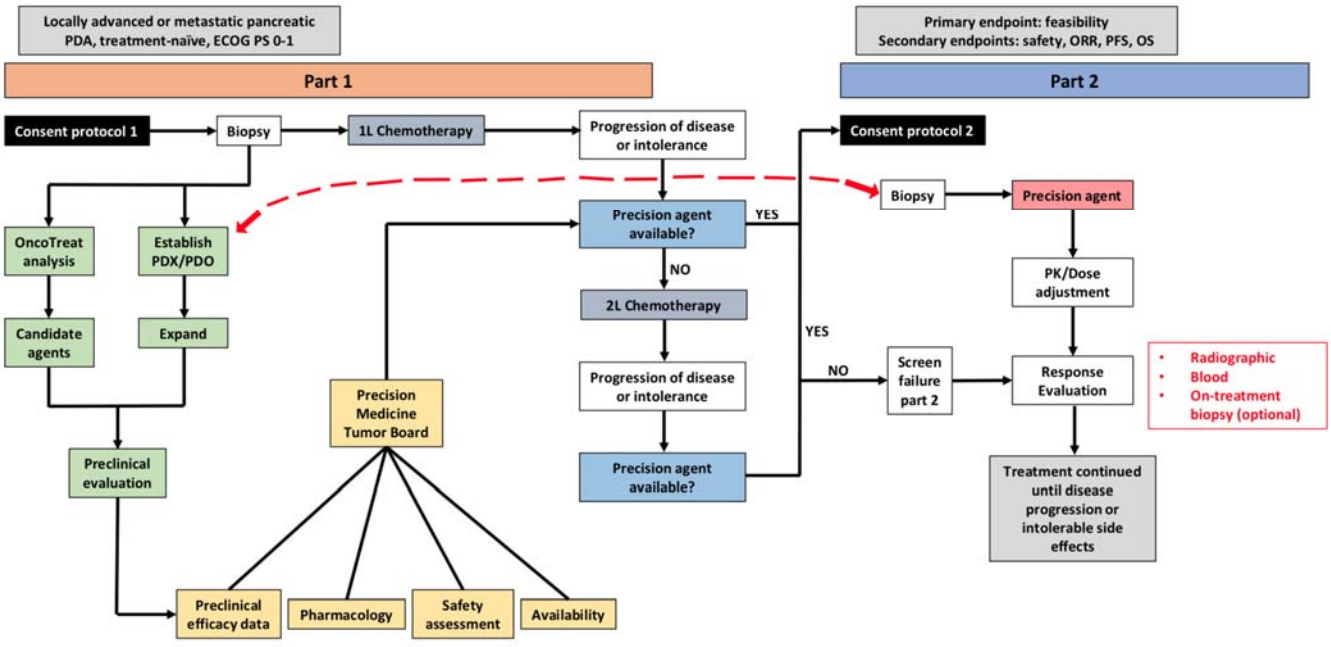


Figure 1. Phase Ib Trial Schema.

2. STUDY OBJECTIVES AND ENDPOINTS

2.1 Primary Objectives & Endpoints

2.1.1 Objectives:

The co-primary objectives of this study are:

- To evaluate the overall feasibility of using the OncoTreat technology to inform rational selection of off-label FDA-approved or investigational drugs in subjects with advanced pancreatic cancer (PDA) who have progressed on one or two prior lines of systemic therapy. This will include the percentage of subjects who begin an OncoTreat-prioritized agent, defined as having received a single dose of study drug, and the "match rate," defined as the fraction of subjects who match with at least one OncoTreat-priotitized agent (p-value $< 1 \times 10^{-5}$).
- To evaluate the clinical efficacy of OncoTreat by assessing the objective response rate (ORR) for subjects with advanced PDA treated with OncoTreat-prioritized drugs after disease progression on one or two prior lines of systemic therapy according to Response Evaluation Criteria in Solid Tumors (RECIST) v1.1 criteria.

2.1.2 Endpoints:

The co-primary endpoints of interest are:

- The percentage of subjects who begin an OncoTreat-prioritized agent, defined as having received a single dose of study drug, and the "match rate," defined as the fraction of subjects who match with at least one OncoTreat-priotitized agent (p-value $< 1 \times 10^{-5}$).
- ORR according to RECIST v1.1 criteria.

2.2 Secondary Objectives & Endpoints

**Note: The secondary objects only apply for patients who are initiated on treatment with an OncoTreat-prioritized FDA-approved or early-phase investigational drug.*

2.2.1 Objectives:

The secondary objectives of this study are to:

- To describe the safety and tolerability of using OncoTreat to inform rational treatment decision in subjects with advanced PDA who have progressed on one or two prior lines of therapy.
- To assess the efficacy of OncoTreat by estimating the disease control rate (DCR) at 16 and 24 weeks, the median progression-free survival (PFS), the overall survival (OS), and the CA 19-9 tumor marker response.
- To assess the pharmacokinetics of each OncoTreat-prioritized agent in subjects with advanced PDA.

2.2.2 Endpoints:

Secondary endpoints of interest include:

- Safety/tolerability
    - Incidence of Grade 3/4 adverse events (AEs) in terms categorized and graded according to the National Cancer Institute Terminology Criteria for Adverse Events (NCI CTCAE v4.0).
    - Incidence of dose modifications, interruptions, and discontinuations.
    - *Note*: OncoTreat-prioritized agents will be dosed at the FDA-approved dose (if available) or the recommended phase 2 dose for investigational agents. Safety endpoints as they pertain to specific drugs will not be assessed in this prospective study, as only a very small number of patients will be assigned to any one of these drugs, all of which are FDA-approved or have been studied in early phase clinical trials and have track records of safety/tolerability.
- DCR, defined as the proportion of subjects with a complete or a partial response as the best response or stable disease, according to RECIST v1.1 criteria.
- PFS, defined as the time from first dose of OncoTreat-prioritized drug to the first occurrence of disease progression or death from any cause (whichever occurs first), using RECIST v1.1 criteria.
- OS, defined as the time from first dose of OncoTreat-informed study drug to death due to any cause.
- Tumor marker response of CA 19-9, defined as ≥ 50% in CA 19-9 in relation to the baseline level at least once during the treatment period.
- Serum concentration or PK parameters for each OncoTreat-prioritized agent.
- Relationship between serum concentration or PK parameters and endpoints of interest.

2.3 Exploratory Objectives & Endpoints

2.3.1 Objectives:

The exploratory objectives of this study are to:

- To explore the relationship between drug exposure, pharmacodynamics, efficacy, and safety of each OncoTreat-prioritized agent. Pharmacodynamic response will be evaluated by comparing the master regulator signature of a given subject's tumor using VIPER analysis, comparing pre-treatment and on-treatment tumor samples. The changes in master regulator activity between these two samples will be compared to that predicted from cell-based and PDX-based treatment studies.
- To assess whether the master regulator signature, using VIPER analysis, in a given subject's tumor changes in response to standard-of-care treatment by comparing initial and secondary biopsies collected for each subject before and after one or two lines of systemic therapy (chemotherapy or clinical trial). These data will be used to determine whether the predicted sensitivity of a patient's tumor to the first- or second-line therapy that they receive is predictive of the actual clinical outcomes that are observed during treatment.
- Using the same data as in the bullet point above, assess the frequency with which chemotherapy treatments alter the predicted sensitivity of tumors to other OncoTreat-prioritized agents (p-value $< 1 \times 10^{-5}$).
- To compare the standard-of-care clinical DNA mutation analysis results with matched master regulator activity profiles to correlate any clinically actionable genomic information with the top OncoTreat-prioritized drugs.
- To evaluate the feasibility of using the OncoTreat technology on peripheral blood circulating tumor cells (CTCs) by comparing master regulators generated from CTCs to those obtained from tumor tissue in matched pre- and on-treatment samples with an OncoTreat-informed agent.
- To identify associations between master regulator profiles and additional clinical, epidemiological, physiological, imaging, or molecular features of the subject or subject's tumor.
- To identify potential biomarkers, beyond the master regulator signature, using additional genomic, RNA, protein, and imaging platforms that are predictive or prognostic of response to each OncoTreat-prioritized agent (p-value $< 1 \times 10^{-5}$).

2.3.2 Endpoints:

Exploratory endpoints of interest include:

- Pharmacodynamic response will be assessed by determining the differentially activated and repressed master regulator proteins between the pre-treatment (second) biopsy and the on-treatment (third) biopsy, and comparing these changes to those predicted by OncoTreat using a modified gene set enrichment analysis with a threshold Fasle Discovery Rate of $p = 0.05$.
- Paired-sample VIPER analysis using Master Regulator profiles from the first and second biopsies.

- The fraction of drugs predicted for each patient using the first biopsy that are still predicted in the second biopsy.
- The DIGGIT algorithm will be used to associate DNA alterations, found from enomic profiling using next-generation sequencing (NGS) assays such as CCCP or Foundation One, with master regulator activity.
- Percentage of OncoTreat-prioritized agents (p-value < 1 x 10^{-5}) that overlap between tumor biopsy specimens and peripheral blood CTCs.
- Relationship between biomarkers in blood and tumor tissue and efficacy, safety, PK, or other biomarker endpoints.

3. BACKGROUND & RATIONALE

3.1 Pancreatic Cancer

Pancreatic ductal adenocarcinoma (PDA) is a major health problem worldwide. It is predicted to be the 2nd leading cause of cancer-related death in the United States by 2030 and carries a dismal 5-year survival of 8.2% despite 50 years of research and therapeutic developments [1, 2]. During 2018, it is estimated that 55,440 people will be diagnosed with PDA and approximately 44,330 people will die from PDA in the United States [2].

Currently, surgical resection offers the only therapeutic means of cure. However, only 15-20% of patients have resectable disease. Even among the small subset of patients who are suitable for surgical resection at the time of diagnosis, complete resection is followed by recurrence in >90% of patients without further systemic therapy, with a median time to recurrence of 6.9 months [3]. In the metastatic setting, the 5-year overall survival is 2% [4]. Therefore, PDA is an unmet need that desperately requires better medical intervention.

Despite recent advances in systemic therapy, upfront cytotoxic chemotherapy for PDA has been disappointing with response rates of 20-30% for the most active regimens and no impact on the 5-year survival rate [5, 6]. The majority of patients will progress after a median of 5-7 months. Data from the second-line NAPOLI-1 study showed that 5-fluorouracil in combination with nanoliposomal irinotecan improved both median PFS and OS compared with 5-fluorouracil alone (3.1 vs 1.5 months and 6.1 vs 4.2 months, respectively) [7]. There is currently no standard-of-care treatment regimen available for the third-line setting, and consensus guidelines recommend best supportive care or participation in a clinical trial [NCCN 2018]. Thus all PDA patients require systemic chemotherapy, and more effective regimens are urgently needed [8].

3.2 Targeted Therapy in Pancreatic Cancer

For at least two decades, there has been an intensive focus on identifying and targeting specific mutations or pathways in PDA. The prevailing model has been that mutated oncogenic drivers are the ideal cancer drug targets (as epitomized by the responsiveness of c-Kit mutant GIST to imatinib). However, few such targets are present in PDA aside from mutant K-ras, which has proven pharmacologically intractable [9]. Indeed, best estimates suggest that only around 15% of PDA tumors harbor alterations that can be matched by current precision therapies. Moreover, many examples exist of tumors bearing targetable mutations that nonetheless fail to respond to

matched therapies and, conversely, of tumors that are unexpectedly sensitive to targeted drugs in the absence of a known relevant oncoprotein [10, 11]. For example, treatment of EGFR-mutant pancreatic tumors with the EGFR inhibitor erlotinib provides at best a modest benefit, despite substantial efficacy in lung cancer [12]. These observations imply that the signaling context of a mutation is highly relevant to therapeutic response. It is our **hypothesis** that the context itself– that is, the regulatory state of the tumor cell– is a relevant and promising therapeutic target (**Figure 2**).

A novel systems biology approach, developed by Andrea Califano (co-PI) at Columbia University Medical Center, is uniquely positioned to extend the frontier of personalized medicine for patients with treatment refractory cancers, including advanced PDA. The OncoTreat algorithm is able to prioritize potentially effective FDA-approved and investigational oncology drugs, on a patient-by-patient basis, for patients currently lacking effective treatment options. In this prospective study, we will investigate the feasibility of using OncoTreat to inform rational selection of off-label FDA-approved oncology drugs for patients with advanced PDA who have received one or two prior lines of systemic therapy.

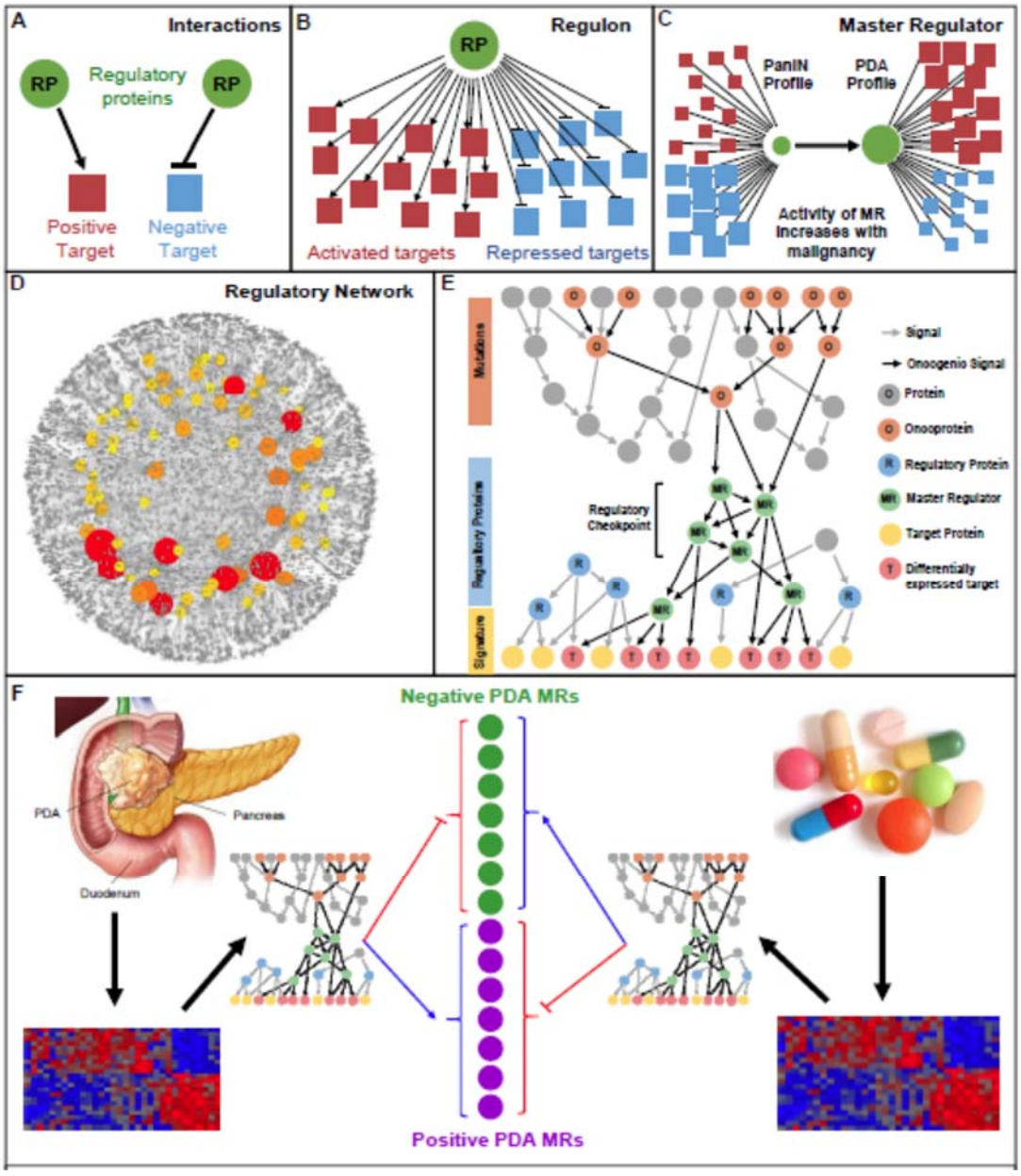


Figure 2. Regulatory Network Concepts and OncoTreat Framework. A) ***Regulatory Proteins*** (e.g. transcription fractions, co-regulators, and others) are proteins that alter the transcript abundance of target genes. **B)** The collection of genes targeted by a regulatory protein is a ***regulon***. Their relative abundances define the ***activity*** of a regulatory protein. C) A regulatory protein whose activity is altered during tumor development is a ***Master Regulator (MR)*** of malignancy. D) A ***regulatory network*** is a global compilation of regulatory genes and their targets (colored circles represent major Mastor Regulators of PDA). **E)** MRs occupy critical nodes within signaling networks. While many upstream signaling molecules may acquire oncogenic mutations, they must ultimately affect the activity of a limited set of MRs that effect execution of their function. **F)** The MRs that promote or restrain malignancy in an individual patient's tumor are identified from an expression profile using the VIPER algorithm. Similarly, the MRs altered by treatment with a drug are identified through high throughput RNA-sequencing of human PDA cell lines. The OncoTreat framework functions identifying drugs whose activity specifically affects the MRs of an individual patient. That is, drugs that inhibit the patient's positive cancer MRs and activate the patient's negative cancer MRs.

3.3 OncoTreat Technology

In the emerging field of personalized cancer medicine, therapies are prioritized based on the molecular characteristics of an individual patient's tumor. In current clinical practice, this approach centers on the concept of oncogene addiction, whereby tumors become dependent on their mutated oncogenes to maintain the malignant phenotype [13] and therapies are selected based on genetic mutations identified by next generation sequencing platforms. However, the limitations of this approach are now increasingly recognized. In the MD Anderson experience, which parallels that of many cancer centers, only 39% of cancer patients were found with at least one mutation in a potentially actionable gene, 11% enrolled on a clinical trial targeting the alteration, and only a small fraction of the patients derived clinical benefit from this approach [14]. Importantly, aberrant protein activity, the central determinant of drug response in cancer, results from a complex array of biologic determinants, only one of which is genetic mutation in the corresponding gene. Genetic and epigenetic events in cognate binding partners [15], competitive endogenous RNAs [16], and upstream regulators [17], as well as post-translational modifications, complex formation and sub-cellular localization, all contribute to dysregulated protein activity. This complexity likely explains an important observation – although cells with activating mutations in a specific oncogene are generally more sensitive to corresponding targeted inhibitors, cells lacking these mutations can present equivalent sensitivity [10, 11]. Similarly, in clinical practice, and as reported in the MD Anderson experience, many tumors with a mutated oncogene fail to respond to seemingly appropriate targeted therapy.

Over the past decade, Andrea Califano has developed a pipeline of experimentally–validated computational tools (referred to collectively as OncoTreat) that is designed to use RNA profiles, rather than DNA mutations, to examine the regulatory state of an individual patient's tumor and predict a matching treatment. Using a novel systems-biology based approach, *Virtual Inference of Protein activity by Enriched Regulon analysis (VIPER)* infers the activity of regulatory proteins in individual tumor samples based on the gene expression of their transcriptional targets [18]. Conceptually, this approach recognizes that the expression of the transcriptional targets of a protein is a more informative reporter of that protein's activity that the presence or absence of a genetic alteration. In practice, this approach requires that context (tumor) specific regulatory models be established that define relationships between transcriptional regulators, regulatory proteins, signaling proteins, and transcripts in that particular tissue context. These models (termed *interactomes*) can now be defined by analyzing large sets of tissue-matched gene expression profiles such as those offered by The Cancer Genome Atlas (TCGA) [19] using validated computational models in a systems biology based approach. Then, at the single tumor level and using RNA-Seq data derived from a tumor biopsy, the VIPER algorithm is used to infer the activity of proteins by analyzing gene expression of their transcriptional agents, as defined by the tumor-specific interactome.

VIPER thereby identifies key proteins, termed *master regulators* (MRs), which play a necessary role in implementing a tumor's transcriptional identity. Surprisingly, these MRs are almost never mutated (that is, they are not conventional oncogenes) and are therefore not identified by somatic genome sequencing of tumors. Rather, they constitute *bottlenecks* within complex networks of molecular interactions that are responsible for integrating the effect of multiple upstream pathway mutations in order to activate the regulatory programs (cancer hallmarks) that are

necessary for cancer cell survival. Extensive research has demonstrated that genetic or pharmacological targeting of master regulator proteins induces collapse of the corresponding tumor bottleneck, which is catastrophic for the tumor, making it virtually impossible for tumor cells to survive [20-24]. As part of this technology, a drug perturbation database is also developed for each tumor type. Here the effect of approximately 500 candidate pharmacologic agents, including FDA-approved drugs and investigational compounds in late stage development (phase II-III), on regulatory protein activity is evaluated by VIPER analysis. This need only to be done once to define the tumor context-specific mechanism of action for each of the compounds, based on drug effect on MR protein activity (**Figure 2**). Then, OncoTreat technology aligns each compound mechanism of action to the master regulators of each individual tumor, prioritizing them by their ability to revert the activity of aberrantly activated or inactivated master regulator proteins.

Together, the OncoTreat methodologies have led to the elucidation of novel tumor dependencies and drug resistance mechanisms across numerous human malignancies – from leukemia [25, 26], and lymphoma [20, 27], to prostate cancer [23, 28], breast cancer [17, 24], and glioma [16, 17, 22, 29] - with results published in high-impact peer-reviewed journals including *Cell*, *Nature*, and *Clinical Cancer Research*. These studies were instrumental in the generation of mechanistic, hypothesis-driven clinical trials, including the use of combination therapy in trastuzumab-resistant ErbB2 positive breast cancer [24] (NCT02066532) and the use of ACY-1215 (Ricolinostat) + Nab-paclitaxel in metastatic breast cancer [30] (NCT02632071). Additional trials are currently in development, such as to evaluate entinostat in enteropancreatic neuroendocrine tumors (EP-NET). A comprehensive clinical trial program of the OncoTreat method (the "N-of-1" Trial) has already been established at Columbia (IRB-AAAN7562). Moreover, the OncoTreat framework for the prioritization of drugs to target tumor checkpoints was validated in a recent study in gastroenteropancreatic neuroendocrine tumors (GEP-NETs) [31].

The aberrant activity of VIPER-inferred master regulator proteins has been shown to be necessary for implementing the transcriptional identity of a tumor cell, and pharmacologic inhibition or genetic silencing of these master regulators resulted in loss of tumor cell viability. Thus, master regulators represent an obvious class of causal dependencies for targeted therapy. Critically, many of the master regulator proteins identified by this analysis are not canonical mutated oncoproteins, but are instead located downstream from such oncoproteins, thus representing proteins whose activity is post-translationally dysregulated by various alterations in upstream pathways [17]. These master regulators would therefore not be identified by the sequencing techniques currently central to personalized cancer medicine. In summary, OncoTreat is a clinical platform which uses *VIPER* to identify the master regulators in a tumor and refers to the drug perturbation database to prioritize drugs and drug combinations based on their ability to revert the activity of master regulator proteins and thus destabilize the tumor state. This protocol will enable the first implementation of the OncoTreat method, a NY State CLIA-certified assay made available by the Department of Pathology at Columbia University Medical Center, in patients with pancreatic ductal adenocarcinoma.

3.4 OncoTreat and Pancreatic Cancer

The Olive Lab at Columbia University Medical Center has invested five years' effort in building the preliminary datasets required as input for a pancreatic implementation of OncoTreat. A major challenge was the large and variable contribution of stromal cells to the bulk mass of a tumor which alter the RNA profiles of bulk tumor tissue and confound computational analyses. We used laser capture microdissection (LCM) to isolate purified samples of malignant epithelium and nearby stroma from 197 human pancreatic tumors (**Figure 3**). RNA sequencing of these samples yielded high quality expression profiles for both compartments. As "benign controls," we also profiled the epithelium and stroma of 26 PanIN-1 samples and 19 IPMN-adenomas.

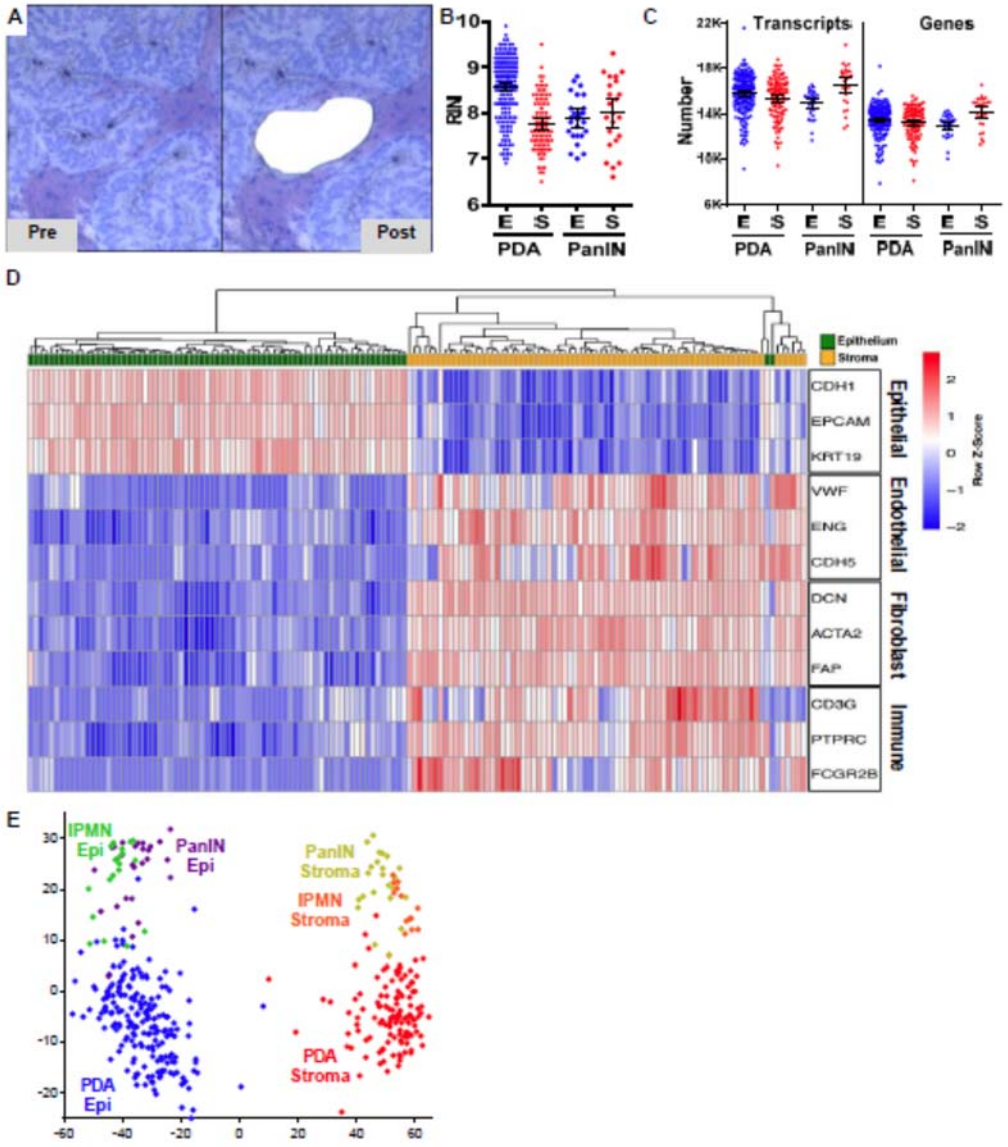


Figure 3. Preliminary Data. (N = 203 PDA Epithelium, 99 PDA Stroma, 26 PanIN-1a Epithelium, and 21 PanIN-1a Stroma). **A)** Cresyl violet stained section of human PDA before and after LCM to isolate epithelial cells. **B)** RNA Integrity (RIN) values from all LCM-RNA-Seq samples showing excellent RNA quality. **C)** ~14,000 genes and ~16,000 transcripts were detected per sample. **D)** Heatmap of normalized expression of selected epithelial and

stromal marker genes shows near perfect distinction of microdissected samples. **E)** Principal Component Analysis (PCA) performed on all LCM-RNA-seq datasets spontaneously distinguishes sample types.

How OncoTreat works:
OncoTreat is a computational pipeline that begins with a tumor expression profile and ends with a list of several candidate treatment regimens. Critically, the OncoTreat assay has now received New York State CLIA certification and is offered through Columbia University Irving Medical Center Pathology's Personalized Genomics Laboratory. The assay is highly reproducible; indeed, flash-frozen and paraffin embedded tissue produce virtually identical results.

OncoTreat is based on two conceptual breakthroughs. The first is to focus on the activity of regulatory proteins rather than DNA mutations. Regulatory proteins are proteins such as transcription factors, cofactors, and chromatin modifiers that have a strong effect on the transcript abundance of other genes. If one knows all of the target genes activated and repressed by a regulatory protein, then its ACTIVITY may be inferred as a function of the expression of that group of targets; higher activity elevates the expression of activated targets while decreasing the expression of repressed targets (**Figure 4**).

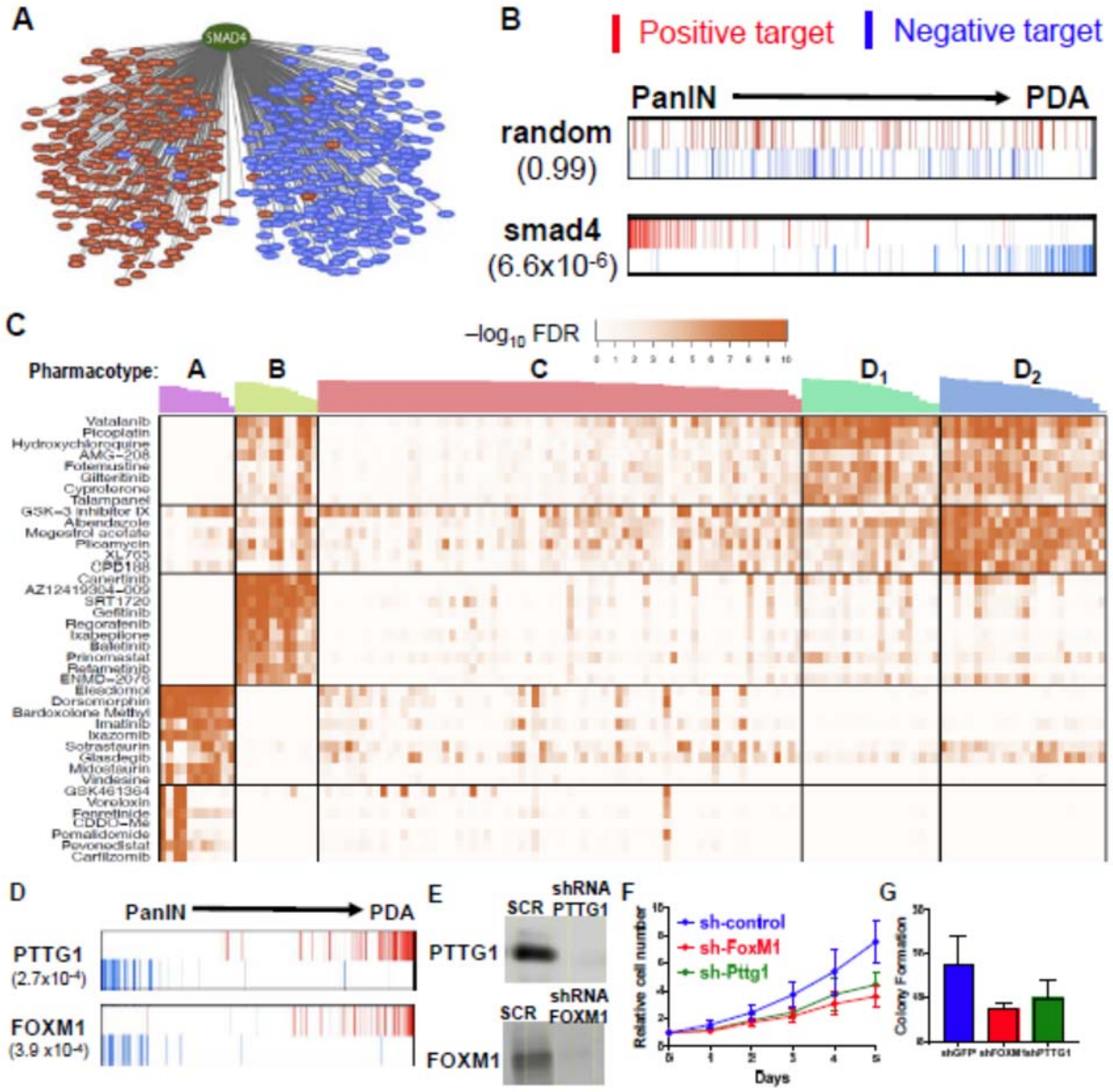


Figure 4. Preliminary Master Regulator and OncoTreat Results. A) The regulon for Smad4 inferred from the PDA epithelial regulatory network included over 200 positive and negative target genes. **B)** Master Regulator analysis found Smad to be the third-most repressed Master Regulator between PanIN and PDA epithelial samples, consistent with the high frequency of Smad4 mutations in PDA patients. Ranked differential gene expression for each target gene is represented as the distribution of positive (red) and negative (blue) lines along a row. Shown are distributions for a random gene that is not differentially activated between PanIN-1 vs. PDA as well as Smad4, in which negative targets are more highly expressed in PDA and positive targets are more highly expressed in PanIN-1. False discovery rate shown in parentheses. **C)** PLATE-Seq was performed on Aspc1 cells treated with 336 different FDA-approved or late clinical trial agents, and VIPER used to determine their effect on the activities of the top 50 MRs for each of 137 PDA patients from TCGA. Color indicates the extent of inhibition of patient-specific MR activity by each agent, with darker orange indicating greater inhibition. Five clusters of patients were observed, but two of them are merged because they share many predicted sensitivities (D_1/D_2). **D)** Validation of individual MRs. We identified foxm1 and pttg1 as epithelial MRs associated with PanIN to PDA progression. E) Western blots show effective shRNA knockdown of PTTG1 and FOXM1 with the pTRIPZ vector in Aspc1 cells,

leading to reduced proliferation and F) reduced colony formation G) relative to a scrambled control following induction with doxycycline. Please note that this depicts the effect of altering expression of just two MRs. The effect of inverting the activity of the entire Top50 MRs for a patient is expected to be much greater.

The second conceptual breakthrough is that the activity of a drug can also be defined by its impact on the activity of regulatory proteins. This provides several advantages. First one doesn't need to restrict precision medicine efforts to drugs with one specific target protein. Multi-targeted agents, agents with unknown targets, off-target effects, even cytotoxic agents, can all be profiled for their effects on MRs. Thus, OncoTreat functions by matching the profile of regulatory proteins active in a patient's tumor with the drugs that reverse the activity of those same regulatory proteins. This is achieved through the following steps:

Step 1: Construct a "regulatory network" – a map of transcriptional relationships – for PDAC (**Figure 2**). A regulatory network is a global list of target genes for all of the regulatory proteins (i.e. transcription factors, cofactors, and other proteins whose activity closely alters transcript abundance) expressed in pancreatic cancer. Networks are derived de novo using ARACNe, an information theoretic algorithm developed by the Califano group, from large collections of expression profiles, and do NOT rely on prior knowledge of signaling pathways.

Step 2: Identify patient-specific MRs. Using the information from the PDAC regulatory network, it is possible to calculate the activity of each MR in patient's RNA-seq expression profile. The VIPER algorithm compares the activity of each regulatory protein to a reference signature representing non-pancreatic lineages. The idea is that the pancreatic lineage is part of what fundamentally defines pancreatic cancer. The top 50 MRs for each patient are integrated as the "target" of subsequent drug screening steps.

Step 3. Determine the impact of drugs on each MR. We next perform in vitro screening to determine how different drugs affect the activity of every regulatory protein in the genome. Using a high-throughput RNA-Seq assay developed at Columbia called PLATE-SEQ, we screened a set of drugs (121 FDA-approved drugs and 228 Phase 2 and 3 investigational oncology agnets) for their effects on regulatory protein activity in Aspc1 cells (selected as the best match to the majority of patients in our cohort). These data may now be mined repeatedly to find agents for each patient.

Step 4. Candidate drug selection. By integrating the above datasets, we can identify drugs that shut down the activity of the specific MRs active in an individual patient's profile. This analysis is integrated across the top 50 MRs for each patient. Successful matches are those drugs that reverse the activity of many or all of these top 50 MRs.

We have performed a preliminary analysis of the OncoTreat methodology on existing pancreatic cancer tumor profiles and found at least one matching drug for 85% of patients (using a conservative threshold FDR of 1×10^{-5}). Moreover, at least 50% of patients have 5 or more matched drugs using the OncoTreat approach, providing an opportunity to screen multiple candidate agents for a given patient using patient-derived models such as PDXs and organoids.

3.5 Rationale for this Clinical Study

There are a number of clinical trials that focus on a drug and then select the rare patient whose tumors harbor a matching alteration. By contrast, we propose a prospective precision medicine trial that enrolls advanced PDA patients and then matches them to one of several hundred FDA-approved or investigational agents. We will assess the feasibility of applying the OncoTreat algorithm to help inform treatment decisions in subjects with recurrent locally advanced, unresectable or metastastic PDA. We hypothesize that OncoTreat will be effective in identifying the master regulators on a tumor-by-tumor basis and determining novel treatments for these patients where limited options exist.

Beyond initial cytotoxic chemotherapy, there is only one FDA-approved treatment regimen (5-fluoruracil in combination with nanoliposomal irinotecan) in the second-line setting. The drug sensitivity profile (meeting a specified statistical threshold) generated by the OncoTreat analysis, performed on tissue obtained from Protocol Part 1 (AAAR6703), will be reviewed at the Precision Medicine Tumor Board (PMTB). If an OncoTreat-priotized FDA-approved or early investigational drug is identified, subjects will consent to be treated on this protocol with the OncoTreat-prioritized drug as their next treatment. A pre study treatment biopsy will be obtained to confirm the prior OncoTreat analysis. Standard response criteria measurements per RECIST v1.1 will be applied, and an optional biopsy during the first month of treatment on the OncoTreat-prioritized drug will be performed.

Unfortunately, not all OncoTreat-prioritized drugs will be available to the patient, but a good faith effort will be made to obtain drugs either through funding from the Lustgarten Foundation, insurance coverage for off-label indications, pharmaceutical patient assistance programs, or single-patient IND.

3.6 Rationale for Testing Capecitabine

Capecitabine, both as a single agent and in combination with other chemotherapeutic agents, has been evaluated in pancreatic cancer patients. A phase II trial of single-agent capecitabine was evaluated in 42 patients with advanced or metastatic treatment-naive pancreatic cancer [32]. Capecitabine was dosed at 2500 mg/m^2 per day in 2 divided doses on the first 2 weeks of each 3-week cycle. The primary endpoint was response rate. A partial response was seen in 3 of 42 patients, and stable disease was achieved in 41%. Median survival was 182 days. With response to clinical benefit, positive responses were seen in pain intensity for 29% and analgesic usage in 12%. Overall the clinical benefit response rate was 24%. Adverse effects were predominantly gastrointestinal events (nausea, diarrhea, and vomiting) and hand-foot syndrome, with 2 patients experiencing grade 4 diarrhea.

Wasif Saif et al reported on two gemcitabine-refractory pancreatic cancer patients who derived long-term survival on capecitabine (50 months and 24 months, respectively) [33].

This precision medicine trial will use OncoTreat analysis and PDX/PDO models to select the patient predicted to respond to capecitabine.

3.7 Rationale for Testing Imatinib

Imatinib is a potent and selective inhibitor of the protein tyrosine kinase Bcr-Abl, platelet-derived growth factor receptors (PDGFRα and PDGFRβ) and KIT. Pancreatic adenocarcinoma expresses PDGFRα. Use of imatinib in xenograft models resulted in transient and reversible reduction in tyrosine phosphorylation of PDGFRα without impacting the level of expression (PMID 25985771).

Given its activity against both c-KIT and PDGFR kinases and its remarkable safety profile, imatinib has been tried in several solid tumors. The results, however, have often been deceiving.

In a small study of 26 patients with unresectable pancreatic cancer, patients were randomized to either gemcitabine 1000 mg/m2 weekly or imatinib 800 mg daily [34]. Expression of KIT and PDGFRβ were determined by immunohistochemistry. No objective responses were seen in either roup. Median time to progression was 77 and 29 days (P=0.411) and median survival time was 140 and 160 days (P=0.517) for gemcitabine and imatinib, respectively. Survival and treatment responses were independent of KIT and PDGFRβ expression in patients treated with imatinib. Grade 3/4 toxicities of imatinib were anemia, elevated liver enzymes, vomiting, and dyspnea. Patients treated with imatinib also reported more frequent diarrhea and/or altered bowel function. Quality of life was similar in both groups.

While imatinib has not demonstrated efficacy in advanced pancreatic cancer, we will utilize OncoTreat analysis and PDX/PDO models as a novel precision medicine approach to select the patient(s) predicted to respond to imatinib.

3.8 Rationale for Testing Ruxolitinib

Aberrant activation of the Janus-associated kinase (JAK)/signal transducer and activator of transcription (STAT) pathway is associated with increased malignant cell proliferation and survival. Ruxolitinib (Jakafi ®) is a potent, orally bioavailable inhibitor of JAK1 and JAK2 kinases in the JAK/STAT pathway. It is approved for the treatment of intermediate-risk or high-risk primary or secondary myelofibrosis, polycythemia vera (in patients with inadequate response or intolerance to hydroxyurea), and graft-versus-host disease.

Cytokine-mediated signaling via the JAK/STAT pathway is central to tumor growth, survival, and systemic inflammation, which is associated with cancer cachexia, particularly in pancreatic cancer. Preclinical studies have demonstrated antiproliferative effects of JAK/STAT pathway inhibition in both in vitro and in vivo models of cancer, including pancreatic cancer. In genetically engineered murine models of pancreatic cancer, ruxolitinib inhibited tumor angiogenesis, controlled disease progression, and improved survival [35]. Ruxolitinib has also been shown to block tumor growth in a syngeneic murine PAN02 pancreatic model (Koblish HK et al., *Cancer Res*, Abstract 1336, 2015). By selectively inhibiting JAK2V617F, STAT5, and ERK1/2 phosphorylation, ruxolitinib was shown to reduce cellular proliferation and induce apoptosis in JAK2V617F^{+} Ba/F3 cells [36].

Pancreatic cancer results in a systemic inflammatory response which is partially mediated by the JAK/STAT pathway [37]. This systemic inflammatory response has been associated with a more

pronounced symptom burden, including cachexia syndrome, and poorer survival outcomes [38, 39]. The rationale for evaluating the therapeutic utility of JAK inhibition in patients with pancreatic cancer has strong scientific merit.

The effectiveness of ruxolitinib in patients with metastatic pancreatic cancer was evaluated in the phase II RECAP trial that included 127 patients who had previously been treated with gemcitabine [40]. Patients were randomized (double-blind) 1:1 to ruxolitinib 15 mg twice daily plus capecitabine 1000 mg/m^2 twice daily (n=64) versus capecitabine 1000 mg/m^2 twice daily plus placebo (n=63). The overall survival was 4.5 months in the ruxolitinib group and 4.3 months in the control group – not a significant difference. However, the investigators, in a prespecified subgroup analysis, did detect a significant difference in the 60 patients with high blood levels of C-reactive protein (CRP). The median survival for patients in this subgroup who received ruxolitinib was 2.7 months, and the objective response rate was 6.5%, versus 1.8 months and 3.4% for patients who received a placebo. The most common side effect for patients treated with ruxolitinib was anemia followed by fatigue and abdominal pain.

Based on promising preliminary phase II data, two randomized phase II studies, JANUS I and JANUS 2, were conducted to evaluate ruxolitinib in combination with capecitabine in patients with advanced pancreatic cancer [41]. Previously treated patients with a CRP >10 mg/L were randomized 1:1 to 21-day cycles of ruxolitinib 15 mg twice daily plus capecitabine 2000 mg/m^2 per day (days 1-14) or placebo plus capecitabine. Both studies were terminated following a planned interim futility/efficacy analysis of JANUS 1.

Although phase III results showed no added benefit of ruxolitinib over capecitabine in patients with advanced pancreatic cancer, we will utilize OncoTreat analysis and PDX/PDO models as a novel precision medicine approach to select the patient(s) predicted to respond to ruxolitinib.

4. STUDY DESIGN

This is a single center, non-randomized, open-label phase Ib study conducted at Columbia University Medical Center to assess the safety and feasibility of the OncoTreat framework while also gathering preliminary data on efficacy.

4.1 General Design

(1) Subjects with newly diagnosed, treatment-naïve locally advanced, unresectable or metastatic pancreatic adenocarcinoma will be enrolled on Part 1 (AAAR6703). Written consent will be obtained. Tumor tissue, from either the primary or metastatic site, will be obtained. Tumor tissue acquision, handling, and processing are described in the laboratory manual. The subject will then begin first-line systemic treatment at the treating physician's discretion (standard-of-care chemotherapy or clinical trial).

(2) RNA-Seq and OncoTreat analysis are performed on the pre-treatment biopsy. At least 0.3 cm^3 of fresh frozen tissue is to be submitted (additional tissue will be utilized in the generation of PDX and PDO models, as described below). Prior to performance of NY State CLIA-certified RNA-Seq, the presence of >70% tumor on H&E stained sections will be confirmed by the

Department of Pathology, and the RNA integrity number of extracted RNA will be confirmed as being RIN > 6.

(3) In order to provide preclinical validation data for each subject, we will effectly perform a separate preclinical trial while each subject receives first-line treatment. A the time of initial biopsy, additional tissue material (designated "P0" samples) will be transferred to the Olive laboratory and utilized to generate patient-derived xenografts (PDXs) through orthotopic implantation into the pancreas of NSG mice. If available, additional tissue may be used for the generation of patient-derived organoids (PDOs) in the laboratory of Christine Chio, for tissue banking, and for cryopreservation. For PDX models, the initial samples will be implanted into up to five animals, and engraftment and outgrowth will be monitored weekly by high resolution ultrasound. Subsequent screening will proceed using whichever model expands more quickly.

(4) Upon outgrowth of a PDX model (designated "P1" samples), or alternatively the expansion of a PDO model (designated P1 organoids), samples will be expanded into additional recipient NSG mice to generate "P2 tumors." These P2 tumors will be utilized to evaluate candidate treatments identified through OncoTreat. We will execute the preclinical studies via the "Mouse Hospital" infrastructure with the Olive Laboratory, which includes expertise in small animal imaging, surgery, drug administration, tissue sampling, histopathology, and pharmacology. Details describing the establishment of orthotopic PDX/PDO models, treatment tolerability studies, survival studies, and PK studies will be described in Appendix D. The outcome of this stage will be a data package summarizing the results of different drug treatments for each patient, which will be provided to the Precision Medicine Tumor Board.

(5) The results of these preclinical studies will be evaluated by the Precision Medicine Tumor Board (PMTB), which will include experts in gastrointestinal (GI) medical oncology, GI surgical oncology, radiology, pharmacology, molecular biology, genetics, and computational biology. Additionally, there will be at least four non-Columbia individuals on the PMTB, including two oncologists, one pathologist, and one patient advocate. The PMTB will review all of the data and prioritize a treatment based on the following criteria:

- Patient safety
- Preclinical efficacy
- Pharmacological properties
- Drug availability. Efforts will be made to obtain the selected OncoTreat-prioritized drug through off-label insurance coverage, pharmaceutical patient assistance programs, or single-patient INDs.

NOTE: It is anticipated that for some number of subjects, either their PDX/PDO models will fail to establish or the preclinical studies will not be completed before the patient progresses on second-line therapy. In this scenario, based on numerous studies in hematologic and other solid tumor malignancies using the OncoTreat technology (8-19) and the lack of an established standard third-line therapy for pancreatic cancer, we will calculate which of our established tumor models is the closest match to that patient's tumor and use that as a surrogate instead to turn the preclinical experiments. This data will then be forwarded to the PMTB for review and

consideration. If an OncoTreat-informed drug is identified and treatment is recommended by the PMTB, the subject may proceed with consent for Part 2.

The PMTB will convene at CUMC on a monthly or as needed basis. A quorum of at least 5 members, including the principal investigator, one additional medical oncologist and one non-Columbia individual, must be present for each meeting. Teleconference is permitted if a PMTB member is located off campus.

(5) If the PMTB recommends a FDA-approved or investigational oncology drug, and the drug is available, the subject will consent to Part 2 and begin the study agent at the time of disease progression or intolerance to therapy. A pre-study treatment biopsy will be required to confirm that the MR dependencies of the subject's tumor have not changed over time or in response to first-line therapy (a theoretical concept that needs to be formally assessed).

If no FDA-approved or investigational agents are identified at the time of first disease progression or intolerance to first-line therapy, or the OncoTreat-prioritized drug is not available in a time manner for administration, the subject will continue on to physician's choice second-line treatment (chemotherapy or clinical trial). The subject will be followed for response evaluation. At the time of second disease progression or intolerance to treatment, the subject will be re-evaluated for enrollment on Part 2. If a FDA-approved or investigational agent is identified, and the drug is available, the subject may consent to Part 2 and begin the study agent following a pre-study treatment biopsy. If no FDA-approved or investigational agents are identified, or the OncoTreat-prioritized drug is not available, the subject will screen fail for Part 2 and will be followed for response evaluation.

(6) The dosing and schedule of the OncoTreat-prioritized drug will be described in the subprotocol for each agent. For each of the FDA-approved drugs, the dose and dosing schedule will be aligned with the currently approved dose and schedule in the product U.S. Package Insert (USPI) and Investigator's Brochures (IBs). For the investigational agents, the dose and dosing schedule will be aligned with the current recommend phase 2 dose (R2PD).

(7) Subjects who are initiated on an OncoTreat-prioritized drug will have an optional on-treatment 'pharmacodynamic' biopsy performed within one month of initiating treatment and within 24 hours of the most recent dose. This biopsy will be performed as 'proof-of-principle' that OncoTreat prioritized drugs result in collapse of the master regulator architecture (also known as master regulator signature) of the tumor, *in vivo*.

(8) Peripheral blood will be drawn for routine laboratory testing, tumor markers (CA 19-9), and research testing for biomarker analysis. Pharmacology measurements will also be drawn weekly during Cycle 1 of treatment. Serge Cremers, the Director of Clinical Pharmacology and Toxicology Laboratory (CPTL), will make available CLIA assays for every FDA-approved drug and will provide assays for any investigational agents that match to the subjects in the study. Pharmacokinetic (PK) results and toxicity may be used by the treating physician, after discussion with the Investigator, to modify dosing schedule as appropriate.

(9) Adverse events will be monitored and recorded (Section 8).

(10) Subjects will undergo routine standard-of-care radiographic assessments (CT preferred) at baseline and every 8 weeks with response measured per RECIST v1.1 (Section 10).

(11) Subjects will continue on study treatment until radiographic disease progression or death, intolerance to the study drug, or withdrawal of consent. Patients will not be permitted to continue study treatment after disease progression per RECIST v1.1 criteria (Section 10).

4.2 Number of Patients

Thirty subjects will be enrolled on Part 1 and, therefore, up to a maximum of 30 subjects will receive OncoTreat-prioritized agents on Part 2. No subjects will be replaced.

4.3 Study Drugs

4.3.1 Capecitabine

The cycle length will be 21 days, and 1000 mg/m^2 given orally twice per day, usually separated 12 hours apart, on Days 1-14 of a 21-day cycle. Capecitabine should be taken with water within 30 minutes after a meal.

4.3.2 Imatinib

The cycle length will be 28 days. Patients will receive imatinib 400 mg orally once daily. A dose increase up to 800 mg daily (given as 400 mg twice daily) may be considered by the investigator, as clinically indicated, in patients showing clear signs or symptoms of disease progression at a lower dose and in the absence of severe adverse drug reactions.

4.3.3 Ruxolitinib

The cycle length will be 28 days. Patients will receive ruxolitinib 15 mg orally twice per day. A dose increase up to a maximum of 25 mg twice daily may be considered by the investigator, as clinically indicated, in patients showing clear signs or symptoms of disease progression at a lower dose and/or in the absence of severe adverse drug reactions.

4.4 Tumor Biopsies

A tissue sample, from either the primary or metastatic site, will be obtained from all treatment-naïve subjects at the time of initial presentation. This biopsy, therefore, will serve both diagnostic and research purposes prior to initiation of first-line chemotherapy. Tumor tissue acquision, handling, and processing are described in the laboratory manual.

Subjects for whom an OncoTreat-prioritized agent is identified will undergo a repeat biopsy prior to starting the study drug. This will confirm no change in master regulator profile, which is not expected, and that the OncoTreat-prioritized agent identified from the pre-treatment sample is no different than the agent identified now following treatment with chemotherapy.

The window for each biopsy is outlined in the study calendar (Section 9).

4.5 Pharmacology: Pharmacodynamics and Pharmacokinetics

4.5.1 Capecitabine

Capecitabine (N-[1-(5-deoxy-β-D-ribofuranosyl)-5-fluoro-1,2-dihydro-2-oxo-4-pyridinyl]-*n*-pentyl carbamate) is a crystalline substance with a molecular weight of 359.35. It is highly soluble in water and stable in tablet form for at least 9 months [42]. Capecitabine is metabolized to the only active compound, FU, via 3 metabolic steps. Once capecitabine is absorbed through the intestine, liver carboxylesterase converts it to 5'-deoxy-5-fluorocytidine (5'-DFCR). 5'DFCR is then metabolized to 5'DFUR by cytidine deaminase, a ubiquitous enzyme with high concentrations in the liver, plasma, and tumor tissue. Finally, 5'DFUR is converted to the active drug FU by thymidine phosphorylase, found in amounts 3 to 10 times higher in various solid tumors compared with normal adjacent tissue [42-44]. The localization of this enzyme to the liver and tumor tissue allows for targeted intratumor release of FU.

Relative FU concentrations in colorectal tumor tissue, adjacent healthy tissue, and plasma were compared in 19 patients undergoing surgical resection of their primary and/or liver metastasis [44]. Capecitabine 1255 mg/m^2 twice daily given orally within 30 minutes of food was administered 5 to 7 days before surgery. Samples of the aforementioned tissue from each patients were evaluated for concentrations of capecitabine and its metabolites as well as enzymes involved in its metabolism. Activity of thymidine phosphorylase was almost 4 times higher in colorectal tumor compared with healthy tissue. Consequently, concentrations of FU were 3.2 times higher in primary colorectal tumors compared with surrounding healthy tissue ($P = 0.002$). The tumor tissue: plasma ratio for the active drug was 21.4 and normal tissue:plasma ratio was 8.89. This selectivity was not demonstrated between liver metastasis and healthy liver tissue, however, with a thymidine phosphorylase activity ratio close to 1 and FU concentration ratio of 1.41 ($P = 0.49$) [44].

Capecitabine has almost 100% oral bioavailability and exhibits linear increases in C_{max} and AUC with dosage increases [45]. After 2 doses of 1250 mg/m^2, the drug undergoes rapid absorption, with peak plasma levels of 3.9 mg/L achieved in 1.5 to 2 hours. In comparison, C_{max} for the active metabolite FU was lower at 0.66 mg/L with a similar T_{max} of 2 hours. AUC values for the parent drug and active metabolite for the same dosage were 5.96 mg and 1.34 mg h/L, respectively. In a study of 34 patients undergoing 14 continuous days of capecitabine therapy at daily doses ranging from 502 to 3514 mg/m^2, no significant accumulation of capecitabine or its metabolites was seen [45, 46].

A crossover study evaluated the effect of food on capecitabine pharmacokinetics in 11 patients with colorectal cancer [47]. Doses of 666 or 1255 mg/m^2 give twice daily were administered either after an overnight fast or within 30 minutes of finishing a standard breakfast on days 1 and 8. The AUC of capecitabine decreased in the presence of food but the AUC of the cytotoxic drug FU was only minimally affected. Dosing with food is still recommended, as this was how the drug was dosed in clinical trials and because the reported response rate and toxicity profiles reflect the administration of capecitabine within 30 minutes of finishing a meal.

The predominant route of elimination is renal. The use of capecitabine in patients with renal impairment was compared in 24 patients with various solid tumors [48]. Capecitabine was dosed orally at 1250 mg/m2 twice daily for 2 weeks followed by a 1-week rest period. All 4 patients with severe renal dysfunction (defined as CrCl <30 mL/min) experienced serious grade 3 or 4 adverse effects. From these results, it is recommended that patients with CrCl 30 to 50 mL/min receive 75% of the recommended starting dose of capecitabine and that use of this agent be avoided in patients with CrCl <30 mL/min because of the potentially increased risk of adverse events.

No dosing adjustments for patients with hepatic dysfunction are recommended with capecitabine.

4.5.2 **Imatinib**

Pharmacokinetic studies of imatinib in healthy volunteers and patients with CML, GIST, and other cancers show that orally administered imatinib is well-absorbed, and has an absolute bioavailability of 98% irrespective of oral dosage form or dosage strength [49]. Food has no relevant impact on the rate or extent of bioavailability. The terminal elimination half-life is approximately 18 hours. Imatinib plasma concentrations predictably increase by 2- to 3-fold when reaching steady state with 400 mg once daily administration.

Imatinib is approximately 95% bound to human plasma proteins, mainly albumin and α_1-acid glycoprotein [50]. The drug is eliminated predominantly via the bile in the form of metabolites. The fecal to urinary excretion ratio is approximately 5:1 [51].

Imatinib is metabolized in the liver mainly by the cytochrome P450 (CYP) 3A4 or CYP3A5 and can competitively inhibit the metabolism of drugs that are CYP3A4 or CYP3A5 substrates. Interactions may occur between imatinib and inhibitors or induces of these enzymes, leading to changes in the plasma concentration of imatinib as well as coadministered drugs. Major induces of CYP3A4 and CYP3A5 activity may increase metabolism and decrease exposure to imatinib (e.g. carbamazepine, dexamethasone, barbituates, phenytoin, rifampin, and St. John's Wort). Concomintant CYP3A4 and CYP3A5 inhibitors should be administered with caution (e.g. cimetidine, erythromycin, clarithromycin, ketoconazole, itraconazole, cyclosporine, grapefruit juice).

Hepatic and renal dysfunction, and the presence of liver metastases, may result in more variable and increased exposure to the drug, although typically not necessitating dosage adjustment.

4.5.3 **Ruxolitinib**

Ruxolitinib inhibits Janus Associated Kinases (JAKs) JAK1 and JAK2 which mediate the signaling of a number of cytokines and growth factors that are important for hematopoiesis and immune function. JAK signaling involves recruitment of STATs (signal transducers and activators of transcription) to cytokine receptors, activation and subsequent localization of STATs to the nucleus leading to modulation of gene expression.

Ruxolitinib inhibits cytokine induced STAT3 phosphorylation in whole blood from healthy subjects with myelofibrosis patients. Ruxolitinib administration resultsin maximal inhibition of STAT3 phosphorylation 2 hours after dosing which returns to near baseline by 10 hours in both healthy subjects and myelofibrosis patients.

The pharmacokinetics of ruxolitinib was evaluated in healthy volunteers in two double-blind, randomized, placebo-controlled studies [52]. Ruxolitinib is rapidly absorbed after oral administration with maximal plasma concentration (C_{max}) achieved within 1 to 2 hours post-dose. Based on a mass balance study in humans, oral absorption of ruxolitinib is estimated to be at least 95%. Mean ruxolitinib C^{max} and total exposure (AUC) increases proportionally over a single dose range of 5 to 200 mg. There is no clinically relevant changes in the pharmacokinetics of ruxolitinib upon administration with a high-fat meal, with the mean C_{max} moderately decreased (24%) and the mean AUC nearly unchanged (4% increase).

For pharmacodynamics evaluations, investigators have relied on measuring the levels of downstream targets of JAK signaling and assessing the change in inflammatory markers in response to therapy. In the initial phase I/II reported by Verstovsek and colleagues [53], maximal mean inhibition of *p*-STAT-3 expression ranged from ~40% at the lowest dose tested to >90% inhibition at the highest dose tested, and returned to baseline levels by 24 hours. A dose- and time-dependent reduction of phosphorylated STAT-3 was observed with ruxolitinib treatment and was repoted in patients with JAK2V617 and wild-type JAK2. Elevated baseline levels of IL-6, IL-ra, IL-8, MIP-1β, TNF-α, and CRP were all dramatically reduced with ruxolitinib treatment in myelofibrosis patients.

In vitro studies suggest that CYP3A4 is the major enzyme responsible for metabolism of ruxolitinib. Ruxolitinib is the predominant entity in humans representing approximately 60% of the drug-related material in circulation. Two major and active metabolites were identified in plasma of healthy subjects representing 25% and 11% of parent AUC. These two metabolites have one-fifth and one-half of ruxolitini's pharmacological activity, respectively. The sum total of all active metabolites contributes 18% of the overall pharmacodynamics of ruxolitinib.

Following a single oral dose of [^{14}C]-labeled ruxolitinib in healthy adult subjects, elimination was predominantly through metabolism with 74% of radioactivity excreted in urine and 22% excretion via feces. Unchanged drug accounted for less than 1% of the excreted total radioactivity. The mean elimination half-life of ruxolitinib is approximately 3 hours and the mean half-life of ruxolitinib + metabolites is approximately 5.8 hours.

4.6 Rationale for Study Design

4.6.1 Rationale for Capecitabine Dosage and Schedule

The dose of capecitabine approved by the US Food and Drug Administration (FDA) for both metastatic colorectal and breast cancer is 1250 mg/m2 given orally twice per day, usually separated by 12 hours for the first 2 weeks of every 3 week cycle.

The most common dose-limited adverse effects associated with capecitabine monotherapy are hyperbilirubinemia, diarrhea, and hand-footy syndrome. Myelosuppression, fatigue and weakness, abdominal pain, and nausea have also been reported.

Approximately 26% - 65% of breast cancer patients had their doses reduced by at least 20% in the clinical trials [54, 55]. For this reason, many investigators evaluated capecitabine at a lower starting dose of 1000 mg/m^2 twice daily and demonstrated similar efficacy to the approved dose and more favorable side effect profile with an incidence of dose reduction ranging from 16 to 34% in phase II trials [56, 57]. A meta-analysis of phase II/III trials comparing toxicity profiles between 1000 mg/m^2 twice daily (lower dose) and 1250 mg/m^2 twice daily (standard dose) capecitabine in breast cancer found that the 1000 mg/m^2 twice daily had a clinically meaningful and significantly better toxicity profile than the 1250 mg/m^2 twice daily regimen [58].

4.6.2 **Rationale for Imatinib Dosage and Schedule**

Imatinib has been the standard-of-care for patients with CML since 2001. In the original phase I trial investigating imatinib therapy, there was no maximum tolerated dose that was reached. Therefore, researchers chose 400 mg because it was a convenient, safe, and active dose. Cumulative complete response achieved in CML patients after 7 years of imatinib therapy is 87%. At 7 years, the overall survival rate is 86% and 94% if only CML-related deaths are considered. One of the key studies for imatinib is the International Randomized Study of Interferon Versus STI571 (IRIS) study, which was a phase III, randomized, open-label trial that compared the standard-of-care at that time, which was interferon and low-dose cytarabine, with imatinib 400 mg daily in patients who had early chronic phase CML [59]. The study was clearly in favor of imatinib in response rates. The study did not show a survival advantage for imatinib because 90% of patients who were on the interferon and cytarabine arm crossed over to imatinib therapy after a median of 9 months.

The first rationale for higher imatinib doses can be explained by a clear dose-response relationship, which was shown in a phase I study where patients responded more when given higher doses of imatinib [60]. Second, some mutations of BCR-ABL can be overcome by high-dose imatinib. Specific mutations can still be mildly sensitive to imatinib, and in these cases, a dose increase may be effective. Third, studies have shown that the dose of 600 mg imatinib given to CML patients in the accelerated phase was independently associated with significantly better time to transformation and better survival, compared to patients who received imatinib 400 mg [61].

The key study investigating standard dose imatinib versus high dose imatinib is the Tyrosine Kinase Inhibitor Optimization and Selectivity (TOPS) trial, a prospective, open-label, randomized (2:1 ratio) phase III trial that studied the efficacy of imatinib 400 mg versus 800 mg in chronic phase CML patients [62]. In the intent-to-treat population, a significantly higher rate of complete cytogenetic response and major molecular response in the high-dose arm was recorded at 6 months. However, at 12 months, although the response rates were still higher than those of the standard dose arm, the differences were not significant.

In patients with advanced or metastatic GIST, who were treated with imatinib 400 mg once daily, mean plasma tough level (C_{trough}) was higher in patients who responded to treatment. A target threshold of >1,100 ng/mL has been defined [63-65]. These results are similar to results previously found in patients with CML [66-68].

We will use therapeutic drug monitoring (TDM) in this clinical trial with the hope of establishing exposure-response relationship.

4.6.3 **Rationale for Ruxolitinib Dosage and Schedule**

A phase I/II study of ruxolitinib was conducted in JAK2V617F-positive and –negative myelofibrosis patients at ascending doses starting at oral doses with a twice daily schedule of 10 mg, 15 mg, 20 mg, and 50 mg, and once-daily dosing at 25 mg, 50 mg, 100 mg, and 200 mg [53]. Ruxolitinib at 25 mg twice daily or 100 mg once a day was established as the maximum tolerated dose based on the dose-limiting toxicity of reversible thrombocytopenia. At 15 mg twice a day, ruxolitinib treatment was associated with sustained reductions of splenomegaly, resolution of constitutional symptoms, improvement in exercise tolerance and performance status, and meaningful weight gain. Durable improvements in symptoms and splenomegaly were seen in both JAK2-mutated and wild-type patients, and 52% of treated patients had a rapid objective response in splenomegaly >50% reduction for ≥12 months. Marked depression in the heightened expression of proinflammatory cytokines were seen with ruxolitinib treatment and correlated with improvement in night sweats, fevers, fatigue, weight loss, and pruritus. After 12 cycles of therapy, there was a mean maximal suppression of JAK2V617F allele burden by a modest 13% with ruxolitinib treatment.

Ruxolitinib was subsequently approved for the treatment of intermediate- and high-risk patients with myelofibrosis based on two randomized, phase III studies: COMFORT-I [69] and COMFORT-II [70]. In COMFORT-I, starting doses in the patients randomized to ruxolitinib were 15 mg twice daily for patients with baseline platelet counts of 100,000 – 200,000 and 20 mg twice daily for those with platelet counts >200,000. There was no protocol-mandated dose change for anemia. Grade 3/4 anemia was the most frequent hematolgic adverse event and was observed in 45% and 19.2% of patients in the ruxolitinib and placebo arms, respectively. Grade 3/4 neutropenia was observed in 7.1% and 2% of patients in the ruxolitinib and placebo arms, respectively. The most common nonhematologic adverse event seen with any grade in the ruxolitinib-treated group was diarrhea (23.2% compared with 21.2% in the placebo group). Overall, ruxolitinib was a well-tolerated drug.

Mesa et al. (Blood 2013) found that approximately 70% of patients who received ruxolitinib in COMFORT-I had a dose adjustment within the first 12 weeks of initiating ruxolitinib treatment. At week 24, 77% of patients with baseline platelet counts of 100,000 – 200,000 and 39% of those with baseline platelet counts >200,000 had titrated to a reduced ruxolitinib dose relative to their starting dose. With longer-term follow-up (median 149 weeks), the mean dose over time in patients who continued on study was ~10 mg twice daily for patients starting at 15 mg twice daily and ~15 mg twice daily for those starting at 20 mg twice daily.

In both COMFORT studies, ruxolitinib demonstrated marked and sustained clinical benefits in spleen size and improvement in symptom burden, and was generally well tolerated. Anemia and thrombocytopenia were the most frequent hematologic adverse events. The recommended starting dose of ruxolitinib is based on the platelet count (https://www.accessdata.fda.gov/drugsatfda_docs/label/2011/202192lbl.pdf).

4.7 Formulation, Packing, Handling, and Administration

4.7.1 Capecitabine

Capecitabine tablets should be swallowed whole with water within 30 minutes after a meal.

Capecitabine is supplied as biconvex, oblong film-coated tablets for oral administration. Each light peach-colored tablet contains 150 mg of capecitabine and each peach-colored tablet contains 500 mg of capecitabine.

Capecitabine should be stored at 25°C (77°F). Excursions permitted to 15°C to 30°C (59°F to 86°F). Keep tightly closed.

Care should be exercised in the handling of capecitabine. Capecitabine tablets should be cut or crushed. Procedures for the proper handling and disposal of anticancer drugs should be considered. Any unused product should be disposed of in accordance with local requirements, or drug take back programs.

4.7.2 Imatinib

Imatinib should be taken with a meal and a large glass of water. Imatinib tablets should not be crushed. Direct contact of crushed tablets with the skin or mucous membranes should be avoided. If such contact occurs, wash thoroughly as outlined in the package insert.

If a dose is missed, patients should be advised to take their dose as soon as possible unless it is almost time for their next dose in which case the missed dose should not be taken. A double dose should not be taken to make up for any missed dose.

Imatinib can be dissolved in water or apple juice for patients having difficulty swallowing. The required number of tablets should be placed in the appropriate volume of beverage (approximatel 50 mL for a 100 mg tablet and 200 mL for a 400 mg tablet) and stirred with a spoon. The suspension should be administered immediately afer complete disintegration of the tablet(s).

Tablets come in 100 mg very dark yellow to brownish orange, film-coated tablets, round, biconvex with bevelled edges as well as 400 mg tablets with very dark yellow to brownish orange, film-coated tablets, ovaloid, biconvex with bevelled edges.

Imatinib should be stored at 25°C (77°F). Excursions permitted to 15°C to 30°C (59°F to 86°F). Protect from moisture. Dispense in a tight container.

Procedures for the proper handling and disposal of anticancer drugs should be considered.

4.7.3 **Ruxolitinib**

Ruxolitinib is dose orally and can be administered with or without food.

If a dose is missed, the patient should not take an additional dose, but should take the next usual prescribed dose.

Ruxolitinib phosphate is a kinase inhibitor. It is a white to off-white to light pink powder and is soluble in aqueous buffers across a pH range of 1 to 8.

Each ruxolitinib tablet contains ruxolitinib phosphate equivalent to 5 mg, 10 mg, 15 mg, 20 mg, and 25 mg of ruxolitinib free base together with microcrystalline cellulose, lactose monohydrate, magnesium stearate, colloidal silicon dioxide, sodium starch glycolate, povidone and hydroxypropyl cellulose.

Dosage Forms and Strengths
5 mg tablets – round and white with "INCY" on one side and "5" on the other
10 mg tablets – round and white with "INCY" on one side and "10" on the other
15 mg tablets – oval and white with "INCY" on one side and "15" on the other
20 mg tablets – capsule-shaped and white with "INCY" on one side and "20" on the other
25 mg tablets – oval and white with "INCY" on one side and "25" on the other

Ruxolitinib should be stored at room temperature 20°C to 25°C (68°F to 77°F); excursions permitted between 15°C and 30°C (59°F and 86°F).

4.8 Pharmacology Assessments

Serum concentration and pharmacokinetic (PK) parameters of OncoTreat-prioritized agents undergoing trial in subjects with advanced pancreatic cancer are going to be measured in the Clinical Pharmacology and Toxicology Laboratory (CPTL), Department of Pathology and Cell Biology, Columbia University Irving Medical Center (CUIMC). CPTL is a New York State CLIA-certified lab dedicated mostly to assay development for drugs in different biological specimens using advanced technology such as ultra-performance liquid chromatography tandem mass spectrometry (LC-MS/MS). In support of OncoTreat, CPTL is going to develop and offer assays for those FDA-approved drugs as well as investigational agents that are prescribed based on this methology. Assays will be developed using LC-MS/MS and target ranges for the Therapeutic Drug Monitoring (TDM) of these various compounds will be derived from the literature. The CPTL has already developed an LC-MS/MS method for the simultaneous quantification of capecitabine, imatinib, and ruxolitinib (see complete details of methods in Appendix 17.5):

Table 1: LC-MS/MS assay method overview

	MRM	RT, min
Imatinib	494.121>217.019*	2.31
	494.121>934.045	

IS: 13CD3-Imatinib	498.195>394.091*	2.31
	498.195>221.044	
Ruxolitinib	307.127>186.228	2.65
	307.127>131.73	
IS: Ruxolitinib-D9	316.133>186.228	2.61
	316.133>131.73	
Capecitabine	360.181>244.036	2.96
	360.181>173.994	
IS: Ruxolitinib-D9	316.133>186.228	2.61
	316.133>131.73	
*Transition used for quantification		

4.8.1 **Capecitabine**

Capecitabine is the oncology pro-drug of 5-fluorouracil (5FU). Capecitabine is administered orally and undergoes mainly hepatic metabolism where it is first biotransformed into 5'-deoxy-5-fluorocytidine (5'-DFCR) by carboxylesterases and subsequently to 5'-deoxy-5-fluorouridine (5'-DFUR) by cytidine deaminase. Metabolism of capecitabine involves different enxymes, where thymidine phosphorylase converts 5'-DFUR to the active drug 5FU [71].

Concentration levels of capecitabine and both 5'-DFCR and 5'-DFUR are measured in PK studies [72]. Several PK studies were assessed previously in serum for capecitabine, where t_{max} ranged from 0.5 to 2 hours, with a C_{max} of 5000-9500 ng/mL and $t_{1/2}$ of 34-50 min. After the administration of a standard dose of capecitabine, it might be expected serum concentrations within 5-10,000 ng/mL.

Dosage: Capecitabine 1000 mg/m^2 given orally twice daily, separated 12 hours apart, on Days 1-14 of a 21-day cycle, to be taken with water within 30 minutes after a meal.

Sampling: Based on a 21-day cycle, as mentioned above, peak levels will be measured 2 hours after administration of capecitabine on day 1 and cycle 1 day 8.

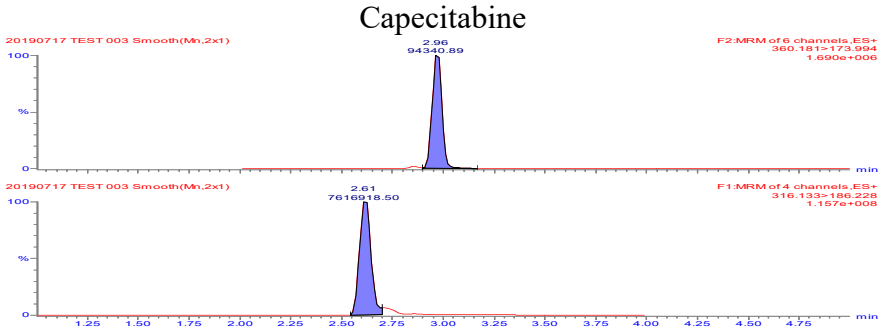
Sample Handling: Peripheral blood will be collected in a tube containing 500 nM tetrahydrouridine (THU) to avoid *ex vivo* conversion of 5'-DFCR to 5'-DFUR [73] and will be allowed to clot for 30-60 min followed by centrifugation at 3000 rpm, 4°C, for 10 min.

Sample Storage: Serum samples will be stored at -80°C.

Extraction: Capecitabine will be extracted from the serum samples using the above mentioned protein precipitation extraction procedure. Minimum volume of blood sample considering duplicates for TDM (and PK) studies should be no less than 300 µL (120 µL is needed for one extraction).

Quantification: Concentration of capecitabine will be measured by a New York State Department of Health (and thereby CLIA-) approved LC-MS/MS assay developed for the

simultaneous quantification of OncoTreat-prioritized drugs. The method is based on an existing New York State DOH-approved assay for imatinib developed by CPTL for its clinical services, with only minor modifications.



Target Range: The target ranges for peak levels will be 5,000 – 10,000 ng/mL for capecitabine in serum.

4.8.2 **Imatinib**

Imatinib is an oral tyroskine kinase inhibitor (TKI) used for the treatment of gastrointestinal stromal tumor (GIST), chronic myeloid leukemia (CML) and Philadelphia positive (Ph+) acute lymphocytic leukemia (ALL). Imatinib is administrated as imatinib mesylate, which is rapidly converted by non specific esterases into imatinib. Imatinib undergoes CYP-mediated metabolism with CYP3A4 and CYP3A5 being the major enzymes involved. N-demethylimatinib is the predominant metabolite and it was observed with pharmacological activity similar to that for the parent drug [74]. Imatinib has a $t_{1/2}$ of approximately 20 hours, and a t_{max} of 2-4 hours with a C_{max} around 3,200 ng/mL.

Dosage: Imatinib 400 mg daily. A dose increase up to 800 mg daily (given as 400 mg twice daily) may be considered by the investigator, as clinically indicated, in patients showing clear signs or symptoms of disease progression at a lower dose and in the absence of severe adverse drug reactions.

Sampling: Trough levels of imatinib will be measured on day 2 at 24 ± 3 hours post-dose [67, 75].

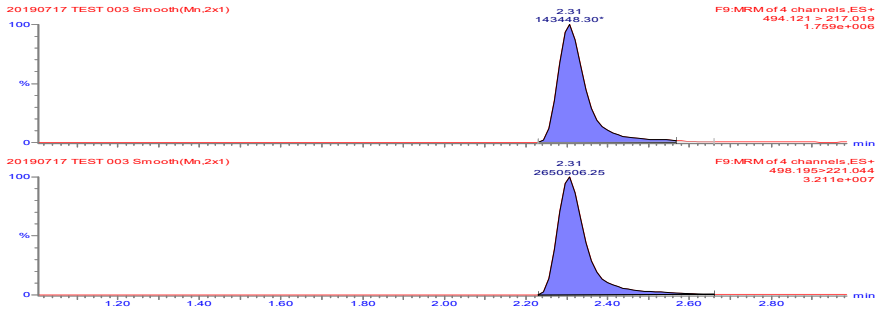
Sample Handling: Peripheral blood will be collected in a tube and will be allowed to clot for 30-60 min following by centrifugation at 3000 rpm, 4°C, for 10 min.

Sample Storage: Serum samples will be stored at -80°C.

Extraction: Imatinib will be extracted from the serum samples using the above mentioned protein precipitation extraction procedure. Minimum volume of blood sample considering duplicates for TDM (and PK) studies should be no less than 300 µL (120 µL is needed for one extraction).

Quantification: Concentration of imatinib will be measured by a New York State Department of Health (and thereby CLIA-) approved LC-MS/MS assay developed for the simultaneous quantification of OncoTreat-prioritized drugs. The method is based on an existing New York State DOH-approved assay for imatinib developed by CPTL for its clinical services, with only minor modifications.

Imatinib



Target Range: The target range for imatinib trough levels will be the same as currently used clinically and is 1,000 – 3,200 ng/mL in serum.

4.8.3 **Ruxolitinib**

Ruxolitinib inhibits the Janus-associated kinase-1 and -2 (JAK 1/2) enzymes and is FDA-approved for the treatment of myelofibrosis, polycythemia vera, and graft-versus-host disease. CYP3A4 is the major enzyme mediating hydroxylation of ruxolitinib. Different formulations are available for this drug from 5 mg to 20 mg tablets. Ruxolitinib has a $t_{1/2}$ of 2.6 – 5 hours, a t_{max} of 1 – 3 hours, and reaches a C_{max} of 60 or 570 ng/mL for doses of 5 mg or 50 mg, respectively [76, 77].

Dosage: Ruxolitinib 15 mg BID given orally twice per day. A dose increase up to 25 mg BID may be considered by the investigator, as clinically indicated, in patients showing clear signs or symptoms of disease progression at a lower dose and in the absence of severe adverse drug reactions.

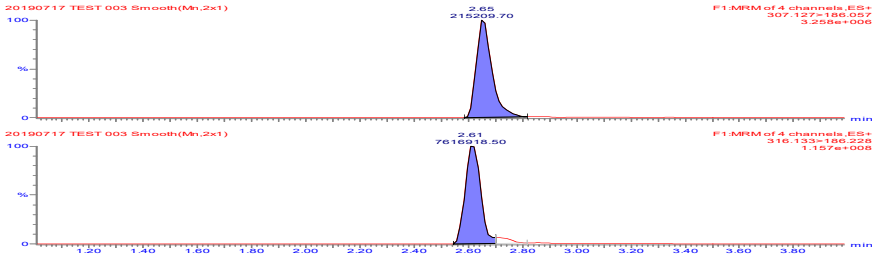
Sampling: Trough levels of ruxolitinib will be measured on day 2 at 24 ± 3 hours post-dose. On day 2, the trough level is expected at a concentration around 3 ng/mL [77].

Sample Handing: Peripheral blood will be collected in a tube and will be allowed to clot for 30 - 60 min following by centrifugation at 3000 rpm, 4°C, for 10 min.

Extraction: Ruxolitinib will be extracted from the serum samples using the above mentioned protein precipitation extraction procedure. Minimum volume of blood sample considering duplicates for TDM (and PK) studies should be no less than 300 µL (120 µL is needed for one extraction).

Quantification: Concentration of ruxolitinib will be measured by a New York State Department of Health (and thereby CLIA-) approved LC-MS/MS assay developed for the simultaneous quantification of OncoTreat-prioritized drugs. The method is based on an existing New York State DOH-approved assay for imatinib developed by CPTL for its clinical services, with only minor modifications.

Ruxolitinib



Target Range: The target range for ruxolitinib trough concentration in serum will be 15 - 60 ng/mL.

4.9 Dose Modifications

4.9.1 General Notes Regarding Dose Modification

Reasons for dose modification or delays, the supportive measures taken, and the outcomes will be documented in the CRF. The severity of adverse events will be graded according to the NCI CTCAE v5.0 grading system.

- For any concomintant conditions already apparent at baseline, the dose modifications will apply according to the corresponding shift in toxicity grade, if the investigator feels it is appropriate. For example, if a patient has Grade 1 asthenia at baseline that increases to Grade 2 during treatment, this will be considered a shift of one grade and treated as Grade 1 toxicity for dose-modification purposes.
- When several toxicities with different grades of severity occur at the same time, the dose modifications should be according to the highest grade observed.

4.9.2 Guidelines for Management of Adverse Events with Capecitabine

Table 2 Recommended dose modifications for capecitabine:

Toxicity (NCI Grade)	During a Course of Therapy	Dose Adjustment for Next Treatment (% of starting dose)
Grade 1	*Maintain dose level*	*Maintain dose level*
Grade 2		
1st appearance	*Interrupt until resolved to grade 0-1*	*100%*
2nd appearance		*75%*
3rd appearance		*50%*
4th appearance	*Discontinue treatment permanently*	-
Grade 3		

1st appearance	*Interrupt until resolved to grade 0-1*	75%
2nd appearance		50%
3rd appearance	*Discontinue treatment permanently*	-
Grade 4		
1st appearance	*Discontinue permanently OR If investigator deems it to be in the patient's best interest to continue, interrupt until resolved to grade 0-1*	50%

4.9.3 **Guidelines for Management of Adverse Events with Imatinib**

4.9.3.1 Dose Adjustments for Hepatotoxicity and Non-Hematologic Adverse Reactions

- If elevations in bilirubin >3 X institutional ULN or in liver transaminases >5X institutional ULN, imatinib should be withheld until bilirubin levels have returned to a <1.5 X institutional ULN and transaminase levels to <2.5 X institutional ULN. Treatment may then be continued at a reduced dose (i.e. 400 mg to 300 mg or 800 mg to 600 mg).
- If a severe non-hematologic adverse reaction develops (such as severe hepatotoxicity or severe fluid retention), imatinib should be withheld until the event has resolved. Thereafter, treatment can be resumed as appropriate depending on the initial severity of the event.

4.9.3.2 Dose Adjustment for Hematologic Adverse Reactions

Dose reduction or treatment interruptions for severe neutropenia and thrombocytopenia are recommended if ANC <1,000 and/or platelets <50,000. First, stop imatinib until ANC ≥1,500 and platelets ≥75,000. Next, resume treatment with imatinib at the original starting dose of 400 mg or 600 mg (including if previously taking 800 mg). If the recurrence of ANC <1,000 and/or platelets <50,000, repeat step 1 and resume imatinib at a reduced dose (300 mg if starting dose was 400 mg, 400 mg if starting dose was 600 mg or 800 mg).

4.9.3.3 Dose Modifications for Concomitant Strong CYP3A4 Inhibitors

The use of concomitant strong CYP3A4 inducers should be avoided (e.g., dexamethasone, phenytoin, carbamazepine, rifampin, rifabutin, rifampicin, phenobarbital). If patients must be co-administered a strong CYP3A4 inducer, based on pharmacokinetic studies, the dosage of imatinib should be increased by at least 50%, and clinical response should be carefully monitored.

4.9.4 **Guidelines for Management of Adverse Events with Ruxolitinib**

4.9.4.1 Dose Modification Guidelines for Thrombocytopenia

Treatment Interruption: Interrupt treatment for platelets less than 50,000. After recovery of platelet counts above this level, dosing may be restarted or increased following recovery of platelet counts to acceptable levels. Table 3 illustrates the maximum allowable dose that may be used in restarting ruxolitinib after a previous discontinuation.

Table 3: Maximum Restarting Doses for Ruxolitinib After Safety Interruption

Current Platelet Count	**Maximum Dose When Restarting Ruxolitib Treatment***
Greater than or equal to 125,000	20 mg twice daily
100,000 to less than 125,000	15 mg twice daily
75,000 to less than 100,000	10 mg twice daily for at least 2 weeks; if stable, may increase to 15 mg twice daily
50,000 to less than 75,000	5 mg twice daily for at least 2 weeks; if stable, may increase to 10 mg twice daily
Less than 50,000	Continue hold
*Maximum doses are displayed. When restarting, begin with a dose at least 5 mg twice daily below the dose at interruption.	

Dose Reductions: Dose reductions should be considered if the platelet counts decrease as outlined in Table 4 with the goal of avoiding dose interruptions for thrombocytopenia.

Table 4: Dosing Recommendations for Thrombocytopenia

	Dose at Time of Platelet Decline				
Platelet Count	**25 mg twice daily**	**20 mg twice daily**	**15 mg twice daily**	**10 mg twice daily**	**5 mg twice daily**
	New Dose	**New Dose**	**New Dose**	**New Dose**	**New Dose**
100,000 to less than 125,000	20 mg twice daily	15 mg twice daily	No change	No change	No change
75,000 to less than 100,000	10 mg twice daily	10 mg twice daily	10 mg twice daily	No change	No change
50,000 to less than 75,000	5 mg twice daily	5 mg twice daily	5 mg twice daily	5 mg twice daily	No change
Less than 50,000	Hold	Hold	Hold	Hold	Hold

4.9.4.2 Dose Modification Based on Response

If efficacy is considered insufficient and platelet and neutrophil counts are adequate, doses may be increased in 5 mg twice daily increments to a maximum of 25 mg twice daily. Doses should not be increased during the first 4 weeks of therapy and not more frequently than every 2 weeks.

4.9.4.3 Dose Adjustment with Concomitant Strong CYP3A4 Inhibitors

On the basis of pharmacokinetic studies in healthy volunteers, when administering ruxolitinib with strong CYP3A4 inhibitors (such as but not limited to boceprevir, clarithromycin, conivaptan, grapefruit juice, indinavir, itraconazole, ketoconazole, lopinavir/ritonavir, mibefradil, nefazodone, nelfinavir, posaconazole, ritonavir, saquinavir, telaprevir, telithromycin, voriconazole), the recommended starting dose is 10 mg twice daily for patients with a platelet count greater than or equal to 100,000. Additional dose modifications should be made with careful monitoring of safety and efficacy.

5. SUBJECT SELECTION AND WITHDRAWAL

Male and female subjects with locally advanced, unresectable or metastatic pancreatic ductal adenocarcinoma after progression on first- or second-line systemic therapy who are considered incurable will be enrolled on this trial.

5.1 Inclusion Criteria

In order to be eligible for participation in this trial, the subject must:

- Be willing and able to provide written informed consent for the trial.
- Age ≥18 years of age on day of signing informed consent.
- Have histologically or cytologically confirmed diagnosis of pancreatic ductal adenocarcinoma.
- Have a predicted life expectancy of greater than 3 months.
- Have measurable disease based on RECIST v1.1.
- Have a performance status of 0 or 1 using the ECOG Performance Scale
- Have documented radiographic or clinical progression to or documented intolerance to 5-FU- or gemcitabine-based systemic therapy for locally advanced, unresectable or metastastic disease. Patients for whom targeted therapy.
- MSI-H/dMMR or NTRK-fusion positive tumors –
    - Subjects must have received prior treatment with approved drugs for tumors harboring these aberrations.
- Have a negative urine or serum pregnancy test within 72 hours prior to receiving the first dose of study medication (female subjects of childbearing potential). If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.
- Be willing to use an adequate method of contraception for the course of the study through 120 days after the last dose of study medication (male and female subjects of childbearing potential).
- Demonstrate adequate organ function as defined below in Table 5.

Table 5 Adequate Organ Function Laboratory Values

System	Laboratory Value

Hematological	
Leukocytes	≥2,000 /mcL
Absolute neutrophil count (ANC)	≥1,500 /mcL
Platelets	≥100,000 /mcL
Hemoglobin	≥9 g/dL without transfusion or EPO dependency within 7 days
Renal	
Creatinine **OR** Measured or calculated[a] creatinine clearance (GFR can also be used in place of creatinine or CrCl)	≤1.5 X upper limit of normal (ULN) **OR** ≥60 mL/min for subject with creatinine levels >1.5 X institutional ULN
Hepatic	
Total bilirubin	≤2 mg/dL or direct bilirubin ≤ ULN for those with total bilirubin >2 X ULN Subjects with Gilbert Syndrome will be eligible if total bilirubin is <3.0 mg/dL
AST (SGOT) and ALT (SGPT)	≤2.5 X ULN **OR** ≤5 X ULN for subjects with liver metastases
Albumin	>3.0 mg/dL
Coagulation	
INR or PT aPTT	≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or aPTT is within therapeutic range of intended use of anticoagulants

5.2 Exclusion Criteria

The subject must be excluded from participating in the trial if the subject:

- Is currently participating and receiving study therapy or has participated in a study of an investigational agent and received study therapy, or herbal/complementary oral or IV medicine, within 2 weeks of the first dose of treatment.
- All toxicities attributed to prior anti-cancer therapy other than alopecia and fatigue must have resolved to Grade 1 (NCI CTCAE v4.0) or baseline prior to administration of first dose of study drug. Subjects with toxicities attributed to prior anti-cancer therapy that are not expected to resolve and result in long-lasting sequalae, such as chronic neuropathy after platinum-based therapy, are permitted to enroll.

- Has received chemotherapy or radiotherapy within 14 days of first dose of study medication.
- Has a solid organ or hematologic transplant.
- Has experienced weight loss >10% over 2 months prior to first dose of study therapy.
- Has a diagnosed additional malignancy within 2 years prior to first dose of trial treatment with the exception of curatively treated basal cell carcinoma of the skin, squamous cell carcinoma of the skin or curatively resected in situ breast cancers. Subjects with another malignancy diagnosed >2 years prior to the first dose of trial medication who were treated with curative intent and are not undergoing active therapy will be eligible.
- Has an active infection requiring systemic therapy.
- Has clinically relevant ascites (defined as requiring paracentesis within 21 days of first dose of study drug) or with moderate radiographic ascites. A minimal amount of radiographic ascites is allowed.
- Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the subject's participation for the full duration of the trial, or is not in the best interest of the subject to participate, in the opinion of the investigator, including dialysis.
- Has a known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.
- Is pregnant or breastfeeding, or expecting to conceive or father children within the projected duration of the trial, starting with the screening visit through 120 days after the last dose of trial treatment.

5.2.1 Exclusion Criteria Specific to Capecitabine

The subject must be excluded from receiving capecitabine if the subject:

- Requires concomitant use of phenytoin and/or warfarin due to reported increases in serum phenytoin levels and the international normalized ratio (INR) in patients receiving concomitant phenytoin and warfarin, respectively.
- Has a known hypersensitivity to capecitabine or to any of its components.
- Has a known hypersensitivity to 5-fluorouracil.

5.2.2 Exclusion Criteria Specific to Imatinib

The subject must be excluded from receiving capecitabine if the subject:

- Requires concomitant use of anticoagulation with warfarin. Patients who require anticoagulation should receive low molecular weight heparin.
- Has a left ventricular ejection fraction (LVEF) of < 45%
- Has New York Heart Association (NYHA) class III or IV symptoms
- Has had major surgery within 2 weeks prior to study entry
- Has a history of ventricular tachycardia, ventricular fibrillation, ventricular flutter, Torsades de Pointes, or long QT syndrome

5.2.3 Exclusion Criteria Specific to Ruxolitinib

- Has a history of hypersensitivity to ruxolitinib or to any medicine with similar chemical compounds
- Has a baseline corrected QT interval (QTc) > 470 ms

5.3 Inclusion of Women and Minorities

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

Accrual Targets					
Ethnic Category	**Sex/Gender**				
	Females		**Males**		**Total**
Hispanic or Latino	7	+	8	=	15
Not Hispanic or Latino	8	+	7	=	15
Ethnic Category: Total of all subjects	15	+	15	=	30
Racial Category					
American Indian or Alaskan Native	3	+	3	=	6
Asian	3	+	3	=	6
Black or African American	3	+	3	=	6
Native Hawaiian or other Pacific Islander	3	+	3	=	6
White	3	+	3	=	6
Racial Category: Total of all subjects	15	+	15	=	30

5.4 Subject Recruitment

This study will be conducted at Columbia University Medical Center. Thirty subjects will be needed to meet the primary study endpoint. The amount of time required to complete this trial will depend on the rapidity of accrual. We estimate evaluation of 3-5 subjects of a month in our Pancreas Center with enrollment of 1-2 subjects a month to complete accrual over 30 months.

Subjects will be followed for 30 additional days after completion or early discontinuation of treatment for safety follow-up, after which they will be off the active treatment phase of the study. Post-treatment long-term follow-up for disease status and survival will proceed until the subject has withdrawn consent, is lost to follow-up, has died, or until the Sponsor makes a decision to close the study.

Participation is voluntary. The patient must be aware of the neoplastic nature of his/her disease and willingly consent after being informed of the procedures to be followed, the experimental

nature of the therapy, alternatives, potential benefits, side effects, risks, and discomforts. The investigatory will make certain that an appropriate informed consent process is in place to ensure that potential research subjects, or their authorized representatives, are fully informed about the nature and objects of the clinical study, the potential risks and benefits of the study participation and their rights as research subjects.

5.5 Treatment Discontinuation

Subjects may withdraw from treatment at any time for any reason. A subject may be dropped from the trial at the discretion of the investigator should any untoward effect occur. Additionally, a subject may be withdrawn by the investigator or Sponsor if enrollment into the trial is inappropriate, the trial plan is violated, or for administrative and/or other safety reasons.

The investigator must discontinue study treatment for any of the following reasons:

- Subject or or legal representative desires discontinuation of treatment (i.e., withdraws consent for treatment)
- Radiographic disease progression (if confirmed by RECIST v1.1)
- Unacceptable toxicity
- Noncompliance with study procedures, including administration of nonprotocol therapies
- Requirement for alternative therapy
- Intercurrent illness or worsening of a chronic condition that prevents further administration of treatment
- The subject has a confirmed positive serum pregnancy test
- Subject is lost to follow-up

The reason for withdrawal from treatment will be documented in the CRF. Posttreatment follow-up for disease status and survival will continue until death unless any of the criteria for early study withdrawal are met (Section 10.9)

Discontinuation of treatment due to progression should be recorded on the Treatment Disconinuation CRF as “Disease Progression” and not as an AE or a SAE (unless the event meets the criteria for SAE as outlined in Section 8.1).

If the reason for withdrawal is AE, the subject will be followed by the Investigator as described in Section 8 until such events resolve, stabilize, and, according to the Investigator’s judgment, there is no need for further follow-up.

5.6 Early Study Withdrawal

When a subject discontinues/withdraws prior to trial completion, all applicable activities scheduled for the final trial visit should be performed at the time of discontinuation. Any AEs which are present at the time of discontinuation/withdrawal should be followed in accordance with the safety requirements outlined in Section 8 Assessing and Recording Adverse Events.

Subjects who discontinue for toxicity but do not withdraw consent from the study will continue to be followed for post-study treatment and survival as described in the Trial Flow Chart (Section 9).

5.7 Data Collection and Follow-Up of Withdrawan Subjects

The End of Treatment and Follow-Up visit procedures are listed in Section 9 (Trial Flow Chart) and Section 9.2 (Administrative Procedures). After the end of treatment, each subject will be followed for 30 days for adverse event monitoring (SAEs will be collected for 30 days after the end of treatment as described in Section 8). Subjects who discontinue for reasons other than progressive disease will have post-treatment follow-up for disease status until disease progression, initiating a non-study cancer treatment, withdrawing consent, or becoming lost to follow-up. After documented disease progression, each subject will be followed by telephone for survival until death, withdrawal of consent, or the end of the study, whichever occurs first.

5.8 Post-Treatment Visits

5.8.1 Safety Follow-Up Visits

The mandatory Safety Follow-Up Visit should be conducted approximately 30 days after the last dose of trial treatment or before the initiation of a new anti-cancer treatment, whichever comes first.

All AEs that occur prior to the Safety Follow-Up Visit should be recorded. Subjects with an AE of Grade ≥ 2 will be followed until the resolution of the AE to Grade 0-1 or until the beginning of a new anti-cancer therapy, whichever occurs first. SAEs that occur within 90 days of the end of treatment or before initiation of a new anti-cancer treatment should also be followed and recorded.

5.8.2 Follow-Up Visits

Subjects who discontinue trial treatment for reasons other than disease progression will move into the follow-up phase and should be assessed Q8W by radiologic imaging to monitor disease status. Every effort should be made to collect information regarding disease status until the start of a new anti-cancer therapy, disease progression, death, or the end of the study.

Information regarding post study anti-cancer treatment will be collected if new treatment is initiated.

5.8.3 Survival Follow-Up

Subjects who experience disease progression (by site assessment) or start a new anti-cancer therapy, will move into the survival follow-up phase. Subjects should be contacted (e.g., by telephone or visit) approximately every 12 weeks to assess for survival status until death, withdrawal of consent, or the end of the study, whichever occurs first. This will be done via telephone calls, subject medical records, and/or clinical visits. If the subject specifically withdraws consent from survival follow-up, the study staff may use a public information source (e.g. county records) to obtain information about survival status only.

Post study anti-cancer therapy will be collected during survival follow-up.

6. REGISTRATION PROCEDURES

6.1 CUMC Research Participant Registration

Confirm eligibility as defined in the section entitled Criteria for Subject Eligibility.

Obtain informed consent, by following procedures defined in section entitled Informed Consent Procedures, along with applicable institutional policies and federal regulations.

Only Investigators/Research personnel properly trained and delegated to consent subjects for this protocol will participate in the consenting process. Furthermore, properly delegated/trained Physician Investigators (e.g., MD, MD PhD) are required to sign/verify a protocol specific Eligibility Checklist for each subject enrolled on the study, in addition to providing the relevant source documentation confirmation subject eligibility.

All participants must be centrally registered through the Central Registration Office within Herbert Irving Comprehensive Cancer Center at CUMC prior to initiation of study treatment.

Registration hours are available Monday through Friday from 9:00am – 5:00pm EST (excluding holidays and weekends). Same day patient registrations (and after hour registrations) will be accommodated on a case by case basis provided that the study team has expressed all time sensitive registration concerns/cases in a timely manner to the Central Registration Office.

CPDM Central Registration Procedures:

Within 48 hours of obtaining consent (excluding holidays and weekends), a completed/signed IRB approved informed consent HIPAA form, and demographics forms must be submitted to the CPDM Central Registration Office via an email to CPDMRegistration@columbia.edu or fax to 212.305.5292, with the subject line "AAAxxxxx Pending Subject Registration Request (PHI)". Upon receipt, applicable subject information as well as a "pending eligibility" status will be entered into HICCC's institutional database. This status will remain until further source documentation is made available to confirm overall patient eligibility. Required materials for all pending registration submissions are as follows:

- Completed/signed IRB approved/stamped Informed Consent Forms, including additional study ICFs (e.g., tissue, DNA, etc.), as applicable.
- The completed/signed IRB approved HIPAA Authorization form
- Completed/signed CPDM ICF checklist
- Completed/signed HICCC personal census form
- Completed/signed CPDM Demographics Note to File

In order to confirm eligibility status, Investigators/designees (e.g., study specific Clinical Research Coordinator/Research Nurse, etc.) must submit the following documentation to the Central Registration Office via email or fax:

- The completed/signed study specific Eligibility Checklist (signed by an Physician level Investigator)

- Copies of source documentation necessary for each item to be verified on the CPDM specific Eligibility Checklist, including but not limited to:

  - Copy of required laboratory test and procedure reports (e.g., hematology, serum chemistry, pregnancy test when applicable, MRI reports, CT/bone scans, etc.)

  - Copy of pathology and surgical reports

  - Copy of clinic note(s) or other appropriate medical records capturing the consent process information, along with providing source documentation of any other items needed for screening/eligibility that are not captured in other source document forms (e.g., positive investigator statements of unique eligibility items not captured via other direct source documentation, concomitant medication lists, etc.)

  - Protocol deviation/waiver approvals (if applicable)

- **Please note**: subject line of email or fax should include the following: "AAAxxxxx Complete Subject Registration Request (PHI)".

Upon receipt of the above mentioned documentation, participant eligibility information will be verified by a qualified Central Registration Registrar. If any questions arise during the review process, queries in the form of emails will be addressed to the applicable study team personnel for clarification prior to enrollment. .All applicable finalized registration/eligibility information will then be entered into HICCC's institutional CTMS database by the Central Registration Registrar. Upon completion, an official subject registration notification email will be sent to the PI/research team which will include eligibility/enrollment status, as well as subject ID information. Protocol therapy may not be initiated prior to receipt of this notification from the Central Registration Office.

All screen fail/ineligible subjects, as well as subject's who withdraw consent prior to enrollment/initiation of protocol therapy must be submitted to the Central Registration office in a manner analogous to the procedures noted above. Applicable source documentation will be required within the corresponding submissions.

7. TREATMENT PLAN

7.1 Study Treatment

The results of the OncoTreat analysis will include on FDA-approved or investigational oncology drugs. These results, along with the preclinical validation studies using PDX/PDO models, will be discussed at the PMTB. Details of the trial design are discussed in Section 4.1.

7.2 Duration of Therapy

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

- Disease progression
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse events(s)
- Patient decides to withdraw from the study
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator

7.3 Duration of Follow Up

Patients will be followed for 4 weeks after completion or removal from study or until death, whichever occurs first. Patients removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event.

7.4 Criteria for Removal from Study

Patients will be removed from study when any of the criteria listed in Section 5.4 applies. The reason for study removal and the date the patient was removed will be documented in the Case Report Form.

8. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

8.1 Definitions

8.1.1 Adverse Event

An adverse event (AE) is any untoward or unfavorable medical occurrence in a human subject, including abnormal sign, symptom or disease, temporally associated with the subject's participation in research, whether or not considered related to the subject's participation in the research. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- Results in study withdrawal
- Is associated with a serious adverse event
- Is associated with clinical signs or symptoms
- Leads to additional treatment or to further diagnostic tests
- Is considered by the investigator to be of clinical significance

8.1.2 Serious Adverse Event

Adverse events are classified as serious or non-serious. A serious adverse event (SAE) is any AE that is:

- Fatal
- Life-threatening
- Requires inpatient hospitalization/prolongation of existing hospitalization, unless:
    - Routine treatment or monitoring of the studied indication, not associated with any deterioration in condiction (procedures such as central line placements, paracentesis, pain control)
    - Elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since the start of study drug
    - Treatment on an emergency outpatient basis for an event not fulfilling any of the definitions of an SAE given above/below and not resulting in hospital administrations
    - Social reasons and respite care in the absence of any deterioration in the patient's general condition
- Results in persistent or significant disability or incapacity
- A congenital anomaly or birth defect
- An important medical event

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious events should be regarded as non-serious adverse events.

8.1.3 **Unanticipated Problem**

An unanticipated problem (UP) is any incident, experience or outcome involving risks to subjects or others in any human subjects research that meets all of the following criteria:

- Unexpected (in terms of nature, severity or frequency) given (a) the research procedures that are described in the IRB-approval protocol and informed consent document, and (b) the characteristics of the subject population being studied;
- Related or possibly related to participation in such research (e.g., there is a reasonable possibility that the incident, experience or outcome may have been caused by the procedures involved in such research); and
- Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic or social harm) than was previously known or recognized.

8.2 Adverse Event Reporting Period

The study period during which adverse events must be reported is normally defined as the period from the initiation of any study procedures (e.g., after the first dose of study treatment) to the end of the study treatment (e.g., last dose of study treatment) and/or follow-up. For this study, the study treatment follow-up is defined as 30 days following the last administration of study treatment, or 30 days following the decision to remove the subject from study treatment, whichever is earliest.

8.2.1 Baseline/Prexisting Condition

A baseline/preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or if the character of the condition worsens during the study period.

8.2.2 General Physical Examination Findings

At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

8.2.3 Post-Study Adverse Event

All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study.

8.2.4 Abnormal Laboratory Values

A clinical laboratory abnormality should be documented as an adverse event if any one of the following conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality.
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management (e.g., change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc).

8.2.5 Hospitalization, Prolonged Hospitalization, or Surgery

Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for an adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should **not** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

8.3 Evaluating Adverse Events

An investigator who is a qualified physician will evaluate all adverse events according to the NCI Common Terminology for Adverse Events (CTCAE), version 4.0. Any adverse event which changes CTCAE grade over the course of a given episode will have each change of grade recorded on the adverse event case report forms/worksheets.

All adverse events regardless of CTCAE grade must also be evaluated for seriousness.

8.4 Recording of Adverse Events

At each contact with the subject, the investigator must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should recorded in the source document, though should be grouped under one diagnosis.

All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately.

8.5 Reporting of Serious Adverse Events

8.5.1 IRB Notification by Sponsor-Investigator

Reports of all events (including follow-up information) that meet the definition of an unanticipated problem posing risk to subjects or others must be submitted to the IRB within one week (5 business days) following the occurrence of the unanticipated problem or the principal investigator's acquiring knowledge of the unanticipated problem in accordance with IRB policy. Additionally, the sponsor-investigator will submit a summary of all Unanticipated problems that occurred since the beginning of the study at the time of continuing review. Copies of each report and documentation of IRB notification and receipt will be kept in the Regulatory binder.

8.5.2 SAE Reporting to Incyte

The Principal Investigator (PI) must report all Serious Adverse Events (SAEs) to Incyte within 24 hours of learning of an event, regardless of the PIs causality assessment. This notification should be provided on a completed Serious Adverse Event (SAE) form. SAE reporting for each subject begins the day the informed consent is signed by the patient and within 30 days after subject has completed or discontinued from the study or has taken last dose of the study drug, or as described in the protocol.

SAEs, occurring using Incyte Study drug, are reported in accordance with the effective protocol. SAEs occurring with any other commercial drug are reported to the manufacturer of that drug in accordance with regulations and protocol.

Initial SAEs and/or subsequent follow-up reports should be reported via email to SafetyReporting@Incyte.com or fax (+) 1-866-981-2057. SAE reports should be for a single subject. SAE forms should be sent with a cover sheet and any additional attachments.

All adverse event information is reported to Incyte on the Principal Investigator's/Institution's Adverse Event Report Form, or a CIOMS-I or MedWatch Form FDA 3500A, or on an Adverse Event Report Form which may be provided by Incyte upon request. The Principal Investigator does not provide medical records (e.g., discharge summary) to Incyte, unless specifically requested.

8.5.2 8.5.3 FDA Notification by Sponsor-Investigator

The Columbia University Medical Center Sponsor-Investigator, as holder of the IND, will be responsible for all communication with the FDA. Columbia University Medical Center Principal Investigator will report to the FDA, regardless of the site of occurrence, any adverse event that is serious, unexpected and there is evidence to suggest a causal relationship between the drug and the adverse event. These must be reported to the FDA and any affiliate sites as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting. The Sponsor-Investigator will also submit an IND annual report to the FDA in accordance with 21.CFR 312.33.

The Columbia University Medical Center Sponsor Investigator must report to the FDA and any affiliate site investigators as follows:

- Any unexpected fatal or life-threatening event must be reported as soon as possible, but no later than 7 calendar days after the sponsor investigator initial receipt of the information
- Any findings from epidemiological studies, pooled analysis of multiple studies, or clinical studies, whether or not conducted under an IND, and whether or not conducted by the sponsor-investigator, that suggest a significant risk in humans exposed to the drug must be reported as soon as possible but no later than 15 calendar days after the sponsor-investigator determines that the information qualifies for reporting

- Any findings from animal or in vitro testing whether or not conducted under an IND, and whether or not conducted by the sponsor-investigator, that suggest a significant risk in humans exposed to the drug must be reported as soon as possible but no later than 15 calendar days after the sponsor-investigator determines that the information qualifies for reporting
- Any clinically important increase in the rate of a serious suspected adverse reactions over that listed in the protocol or Investigator Brochure
- Expected SAEs and AEs will be included in the IND Annual Reports

Follow-up information to a safety report should be submitted as soon as the relevant information is available. However, if the results of a sponsor's investigation show that an adverse drug experience not initially determined to be reportable are so reportable, the sponsor investigator must report such experience as soon as possible, but no later than 15 calendar days after the determination is made.

All other serious unexpected experiences associated with the use of the study treatment will be reported to FDA as soon as possible but in no event later than 15 calendar days after initial receipt of the information.

8.5.4 DSMC Reporting by the Sponsor-Investigator

Serious adverse events not constituting unanticipated problems are to be reported to the HICCC DSMC. Reporting should occur within 24 hours of knowledge of the SAE occurring at our institution or affiliate sites.

8.5.5 Reporting to Drug Manufacturer by Sponsor-Investigator

The Sponsor-Investigator will report to the investigational agent manufacturer any serious adverse events that meet the reporting criteria to the Institutional Review Board and/or FDA as described in Section 8.5 within 72 hours of becoming aware of it, so that these reports can be evaluated and included in the Investigator's Brochure and for IND safety submissions per regulations. Reporting will occur by sending the reporting form along with any additional documentation sent to the regulatory authorities.

At the the time of IRB renewal or at the request of the manufacturer, the Sponsor-Investigator will submit a summary of all Serious Adverse Events that have occurred inclusive of all sites to manufacturer.

8.5.5

8.6 Reporting Process

Adverse events may be submitted on FDA Form 3500A, the HICCC DSMC Serious Adverse Event Reporting Form, or in a narrative format.

8.7 Reporting of Pregnancy to Incyte

An "Initial Pregnancy Report" or equivalent must be completed in full and emailed to

SafetyReporting@Incyte.com or faxed to (+) 1-866-981-2057 within 24 hours of discovery of a pregnancy of a subject who has taken the Incyte product or the pregnancy of a partner for a subject who has taken the Incyte product. The "Follow-up Pregnancy Report Form" or equivalent must be completed and emailed to SafetyReporting@Incyte.com or faxed to (+) 1-866-981-2057 within 30 days after delivery, so that Incyte is provided with information regarding the outcome of the pregnancy. If the pregnancy results in any events which meet the serious criteria (i.e., miscarriage or termination), the SAE reporting process needs to be followed and the timelines associated with a SAE should be followed.

9. STUDY CALENDAR

The screening period for a particular subject commences when the subject undergoes the first study-specific screening assessment. Written informed consent must be obtained before any protocol-specific tests or procedures may be conducted. After informed consent is obtained, baseline evaluations are to be conducted within 2 weeks prior to start of protocol therapy. Routine laboratories – hematology, serum chemistries, and coagulation – performed prior to informed consent may be used if within a 2 week period to start of study treatment. Baseline radiographic evaluations must be done $\leq$ 4 weeks prior to the start of therapy. In the event that the patient's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy.

9.1 Study calendar

Table 6 Trial Flow Chart (Part 2 Only)

Trial Period:	**Screening Phase**	**Treatment Weeks[a]**								**End of Treatment (last dose) or Discontinuation**	**Post-Treatment**		
Treatment Week:		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**		**Safety Follow-Up (30 days from last dose)**	**Follow-Up Visits[b] (Q8W)**	**Survival Follow-Up (Phone)**
Scheduling Window (Days):	**-28 to -1**	**Details are described in the study calendars for each agent.**											
Administrative Procedures													
Informed Consent[c]	X												
Inclusion/Exclusion Criteria	X												
Demographics and Medical History	X												
Prior and Concomitant Medication Review	X	Details are described in the study calendars for each agent.								X	X		
Clinical Procedures/Assessments													
Review Adverse Events	X	Details are described in the study calendars for each agent.								X	X		
12-Lead ECG and echocardiogram[p]	X												
Physical Examination	X	Details are described in the study calendars for each agent.								X			
Ht (V1 only), Wt, & Vital Signs (T/P/RR/BP)	X	Details are described in the study calendars for each agent.								X			
ECOG Performance Status	X[m]	Details are described in the study calendars for each agent.								X			
OncoTreat-Prioritized Agent		Details are described in the study calendars for each agent.											
Post-Study Anticancer Therapy Status												X	Q12W
Survival Status													Q12W
Laboratory Procedures/Assessments													
Pregnancy Test – Urine or Serum β HCG[d]	X												
PT/INR and aPTT[e]	X												
CBC with Differential[e]	X	Details are described in the study calendars for each agent.								X			
Comprehensive Metabolic Panel[e]	X	Details are described in the study calendars for each agent.								X			
HBV, HCV, HIV[e,f]	X												
Efficacy Measurements													
Tumor Imaging	X[g]	X[h]								X[i]			
Tumor markers (CA 19-9 and CEA)[e]	X	X[n]								X			
Tumor Biopsies/Pharmacokinetics/Correlative Blood Studies													
Newly Obtained Tissue Collection	X[j]	X[k]								X			
Pharmacokinetics[o] (Blood Collection)		Details are described in Section 4.8											
Correlative Studies (Blood Collection)		X[l]								X			

a. Unless otherwise specified, procedures/assessments are to be performed prior to dose administration on Day 1 of each cycle.
b. Subjects who discontinue study treatment without documented disease progression should continue to be monitored for disease by radiologic imaging every 8 weeks (±7 days) until (1) the start of new anti-cancer treatment, (2) disease progression, (3) death, or (4) the end of the study, whichever occurs first.
c. Informed consent will include consent for correlative studies including biopsies and serum collections.
d. For women of reproductive potential, a negative pregnancy test should be performed within 72 hours prior to first dose of trial treatment. Pregnancy tests (serum and/or urine) should be repeated if required by local guidelines.
e. Laboratory screening tests should be performed within 14 days prior to first dose of study treatment. For all subjects, unresolved abnormal labs resulting in drug-related AEs should be followed until resolution.
f. All subjects will be tested for HIV, HBV, and HCV prior to enrollment. Those who are positive for HIV, untreated active Hepatitis B, and dual infection with HBV/HCV will be excluded.
g. Tumor imaging at screening: Imaging will be performed within 28 days prior to the first dose of OncoTreat-prioritized drug. Imaging should include CT chest, abdomen, and pelvis or MRI if CT is clinically contraindicated.
h. The first on-study tumor imaging will be performed after 8 weeks (±7 days) of treatment and will continue every 8 weeks (±7 days) thereafter (or more frequently if clinically indicated). Timing of imaging follows calendar days and should not be adjusted due to dose interruptions. The same imaging technique, acquisition, and processing parameters for a subject should be used throughout the trial if possible.
i. Subjects without confirmed PD who discontinue treatment will have imaging performed at the time that study treatment is discontinued (i.e. date of discontinuation ± 4 week window). If a scan was obtained within 4 weeks prior to discontinuation of treatment, then imaging at treatment discontinuation is not required. Subjects who discontinue treatment after confirmed PD will not need further imaging performed during the follow-up period.
j. Following disease progression/intolerance to standard of care therapy, a fresh tumor biopsy (primary or metastatic site) is required to be performed after the last dose of systemic therapy. OncoTreat analysis will confirm the master regulator profile and the recommended OncoTreat-informed drug.
k. An optional on-treatment tumor biopsy is requested and will be performed within one month of initiation of an OncoTreat-prioritized drug (28 days ±7 days). The biopsy is to be done within 24 hours of administration of the most recent dose. An optional end-of-treatment tumor biopsy is also requested (±7 days of completing OncoTreat-prioritized drug).
l. Collect on Cycle 1 Day 1 and Cycle 2 Day 1 (prior to study treatment administration).
m. Screening ECOG performance status should be performed within 3 days prior to the first dose of the OncoTreat-prioritized agent.
n. Serum CA 19-9 will be collected prior to dose administration on Day 1 of each cycle.
o. On days with PK assessments, patients should not eat breakfast or take the OncoTreat-prioritized agent at home. Upon arrival to the clinic, whole blood will be obtained pre-morning dose for imatinib and ruxolitinib. Patients will then take the medication as instructed by the clinic staff. For capecitabine, patients will take the medication as instructed by the clinic staff then will have whole blood obtained for PK analysis. See Section 4.8 for details.
   Capecitabine: cycle 1 day, cycle 1 day 8
   Imatinib: cycle 1 day 2 (24 hours ± 3 hours after cycle 1 day 1 dose administration)
   Ruxolitinib: cycle 1 day 2
p. Baseline transthoracic echocardiogram (TTE) is required for all subjects on the imatinib-treated arm.

9.1.1 Study calendar for capecitabine

For complete details, please see the general study calendar (Table 4).

Table 7 Capecitabine study calendar

Trial Period:	**Screening Phase**	**Treatment Weeks**									**End of Treatment (last dose) or Discontinuation**	**Post-Treatment**		
		Capecitabine 1000 mg/m^2 D1-14 every 21 days												
		Cycle 1			**Cycle 2**			**Cycle 3**						
Treatment Week:		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**		**Safety Follow-Up (30 days from last dose)**	**Follow-Up Visits (Q8W)**	**Survival Follow-Up (Phone)**
Scheduling Window (Days):	**-28 to -1**	± 3d			± 3d			± 3d						
Informed Consent	X													
Inclusion/Exclusion Criteria	X													
Demographics and Medical History	X													
Prior and Concomitant Medication Review	X	X			X			X then every 21 days			X	X		
Review Adverse Events	X				X			X then every 21 days			X	X		
12-Lead ECG	X													
Physical Examination	X	X			X			X then every 21 days			X			
Ht (V1 only), Wt, & Vital Signs (T/P/RR/BP)	X	X			X			X then every 21 days			X			
ECOG Performance Status	X	X			X			X then every 21 days			X			
Dispense capecitabine		X			X			X then every 21 days						
Post-Study Anticancer Therapy Status													X	Q12W
Survival Status														Q12W
Pregnancy Test – Urine or Serum β HCG	X													
PT/INR and aPTT[e]	X													
CBC with Differential	X	X			X			X then every 21 days			X			
Comprehensive Metabolic Panel	X	X			X			X then every 21 days			X			
HBV, HCV, HIV	X													
Tumor Imaging	X								X then every 8 weeks		X			
CA 19-9 and CEA	X	X			X			X then every 21 days			X			
Newly Obtained Tissue Collection	X					X					X			
Pharmacokinetics (Blood Collection)	Details are described in Section 4.8													
Correlative Studies (Blood Collection)		X			X				X		X			

9.1.2 **Study calendar for imatinib**

For complete details, please see the general study calendar (Table 4).

Table 8 Imatinib study calendar

Trial Period:	**Screening Phase**	**Treatment Weeks[a]**								**End of Treatment (last dose) or Discontinuation**	**Post-Treatment**		
		Imatinib 400 mg daily											
Treatment Week:		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**		**Safety Follow-Up (30 days from last dose)**	**Follow-Up Visits[b] (Q8W)**	**Survival Follow-Up (Phone)**
Scheduling Window (Days):	**-28 to -1**	± 3d				± 3d							
Informed Consent	X												
Inclusion/Exclusion Criteria	X												
Demographics and Medical History	X												
Prior and Concomitant Medication Review	X	X		X		X then every 28 days				X	X		
Review Adverse Events	X			X		X then every 28 days				X	X		
12-Lead ECG	X												
Physical Examination	X	X		X		X then every 28 days				X			
Ht (V1 only), Wt, & Vital Signs (T/P/RR/BP)	X	X		X		X then every 28 days				X			
ECOG Performance Status	X	X		X		X then every 28 days				X			
Dispense imatinib		X				X then every 28 days							
Post-Study Anticancer Therapy Status												X	Q12W
Survival Status													Q12W
Pregnancy Test – Urine or Serum β HCG	X												
PT/INR and aPTT[e]	X												
CBC with Differential	X	X		X		X then every 28 days				X			
Comprehensive Metabolic Panel	X	X		X		X then every 28 days				X			
HBV, HCV, HIV	X												
Tumor Imaging	X								X then every 8 weeks	X[i]			
Tumor markers (CA 19-9 and CEA)	X	X		X		X then every 28 days				X			
Newly Obtained Tissue Collection	X					X				X			
Pharmacokinetics (Blood Collection)		Details are described in Section 4.8											
Correlative Studies (Blood Collection)		X				X				X			

9.1.3 **Study calendar for ruxolitinib**

For complete details, please see the general study calendar (Table 4).

Table 9 Ruxolitinib study calendar

Trial Period:	**Screening Phase**	**Treatment Weeks**									**End of Treatment (last dose) or Discontinuation**	**Post-Treatment**		
		Ruxolitinib 15 mg BID												
Treatment Week:		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**		**Safety Follow-Up (30 days from last dose)**	**Follow-Up Visits (Q8W)**	**Survival Follow-Up (Phone)**
Scheduling Window (Days):	**-28 to -1**	± 3d			± 3d			± 3d						
Informed Consent	X													
Inclusion/Exclusion Criteria	X													
Demographics and Medical History	X													
Prior and Concomitant Medication Review	X	X		X		X				X then every 4 weeks	X	X		
Review Adverse Events	X			X		X				X then every 4 weeks	X	X		
12-Lead ECG and echocardiogram	X													
Physical Examination	X	X		X		X				X then every 4 weeks	X			
Ht (V1 only), Wt, & Vital Signs (T/P/RR/BP)	X	X		X		X				X then every 4 weeks	X			
ECOG Performance Status	X	X		X		X				X then every 4 weeks	X			
Dispense ruxolitinib		X				X				X then every 4 weeks				
Post-Study Anticancer Therapy Status													X	Q12W
Survival Status														Q12W
Pregnancy Test – Urine or Serum β HCG	X													
PT/INR and aPTT	X													
CBC with Differential	X	X		X		X		X		X then every 2 weeks	X			
Comprehensive Metabolic Panel	X	X		X		X					X			
HBV, HCV, HIV	X													
Lipid panel	X									X then every 9 weeks				
Tumor Imaging	X										X			
Tumor markers (CA 19-9 and CEA)	X	X		X		X				X then every 4 weeks	X			
Newly Obtained Tissue Collection	X					X					X			
Pharmacokinetics (Blood Collection)		Details are described in Section 4.8												
Correlative Studies (Blood Collection)		X				X					X			

9.2 Trial Procedures

The Trial Flow Chart (Section 9) summarizes the trial procedures to be performed at each visit. Individual trial procedures are described in detail below. It may be necessary to perform these procedures at unscheduled time points if deemed clinically necessary by the investigator. Furthermore, additional evaluations/testing may be deemed necessary by the Sponsor for reasons related to safety. In some cases, such evaluation/testing may be potentially sensitive in nature (e.g. HIV, Hepatitis C, etc.), and thus local regulations may require that additional informed consent be obtained from the subject. In these cases, such evaluations/testing will be performed in accordance with those regulations.

9.3 Administrative Procedures

9.3.1 Informed Consent

The investigator must obtain documented consent from each potential subject or each subject's legally acceptable representative prior to participating in a clinical trial.

Consent must be documented by the subject's dated signature or by the subject's legally acceptable representative's dated signature on a consent form along with the dated signature of the person conducting the consent discussion. A copy of the signed and dated consent form should be given to the subject before participation in the trial.

The initial informed consent form, any subsequent revised written informed consent form, and any written information provided to the subject must receive the IRB/ERC's approval/favorable opinion in advance of use. The subject, or his/her legally acceptable representative, should be informed in a timely manner if new information becomes available that may be relevant to the subject's willingness to continue participation in the trial. The communication of this information will be provided and documented via a revised consent form or addendum to the original consent form that captures the subject's dated signature or by the subject's legally acceptable representative's dated signature.

The informed consent will adhere to IRB/ERC requirements, applicable to state laws, and federal regulations.

9.3.2 Inclusion/Exclusion Criteria

All inclusion and exclusion criteria will be reviewed by the investigator or qualified designee to ensure that the subject qualifies for the trial (see Section 5).

9.3.3 Medical History

A medical history will be obtained by the investigator or qualified designee. Medical history will include all active conditions and any condition diagnosed within the prior 10 years that is considered to be clinically significant by the investigator. Details regarding the disease for which the subject has enrolled in this study will be recorded separately and not listed as medical history.

9.3.4 Prior and Concomitant Medications

Concomitant therapy includes any medication (e.g. prescription drugs, over-the-counter drugs, herbal or homeopathic remedies, nutritional supplements) used by a patient from 7 days prior to screening to the treatment discontinuation visit. All such medications should be reported to the investigator and recorded to the Concomitant Medications Case Report Form (CRF). All medications related to reportable SAEs and ECIs should be recorded.

9.3.5 Disease Details and Treatments

The investigator or qualified designee will obtain prior and current details regarding disease status.

Prior Treatment Details: The investigator or qualified designee will review all prior cancer treatments including systemic treatments, radiation, and surgeries.

Subsequent Anti-Cancer Therapy Status: The investigator or qualified designee will review all new anti-neoplastic therapy initiated after the last dose of trial treatment. If a subject initiates a new anti-cancer therapy within 30 days after the last dose of trial treatment, the 30-day Safety Follow-Up visit must occur before the first dose of the new therapy. Once new anti-cancer therapy has been initiated, the subject will move into survival follow-up.

9.3.6 Long-Term Follow Up

After completion of the posttreatment safety visit, subjects will be followed for disease and survival status by telephone contact or other method every three months for one year from treatment discontinuation, then every six months thereafter. Long-term follow-up will continue until the subject has withdrawn consent for further participation, is lost to follow-up, has died, or the Sponsor makes a decision to close the study.

9.4 Clinical Procedures and Assessments

9.4.1 Adverse Event (AE) Monitoring

The investigator or qualified designee will assess each subject to evaluate for potential new or worsening AEs as specified in the Trial Flow Chart (Section 9) and more frequently if clinically indicated. AEs will be graded and recorded throughout the study and during the follow-up period according to NCI CTCAE Version 4.0 (Appendix B). Toxicities will be characterized in terms regarding seriousness, causality, toxicity grading, and action taken with regard to trial treatment.

Refer to Section 8 for detailed information regarding the assessment and recording of AEs.

9.4.2 Physical Exam

The investigator or qualified designee will perform a complete physical exam during the screening period. Clinically significant abnormal findings should be recorded as medical history. A physical exam should be performed as specified in the Trial Flow Chart (Section 9). For subsequent cycles, the investigator or qualified designee will perform a directed physical exam as clinically indicated prior to dosing on Day 1 of each treatment cycle. After

the first dose of trial treatment, new clinically significant abnormal findings should be recorded as AEs.

9.4.3 Vital Signs

The investigator or qualified designee will take vital signs at screening, prior to the administration of each dose of trial treatment, and at treatment discontinuation as specified in the Trial Flow Chart (Section 9). Vital signs should include temperature, pulse, respiratory rate, weight, and blood pressure. Height will be measured at screening only.

9.4.4 Eastern Cooperative Oncology Group (ECOG) Performance Status

The investigator or qualified designee will assess ECOG status (Appendix A) at screening, prior to the administration of each dose of trial treatment, and at discontinuation of trial treatment as specified in the Trial Flow Chart (Section 9).

9.4.5 Tumor Imaging and Assessment of Disease

CT chest, abdomen, and pelvis with contrast will be performed at baseline screening, prior to the administration of study drugs, and every 8 weeks thereafter. Subjects will be evaluated for diease response and progression according to RECIST v1.1. Disease status categories include complete response (CR), partial response (PR), stable disease (SD), and progression of disease (PD).

9.4.6 Tumor Tissue Collection, Pharmacokinetics, & Correlative Studies Blood Sampling

All patients will have tumor biopsy specimens obtained at baseline prior to study drug administration and then at week 4 (optional) to assess for successful collapse of the master regulator siguature in a given patient's tumor. A biopsy will also be requested (optional) at the time of disease progression. Tissue collection, handling, processing, and storage instructions are described in the separate study manual.

Peripheral blood will be collected for pharmacokinetic analysis only during Cycle 1. The timing of blood sampling will vary by study drug (Section 4.8). The exact times of medication administration and the times of these blood samples must be recorded on the appropriate CRF. A validated assay will be used to measure plasma drug concentration. Blood processing, handling, and storage instructions are described in Section 4.8 as well as in the separate study manual.

Peripheral blood will be collected pre-treatment, cycle 1 day 1, and cycle 2 for future evaluation of biomarkers. The analyses may include genomics, transcriptomics, and proteomics. Assessment of ctDNA and OncoTreat analysis on circulating tumor cells (CTCs) may also be performed.

9.4.7 Laboratory Procedures/Assessments

Details regarding specific laboratory procedures/assessments to be performed in this trial are provided below. Laboratory tests for hematology, chemistry, urinalysis, and others are specified in the Trial Flow Chart (Section 9).

Laboratory tests for screening should be performed within 7 days prior to the first dose of study treatment. After Cycle 1, pre-dose laboratory procedures can be conducted up to 72 hours prior to dosing. Results must be reviewed by the investigator or qualified designee and found to be acceptable prior to each dose of trial treatment.

10. MEASUREMENT OF EFFECT

10.1 Antitumor Effect – Solid Tumors

For the purposes of this study, patients should be re-evaluated for response every 8 weeks. In addition to a baseline scan, confirmatory scans should also be obtained 8 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 revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [Eur J Ca 45:228-247, 2009]. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

10.2 Definitions

Evaluable for toxicity: All patients will be evaluable for toxicity from the time of their first treatment with an OncoTreat-prioritized agent.

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

Evaluable for feasibility – primary outcome: All subjects who receive a single dose of study drug will be included in the primary outcome analysis of feasibility.

10.3 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 for non-nodal lesions and short axis for nodal lesions) as $\geq$ 20 mm by chest x-ray, as $\geq$ 10 mm with CT scan, or $\geq$ 10 mm with 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. If the investigator thinks it appropriate to include them, the conditions under which such lesions should be considered must be defined in the protocol.

Malignant lymph nodes: To be considered pathologically enlarged and measurable, a lymph node must be $\geq$ 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, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts. Cystic lesions thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

Target lesions: All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as target lesions and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

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

10.4 Methods for Evaluation of Measurable Disease

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

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

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

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

Tumor markers: Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

10.5 Response Criteria – RECIST Criteria

10.5.1 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.

10.5.2 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).

10.5.3 Evaluation of Best Overall Response

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

Table 10 Disease Assessment For Patients with Measurable Disease

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

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

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

10.6 Duration of Response

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

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

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

10.7 Analysis of Endpoints

Efficacy and safety endpoints that will be evaluated are listed below:

Efficacy

10.7.1 Objective Response Rate (ORR)

ORR is defined as the proportion of the subjects in the analysis population who have a CR or PR. Responses are based on assessments per RECIST 1.1.

10.7.2 Progression-Free Survival (PFS)

PFS is defined as the duration of time from the first day of trial treatment to the first documented disease progression per RECIST 1.1 or death due to any cause, whichever occurs first.

10.7.3 Disease Control Rate (DCR)

DCR is defined as the percentage of subjects who have achieved CR, PR, and SD. Responses are based on assessments per RECIST 1.1.

10.7.4 Overall Survival (OS)

OS is defined as the time from first dose of study medication to death due to any cause.

Safety

10.7.5 Safety Endpoints

The safety analysis will be based on subjects who experienced toxicities as defined by CTCAE criteria. Safety will be assessed by quantifying the toxicities and grades experienced by subjects who have received the study drug, including serious adverse events (SAEs) and events of clinical interest (ECIs).

Safety will be assessed by reported adverse experiences using CTCAE, Version 4.0. The attribution to study treatment, time-of-onset, duration of the event, its resolution, and any concomitant medications administered will be recorded. AEs will be analyzed including but not limited to all AEs, SAEs, fatal AEs, and laboratory changes.

10.8 Pharmacokinetic Outcome Measures
Pharmacokinetic (PK) samples will be collected for all subjects (see Appendix ___ for sample collection details) and include the following:

10.9 Analysis of Exploratory Endpoints

10.10 Unblinding Procedures
Not applicable – this is not a blinded study.

10.11 Stopping Rules
Early trial termination will be the result of the criteria specified below:

- Quality or quantity of data recording is inaccurate or incomplete
- Poor adherence to protocol and regulatory requirements
- Incidence or severity of adverse drug reaction in this or other studies indicates a potential health hazard to subjects
- Plans to modify or discontinue the development of the study drug

11. DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 8 (Adverse Events: List and Reporting Requirements). The Data Safety Monitoring Plan is described in Section 11.3.

11.1 Data Collection
The Herbert Irving Comprehensive Cancer Center has an electronic clinical trials and data management system (CTMS) that will be used for data collection. CRFs for the study will be built into the CTMS, Velos, for data entry. The system has full auditing capabilities which is web-based and housed on a server in a fully HIPAA compliant server room with restricted access and video camera monitoring. All users must login with their own application username and password. Users off campus must first access the Virtual Private Network with their assigned campus username and password and then use their application credentials. Users are only able to see study information if they are indicated as study personnel in our electronic IRB system. Users are limited to access based on the role assigned in their corresponding protocol. Subject data is entered directly into the system, which (in the case of Columbia subjects) confirms the correct identity of patients via an interface with the electronic medical patient index. Staff with the appropriate IRB defined roles can run reports within the system for reporting purposes.

11.2 Data Reporting
Case Report Forms (CRFs) will be completed for each subject enrolled into the clinical study through the CTMS. It is the investigator's responsibility for ensuring that all clinical and laboratory data entered on the corresponding CRFs are complete, accurate and authentic.

11.3 Data and Safety Monitoring Committee

The NCI-approved Data Safety and Monitoring Committee (DSMC) of the Herbert Irving Comprehensive Cancer Center (HICCC) will monitor every subject who receives treatment on this protocol for toxicity. This protocol will adhere to the policies of the currently approved HICCC Data and Safety Monitoring Plan (DSMP), which is in accordance with NCI and CUMC-IRB policy and guidelines. The committee is chair is appointed by the HICCC Director. The committee consists of HICCC faculty and staff with expertise in oncology, research pharmacy, research nursing, and data management. The DSMC convenes twice a month to review patient safety and the conduct of the trial. The PI will submit data and safety monitoring reports to the DSMC at a frequency to be determined by the DSMC based on risk to the subjects.

At the time of renewal, the study team will submit the most recent DSMC approval letter for safety review to the CUMC IRB. Any modifications that are required by the DSMC to ensure patient safety will be submitted to the IRB. All protocol deviations, violations, and eligibility waivers will be submitted to and approved by the DSMC prior to being reported to the IRB. All study data reviewed and discussed during these meetings will be kept confidential.

11.4 Quality Control and Quality Assurance

Independent monitoring of the clinical study for protocol and GCP compliance will be conducted periodically by the CPDM Compliance Core on behalf of the HICCC DSMC. Additionally, the Compliance Oversight Committee of the IRB at Columbia University Medical Center may audit the study at any time per institutional policies and procedures. The investigator-sponsor and Columbia University Medical Center will permit direct access of the study monitors and appropriate regulatory authorities to the study data and to the corresponding source data and documents to verify the accuracy of this data.

A risk-based approach will be used by the Compliance Core to determine the frequency, number of subject charts, and data elements to be monitored. The Compliance Coordinator will review the study status and summarize enrollment, toxicities, SAEs/UPs, dose escalation, statistical endpoints (e.g., stopping rules), etc. for the full DSMC membership at the regularly scheduled meetings.

Internal On-site Monitoring:

- Initial, recurrent, and close-out on-site monitoring visits will also be conducted at remote clinical sites, as appropriate/feasible. Other sites will have monitoring performed remotely (see below for further details).
- The study Monitoring Visit Log will be completed and signed by the monitor and the PI/CRNP/CRN and/or CRC and will be filed in the regulatory binder.
- The Compliance Coordinator will communicate with the site coordinator/Site Principle Investigator to schedule the monitoring visit and arrange for access to study materials and documentation.
- The assigned Compliance Coordinator will monitor IIT trials within 1 month after the first subject is enrolled and throughout the life of the study to ensure that the study is being conducted in accordance with the protocol, GCP, applicable federal and local regulations, and per all applicable SOPs. The Compliance Coordinator is responsible to notify the PI and CRNP/CRN/CRC of upcoming monitor visits and convey what

information and documentation will be required for the visit(s). The Compliance Coordinator is responsible for verifying that informed consent is properly obtained, eligibility is met (via the central registration process), and all study procedures are conducted according to the study protocol. The Compliance Coordinator will also verify that the data reported in the CRF's accurately reflect source documents, that all toxicities have been reported to date, and that all SAE's/UPs/deviations/violations have been reported according to local IRB and HICCC DSMC requirements. The Compliance Coordinator will issue queries and ensure resolution in a timely and efficient manner. The Compliance Coordinator will also monitor for applicable regulatory compliance and research pharmacy compliance (if applicable) and communicate any deficiencies as appropriate.

11.5 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (e.g., that the subject is alive) at the end of their scheduled study period.

The subject binders will be maintained with in the CPDM offices, a secured floor within the Herbert Irving Pavilion and only the investigator and study staff will have access to the file.

11.6 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

11.7 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on

the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A".

11.8 Records Retention

Records relating to a specific research activity, including research records collected by investigators, must be maintained for at least three years after completion of the research (45 CFR 46.115(b); 21 CFR 56.115(b); 21 CFR 312.62). This minimum retention period applies whether or not any subjects were enrolled in the study. If the research is FDA regulated, records should be retained for at least two years after approval of the investigational agent by FDA; if it is not approved, records should be retained at least two years after the study is terminated and FDA is notified (note the additional requirement below for clinical research studies). Clinical records, including consent forms that document clinical intervention or clinical diagnostic procedure research-related procedures, must be retained in medical records by the institution for at least seven years, per CUMC and NYP policy which is based on state law.

12. STATISTICAL CONSIDERATIONS

This is a single arm, phase Ib study with 2 co-primary endpoints:

- Feasibility: The primary objective of this trial is to determine the feasibility of the OncoTreat platform in guiding therapy. The primary outcome is whether a subject begins therapy on Part 2. Previous attempts to match subjects based on DNA profiling have yielded enrollment rates of 3-5% [14, 78]. We hypothesize that we will validate OncoTreat matches for 50% of subjects and that at least 30% of enrolled subjects (60% of matches) will begin therapy on Part 2. With 30 enrolled patients on Part 1, we will have 80% power to detect a treatment rate of 30% on Part 2 compared to a historical treatment rate of 5% with alpha of 0.05. We will also assess the "match rate" – the fraction of patients who match with at leaset one drug. With 30 enrolled subjects, we will have 97% power with alpha = 0.05 to detect a 50% match rate (15 of 30 subjects) compared to a (liberal) estimated match rate of 20% based on DNA profiling in PDA.
- Efficacy: The primary clinical efficacy endpoint is the **objective response rate (ORR)** for OncoTreat-prioritized drugs, which corresponds to the number of patients whose tumors demonstrate a 30% or greater reduction from baseline in the sum of the diameters of all target lesions amongst all evaluable patients, as assessed by standard-of-care CT scans. We target estimating the ORR and the corresponding 95% confidence interval.

Secondary endpoints will include: 1) **disease control rate (DCR) at 16 and 24 weeks**, which corresponds to the number of patients whose tumors demonstrate either an objective response or stable disease, defined as a no more than 20% increase from baseline in the sum of the diameters of all target lesions; 2) **median progression-free survival (mPFS)**, defined as the median duration prior to the development of disease progression or patient death; 3) **overall survival (OS)**, defined as time from first dose of OncoType-informed drug to death due to any cause; and 4) **safety and tolerability**, defined as the incidence of Grade 3/4 AEs in terms categorized and graded according to NCI CTCAE v4.0. We will estimate DCR, mPFS, and OS and their 95% confidence interval.

The phase III trial (NAPOLI-1) that resulted in the FDA-approval of nanoliposomal irinotecan and 5-fluorouracil in the second-line setting demonstrated a median overall survival of 6.1 months versus 4.2 months for 5-fluorouracil monotherapy (primary endpoint), mPFS of 3.1 months, and an ORR of 16% (compared to 1% in the 5-fluorouracil arm) [7]. Fifty-one (12%) patients had not had previous treatment for metastatic disease, 234 (56%) had received one previous line of metastatic treatment, and 132 (32%) had previously received two or more lines of metastatic treatment. The disease control rate was less than 20% and did not differ between treatment groups. Thus, based on historical controls, the ORR for treatment-resistant metastatic PDAC is expected to be no more than 16%.

Using the above mentioned rates, a sample size of n = 9 achieves at least a 80% power to detect a difference of 25% in ORR (30% vs 5%) using a one-sided binomial test with a target alpha of 0.1. To be conservative, we use the minimum number of patients (9) expected to be treated in this calculation. If at the end of the study the total number of objective responses is greater than or equal to 2, we will be able to reject the historical rate of 5% at the significance level of 0.05 with power of 69% and OncoTreat will have demonstrated a clear signal of clinically meaningful improvement in the primary endpoint for patients with advanced, previously-treated PDA. Assuming about 30% of enrolled patients will receive an OncoTreat-informed treatment, N = 30 subjects is an appropriate sample size for this prospective study.

13. PROTECTION OF HUMAN SUBJECTS

This study is to be conducted in accordance with applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be obtained before commencement of this study.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the IRB-approved consent form, must be obtained before that subject is submitted to any study procedure. This consent form must be signed by the subject or legally acceptable surrogate, as outlined in the IRB approved protocol, and the investigator-designated research professional obtaining the consent.

14. STUDY FINANCES

14.1 Conflict of Interest

Any investigator who has a conlict of interest with this study (patent ownership, royalities, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by the Columbia University Conflict of Interest Committee with a Committee-

sanctioned conflict management plan that has been reviewed and approved prior to participation in this study. All CUMC investigators will follow the University conflict of interest policy.

14.2 Subject Stipends or Payments

There are no subject payments or stipends.

15. PUBLICATION PLAN

Neither the complete nor any part of the results of the study carried out under this protocol, nor any of the information provided by the sponsor for the purposes of performing the study, will be published or passed on to any third party without the consent of the study sponsor. Any investigator involved with this study is obligated to provide the sponsor with complete test results and all data derived from the study.

16. REFERENCES

1. Miller, K.D., et al., *Cancer treatment and survivorship statistics, 2016.* CA Cancer J Clin, 2016. **66**(4): p. 271-89.
2. Siegel, R.L., K.D. Miller, and A. Jemal, *Cancer statistics, 2018.* CA Cancer J Clin, 2018. **68**(1): p. 7-30.
3. Oettle, H., et al., *Adjuvant chemotherapy with gemcitabine vs observation in patients undergoing curative-intent resection of pancreatic cancer: a randomized controlled trial.* JAMA, 2007. **297**(3): p. 267-77.
4. Malvezzi, M., et al., *European cancer mortality predictions for the year 2014.* Ann Oncol, 2014. **25**(8): p. 1650-6.
5. Conroy, T., et al., *FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer.* N Engl J Med, 2011. **364**(19): p. 1817-25.
6. Von Hoff, D.D., et al., *Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine.* N Engl J Med, 2013. **369**(18): p. 1691-703.
7. Wang-Gillam, A., et al., *Nanoliposomal irinotecan with fluorouracil and folinic acid in metastatic pancreatic cancer after previous gemcitabine-based therapy (NAPOLI-1): a global, randomised, open-label, phase 3 trial.* Lancet, 2016. **387**(10018): p. 545-557.
8. Sohal, D.P., et al., *Pancreatic adenocarcinoma: treating a systemic disease with systemic therapy.* J Natl Cancer Inst, 2014. **106**(3): p. dju011.
9. Stephen, A.G., et al., *Dragging ras back in the ring.* Cancer Cell, 2014. **25**(3): p. 272-81.
10. Basu, A., et al., *An interactive resource to identify cancer genetic and lineage dependencies targeted by small molecules.* Cell, 2013. **154**(5): p. 1151-1161.
11. Barretina, J., et al., *The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity.* Nature, 2012. **483**(7391): p. 603-7.
12. Moore, M.J., et al., *Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group.* J Clin Oncol, 2007. **25**(15): p. 1960-6.
13. Weinstein, I.B., *Cancer. Addiction to oncogenes--the Achilles heal of cancer.* Science, 2002. **297**(5578): p. 63-4.
14. Meric-Bernstam, F., et al., *Feasibility of Large-Scale Genomic Testing to Facilitate*

Enrollment Onto Genomically Matched Clinical Trials. J Clin Oncol, 2015. **33**(25): p. 2753-62.
15. Wang, X., J.R. Haswell, and C.W. Roberts, *Molecular pathways: SWI/SNF (BAF) complexes are frequently mutated in cancer--mechanisms and potential therapeutic insights.* Clin Cancer Res, 2014. **20**(1): p. 21-7.
16. Sumazin, P., et al., *An extensive microRNA-mediated network of RNA-RNA interactions regulates established oncogenic pathways in glioblastoma.* Cell, 2011. **147**(2): p. 370-81.
17. Chen, J.C., et al., *Identification of causal genetic drivers of human disease through systems-level analysis of regulatory networks.* Cell, 2014. **159**(2): p. 402-14.
18. Alvarez, M.J., et al., *Functional characterization of somatic mutations in cancer using network-based inference of protein activity.* Nat Genet, 2016. **48**(8): p. 838-47.
19. Forbes, S.A., et al., *COSMIC: mining complete cancer genomes in the Catalogue of Somatic Mutations in Cancer.* Nucleic Acids Res, 2011. **39**(Database issue): p. D945-50.
20. Compagno, M., et al., *Mutations of multiple genes cause deregulation of NF-kappaB in diffuse large B-cell lymphoma.* Nature, 2009. **459**(7247): p. 717-21.
21. Bisikirska, B., et al., *Elucidation and Pharmacological Targeting of Novel Molecular Drivers of Follicular Lymphoma Progression.* Cancer Res, 2016. **76**(3): p. 664-74.
22. Carro, M.S., et al., *The transcriptional network for mesenchymal transformation of brain tumours.* Nature, 2010. **463**(7279): p. 318-25.
23. Aytes, A., et al., *Cross-species regulatory network analysis identifies a synergistic interaction between FOXM1 and CENPF that drives prostate cancer malignancy.* Cancer Cell, 2014. **25**(5): p. 638-651.
24. Rodriguez-Barrueco, R., et al., *Inhibition of the autocrine IL-6-JAK2-STAT3-calprotectin axis as targeted therapy for HR-/HER2+ breast cancers.* Genes Dev, 2015. **29**(15): p. 1631-48.
25. Piovan, E., et al., *Direct reversal of glucocorticoid resistance by AKT inhibition in acute lymphoblastic leukemia.* Cancer Cell, 2013. **24**(6): p. 766-76.
26. Della Gatta, G., et al., *Reverse engineering of TLX oncogenic transcriptional networks identifies RUNX1 as tumor suppressor in T-ALL.* Nat Med, 2012. **18**(3): p. 436-40.
27. Bisikirska, B., et al., *Systematic Elucidation and Pharmacological Targeting of Follicular Lymphoma Progression Drivers: A New Spin on Precision Cancer Medicine.* Cancer Res, 2015. **In press**.
28. Mitrofanova, A., et al., *A systems biology approach to predict drug response for human prostate cancer based on in vivo preclinical analyses of mouse models.* Cell Reports, 2015. **12**: p. 1–12.
29. Chudnovsky, Y., et al., *ZFHX4 interacts with the NuRD core member CHD4 and regulates the glioblastoma tumor-initiating cell state.* Cell Rep, 2014. **6**(2): p. 313-24.
30. Putcha, P., et al., *HDAC6 activity is a non-oncogene addiction hub for inflammatory breast cancers.* Breast Cancer Res, 2015. **17**(1): p. 149.
31. Alvarez, M.J., et al., *A precision oncology approach to the pharmacological targeting of mechanistic dependencies in neuroendocrine tumors.* Nat Genet, 2018. **50**(7): p. 979-989.
32. Cartwright, T.H., et al., *Phase II study of oral capecitabine in patients with advanced or metastatic pancreatic cancer.* J Clin Oncol, 2002. **20**(1): p. 160-4.
33. Saif, M.W., et al., *Long-term survival on capecitabine in two gemcitabine refractory pancreatic cancer patients. Is there a pharmacogenetic explanation?* JOP, 2007. **8**(6): p.

799-805.
34. Chen, J., et al., *The tyrosine kinase inhibitor imatinib fails to inhibit pancreatic cancer progression.* Cancer Lett, 2006. **233**(2): p. 328-37.
35. Gore, J., et al., *TCGA data and patient-derived orthotopic xenografts highlight pancreatic cancer-associated angiogenesis.* Oncotarget, 2015. **6**(10): p. 7504-21.
36. Quintas-Cardama, A., et al., *Preclinical characterization of the selective JAK1/2 inhibitor INCB018424: therapeutic implications for the treatment of myeloproliferative neoplasms.* Blood, 2010. **115**(15): p. 3109-17.
37. Lesina, M., et al., *Stat3/Socs3 activation by IL-6 transsignaling promotes progression of pancreatic intraepithelial neoplasia and development of pancreatic cancer.* Cancer Cell, 2011. **19**(4): p. 456-69.
38. Tan, C.R., et al., *Pancreatic cancer cachexia: a review of mechanisms and therapeutics.* Front Physiol, 2014. **5**: p. 88.
39. Imrie, C.W., *Host systemic inflammatory response influences outcome in pancreatic cancer.* Pancreatology, 2015. **15**(4): p. 327-30.
40. Hurwitz, H.I., et al., *Randomized, Double-Blind, Phase II Study of Ruxolitinib or Placebo in Combination With Capecitabine in Patients With Metastatic Pancreatic Cancer for Whom Therapy With Gemcitabine Has Failed.* J Clin Oncol, 2015. **33**(34): p. 4039-47.
41. Hurwitz, H., et al., *Ruxolitinib + capecitabine in advanced/metastatic pancreatic cancer after disease progression/intolerance to first-line therapy: JANUS 1 and 2 randomized phase III studies.* Invest New Drugs, 2018. **36**(4): p. 683-695.
42. Budman, D.R., et al., *Preliminary studies of a novel oral fluoropyrimidine carbamate: capecitabine.* J Clin Oncol, 1998. **16**(5): p. 1795-802.
43. Miwa, M., et al., *Design of a novel oral fluoropyrimidine carbamate, capecitabine, which generates 5-fluorouracil selectively in tumours by enzymes concentrated in human liver and cancer tissue.* Eur J Cancer, 1998. **34**(8): p. 1274-81.
44. Schuller, J., et al., *Preferential activation of capecitabine in tumor following oral administration to colorectal cancer patients.* Cancer Chemother Pharmacol, 2000. **45**(4): p. 291-7.
45. Reigner, B., K. Blesch, and E. Weidekamm, *Clinical pharmacokinetics of capecitabine.* Clin Pharmacokinet, 2001. **40**(2): p. 85-104.
46. Mackean, M., et al., *Phase I and pharmacologic study of intermittent twice-daily oral therapy with capecitabine in patients with advanced and/or metastatic cancer.* J Clin Oncol, 1998. **16**(9): p. 2977-85.
47. Reigner, B., et al., *Effect of food on the pharmacokinetics of capecitabine and its metabolites following oral administration in cancer patients.* Clin Cancer Res, 1998. **4**(4): p. 941-8.
48. Poole, C., et al., *Effect of renal impairment on the pharmacokinetics and tolerability of capecitabine (Xeloda) in cancer patients.* Cancer Chemother Pharmacol, 2002. **49**(3): p. 225-34.
49. Gschwind, H.P., et al., *Metabolism and disposition of imatinib mesylate in healthy volunteers.* Drug Metab Dispos, 2005. **33**(10): p. 1503-12.
50. Peng, B., et al., *Absolute bioavailability of imatinib (Glivec) orally versus intravenous infusion.* J Clin Pharmacol, 2004. **44**(2): p. 158-62.
51. Baker, D.E., *Imatinib mesylate.* Rev Gastroenterol Disord, 2002. **2**(2): p. 75-86.

52. Shi, J.G., et al., *The pharmacokinetics, pharmacodynamics, and safety of orally dosed INCB018424 phosphate in healthy volunteers.* J Clin Pharmacol, 2011. **51**(12): p. 1644-54.
53. Verstovsek, S., et al., *Safety and efficacy of INCB018424, a JAK1 and JAK2 inhibitor, in myelofibrosis.* N Engl J Med, 2010. **363**(12): p. 1117-27.
54. Pallis, A.G., et al., *A multicenter randomized phase III trial of vinorelbine/gemcitabine doublet versus capecitabine monotherapy in anthracycline- and taxane-pretreated women with metastatic breast cancer.* Ann Oncol, 2012. **23**(5): p. 1164-9.
55. Miller, K.D., et al., *Randomized phase III trial of capecitabine compared with bevacizumab plus capecitabine in patients with previously treated metastatic breast cancer.* J Clin Oncol, 2005. **23**(4): p. 792-9.
56. El-Helw, L. and R.E. Coleman, *Reduced dose capecitabine is an effective and well-tolerated treatment in patients with metastatic breast cancer.* Breast, 2005. **14**(5): p. 368-74.
57. Baselga, J., et al., *Sorafenib in combination with capecitabine: an oral regimen for patients with HER2-negative locally advanced or metastatic breast cancer.* J Clin Oncol, 2012. **30**(13): p. 1484-91.
58. Nishijima, T.F., M. Suzuki, and H.B. Muss, *A comparison of toxicity profiles between the lower and standard dose capecitabine in breast cancer: a systematic review and meta-analysis.* Breast Cancer Res Treat, 2016. **156**(2): p. 227-36.
59. Hochhaus, A., et al., *Long-Term Outcomes of Imatinib Treatment for Chronic Myeloid Leukemia.* N Engl J Med, 2017. **376**(10): p. 917-927.
60. Druker, B.J., et al., *Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia.* N Engl J Med, 2001. **344**(14): p. 1031-7.
61. Talpaz, M., et al., *Imatinib induces durable hematologic and cytogenetic responses in patients with accelerated phase chronic myeloid leukemia: results of a phase 2 study.* Blood, 2002. **99**(6): p. 1928-37.
62. Cortes, J.E., et al., *Phase III, randomized, open-label study of daily imatinib mesylate 400 mg versus 800 mg in patients with newly diagnosed, previously untreated chronic myeloid leukemia in chronic phase using molecular end points: tyrosine kinase inhibitor optimization and selectivity study.* J Clin Oncol, 2010. **28**(3): p. 424-30.
63. Demetri, G.D., et al., *Imatinib plasma levels are correlated with clinical benefit in patients with unresectable/metastatic gastrointestinal stromal tumors.* J Clin Oncol, 2009. **27**(19): p. 3141-7.
64. Widmer, N., et al., *Relationship of imatinib-free plasma levels and target genotype with efficacy and tolerability.* Br J Cancer, 2008. **98**(10): p. 1633-40.
65. Farag, S., et al., *Imatinib Pharmacokinetics in a Large Observational Cohort of Gastrointestinal Stromal Tumour Patients.* Clin Pharmacokinet, 2017. **56**(3): p. 287-292.
66. Guilhot, F., et al., *Plasma exposure of imatinib and its correlation with clinical response in the Tyrosine Kinase Inhibitor Optimization and Selectivity Trial.* Haematologica, 2012. **97**(5): p. 731-8.
67. Bouchet, S., et al., *Therapeutic drug monitoring of imatinib in chronic myeloid leukemia: experience from 1216 patients at a centralized laboratory.* Fundam Clin Pharmacol, 2013. **27**(6): p. 690-7.
68. Picard, S., et al., *Trough imatinib plasma levels are associated with both cytogenetic and*

molecular responses to standard-dose imatinib in chronic myeloid leukemia. Blood, 2007. **109**(8): p. 3496-9.
69. Verstovsek, S., et al., *A double-blind, placebo-controlled trial of ruxolitinib for myelofibrosis.* N Engl J Med, 2012. **366**(9): p. 799-807.
70. *Cervantes F, Vannucchi AM, Kiladjian J-J, et al. Three-year efficacy, safety, and survival findings from COMFORT-II, a phase 3 study comparing ruxolitinib with best available therapy for myelofibrosis. Blood. 2013;122(25):4047-4053.* Blood, 2016. **128**(25): p. 3013.
71. Desmoulin, F., et al., *Metabolism of capecitabine, an oral fluorouracil prodrug: (19)F NMR studies in animal models and human urine.* Drug Metab Dispos, 2002. **30**(11): p. 1221-9.
72. Rudek, M.A., et al., *Fixed-dose capecitabine is feasible: results from a pharmacokinetic and pharmacogenetic study in metastatic breast cancer.* Breast Cancer Res Treat, 2013. **139**(1): p. 135-43.
73. Besnard, T., et al., *Optimized blood sampling with cytidine deaminase inhibitor for improved analysis of capecitabine metabolites.* J Chromatogr B Analyt Technol Biomed Life Sci, 2008. **870**(1): p. 117-20.
74. Peng, B., P. Lloyd, and H. Schran, *Clinical pharmacokinetics of imatinib.* Clin Pharmacokinet, 2005. **44**(9): p. 879-94.
75. von Mehren, M. and N. Widmer, *Correlations between imatinib pharmacokinetics, pharmacodynamics, adherence, and clinical response in advanced metastatic gastrointestinal stromal tumor (GIST): an emerging role for drug blood level testing?* Cancer Treat Rev, 2011. **37**(4): p. 291-9.
76. Josephs, D.H., et al., *Clinical pharmacokinetics of tyrosine kinase inhibitors: implications for therapeutic drug monitoring.* Ther Drug Monit, 2013. **35**(5): p. 562-87.
77. Shi, J.G., et al., *The effect of CYP3A4 inhibition or induction on the pharmacokinetics and pharmacodynamics of orally administered ruxolitinib (INCB018424 phosphate) in healthy volunteers.* J Clin Pharmacol, 2012. **52**(6): p. 809-18.
78. Beltran, H., et al., *Whole-Exome Sequencing of Metastatic Cancer and Biomarkers of Treatment Response.* JAMA Oncol, 2015. **1**(4): p. 466-74.
79. Qiu, W. and G.H. Su, *Development of orthotopic pancreatic tumor mouse models.* Methods Mol Biol, 2013. **980**: p. 215-23.
80. Sastra, S.A. and K.P. Olive, *Quantification of murine pancreatic tumors by high-resolution ultrasound.* Methods Mol Biol, 2013. **980**: p. 249-66.
81. Olive, K.P., et al., *Inhibition of Hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer.* Science, 2009. **324**(5933): p. 1457-61.
82. Rhim, A.D., et al., *Stromal elements act to restrain, rather than support, pancreatic ductal adenocarcinoma.* Cancer Cell, 2014. **25**(6): p. 735-47.

17. APPENDICES

17.1 Appendix A: Eastern Cooperative Oncology Group (ECOG) Performance Status

Grade	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.

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).
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.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.
* As published in Am. J. Clin. Oncol.: *Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.* The Eastern Cooperative Oncology Group, Robert Comis M.D., Group Chair.	

17.2 Appendix B: NCI Common Terminology Criteria for Adverse Events (CTCAE) V4.0 Grading Scale

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events version 4.0 will be utilized for adverse event reporting.

http://www.oncology.tv/SymptomManagement/NationalCancerInstituteUpdatesC TCAEtov403.a spx

17.3 Appendix C: Response Evaluation Criteria in Solid Tumors (RECIST) 1.1

Criteria for Evaluating Response in Solid Tumors RECIST version 1.1 will be used in this study for assessment of tumor response. While either CT or MRI may be utilized, as per RECIST 1.1, CT is the preferred imaging technique in this study.

In addition, volumetric analysis will be explored for response assessment.

17.4 Appendix D: Preclinical Studies – PDX/PDO Analyses

The initial biopsy from each newly diagnosed, treatment-naïve subject will be used both for OncoTreat profiling and to establish orthotopic PDX and/or PDO models from each patient in NSG mice (from an established NSG breeding colony). After outgrowth of the primary tumor/organoids, a portion will be banked and the bulk tumor dissected into 1-2 mm cubes for orthotopic re-implantation [79] into NSG recipients. For each primary tumor, we will evaluate a minimum of 6 and up to 10 candidate OncoTreat regimens and 2 controls (gemcitabine and vehicle). For each treatment, we will implant 8 tumors (5 for survival, 3 for PD analysis) plus 6 extra in case any fail to engraft (total = 70).

Tolerable schedules in mice for top-predicted drugs from our preliminary analysis have been developed. Additional agents will be assessed during the period when PDX/O models are engrafting, relying on a combination of published data and experimental assessments in mice using a dose escalation format and daily health, weight, and behavior assessments.

Upon reaching 150 mm^3, tumors will be enrolled and randomized to a treatment arm and treated until the animal meets endpoint criteria (based on a scoring system). Tumor volumes will be monitored twice weekly by 3D ultrasound [80]. The **primary endpoint** will be response rate, as determined by ultrasound-measured tumor volumes. In our (The Olive Laboratory) experience, replicate tumors from the same donor tend to respond uniformly to treatment, making 5 animals sufficient for analysis of response rate and allowing us to evaluate more regimens from one donor sample.

Three animals per treatment group will be euthanized 24 hours after receiving their third treatment dose for assessment of pharmacodynamics response. RNA-seq will be performed on bulk tissue samples, with computational filtering for human reads to assess effects on the epithelial compartment. VIPER analysis will be performed on the tumor profiles to determine whether the treatment reversed the activity of the predicted MRs relative to vehicle-treated tumors. In addition, tumor histopathology will be analyzed by Dr. Alina Iuga in the Department of Pathology. Immunohistochemistry (IHC) will also be performed to assess proliferation (Ki-67, phospho-histone H3) and apoptosis (cleaved caspase 3) [81, 82].

17.5 Appendix E: LC-MS/MS Method

Specimen/*Sample Type:* Serum

Volume: 300µL

Sample Stability: all analytes are stable in serum samples at room temperature for 5 hours, at 4-8 °C for 3 days, and at -80 °C for at least 60 days.

Instrument and materials:

- ACQUITY Xevo TQ-D with ACQUITY UPLC® CSH C18 3.0x100mm 1.7 µm column (Waters Corporation)
- Single-channel pipettes: 20µl, 100µl,200µl and 1000µl air displacement pipettes
- Vortex mixer
- Centrifuge
- Vials Waters Acquity UPLC H-Class System and H-Class Bio System
- Eppendorf Centrifuge 5427 R
- Balance Mettler Toledo AL54

Sample preparation – extraction by protein precipitation

1- In a 1.5 ml eppendorf tube add 120 µl sample (blank, double blank, calibrators, QC low, QC middle, QC high, and patient samples)
2- Add 10 µl ISTD working solution
3- Add 500 µl methanol
4- Mix with vortex for 5 min
5- Centrifuge at 14000 rpm, 15 °C, for 15 min
6- Take 240 µl supernatant and transfer into an UHPLC autosampler vial containing 200 µl water and mix.
7- Place the samples in the autosampler of the instrument and start the analytical run. 10ul of each sample will be injected for LC-MS/MS analysis.

Mobile Phase Preparation:

1. 2M ammonium acetate in water (stock):
   77.08g ammonium acetate
   500 ml water (use up 6 months of being prepared)
2. Mobile Phase A:
   2mM ammonium acetate and 0.1% formic acid (v/v) in water
   1.0 ml 2M ammonium acetate solution (above step 1)
   1.0 ml Formic acid
   998 ml optima grade water
3. Mobile Phase B:
   2mM ammonium acetate and 0.1% formic acid (v/v) in methanol
   1.0 ml 2M ammonium acetate solution (above step 1)
   1.0 ml Formic acid
   998 ml optima grade Methanol
4. Wash Solvent: 50% optima grade acetonitrile / 50% optima grade water
5. Needle Wash: 25% optima grade methanol / 25% optima grade acetonitrile / 50% optima grade 2-propanol
6. Seal Wash: 80% optima grade water / 20% optima grade methanol

Liquid Chromatography settings

File name of inlet method: Assay extension II.acm/.ftn/.qsm

Time (min)	Flow (ml/min)	%A	%B	Curve
0	0.500	85	15	6
0.5	0.500	85	15	6
1.20	0.500	60	40	6
5.00	0.500	5	95	6
5.2	0.500	5	95	6
5.21	0.500	85	15	6
5.50	0.500	85	15	11

Xevo TQD Settings

Ion Mode: ESI(+)
Capillary Voltage: 1.10 kV
Source Temperature: 150˚C
Desolvation Gas Temperature: 600°C
Cone Gas Flow: 50 L/Hr
Desolvation Gas Flow: 900 L/Hr
MS Mode: Multiple Reaction Monitoring (MRM)