

MC1563: Phase 1 Dose Escalation and Expansion Cohort Trial of Carboplatin and Gemcitabine with or without M6620 (VX-970) in First or Second Recurrence Platinum-Sensitive Epithelial Ovarian, Peritoneal, and Fallopian Tube Cancer

MCCC Amendment 7

1	<u>Title page and headers</u>	Updated to reflect the current Protocol Version date submitted in response to an Amendment from CTEP
2	<u>Section 2.51</u>	Change “Pharmacodynamic Assay Development & Implementation Section (PADIS)” to “PADIS, Frederick National Laboratory for Cancer Research (FNLCR)”.
3	<u>Section 9.1.1.2</u>	Replace “Dr. Kinders” with “Dr. Ralph Parchment”.
4	<u>Section 9.1.1.3</u>	Delete entire section and add the below: The pATR and Rad51 IFA assays will be performed in the laboratory of Dr. Parchment at PADIS, Frederick National Laboratory for Cancer Research (FNLCR) under the guidance of Dr. Deborah Wilsker. γH2AX, pNBS1 IFA with βCATN segmentation assay will be performed in the NCLN PD Assay Laboratory at MD Anderson under the guidance of Dr. Kate Ferry-Galow.
5	<u>Section 9.1.1.5</u>	Replace the sentence “Samples will be submitted to Dr. Kinders' laboratory for evaluation of DNA damage markers, and H&E pathology evaluation.” with the below: Samples will be first submitted to Dr. Ralph Parchment’s laboratory at PADIS, Frederick National Laboratory for Cancer Research (FNLCR), then routed to IFA assay performance sites as described in Section 9.1.1.3.
6	<u>Section 9.2.1.3</u>	Replace “Frederick National Laboratory for Cancer Research (FNLCR) Pharmacodynamic (PD) Specimen Central Receiving” with “PADIS, Frederick National Laboratory for Cancer Research (FNLCR) Specimen Central Receiving”. Replace “Email FNCLR PD specimen Control Receiving” with “PADIS, Frederick National Laboratory for Cancer Research (FNLCR) Specimen Central Receiving”.
7	<u>Section 9.2.1.4</u>	Delete entire section and add the below: The pATR and RAD51 IFA assays will be performed at PADIS, Frederick National Laboratory for Cancer Research (FNLCR). The γH2AX, pNBS1 IFA with βCATN segmentation assay will be performed at the NCLN PD Assay Laboratory at MD Anderson.
8	ICD	Version date changed to match new protocol

TITLE: Phase 1 Dose Escalation and Expansion Cohort of Carboplatin and Gemcitabine with or without M6620 (VX-970) in First or Second Recurrence Platinum-Sensitive Epithelial Ovarian, Peritoneal, and Fallopian Tube Cancer

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NCI-Supplied Agent: M6620 (VX-970) (NSC 780162)

Other Agents: Carboplatin (NSC 241240) and Gemcitabine (NSC 613327), commercial supply

IND #: [REDACTED]

IND Sponsor: DCTD, NCI

Protocol Type / Version # / Version Date:

Original / Version 1 / October 20, 2015

Resubmission / Version 2 / January 29, 2016

Resubmission / Version 3 / May 24, 2016

Resubmission / Version 4 / July 11, 2016

Resubmission / Version 5 / August 3, 2016

Resubmission / Version 6 / September 29, 2016

CTSU Activation November 4, 2016

Amendment 1 / Version 7 / February 2, 2017

Amendment 2 / Version 8 / March 12, 2018

Amendment 2 / Version 9 / May 3, 2018

Amendment 2 / Version 10 / May 18, 2018

Amendment 2 / Version 11 / July 17, 2018

Amendment 3 / Version 12 / August 22, 2018

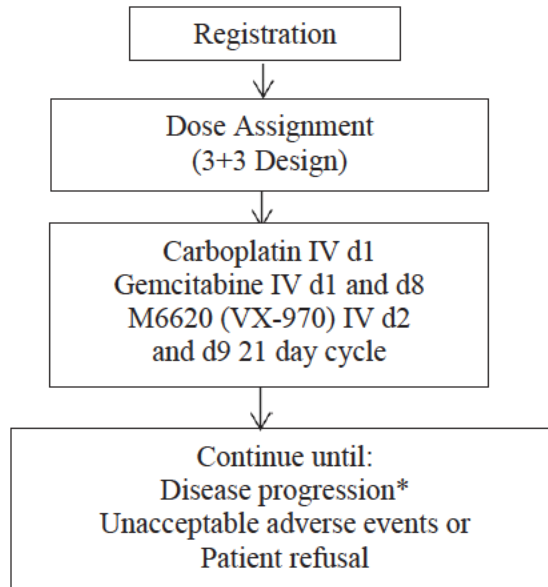
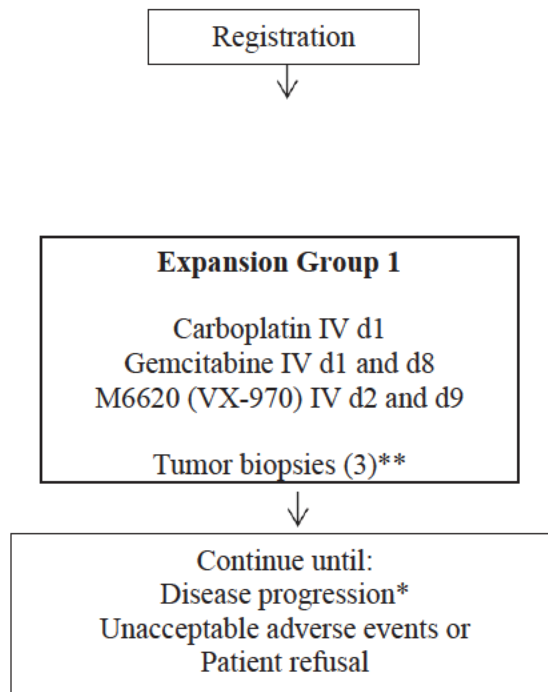
Amendment 4 / Version 13 / August 26, 2019

Amendment 5 / Version 14 / ~~October 18, 2019~~ November 20, 2019

Amendment 6 / Version 15 / July 23, 2020

Amendment 7 / Version 16 / February 25, 2021

SCHEMA

Phase 1 Dose Escalation/Safety Lead-In:**Expansion Cohort:**

*For both dose escalation and expansion cohorts, if a CR is obtained and imaging after two cycles of additional therapy remains CR, consideration should be given to discontinuation of therapy and initiation of observation or PARP inhibitor maintenance. PARP inhibitor maintenance therapy with olaparib, niraparib or

rucaparib per FDA approved indications is allowed but is not part of this protocol. Bevacizumab therapy while on the clinical trial is not allowed.

** Patients will undergo three biopsies. Patients will all have a pretreatment biopsy (up to three weeks prior to initiation of therapy) and be randomly assigned to have either the 18-24 h post carboplatin/gemcitabine biopsy on day 2 cycle 1 or the post-M6620 biopsy in cycle 1 (day 2 6 h after treatment with M6620) and the other biopsy on day 2 in cycle 2.

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1. OBJECTIVES

1.1 Primary Objectives

1.1.1 Phase 1 Dose Escalation/Safety Lead-in

- Assess safety and tolerability of the combination therapy carboplatin, gemcitabine and M6620 (VX-970) in adult women with platinum sensitive recurrent high grade serous or high grade endometrioid ovarian, primary peritoneal or fallopian tube cancer.
- Determine the dose of the triple therapy to be used in the dose expansion cohort of the study.

1.1.2 Expansion Cohort

- Confirm the safety at the maximum tolerated dose (MTD) for the addition of M6620 (VX-970) to carboplatin and gemcitabine in first or second recurrence of platinum sensitive high grade serous or endometrioid ovarian, primary peritoneal or fallopian tube carcinoma.

1.2 Integrated Correlative Study Objectives

1.2.1 Collection of specimens for biomarker studies to provide preliminary proof of mechanism. Assess, in an exploratory fashion, whether the combination of gemcitabine and carboplatin activates the ATR/CHK1 pathway at achievable concentrations and also whether M6620 inhibits the activated pathway.

1.2.2 To determine whether increased DNA damage as assessed by two different multiplex assays correlates with response to combination therapy with M6620 (VX-970).

1.2.3 . To determine whether mutations in homologous recombination repair genes correlate with response to combination therapy with M6620 (VX-970). To ascertain modulation of ATR autophosphorylation and other pharmacodynamic readouts for ATR inhibition by M6620 (VX-970).

1.2.4 To ascertain modulation of ATR autophosphorylation and other pharmacodynamic readouts for ATR inhibition by M6620 (VX-970).

1.3 Secondary Objectives

1.3.1 To determine if the MTD for the combination of carboplatin, gemcitabine and M6620 (VX-970) improves the confirmed response rate in adult women with platinum sensitive recurrent high grade serous or high grade endometrioid ovarian, primary peritoneal or fallopian tube cancer.

1.2.5 1.3.2 To determine the impact of the MTD on overall survival (OS), duration of response, and progression-free survival (PFS).

2. BACKGROUND

2.1 Ovarian, Peritoneal and Fallopian Tube Cancer

Overview of Ovarian Cancer- Unmet Clinical Need

In 2014, ovarian cancer accounted for approximately 21,980 (3%) of new cancer cases and 14,270 (6%) cancer deaths in women [1]. It has the highest mortality of all gynecologic malignancies. In 75% of newly identified cases, the disease is already advanced (stage III or IV) at the time of diagnosis [2]. First line treatment generally consists of a surgical procedure to debulk as much tumor as feasible as well as systemic chemotherapy, most often a platinum salt (cisplatin or carboplatin) and a taxane (most often paclitaxel) administered intravenously or intraperitoneally [2, 3]. Despite this initial rigorous treatment, more than 75% of patients experience a recurrence within the first 20 months of therapy completion [2]. Current management of recurrent ovarian cancer is guided by the interval of time to disease recurrence and is categorized according to the response to platinum therapy. Patients who progress during front line chemotherapy are defined as having platinum refractory disease and those who progress within 6 months after initial therapy are considered platinum resistant. In these cases, the response rate to further therapies ranges from 15-25% [4-6]. Those who recur more than 6 months after completion of initial therapy are considered to be platinum sensitive and a platinum-based chemotherapy regimen is used upon recurrence. Platinum sensitivity portends a slightly better prognosis with a higher response rate to subsequent therapy as well as improved overall survival compared to those with platinum resistant disease [2].

In general, combination therapy is preferred in platinum sensitive recurrence, with three regimens commonly used showing approximately the same response rates, namely carboplatin plus pegylated liposomal doxorubicin [7], carboplatin plus paclitaxel [8], or carboplatin plus gemcitabine [9]. The ideal platinum based combination is not known and the choices depend on physician and patient preferences regarding side effect profile, ease of scheduling and possible patient comorbidities. More recent studies have also evaluated the role of bevacizumab in ovarian cancer [10-14]. Bevacizumab has been shown to improve progression free survival when used in first line therapy with maintenance therapy [10, 11], in combination with carboplatin and gemcitabine in platinum sensitive recurrence [13] as well as with several FDA approved agents in platinum resistant disease [12]. Currently, bevacizumab is only FDA approved in the setting of platinum resistant disease. For this reason, bevacizumab is not being investigated in this trial.

ATR and DNA Damage response

The DNA-damage response (DDR) is the term applied to a complex network of signaling pathways involved in surveillance and repair of DNA damage and transient cell cycle

arrest to ensure genomic stability and cell viability [15-17]. Deficiencies in DDR mechanisms have been shown to contribute to tumor development. The primary sensors of DNA damage and regulators of DDR are ataxia telangiectasia mutated (ATM) and ataxia telangiectasia Rad3-related (ATR) protein kinases. They both contribute to maintaining genome integrity in response to various exogenous and endogenous genotoxic insults, *e.g.*, cytotoxic chemotherapy, ultraviolet light, ionizing radiation (IR), or hypoxia [15, 18, 19]. Although ATR and ATM have broadly overlapping substrate specificities, they have non-redundant functions, which are well coordinated during DDR.

ATR appears to be primarily activated by single-strand DNA (ssDNA) breaks (SSB) during replicative stress while ATM is a main sensor of double-strand DNA (dsDNA) breaks (DSB). The key outcomes of ATR activation are inhibition of cell-cycle progression and suppression of late replicating origin firing [17]. ATR not only helps to stabilize but also restarts stalled replication forks, and suppresses recombination. ATR is recruited to the sites of SSB at stalled replication forks resulting from replication stress [15, 18]. ATR phosphorylates/activates checkpoint kinase 1 (CHK1) at serine 345 (CHK1pS³⁴⁵), which stabilizes stalled replication forks until replication stress is resolved and DNA damage is repaired. Activated CHK1 phosphorylates and inhibits the cell division cycle 25A (CDC25A) phosphatase, which ultimately results in cell cycle arrest in intra-S-phase and/or G2-phase and blocks cells from entering mitosis until DNA is repaired and completely replicated [18, 20]. ATR function is not entirely restricted to CHK1 activation as it has been shown to be independently involved in replication of DNA and regulation of a DNA-damage protein network [15].

Upon detecting DSBs, ATM activates CHK2, which controls p53-dependent G1-phase arrest. Unlike normal cells, cancer cells are often deficient in ATM-CHK2-p53 signaling. It has been hypothesized that loss of the G1 checkpoint renders tumor cells more reliant on the ATR-controlled S/G2 checkpoints for repairing DNA damage and survival [15, 21]. Therefore, in tumor cells with defective p53, ATR inhibition may exacerbate replication stress leading to accumulation of DSBs, collapse of stalled replication forks, and eventually to lethal mitotic catastrophe. In contrast, normal cells that exhibit a low level of replicative stress and have functional ATM are expected to tolerate ATR inhibition. Indeed, preclinical studies have shown that disruption of the ATR pathway can exacerbate replication stress in oncogene-driven tumors and promotes cell killing. In addition, tumor cells, which proliferate rapidly, are more susceptible to the cytotoxic effects of chemotherapy and radiation than slowly proliferating normal cells [15, 16]. However, the effectiveness of such DNA damage-inducing therapies in cancer treatment is attenuated by cells developing drug resistance, leading to tumor recurrence. Acquired resistance to cytotoxic therapies in tumors has been linked to the activation of DDR. There is accumulating preclinical evidence that ATR inhibition can sensitize tumor cells to the effects of radiation or chemotherapy as described below [22].

2.2 M6620 (VX-970)

2.2.1 Mechanism of Action

M6620 (VX-970) (former names VET-0768079 or VE-822) is a highly potent and selective ATP- competitive inhibitor of ATR, with an inhibition constant (K_i) <0.2 nmol/L (nM) [22, 23]. In comparison, M6620 (VX-970) was >100 -fold weaker inhibitor of ATM ($K_i=34$ nM) and >1000 -fold less effective against other closely related kinases, such as DNA-dependent protein kinase (DNA-PK) ($K_i>4$ mcM), mTOR ($K_i>1$ mcM), and PI3K-gamma ($K_i=0.22$ mcM) [22]. Overall, among 291 kinases tested, M6620 (VX-970)'s K_i values were >500 -fold higher for 278 kinases ($K_i>200$ nM), >50 -fold higher for 12 kinases ($K_i>15$ nM), and >25 -fold higher for FLT4 ($K_i=8$ nM) than its K_i for ATR [23]. A cellular 50% inhibition of ATR was attained at a M6620 (VX-970) concentration (IC_{50}) of 0.019 mcM, demonstrating >100 -fold greater selectivity against ATR compared to ATM or DNA-PK (IC_{50} of 2.6 mcM or 18.1 mcM, respectively) [22].

Effect of M6620 (VX-970) on DDR Signaling and DNA Damage

Concurrent treatment of cancer cell lines with M6620 (VX-970) and various DNA-damaging agents led to sustained M6620 (VX-970)-dose-dependent decreases in levels of chemotherapy- induced CHK1pS³⁴⁵, a major substrate of ATR [22, 23]. In the presence of DNA damage, primarily DSBs, histone H2AX is phosphorylated at Ser139 to produce γ H2AX (H2AXpS¹³⁹). Although all three DDR regulatory kinases, ATM, ATR and DNA-PK, phosphorylate H2AX to γ H2AX, they are variably activated during different DNA- damage repair mechanisms (*e.g.*, HR repair, non-homologous end joining [NHEJ] repair, base excision repair due induced by stalled replication forks, *etc.*) [24]. In addition, for efficient DNA-damage repair, the DDR regulatory kinases must be able to access damaged sites in the chromatin environment. ATM has been shown to phosphorylate the heterochromatin protein KAP1 at serine 824 (KAP1pS⁸²⁴) in response to DNA damage [25]. Exposure of lung cancer cell lines as well as primary tumors to M6620 (VX-970) in combination with DNA-damaging agents enhanced levels of the DNA-damage markers, *i.e.*, γ H2AX and KAP1pS⁸²⁴, as compared to DNA-damaging agent alone [23, 26].

Sequential treatment of cells with DNA-damaging agent followed 15 h later by M6620 (VX-970) resulted in an initial inhibition of phospho-CHK1 (for 1 to 2 h) [23]. However, over time, phospho-CHK1 reappeared despite continued exposure to M6620 (VX-970). The rebound of phospho-CHK1 has been attributed to non-specific phosphorylation by an undefined kinase. However, despite the transient inhibition of phospho-CHK1, the sustained accumulation of γ H2AX and KAP1pS⁸²⁴ was observed. Together these data suggest that disruption of ATR-mediated DDR signaling by M6620 (VX-970) leads to sustained accumulation of DNA damage in cancer cells exposed to DNA-damaging agents. Failure to repair chemotherapy-induced DNA damage in the presence of M6620 (VX-970) has been hypothesized to drive enhanced cytotoxicity in cancer cells. These data support using γ H2AX and KAP1pS⁸²⁴ as pharmacodynamic markers of M6620 (VX-970) activity.

M6620 (VX-970)-mediated radiosensitivity of pancreatic ductal adenocarcinoma cells was associated with inhibition of HR repair [22]. M6620 (VX-970) caused increased persistence of γ H2AX levels both *in vitro* and *in vivo*. Adding M6620 (VX-970) to gemcitabine and ionizing radiation (IR) dramatically enhanced antitumor effects, with early and late apoptosis and abrogation of IR-induced G2 checkpoint in cell culture experiments. It has been suggested that by promoting strong S-phase arrest, chemoradiation may further increase dependence of tumor cells on ATR-initiated HR-mediated repair of DSBs and survival.

2.2.2. Nonclinical Studies

In vitro Antitumor Activity

In the absence of exogenous DNA-damaging agents, M6620 (VX-970) demonstrated stronger antiproliferative effects against three cancer cell lines tested (HCT116, HT29, and NCI-H23 with IC₅₀s of 35, 48, and 170 nM, respectively) compared to noncancerous fibroblast and epithelial cells (IC₅₀=110-200 nM) [23]. However, among the three cancer cell lines, potent cytotoxicity by single-agent M6620 (VX-970) was seen only in a colorectal cancer [CRC] cell line HCT116: a 50% effect (death in 50% of cells) was observed at a concentration of 61 nM M6620 (VX-970) (EC₅₀). This suggests that certain cancer cells may be particularly reliant on ATR for survival even in the absence of an exogenous DNA-damaging agent.

In the cell proliferation assay with the HCT116 cell line, M6620 (VX-970) synergized with cisplatin (cross-linking agent), gemcitabine (anti-metabolite), irinotecan (topoisomerase I inhibitor), and etoposide (topoisomerase II inhibitor) [23]. The most dramatic response was observed in combination with cisplatin (a 20-fold lower IC₅₀ compared to the IC₅₀ of cisplatin alone). Preliminary data from cell proliferation studies with M6620 (VX-970) + carboplatin also suggests >10-fold reduction in carboplatin IC₅₀ for two non-small cell lung cancer (NSCLC) cell lines (H23 and HT1299) tested.

The impact of M6620 (VX-970) on chemotherapy-induced cytotoxicity was further examined against a panel of 37 lung cancer cell lines (including squamous NSCLC and small cell lung cancer [SCLC] histotypes) and 15 pancreatic cancer cell lines [23]. Most lung cancer cell lines responded well to M6620 (VX-970) in combination with cisplatin (84% of cell lines) or gemcitabine (76% of cell lines), demonstrating ≥ 3 -fold reduction in the IC₅₀ compared to IC₅₀ of the cytotoxic agent alone [23, 27]. Enhanced sensitivity was also observed with etoposide (53% of cell lines), irinotecan (49% of cell lines) and oxaliplatin (39% of cell lines). About 40% of cell lines were hypersensitized (>10-fold reduction in IC₅₀ observed) to cisplatin by M6620 (VX-970). Marked synergy between the two agents was also seen against four of seven human NSCLC primary tumors tested *in vitro* [27]. The greatest antitumor synergistic effect was observed in tumors with poor response to cisplatin alone. Similarly, most pancreatic cancer lines responded well to combination of M6620 (VX-970) with cisplatin or gemcitabine: antitumor IC₅₀ was ≥ 3 -fold lower for the M6620 (VX-970) + cytotoxic agent in >70% of cell lines as compared to IC₅₀ of cytotoxic agent alone [23].

In addition, significant radiosensitization effects of M6620 (VX-970) were observed against two human pancreatic cancer cell lines with mutant KRAS and mutant p53 (MiaPaCa-2 and PSN1) ($p < 0.05$), but not against non-cancerous fibroblast cell lines

[22]. In addition, M6620 (VX-970) profoundly sensitized pancreatic tumor cells to gemcitabine-based chemoradiation. Impact of defective p53 signaling on sensitivity of cells to M6620 (VX-970) in combination with a cytotoxic agent (cisplatin, gemcitabine, irinotecan, oxaliplatin, or etoposide) was examined in isogenic matched lung cancer cells (wild-type p53 A549 versus A549 transfected with p53 shRNA), using a cell viability assay [23, 26]. Loss of p53 promoted sensitivity to ATR inhibition in combination with all five cytotoxic agents in contrast with the effects in wild-type A549. M6620 (VX-970) also synergized with cisplatin resulting in cytotoxicity in ATM-null primary skin fibroblasts, but no cytotoxicity was observed against wild-type fibroblasts [23]. This suggests that the functional status of the ATM pathway is a contributing factor in the cellular response to the inhibition of ATR.

Of note, the response/p53 status relationship was unclear in the panel of heterogeneous cancer cell lines exposed to M6620 (VX-970) + chemotherapy [27]. Although not significant, there was a trend toward improved response with *TP53* mutations ($p=0.08$) for M6620 (VX-970) combined with cisplatin. Furthermore, no clear relationship between cellular response to M6620 (VX-970) + cisplatin and p53 status was observed in seven primary lung tumors.

In vivo Antitumor Activity

The *in vivo* activity of M6620 (VX-970) was tested in multiple mouse xenograft models derived from human lung cancer cell lines and primary human tumor cells [23, 26]. M6620 (VX-970) potentiated antitumor effects of cisplatin, gemcitabine, irinotecan, and IR in a dose- dependent as well as schedule-dependent manner. Antitumor efficacy correlated with inhibition of phospho-CHK1 and an increase in DNA-damage markers. This supports ATR inhibition as a primary mechanism of action for M6620 (VX-970). Single-agent M6620 (VX-970) had no significant effect on tumor growth in the experimental models. M6620 (VX-970) was generally well tolerated at efficacious doses in combination with DNA-damaging agents. Some body weight loss and enhanced changes in specific peripheral blood cell populations were observed with intensive and sustained dosing of M6620 (VX-970) in combination with cisplatin. This effect could be attributed to an increased growth arrest, which was observed *in vitro* in normal cells for combinations of M6620 (VX-970) with DNA-damaging agents. This effect was reversed when ATR activity was restored. M6620 (VX-970) sensitized pancreatic tumor xenografts to the cytotoxic effects of gemcitabine-based chemoradiation [22]. The combination treatment was effective even at gemcitabine doses with no single-agent activity. M6620 (VX-970) administered in combination with gemcitabine + IR was well tolerated.

In schedule optimization studies, M6620 (VX-970) was administered intravenously (IV) at 20 mg/kg (either as a single injection or as two 10 mg/kg injections 3 days apart) before (-2 h) or after cytotoxic agent (+12, 24, or 48 h) in two human pancreatic cancer and NSCLC xenograft mouse models. M6620 (VX-970) effectively enhanced antitumor activity of gemcitabine or cisplatin when administered 12 to 24 h after a cytotoxic agent. M6620 (VX-970) administered before cytotoxic drug or greater than 48 h after a DNA-damaging agent had no impact on tumor growth compared to the effect of cytotoxic agent alone.

Therapeutic human dose has been estimated based on the efficacious exposure achieved

at 20 mg/kg/week of M6620 (VX-970) (given either as a single IV injection or as two IV injections of 10 mg/kg per week) 12-24 h after cytotoxic agent (gemcitabine or cisplatin) in mice.

The target M6620 (VX-970) plasma exposure, which corresponded to this dose, was an area under the concentration-time curve (AUC) of 4080 ng×h/mL/week. Allometry predicts that a human dose of 2.5 mg/kg (100 mg/m²) will be sufficient to achieve this exposure.

Nonclinical Pharmacokinetics

In all non-clinical species (the mouse, rat, dog, and monkey), M6620 (VX-970) exhibited a high volume of distribution (V_d); tissue exposure, including tumor, was high. In rats, no accumulation or retention was observed in tissues and the elimination half-lives ($t_{1/2}$) were similar across all tissues and whole blood (Investigator's Brochure, 2015). The whole blood $t_{1/2}$ was 11.6 h in rats and 9.8 h in dogs. M6620 (VX-970) was extensively bound to plasma proteins; the free fraction of M6620 (VX-970) was only 2.1% in human blood.

M6620 (VX-970) is primarily eliminated by oxidative metabolism, with a cytochrome P450 (CYP) 3A4 isoform being the principal isoform responsible. Strong inducers or inhibitors of CYP3A4 may alter M6620 (VX-970) kinetics and blood levels. Based on its minimal inhibition or induction effects on CYPs, M6620 (VX-970) is expected to have a low potential for drug-drug interactions. M6620 (VX-970) metabolites were excreted in the urine and bile. All metabolites observed in human hepatocyte incubations were also observed in either rat or dog hepatocyte incubations and in the blood, bile, or urine from rats or dogs. The systemic clearance of M6620 (VX-970) following IV administration was 26 and 13 mL/min/kg in the rat and dog, respectively.

Nonclinical Safety Pharmacology

An in-house manual patch-clamp human ether-a-go-go-related gene (hERG) assay demonstrated moderate inhibition of the hERG channel [23]. However, a telemetry dog study did not demonstrate any cardiovascular (CV) effects at exposures greatly exceeding the target human exposure.

Nonclinical Toxicology

M6620 (VX-970) was administered PO or IV for up to 28 days in rats and dogs. The oral studies used an aggressive dosing regimen (every 2 days) to define the toxicity profile, while IV studies (dosed twice per week) were more representative of the planned clinical dosing schedule [23]. In the rat, the severely toxic dose in 10% of animals (STD₁₀) was 30 mg/kg/day IV. The highest non-severely toxic dose (HNSTD) in dogs was 20 mg/kg/day

IV. The target organs for M6620 (VX-970) toxicity in rats included testes and peripheral blood cell populations (red cell mass, eosinophils, and platelets). Target organs in the dog included the liver, testes, and peripheral blood cell populations (red cell mass and eosinophils); changes in these organs appeared to be reversible after discontinuing of M6620 (VX-970) in both rats and dogs.

M6620 (VX-970) had no cardiovascular liabilities, was not genotoxic in mutagenicity assay, had no hemolytic potential in human blood or compatibility issues in human plasma, and was well tolerated in an acute rabbit parenteral injection study. M6620 (VX-970) does absorb in the ultraviolet (UV) spectrum and has high tissue distribution in rats.

M6620 (VX-970) has yet not been assessed in developmental and reproductive toxicity studies. However, VX 970 inhibits DNA-damage repair and will be administered in conjunction with cytotoxic chemotherapy, thus the potential for teratogenicity should be considered high.

2.2.3 Clinical Studies

The suggested starting dose of M6620 (VX-970) in humans, 18 mg/m² IV, was equivalent to 1/10 of the rat STD₁₀ (30 mg/kg or 180 mg/m²) [23]. This dose represents a more conservative estimate than 37 mg/m² IV which would be an estimate corresponding to the 1/6 of the dog HNSTD (20 mg/kg or 222 mg/m²).

Vertex Pharmaceuticals, Inc. has sponsored the first-in-human M6620 (VX-970) phase 1 study with M6620 (VX-970) being administered in combination with DNA-damaging agents to patients with advanced solid malignancies (Study 001); the study is ongoing [23]. This study evaluates M6620 (VX-970) in combination with either gemcitabine +/- cisplatin or cisplatin +/- etoposide. M6620 (VX-970) is dose-escalated (18, 36, 60, 72 mg/m² IV) following the standard 3+3 design. To allow for the single-agent M6620 (VX-970) PK, a 7-day lead-in treatment period of M6620 (VX-970) before cycle 1 has been included. Combinations of M6620 (VX-970) with gemcitabine or cisplatin are administered on a weekly schedule, with M6620 (VX-970) being dosed 24 h after a DNA-damaging agent.

Vertex Pharmaceuticals, Inc. has also sponsored a second trial, study 002, to evaluate the safety and tolerability of multiple ascending doses of M6620 (VX-970) administered once weekly (Part A1) or twice weekly (Part A2) as a single agent in advanced solid tumors as well as the safety and tolerability of M6620 (VX-970) when administered in combination with carboplatin (Part B1) and carboplatin and paclitaxel (Part B2) in subjects with advanced solid tumors to determine MTD. The study is currently ongoing.

Clinical Pharmacokinetics

Clinical PK have been evaluated both in whole blood and plasma [23]. Preliminary clinical PK data are available from the lead-in period for the first two cohorts (M6620 (VX-970) 18 mg/m² and 36 mg/m²). Mean exposure (AUC) profiles were similar in whole blood and plasma. The terminal elimination t_{1/2} was approximately 16 h across all doses.

Overall, the C_{max} was 1.36x greater and AUC_{0-∞} 1.43x greater in whole blood than in plasma. The results suggest that plasma is an appropriate matrix to characterize the VX-970 PK. M6620 (VX-970) exposures were similar for the agent administered alone and in combination with gemcitabine, suggesting no apparent drug-drug interactions. In the M6620 (VX-970) single dose studies, the plasma C_{max} and AUC_{0-∞} increase in linear fashion with doses up to 480 mg/m².

Clinical Efficacy

Preliminary efficacy data are available for 48 patients treated with M6620 (VX-970) in combination with cisplatin (Study 001 Part A; data cutoff date March 21, 2016), 23 patients treated with M6620 (VX-970) and gemcitabine (Study 001 Part B; data cutoff date February 23, 2016), 17 patients treated with M6620 (VX-970) single agent (Study 002 Part A; data cutoff date: March 14, 2016), and 17 patients treated with M6620 (VX-970) in combination with carboplatin (Study 002 Part B1; data cutoff date: March 14, 2016). In study 002 Part A (single agent M6620 (VX-970)), all 17 subjects who received at least 1 dose of single agent VX- 970 therapy either once weekly (Part A1) or twice weekly (Part A2) were included. Six of the 17 subjects (35.3%) had disease control, defined as CR+PR+SD. One subject with colon cancer had a CR. There were no PRs but 5 of the 17 subjects had stable disease and including patients with ovarian, prostate, gallbladder and gastric carcinomas as well as peritoneal mesothelioma. PFS ranged from 1 day to 582+ days.

In Study 001 Part A (M6620 (VX-970) and gemcitabine combination therapy), 4 of 48 patients had a PR and 29 subjects had SD. PFS for all subjects ranged from 1+ to 443 days. In Study 001 Part B (M6620 (VX-970) plus cisplatin therapy), 16 of 23 patients had disease control. These included four PRs, all of which occurred in patients with platinum resistant malignancies, and 12 subjects with SD. PFS ranged from 17 to 172 days. In Study 002 Part B1 (VX- 970 and carboplatin) 13 of 17 patients had disease control. One patient with ovarian cancer had a PR and 12 patients had SD. PFS ranged from 37 to 234 days.

Clinical Safety

Preliminary safety data for 77 patients receiving M6620 (VX-970) in combination with gemcitabine or cisplatin (Study 001) and 36 patients receiving M6620 (VX-970) alone or in combination with carboplatin (Study 002) can be found in Investigator's Brochure (2016)[23]. No dose-limiting toxicities (DLTs) were observed during either 7- to 14-day or 21-day lead-in periods of M6620 (VX-970) monotherapy in Study 001. DLTs and serious adverse events are summarized in the tables below.

When treated with M6620 (VX-970) in combination with gemcitabine (Study 001 Part A), 26 (52%) subjects had at least one grade ≥ 3 AE that was considered related to the study drug treatment. Grade ≥ 3 AEs that were considered related to study drug and occurred in at least 10% of subjects were neutropenia, thrombocytopenia, increased ALT and fatigue. DLTs of increased ALT, increased AST, increased alkaline phosphatase, thrombocytopenia, and fatigue occurred in 4 of 45 patients in the DLT evaluable set.

In Study 001 Part B (M6620 (VX-970) and cisplatin combination therapy) 17 subjects (63%) had at least one Grade ≥ 3 AE. The majority of Grade ≥ 3 AEs occurred in less than 5% of subjects. Grade ≥ 3 AEs that occurred in at least 10% of subjects were neutropenia and anemia. Ten subjects (37%) had at least one Grade > 3 AE that was considered related to the study drug treatment. Neutropenia was the only Grade > 3 AE that was considered related to study drug treatment and occurred in at least 10% of subjects. DLTs of hypersensitivity and increased ALT occurred in 2 of 26 (7.7%) subjects in the DLT evaluable set.

In study 002 Part A1 (weekly M6620 (VX-970)), the most common AEs regardless of

causality were fatigue (45%), nausea, diarrhea, urinary tract infection (36.4% each) and anemia, headache, and flushing (27.3% each). No grade ≥ 3 AEs were considered related to study drug. No subjects had DLTs. In Study 002 Part A2 (twice weekly M6620 (VX-970)), the only grade ≥ 3 AE considered possibly related to study drug was increased GGT. No subjects had DLTs.

In Study 002 Part B1 (M6620 (VX-970) and carboplatin combination therapy), 6 subjects (31.6%) had at least 1 SAE, and 4 subjects (21%) had at least 1 SAE that was considered related to study drug. Five subjects (26.3%) had at least 1 grade ≥ 3 AE that was considered related to study drug treatment. Neutropenia was the only grade ≥ 3 AE that was considered related to study drug treatment and occurred in at least 10% of the subjects. DLTs of febrile neutropenia, neutropenia, and hypersensitivity occurred in 3 of the 19 patients. Four subjects (21%) had AEs leading to study drug discontinuation, which included infusion-related hypersensitivity and an infusion-related facial flushing (both considered related to study treatment and progressive disease in the liver and shortness of breath (both considered not related to study drug. There were no Grade 5 AEs.

Myelosuppression is an identified risk of M6620 (VX-970) when combined with DNA damaging agents. As of April 29, 2016, 3 subjects had serious myelosuppression events in combination with carboplatin.

As of March 21, 2016, 6 serious infusion reactions to M6620 (VX-970) occurred at doses of VX- 970 ranging from 90 to 210 mg/m². In all cases the reaction occurred during the second infusion of M6620 (VX-970) and caused symptoms that included flushing, erythema, and nausea, in addition to blood pressure abnormalities and mental status changes.

A summary of the DLTs and most common adverse events regardless of causality are listed in the following tables.

Summary of DLTs in Studies 001 and 002

Regimen	DLTs	Percent (%)
M6620 (VX-970) and gemcitabine (Study 001Part A)	AST	4.4
	ALT	4.4
	Alkaline phosphatase	2.2
	Thrombocytopenia	2.2
	Fatigue	2.2
M6620 (VX-970) and cisplatin (Study 001 Part B)	Hypersensitivity	3.8
	ALT	3.8
M6620 (VX-970) (Study 002)	Neutropenia	5.2
	Hypersensitivity	5.2
	Febrile Neutropenia	5.2

Summary of Serious AEs Regardless of Causality in Studies 001 and 002

Regimen	AEs	Percent (%)
M6620 (VX-970) and gemcitabine (Study 001 Part A)	Pyrexia	12.0
	Influenza-like Illness	2.0
	Infections	7.2
	Dehydration	4.0
	Hyponatremia	2.0
	Musculoskeletal pain	4.0
	Pathological fracture	2.0
	Atelectasis	2.0
	Pleuritic pain	2.0
	Pulmonary embolism	2.0
	Thrombocytopenia	4.0
	Anemia	2.0
	Febrile Neutropenia	2.0
	Left Ventricular hypertrophy	2.0
	Pericardial effusion	2.0
	Vomiting	4.0
	Hypersensitivity	4.0
	Body temperature increased	2.0
	Non-small cell lung cancer	2.0
	Hydronephrosis	2.0
	Deep vein thrombosis	2.0
M6620 (VX-970) and cisplatin (Study 001 Part B)	Abdominal pain	3.7
	Diarrhea	3.7
	Small bowel obstruction	3.7
	Hypersensitivity	7.4
	Dyspnea	3.7
	Hypoxia	3.7
	Pyrexia	3.7
	Skin infection	3.7
	ALT	3.7
	AST	3.7
M6620 (VX-970) (Study 002 Part B1)	Febrile Neutropenia	5.3
	Neutropenia	5.3
	Thrombocytopenia	5.3
	Pleuritic Paine	5.3
	Pulmonary Embolism	5.3
	Hypersensitivity	5.3
	Lower respiratory tract	5.3

	infection	
	Infusion related reaction	5.3
	Tumor pain	5.3

One death was reported with single-agent therapy and 19 deaths were reported with combination therapy. None of these deaths were attributable to the study treatment (23).

Safety Summary and Guidance for Investigators (Investigator's Brochure, 2016)

- M6620 (VX-970) absorbs in the UV-visible radiation spectrum and is widely distributed, including skin, so patients receiving M6620 (VX-970) should take protective measures to minimize sun exposure.
- To minimize the possibility of phlebitis, M6620 (VX-970) should be administered through a large-bore catheter into a large-caliber peripheral vein. The intravenous infusion site should be monitored closely for the development of erythema, induration, purulence, tenderness, or warmth. If any subject develops signs or symptoms of inflammation that may progress to phlebitis or that the patient cannot tolerate, standard measures should be employed to ameliorate these symptoms.
- Because the drug-interaction profile of M6620 (VX-970) has not been fully characterized, caution should be used when co-administering medications with M6620 (VX-970). Because M6620 (VX-970) is primarily metabolized by CYP3A4, concomitant administration with strong inhibitors or inducers of CYP3A4 should be avoided.
- Developmental and reproductive toxicity studies have not been conducted yet. Therefore, patients should take stringent measures to avoid bearing children while on study drug and for 6 months after discontinuation of M6620 (VX-970).
- Serious acute hypersensitivity reactions have occurred in a few subjects receiving M6620 (VX-970). These reactions have occurred within minutes of re-exposure to M6620 (VX-970), and in cases to date have occurred during the second infusion. They may include hypotension and mental status changes. All subjects have fully recovered with standard treatment for this reaction, including immediate discontinuation of the inciting infusion and administration of IV corticosteroid and antihistamine, as well as IV fluids and oxygen when clinically indicated.
- There is a potential increase in toxicity of DNA- damaging agents paired with M6620 (VX-970) in patients with evidence of germline defects in their DDR, e.g., patients with Li Fraumeni syndrome or ataxia telangiectasia. M6620 (VX-970) should be used with caution in these patients.

2.3 Gemcitabine and Carboplatin

Gemcitabine

Gemcitabine is a pyrimidine based antimetabolite with activity against recurrent ovarian cancer. The diphosphate metabolite of gemcitabine inhibits ribonucleotide reductase, depleting deoxyribonucleotide levels, and the triphosphate metabolite acts as a substrate for DNA polymerases, causing chain termination [28]. In the presence of these types of replication stresses, the ATR/CHK1 signaling pathway promotes cell survival by blocking additional origin firing and stabilizing the stalled replication forks [29]. Consistent with a role for ATR/CHK1 protecting against gemcitabine toxicity, gemcitabine induces CHK1 activation in various cell lines; and targeted depletion of components of this pathway sensitizes these tumor cells to gemcitabine [29, 30].

Gemcitabine has a wide range of activity in a variety of tumor types, including ovarian cancer. It is also a drug that can be given in patients with underlying mild to moderate hepatic dysfunction.

Gemcitabine will be obtained according to local clinical trial agreement and in accordance with local guidelines. Preparation and administration should follow local prescribing information and local practice. Please see gemcitabine prescribing information for more details on the approved indications, known precautions, warnings, and adverse reactions of gemcitabine (current version of prescribing information is provided in the Pharmacy Binder).

Pharmacokinetic evaluation of gemcitabine indicates that gemcitabine reaches a plateau value 15 minutes after the start of infusion. The elimination of gemcitabine is rapid, with a median half-life of 8 minutes, and is dose dependent. Difluorodeoxyuridine (dFdU), the gemcitabine deaminated uridine metabolite that is the only detectable metabolite in the serum, has a half-life of 14 hours. The peak dFdU concentration generally occurs about 5-15 minutes after the end of the infusion. Clearance of gemcitabine and dFdU is biphasic, with a prolonged terminal elimination phase. The prolonged phase of dFdU elimination is likely a result of renal absorption, a reduced rate of renal excretion at low concentration, and the slow release of the metabolite from tissue stores.

Carboplatin

Carboplatin is a platinum coordination compound that causes predominantly intra- and interstrand DNA cross-links rather than DNA-protein cross-links. Carboplatin is approved for the treatment of ovarian cancer in the U.S. and for the treatment of ovarian cancer and small cell lung cancer in the E.U., where it has also been used as a standard for triple negative breast cancer (TNBC). Carboplatin administration is discussed in Section 8.2, Treatment Administration and Schedule.

Carboplatin will be obtained according to local clinical trial agreement and in accordance with local guidelines. Carboplatin should be prepared and stored according to local prescribing information and local practice. Please see carboplatin prescribing information for more details on the approved indications, known precautions, warnings, and adverse reactions of carboplatin (the current version of prescribing information is provided in the Pharmacy Binder).

Needles or intravenous administration sets containing aluminum parts that may come in contact with carboplatin injection should not be used for the preparation and administration of the drug. Aluminum can react with carboplatin causing precipitate formation and loss of potency.

Dosing of carboplatin will be based on the Calvert formula: carboplatin dose (mg) = (Target AUC) x (GFR + 25). For the purposes of this protocol, the glomerular filtration rate (GFR) is considered to be equivalent to the creatinine clearance (calculated by the method of Cockcroft and Gault, 1976).

To calculate creatinine clearance (CrCl) from serum creatinine

Use current [actual] weight:

$$CrCl = (140 - age) \times (weight \text{ in kg}) \\ (72 \times serum \text{ creatinine}) \times (0.85 \text{ [for females]})$$

To calculate dose of carboplatin (total mg, not mg/m²):

$$\text{mg carboplatin} = (\text{AUC}) \times (\text{CrCl} + 25)$$

Carboplatin dose should be calculated prior to each dose using actual weight and current serum creatinine level according to local prescribing information and local practice.

Please see carboplatin prescribing information for more details on the known precautions, warnings, and adverse reactions of carboplatin.

2.4 Rationale

Combination Carboplatin and Gemcitabine in Platinum Sensitive Ovarian Cancer

As indicated in Section 2.1, the combination of carboplatin and gemcitabine is a commonly used FDA approved regimen for platinum-sensitive recurrent ovarian, peritoneal and fallopian tube malignancies [9]. It is often preferred over carboplatin and paclitaxel, as it is associated with less peripheral neuropathy, which is a significant issue in a large proportion of women with recurrent disease. The combination of carboplatin and gemcitabine was FDA approved in 2006 for platinum sensitive recurrent ovarian cancer based on a multicenter international randomized clinical trial of 356 women with first recurrence of platinum sensitive ovarian cancer [9]. Patients were randomized to receive either gemcitabine 1000 mg/m² on days 1, 8 and carboplatin AUC 4 on day 1 of a 21 day cycle or carboplatin AUC 5 administered on day 1 of each 21 day cycle. The combination therapy led to a significant improvement in PFS. Median PFS was 8.6 months for the combination group and 5.8 months for the carboplatin only group. The overall response rate in the combination group was 46%. Because this combination regimen is considered a standard of care for platinum sensitive ovarian cancer, it has been chosen as the comparator group for this study.

An early event in tumorigenesis involves abrogation of DNA damage responses. As a consequence tumors exhibit increased genomic instability with defects in DNA repair pathways and cell cycle regulation. In particular, mutations in the TP53 render cancer cells deficient in the G1 phase checkpoint DNA damage repair and therefore, more reliant on the S and G2 phase checkpoints for survival. Because it has been reported that TP53

mutations occur in over 95% of serous ovarian cancers, targeting the ATR pathway in this tumor type is of particular interest. In contrast, normal tissue has functional p53 and G1 phase checkpoint repair, making G2 checkpoint abrogation a means of enhancing the therapeutic index of cytotoxic agents with minimal effects on normal tissue.

Gemcitabine and the ATR Pathway

As indicated in the prior section, gemcitabine is a pyrimidine-based antimetabolite with activity against recurrent ovarian cancer. In the presence of this type of replication stress, the ATR/CHK1 signaling pathway promotes cell survival by blocking additional origin firing and stabilizing the stalled replication forks [29]. Consistent with a role for the ATR pathway in protecting against gemcitabine toxicity, gemcitabine induces CHK1 activation in various cell lines, and inhibition of CHK1 or targeted deletion of components of the CHK1 pathway sensitizes these tumor cells to gemcitabine [29, 30]. Moreover, down regulation of ATR, Chk1, or RAD9 using siRNA each sensitizes a variety of cells to gemcitabine [30, 31]. In further studies performed by the Karnitz laboratory, the effects of ATR knockdown were explored in ovarian cancer cell lines and it was found that the ATR inhibitor VE-821 sensitized to a wide variety of genotoxic stresses, including gemcitabine and cisplatin [32]. Since many high grade serous ovarian cancers have HR defects, including but not limited to *BRCA1* or *BRCA2* mutations, the effect of ATR inhibition was investigated as well. ATR inhibition further sensitized cells with HR defects to cisplatin [32].

Effect of M6620 (VX-970) on DNA Damage

In preliminary studies performed by Vertex, treatment of cell lines with various DNA damaging agents, including cisplatin and gemcitabine, led to increased phospho-Ser³¹⁷,³⁴⁵-Chk1, a marker of ATR activity, and activation of proteins that are recruited to sites of DNA damage (phospho-KAP1 and phospho-Ser¹³⁹-H2AX). Concurrent treatment of these cell lines with M6620 (VX-970) and a DNA-damaging agent led to sustained, M6620 (VX-970) dose- dependent decreases in phosphorylations of Chk1, H2AX and KAP1 (compared with DNA damaging agent alone). The failure to repair DNA damage in the presence of VX- 970 is believed to be the driver for the enhanced cytotoxicity in cancer cells that is observed when M6620 (VX-970) is combined with DNA damaging agents. Furthermore, these data support the potential for using DNA damage markers as pharmacodynamic markers of M6620 (VX-970) activity [23].

Optimization of Schedule

The effect of timing of M6620 (VX-970) in combination with DNA damaging agents was assessed with gemcitabine in pancreatic cancer cell lines. Cells were treated with gemcitabine for 24 hours in combination with M6620 (VX-970), with M6620 (VX-970) added at various time points both during and after gemcitabine treatment. Cell viability was measured by MTS assay (96 hours) and the data were subjected to Bliss analysis. Synergy was maximal when VX- 970 was administered 24 hours after starting gemcitabine treatment; later administration was less effective. No synergy was seen when M6620 (VX-970) was administered 48 or more hours after gemcitabine treatment was started. The schedule dependence is attributed to an accumulation of cells in S-phase, and concomitant increase in ATR activity (as measured by Chk1 phosphorylation) that occurs in response to gemcitabine treatment.

The data suggest that administering M6620 (VX-970) after treatment with a DNA damaging agent, to coincide with maximal S-phase accumulation is optimal [23].

2.5 Correlative Studies Background

2.5.1 Multiplex Quantitative IFA Assay (Expansion Cohort)

PADIS, Frederick National Laboratory for Cancer Research (FNLCR) has developed a panel of DNA damage repair markers for use as a multiplex immunofluorescence assay to monitor drug effect in formalin-fixed, paraffin-embedded (FFPE) cell pellets. The multiplex DDR2 assay expands the scope of the previously validated immunofluorescence assay for histone H2AX phosphorylated at Ser139 (γ H2AX) to include three additional biomarkers: Rad51, Nbs1 phosphorylated at Ser343 (pS343-Nbs1), and pATR-Thr1989. γ H2AX is well established as a marker of DNA double-strand breaks and is widely used as a tool in measuring DNA damage responses following drug exposure in both biopsy and circulating tumor cell samples. However, γ H2AX also increases during DNA fragmentation induced by apoptosis, so induction of the marker at later time points cannot be defined purely as a DNA damage response.

Nbs1 is part of the Mre11-Rad50-Nbs1 complex (MRN), which is essential in early DNA damage recognition, recruitment of ATM to the site of damage, repair of double-strand breaks and cell cycle checkpoint activation. The phosphorylation of Nbs1 is critical to the double-strand break DNA damage response. Similar to γ H2AX, Nbs1 is phosphorylated at Ser343 primarily by ATM early in the response to DNA damage. Once phosphorylated, pSer343-Nbs1 plays a role in activation of cell cycle checkpoints and has a direct role in repair of damaged DNA. Rad51 is essential to the HR repair pathway, serving as a recombinase that facilitates strand homology searching and forming foci at double-strand break sites which can be quantified to assess ongoing HR. pThr1989-ATR is an autophosphorylation site critical for ATR function and PADIS has developed a phospho-specific rabbit monoclonal antibody for use as a pharmacodynamic biomarker of ATR activation during DNA damage responses.

This multiplex immunofluorescence assay is incorporated into the protocol as an integrated biomarker. The purpose is to provide proof of mechanism that the predicted DNA repair pathways have been activated by carboplatin + gemcitabine and have been inhibited by M6620 (VX-970). Comparison of tumor samples harvested prior to therapy and again 24 hours after initiation of carboplatin/gemcitabine therapy will allow assessment of the hypothesis that ATR and the DNA damage response have been activated. Comparison of the increment in signal observed after carboplatin/gemcitabine vs. carboplatin/gemcitabine followed by M6620 (VX-970) will allow assessment of the hypothesis that ATR has been inhibited by M6620 (VX-970) *in situ*. These hypotheses are described in greater detail in Section 9 of this protocol.

2.5.2 BROCA Analysis

Ovarian cancer has a significant heritable component. A woman with a first degree relative diagnosed with ovarian cancer has approximately a 3-fold increased risk of developing ovarian cancer compared to the general population. Approximately 10-15% of ovarian cancer patients are *BRCA1* or *BRCA2* mutation carriers [33]. Defects in *BRCA1* or *BRCA2* function result in defective HR repair, leading to i) heightened sensitivity to platinum agents and PARP inhibitors and ii) improvement in overall survival after these treatments. Preclinical data from the Karnitz laboratory also revealed that disabling the HR pathway by depleting *BRCA1* led to further sensitization to platinum in combination with an ATR inhibitor [32]. Of note, a large subset of sporadic

ovarian cancers (up to 50% of all ovarian cancers) exhibits defective HR repair pathway; and these sporadic tumors appear to behave similarly to those with germline *BRCA1* and *BRCA2* mutations [34]. Indeed, this so-called “BRCA-ness” phenotype can arise due to *BRCA1/2* mutations in the tumor, reduced levels of BRCA1 or BRCA2 protein due to promoter methylation, or mutations in up to a dozen other genes encoding proteins in the HR pathway such as *RAD51C* and *RAD51D* [35][36].

BROCA is a validated, highly sensitive approach (based on targeted capture and massively parallel sequencing of selected genomic regions) for sequencing a panel of over 21 tumor suppressor genes, including *BRCA1*, *BRCA2*, and other genes known to be mutated in inherited breast or ovarian carcinoma [36]. We will utilize this approach to analyze tumor and germline DNA for mutations in a panel of genes involved in various aspects of DNA repair or its regulation [36]. In women in the standard treatment group (carboplatin and gemcitabine), this will be assessed using germline DNA and archival tumor DNA. This will be done at the University of Washington under the direction of Elizabeth Swisher. The list of current genes in the BROCA test is attached in Appendix C.

2.5.3 Multiplexed Mass Spectrometry-Based Assay for Quantification of Phospho-signaling in Response to DNA Damage

A lack of analytically robust and multiplexed assays has hampered studies of the large, branched phospho-signaling network responsive to DNA damage. The Paulovich laboratory at the Fred Hutchinson Cancer Research Center has developed and fully analytically characterized a 62-plex assay quantifying protein expression and post-translational modification (phosphorylation and ubiquitination) after induction of DNA damage. The linear range was over 3 orders of magnitude, the median inter-assay variability was 10% CV and the vast majority (~85%) of assays were stable after extended storage. The multiplexed assay was applied in proof-of-principle studies to quantify signaling after exposure to genotoxic stress (ionizing radiation and 4-nitroquinoline 1-oxide) in immortalized cell lines and primary human cells. The effects of genomic variants and pharmacologic kinase inhibition (ATM/ATR) were profiled using the assay. The DDR1 panel includes phospho-Thr1989-ATR, phospho-Ser317-CHK1 (an ATR substrate), phospho-Ser343-NBS1 (an ATM/ATR site), phospho-Ser1524-BRCA1 (an ATM/ATR site), phospho-Ser376-PALB2 (an ATR/ATM site), phospho-Ser635-RAD51D (an ATR/ATM site) and phospho-Ser730-FANCI (an ATM/ATR site).

The purpose of this exploratory endpoint is to provide proof of mechanism that the predicted DNA repair pathways have been activated by carboplatin + gemcitabine and have been inhibited by M6620 (VX-970). Comparison of tumor samples harvested prior to therapy and again 24 hours after initiation of carboplatin/gemcitabine therapy will allow assessment of the hypothesis that ATR and the DNA damage response have been activated. Comparison of the increment in signal observed after carboplatin/gemcitabine vs. carboplatin/gemcitabine followed by M6620 (VX-970) will allow assessment of the hypothesis that ATR has been inhibited by M6620 (VX-970) *in situ*.

3. PATIENT SELECTION

3.1 Eligibility Criteria

- 3.1.1 Histologically confirmed high grade serous or endometrioid ovarian, peritoneal or fallopian tube malignancy that is metastatic and for which curative measures do not exist. The histology can be confirmed from tissue that was taken at the time of diagnosis. A biopsy at the time of recurrence prior to enrollment on study is not required.
- 3.1.2 Patients must have measurable disease, defined as at least one lesion that can be accurately measured in at least one dimension (longest diameter to be recorded for non-nodal lesions and short axis for nodal lesions) as ≥ 20 mm (≥ 2 cm) with conventional techniques or as ≥ 10 mm (≥ 1 cm) with spiral CT scan, MRI, or calipers by clinical exam. See Section 11 for the evaluation of measurable disease.
- 3.1.3 Patients enrolled in the expansion cohort will be required to have archival tumor tissue available for analysis and be willing to have a tumor biopsy at baseline (after registration and prior to starting study treatment), at Cycle 1 Day 2 and at Cycle 2 Day 2. Patients must have platinum sensitive disease and be in their first or second platinum sensitive recurrence. Platinum sensitive disease is defined as recurrence that occurred greater than six months after completion of their last line of platinum based therapy. No non-platinum regimens allowed. Prior therapy with PARP inhibitors as well as bevacizumab is allowed.
- 3.1.4 No more than two prior platinum based regimens. One regimen is defined as the interval of treatment from start of platinum based doublet to finish of that treatment course for the initial therapy or for the recurrent disease episode. If the nonplatinum agent is altered due to any reason other than disease progression, it counts as one regimen. For example, if a patient started on carboplatin and paclitaxel but developed a taxol reaction and was switched to carboplatin and Abraxane, this counts as one prior regimen.
- 3.1.5 Age ≥ 18 years.
Because no dosing or adverse event data are currently available on the use of M6620 (VX-970) in combination with carboplatin and gemcitabine in patients <18 years of age, children are excluded from this study, but will be eligible for future pediatric trials.
- 3.1.6 ECOG performance status ≤ 2 (Karnofsky $\geq 60\%$, see Appendix A).
- 3.1.7 Life expectancy of greater than 6 months.
- 3.1.8 Patients must have normal organ and marrow function as defined below:
- Leukocytes $\geq 3,000/\text{mcL}$
 - Absolute Neutrophil Count (ANC) $\geq 1,500/\text{mcL}$
 - Platelets $\geq 100,000/\text{mcL}$
 - Total Bilirubin Within normal institutional limits
 - AST(SGOT)/ALT(SGPT) $\leq 2 \times$ institutional upper limit of normal (ULN)
 - Creatinine Within normal institutional limits
- OR
- Creatinine Clearance ≥ 50 mL/min/1.73 m² for patients with creatinine levels above institutional normal.

3.1.9 Negative serum pregnancy test result for females of child bearing potential

Note: The effects of M6620 (VX-970) on the developing human fetus are unknown. For this reason and because DNA-damage response (DDR) inhibitors as well as other therapeutic agents used in this trial may have teratogenic potential, women of child-bearing potential must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry, for the duration of study participation, and 6 months after completion of M6620 (VX-970) administration. Should a woman become pregnant or suspect she is pregnant while she is participating in this study, she should inform her treating physician immediately.

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

- 3.2** Of note, if appropriate per the FDA approved indications, patients may receive PARP inhibitor maintenance therapy after the primary endpoint has been reached, if desired. This therapy is not a part of the clinical trial and should be discussed and performed at the discretion of the patient and treating physician.

3.3 Exclusion Criteria

- 3.3.1 Patients with platinum resistant disease or platinum sensitive disease that is past the first or second recurrence.
- 3.3.2 Patients who have had chemotherapy or radiotherapy within 4 weeks prior to entering the study or those who have not recovered from adverse events due to agents administered more than 4 weeks earlier, excluding alopecia. Patients with treatment related effects, such as peripheral neuropathy, that are grade 1 or less are eligible.
- 3.3.3 Prior exposure to gemcitabine.
- 3.3.4 Patients who are concurrently receiving any other investigational agents.
- 3.3.5 Patients with known brain metastases should be excluded from this clinical trial because of their poor prognosis and because they often develop progressive neurologic dysfunction that would confound the evaluation of neurologic and other adverse events.
- 3.3.6 History of allergic reactions attributed to compounds of similar chemical or biologic composition to M6620 (VX-970), carboplatin, gemcitabine or to these specific compounds.
- 3.3.7 M6620 (VX-970) is primarily metabolized by CYP3A4; therefore, concomitant administration with strong inhibitors or inducers of CYP3A4 should be avoided. Because the lists of these agents are constantly changing, it is important to regularly consult a frequently- updated medical reference for a list of drugs to avoid or minimize use of. Appendix B (Patient Drug Information Handout and Wallet Card) should be provided to patients. As part of the enrollment/informed consent procedures, the patient will be counseled on the risk of interactions with other agents, and what to do if new

medications need to be prescribed or if the patient is considering a new over-the-counter medicine or herbal product.

- 3.3.8 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.
- 3.3.9 Pregnant women are excluded from this study because M6620 (VX-970) as a DNA-damage response (DDR) inhibitor may have the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with M6620 (VX-970), breastfeeding should be discontinued if the mother is treated with M6620 (VX-970). These potential risks also apply to the other agents used in this study, such as carboplatin and gemcitabine.
- 3.3.10 Patients with Li Fraumeni syndrome are excluded from the study as M6620 (VX-970) is a DDR inhibitor.
- 3.3.11 Addition of bevacizumab to the treatment in this study is not allowed. If the treating physician feels that the addition of bevacizumab is in the best interest of the patient, the patient should be treated with an FDA approved regimen outside of the present study.

3.4 Inclusion of Women and Minorities

This study will be available to all eligible patients regardless of race or ethnic group. Because this is a clinical trial in primary peritoneal, ovarian and fallopian tube malignancies, all patients in this study will be women.

4. REGISTRATION PROCEDURES

4.1 Investigator and Research Associate Registration with CTEP

Food and Drug Administration (FDA) regulations require IND sponsors to select qualified investigators. NCI policy requires all persons participating in any NCI-sponsored clinical trial to register and renew their registration annually. To register, all individuals must obtain a CTEP Identity and Access Management (IAM) account (<https://ctepcore.nci.nih.gov/iam>). In addition, persons with a registration type of Investigator (IVR), Non-Physician Investigator (NPIVR), or Associate Plus (AP) (*i.e.*, clinical site staff requiring write access to Oncology Patient Enrollment Network (OPEN) or Rave or acting as a primary site contact) must complete their annual registration using CTEP's web-based Registration and Credential Repository (RCR) (<https://ctepcore.nci.nih.gov/rcr>). Documentation requirements per registration type are outlined in the table below.

Documentation Required	IVR	NPIVR	AP	A
FDA Form 1572	✓	✓		
Financial Disclosure Form	✓	✓	✓	
NCI Biosketch (education, training, employment, license, and certification)	✓	✓	✓	
HSP/GCP training	✓	✓	✓	
Agent Shipment Form (if applicable)	✓			
CV (optional)	✓	✓	✓	

An active CTEP-IAM user account and appropriate RCR registration is required to access all CTEP and Cancer Trials Support Unit (CTSUS) websites and applications. In addition, IVRs and NPIVRs must list all clinical practice sites and IRBs covering their practice sites on the FDA Form 1572 in RCR to allow the following:

- Added to a site roster
- Assigned the treating, credit, consenting, or drug shipment (IVR only) tasks in OPEN
- Act as the site-protocol PI on the IRB approval
- Assigned the Clinical Investigator (CI) role on the Delegation of Tasks Log (DTL).

Additional information can be found on the CTEP website at <https://ctep.cancer.gov/investigatorResources/default.htm>. For questions, please contact the RCR **Help Desk** by email at RCRHelpDesk@nih.gov.

4.2 Site Registration

This study is supported by the NCI Cancer Trials Support Unit (CTSUS).

Each investigator or group of investigators at a clinical site must obtain IRB approval

for this protocol and submit IRB approval and supporting documentation to the CTSU Regulatory Office before they can be approved to enroll patients. Assignment of site registration status in the CTSU Regulatory Support System (RSS) uses extensive data to make a determination of whether a site has fulfilled all regulatory criteria including but not limited to the following:

- An active Federalwide Assurance (FWA) number
- An active roster affiliation with the Lead Network or a participating organization
- A valid IRB approval
- Compliance with all protocol specific requirements.

In addition, the site-protocol Principal Investigator (PI) must meet the following criteria:

- Active registration status
- The IRB number of the site IRB of record listed on their Form FDA 1572
- An active status on a participating roster at the registering site

Sites participating on the NCI CIRB initiative that are approved by the CIRB for this study are not required to submit IRB approval documentation to the CTSU Regulatory Office. For sites using the CIRB, IRB approval information is received from the CIRB and applied to the RSS in an automated process. Signatory Institutions must submit a Study Specific Worksheet for Local Context (SSW) to the CIRB via IRBManager to indicate their intent to open the study locally. The CIRB's approval of the SSW is then communicated to the CTSU Regulatory Office. In order for the SSW approval to be processed, the Signatory Institution must inform the CTSU which CIRB-approved institutions aligned with the Signatory Institution are participating in the study.

4.2.1 Downloading Regulatory Documents

Site registration forms may be downloaded from the 9948 protocol page located on the CTSU Web site. Permission to view and download this protocol is restricted and is based on person and site roster data housed in the CTSU RSS. To participate, Investigators and Associates must be associated with the Corresponding or Participating protocol organization in the RSS.

- Go to <https://www.ctsuo.org> and log in using your CTEP IAM username and password.
- Click on the Protocols tab in the upper left of your screen.
- Either enter the protocol number in the search field at the top of the protocol tree, or
- Click on the By Lead Organization folder to expand, then select LAO-MN026 and protocol # 9948
- Click on LPO Documents, select the Site Registration documents link, and download and complete the forms provided. (Note: For sites under the CIRB initiative, IRB data will automatically load to RSS.)

4.2.2 Requirements for Protocol 9948 Site Registration

- IRB approval (For sites not participating via the NCI CIRB; local IRB documentation, an IRB-signed CTSU IRB Certification Form, Protocol of Human Subjects Assurance Identification/IRB Certification/Declaration of Exemption Form, or combination is accepted.)
- Site Initiation Teleconference

4.2.3 Submitting Regulatory Documents

Submit required forms and documents to the CTSU Regulatory Office, where they will be entered and tracked in the CTSU RSS.

CTSU Regulatory Office

1818 Market Street, Suite 3000 1100

Philadelphia, PA 19103

Institutions with patients waiting that are unable to use the Portal should alert the CTSU Regulatory Office immediately at 1-866-651-2878 in order to receive further instruction and support.

4.2.4 Checking **Site** Registration Status

You can verify your site registration status on the members' section of the CTSU website.

- Go to <https://www.ctsu.org> and log in using your CTEP IAM username and password.
- Click on the Regulatory tab at the top of your screen.
- Click on the Site Registration sub tab.
- Enter your 5-character CTEP Institution Code and click on Go.

Note: The status given only reflects compliance with IRB documentation and institutional compliance with protocol-specific requirements as outlined by the Lead Network. It does not reflect compliance with protocol requirements for individuals participating on the protocol or the enrolling investigator's status with the NCI or their affiliated networks.

4.3 Patient Registration

4.3.1 OPEN / IWRS

Patient enrollment will be facilitated using the Oncology Patient Enrollment Network (OPEN). OPEN is a web-based registration system available to users on a 24/7 basis. It is integrated with the CTSU Enterprise System for regulatory and roster data interchange and with the Theradex Interactive Web Response System (IWRS) for retrieval of patient registration/randomization assignment. Patient enrollment data entered by Registrars in OPEN / IWRS will automatically transfer to the NCI's clinical data management system,

Medidata Rave.

The OPEN system will provide the site with a printable confirmation of registration and treatment information. Please print this confirmation for your records.

4.3.2 OPEN/IWRS User Requirements

OPEN/IWRS users must meet the following requirements:

- Have a valid CTEP-IAM account (*i.e.*, CTEP username and password).
- To enroll patients: Be on an ETCTN Corresponding or Participating Organization roster with the role of Registrar. Registrars must hold a minimum of an AP registration type.
- Have regulatory approval for the conduct of the study at their site.

Prior to accessing OPEN/IWRS, site staff should verify the following:

- All eligibility criteria have been met within the protocol stated timeframes. If applicable, all patients have signed an appropriate consent form and HIPAA authorization form.

4.3.3 OPEN/IWRS Questions?

Further instructional information on OPEN is provided on the OPEN tab of the CTSU website at <https://www.ctsu.org> or at <https://open.ctsu.org>. For any additional questions contact the CTSU Help Desk at 1-888-823-5923 or ctsucontact@westat.com.

4.4 General Guidelines

Following registration, patients should begin protocol treatment within 21 days. Issues that would cause treatment delays should be discussed with the Principal Investigator. If a patient does not receive protocol therapy following registration, the patient's registration on the study may be canceled. The Study Coordinator should be notified of cancellations as soon as possible.

5. TREATMENT PLAN

5.1 Agent Administration

Treatment will be administered on an outpatient basis. To minimize the possibility of phlebitis, M6620 (VX-970) should be administered through a large-bore catheter into a large-caliber peripheral vein. The intravenous infusion site should be monitored closely for the development of erythema, induration, purulence, tenderness or warmth. M6620 (VX-970) should not come into contact with 0.9% sodium chloride due to incompatibility. Infuse using an infusion set containing low-sorption or non-PVC, DEHP-free tubing and an in-line 0.2 micro filter.

Reported adverse events and potential risks are described in Section 7. Appropriate dose modifications are described in Section 6. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

Phase 1 Dose Escalation/Safety Lead-In Regimen Description

Dose Level	M6620 (VX-970)	Gemcitabine	Carboplatin
-2	90 mg/m ² , day 2, 9	480 mg/m ² , day 1, 8	AUC 4, day 1
-1	90 mg/m ² , day 2, 9	560 mg/m ² , day 1, 8	AUC 4, day 1
1*	90 mg/m ² , day 2, 9	640 mg/m ² , day 1, 8	AUC 4, day 1
2	90 mg/m ² , day 2, 9	800 mg/m ² , day 1, 8	AUC 4, day 1
3	120 mg/m ² , day 2, 9	800 mg/m ² , day 1, 8	AUC 4, day 1

* Starting dose level

Regimen Description – Expansion Cohort (Experimental Group: M6620 (VX-970) + Gemcitabine + Carboplatin)					
Agent	Premedications; Precautions	Dose	Route	Schedule	Cycle Length
Gemcitabine	See antiemetic regimen and section 8.2.	640 mg/m ² /day in 250 mL 0.9% NaCl	IV over approximately 30 minutes or according to institutional standards	Days 1, 8	21 days (3 weeks)
Carboplatin	Dosed using Calvert Formula with Cockcroft-Gault, actual body weight and maximum CrCL of	AUC 4 (Cockcroft-Gault) in 250 mL 0.9%	IV infusion over approximately 30 minutes or according to institutional standards	Days 1	

	125 mL/min. Creatinine clearance should be estimated using a minimum serum creatinine value of 0.7 mg/dL. Dose rounding to the nearest 10 mg.	NaCl			
M6620 (VX-970)		90 mg/m ² IV in D5W to a concentration between 0.075 mg/ml to 1 mg/ml	IV infusion over 60 (+/- 15) minutes.	Days 2, 9	
*Doses of gemcitabine and M6620 (VX-970) have been determined from the phase 1 dose escalation/safety lead-in.					

Pretreatment Medication

Agent	Dose	Route	Day
Dexamethasone	20 mg	PO or IV PO route preferred, if unable to tolerate, administer same dose IV	Day 1
Dexamethasone	10 mg	PO or IV, PO route preferred, if unable to tolerate, administer same dose IV	Day 2, 9
Granisetron (Kytril)	1 mg	PO or IV PO route preferred, if unable to tolerate, administer same dose IV	Days 1, 8
Palonosetron (Aloxi)	0.25 mg/day	IV push	Day 2, 9
Fosaprepitant (Emend)	150 mg/day	IV	Day 2
Prochlorperazine	10 mg	PO every 6 hours as needed for nausea and vomiting	Take home script
Lorazepam	1 mg/dose	If nausea unrelieved by prochlorperazine	Take home script
Ondansetron	8 mg/dose	PO daily for 2 days, start in AM with breakfast the day after treatment	Take home script
Dexamethasone	4mg	PO daily for 2 days, start in AM with breakfast the day after treatment	Optional take home script to begin on day 3 and day 10

Note: Antiemetics can also be used per institutional guidelines or modified as needed per treating physician

5.2 General Concomitant Medication and Supportive Care Guidelines

5.2.1 M6620 is metabolized by cytochrome P450 (CYP) isoenzyme 3A4 (CYP3A4); exposure to M6620 may be affected by concomitantly administered drugs that are strong inhibitors or inducers of CYP3A4. Because there is a potential for interaction of M6620 with other concomitantly administered drugs, the case report form must capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies. The Principal Investigator should be alerted if the patient is taking any agent known to affect or with the potential for drug interactions. [For example, the potential targets for drug interaction can involve, but are not limited to CYP450, glucuronidation, P-glycoprotein, protein binding, or reduced absorption from proton-pump inhibitors. Check the study agent Investigator's Brochure for potential sources of drug interactions]. The study team should check a frequently-updated medical reference for a list of drugs to avoid or minimize use of. Appendix D (Patient Drug Interactions Handout and Wallet Card) should be provided to patients.

5.2.2 If any subject develops phlebitis or signs of inflammation which the subject cannot tolerate, standard measures should be employed to ameliorate these symptoms, including removal of the infusion catheter and resumption of infusion through a different vein. If any subject develops pruritus or evidence of an allergic reaction, standard measures may be employed to ameliorate these symptoms or prevent recurrence (e.g., premedication with acetaminophen 325 mg, PO approximately 30 minutes before the infusion, 200 mg hydrocortisone IV approximately 60 minutes before infusion, and 25 mg diphenhydramine IV approximately 30 minutes before infusion). Alternative antihistamine and steroid combinations may be considered, as long as they are not prohibited by protocol. If standard procedures to limit symptoms of injection site reaction or pruritus are insufficient, the infusion time may be extended beyond 60 minutes, but no more than 90 minutes.

5.2.3 To decrease the risk of phlebitis, M6620 should be administered through a large-bore catheter into a large-caliber peripheral vein. The intravenous infusion site should be monitored closely for the development of erythema, induration, purulence, tenderness, or warmth.

5.3 Duration of Therapy

In the absence of treatment delays due to adverse event(s), treatment may continue until one of the following criteria applies:

- Disease progression;
- Intercurrent illness that prevents further administration of treatment,
- Unacceptable adverse event(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;

- If a patient has a complete response to therapy and has continued two cycles beyond complete response, discussion between treating physician and investigator regarding discontinuation of therapy may be considered.

5.4 Duration of Follow Up

Patients who begin study treatment will be followed for 3 years after removal from study treatment or until death, whichever occurs first. Patients will be followed periodically until death by telephone call or letter, as well as by using public health records, for example, a local newspaper.

5.5 Criteria for Removal from Study

Patients will be removed from study treatment when any of the criteria listed in Section 5.3 applies. Patients will be removed from the study 3 years from study treatment, death or withdrawal of consent, whichever comes first. The reason for discontinuation of study treatment and the date the patient was discontinued treatment must be documented in the Case Report Form; the reason for removal from study treatment and the date must be documented in the Case Report Form.

Patients who are eligible for PARP inhibitor maintenance therapy may receive this therapy per FDA approval. This is not part of the present study but may be done once the patient has come off study after assessment of the primary endpoint.

6. DOSING DELAYS/DOSE MODIFICATIONS

6.1 Dose Modification Tables for Phase 1 Dose Escalation/Safety Lead In Regimen

Phase 1 Safety Lead-In Regimen Description

Dose Level	M6620 (VX-970)	Gemcitabine	Carboplatin
-2	90 mg/m ² day 2, 9	480 mg/m ² day 1, 8	AUC 4, day 1
-1	90 mg/m ² day 2, 9	560 mg/m ² day 1, 8	AUC 4, day 1
1**	90 mg/m ² day 2, 9	640 mg/m ² day 1, 8	AUC 4, day 1
2	90 mg/m ² day 2, 9	800 mg/m ² day 1, 8	AUC 4, day 1
3	120 mg/m ² day 2, 9	800 mg/m ² day 1, 8	AUC 4, day 1

** starting dose level.

At Time of Treatment

<u>Nausea/ Vomiting*</u>	Management/ Next Dose for M6620 (VX- 970)	Management/ Next Dose for Gemcitabine	Management/ Next Dose for Carboplatin
≤ Grade 1	No change in dose	No change in dose	No change in dose
Grade 2	No change in dose	No change in dose	No change in dose
Grade 3	Hold** until ≤ Grade 2. Resume at one dose level lower, if at dose level 3 or 4 and if indicated.*** If at dose level 2 or lower, continue at same dose.	Hold** until ≤ Grade 2. At first occurrence, reduce gemcitabine by one dose level.*** If at dose level 3 or 4, reduce gemcitabine to dose level 1 at first occurrence. At second occurrence, resume at same dose.	Hold** until ≤ Grade 2. Resume at same dose at first occurrence. At second occurrence, reduce dose to AUC 3, at third occurrence, reduce to AUC 2.
Grade 4	Hold** until ≤ Grade 2. Resume at one dose level lower, if at dose level 3 or 4 and if indicated.*** If at dose level 2 or lower, continue at same dose.	Hold** until ≤ Grade 2. At first occurrence, reduce gemcitabine by one dose level.*** If at dose level 3 or 4, reduce gemcitabine to dose level 1 at first occurrence. At second occurrence, reduce to dose level - 1	Hold** until ≤ Grade 2. Resume at same dose at first occurrence. At second occurrence, reduce dose to AUC 3, at third occurrence, reduce to AUC 2.

* Nausea and vomiting after maximal therapy (see premedication suggestions and institutional guidelines for optimal management of nausea and vomiting).

** Patients with grade 3 toxicity requiring a delay of > 2 weeks should be discussed with the PI. Patients may continue therapy if receiving benefit. Patients with a grade 4 toxicity requiring a delay of > 2 weeks, study should be discontinued.

*** Patients requiring > two dose reductions should be discussed with PI. Patients may continue therapy if receiving benefit.

Recommended management: antiemetics.

At Time of Treatment

<u>Diarrhea</u>	Management/ Next Dose for M6620 (VX- 970)	Management/ Next Dose for Gemcitabine	Management/ Next Dose for Carboplatin
≤ Grade 1	No change in dose	No change in dose	No change in dose
Grade 2	No change in dose	No change in dose	No change in dose
Grade 3	Hold* until < Grade 2. Resume at one dose level lower, if at dose level 3 or 4 and if indicated.** If at dose level 2 or lower, continue at same dose.	Hold* until < Grade 2. Resume at one dose level lower, if indicated.** If at dose level 3 or 4, reduce gemcitabine to dose level 1 at first occurrence and dose level -1 at second occurrence. Third recurrence, reduce to dose level -2.	Hold* until < Grade 2. Resume at same dose at first occurrence. At second occurrence, reduce dose to AUC 3, at third occurrence, reduce to AUC 2.
Grade 4	Hold* until < Grade 2. Resume at one dose level lower, if at dose level 3 or 4 and if indicated.** If at dose level 2 or lower, continue at same dose.	Hold* until < Grade 2. Resume at one dose level lower, if indicated.** If at dose level 3 or 4, reduce gemcitabine to dose level 1 at first occurrence and dose level -1 at second occurrence. Third recurrence, reduce to dose level -2.	Hold* until < Grade 2. Resume at same dose at first occurrence. At second occurrence, reduce dose to AUC 3, at third occurrence, reduce to AUC 2.
* Patients with a grade 3 toxicity requiring a delay of > 2 weeks should be discussed with the PI. Patients may continue therapy if receiving benefit. Patients with a grade 4 toxicity requiring a delay of > 2 weeks, study should be discontinued.			
** Patients requiring > two dose reductions should be discussed with PI. Patients may continue therapy if receiving benefit.			
Recommended management: Loperamide antidiarrheal therapy.			
Dosage schedule: 4 mg at first onset, followed by 2 mg with each loose motion until diarrhea-free for 12 hours (maximum dosage: 16 mg/24 hours)			
Adjunct anti-diarrheal therapy is permitted and should be recorded when used			

Dose Modifications for Neutropenia at Time of Treatment on Day 1 of Any Cycle

<u>Neutropenia</u>	Management/ Next Dose for M6620 (VX-970)	Management/ Next Dose for Gemcitabine	Management/ Next Dose for Carboplatin
≤ Grade 1	No change in dose	No change in dose	No change in dose
Grade 2	Hold until ≤ Grade 1. Resume at same dose level.	Hold until ≤ Grade 1. Resume at same dose level.	Hold until ≤ Grade 1. Resume at same dose level.
Grade 3	Hold* until ≤ Grade 1. Resume at one dose level lower, at dose level 3 or 4 and if indicated.** If at dose level 2 or lower, continue at same dose.	Hold* until ≤ Grade 1. Resume at one dose level lower, if indicated.** If at dose level 3 or 4, reduce gemcitabine to dose level 1 at first occurrence, keep at dose level 1 for second occurrence, reduce to dose level -1 at third occurrence.	Hold* until ≤ Grade 1. Resume at same dose at first occurrence. At second occurrence, reduce dose to AUC 3; at third occurrence, reduce to AUC 2.
Grade 4	Hold* until ≤ Grade 1. Resume at one dose level lower, at dose level 3 or 4 and if indicated.** If at dose level 2 or lower, continue at same dose.	Hold* until ≤ Grade 1. Resume at one dose level lower, if indicated.** If at dose level 3 or 4, reduce gemcitabine to dose level 1 at first occurrence, reduce to dose level -1 for second occurrence, reduce to dose level -2 at second occurrence	Hold* until ≤ Grade 1. Resume at one dose level lower, if indicated.** Resume at same dose at first occurrence. At second occurrence, reduce dose to AUC 3; at third occurrence, reduce to AUC 2.
Patients with a grade 3 toxicity requiring a delay of > 2 weeks should be discussed with the PI. Patients may continue therapy if receiving benefit. Patients with a grade 4 toxicity requiring a delay of > 2 weeks, study should be discontinued.			
** Patients requiring > two dose reductions should be discussed with PI. Patients may continue therapy if receiving benefit.			
Recommended management: If dose delay occurs due to grade 2 or 3 neutropenia, growth factor (G-CSF or GM-CSF) can be used at the discretion of the treating physician with subsequent cycles.			

Dose Modifications for Neutropenia at Time of Treatment on Day 8 of Any Cycle

<u>Neutropenia</u>	Management/ Next Dose for M6620 (VX-970)*	Management/ Next Dose for Gemcitabine	Management/ Next Dose for Carboplatin**
≤ Grade 1	No change in dose	No change in dose	No change in dose
Grade 2	No change in dose	No change in dose	No change in dose
Grade 3	Omit current cycle day 8 dose	Omit current cycle day 8 dose	No change in dose
Grade 4	Omit current cycle day 8 dose	Omit current cycle day 8 dose	Omit current cycle day 8 dose
Recommended management: If dose delay occurs due to grade 2 or 3 neutropenia, growth factor (G-CSF or GM-CSF) can be used at the discretion of the treating physician with subsequent cycles.			
* M6620 (VX-970) dose is given on day 9; however, the CBC from day 8 will be used to determine the day 9 dose			
**Carboplatin is not given on day 8 or day 9 of the cycle.			

Dose Modifications for Thrombocytopenia at Time of Treatment on Day 1 of Any Cycle

<u>Thrombocytopenia</u>	Management/ Next Dose for M6620 (VX-970)***	Management/ Next Dose for Gemcitabine	Management/ Next Dose for Carboplatin
≤ Grade 1	No change in dose	No change in dose	No change in dose
Grade 2	Hold until ≤ Grade 1. Resume at same dose level.	Hold until ≤ Grade 1. Resume at same dose level.	Hold until ≤ Grade 1. Resume at same dose.
Grade 3	Hold* until ≤ Grade 1. Resume at one dose level lower, at dose level 3 or 4 and if indicated. ** If at dose level 2 or lower, continue at same dose.	Hold* until ≤ Grade 1. Resume at one dose level lower, if indicated. ** If at dose level 3 or 4, reduce gemcitabine to dose level 1 at first occurrence,, reduce to dose level -1 at second occurrence and reduce to dose level -2 at second occurrence.	Hold* until ≤ Grade 1. Resume at same dose at first occurrence, AUC 3 at second occurrence, reduce to AUC2 at third recurrence if indicated.**
Grade 4	Hold* until ≤ Grade 1. Resume at one dose level lower, at dose level 3 or 4 and if indicated. ** If at dose level 2 or lower, continue at same dose.	Hold* until ≤ Grade 1. Resume at one dose level lower, if indicated. ** If at dose level 3 or 4, reduce gemcitabine to dose level 1 at first occurrence, reduce to	Hold* until ≤ Grade 1. Reduce to AUC 3 at first occurrence, AUC 2 at second occurrence, maintain at AUC2 at third

		dose level -1 at second occurrence and reduce to dose level -2 at second occurrence.	recurrence if indicated.**
<p>* Patients with a grade 3 toxicity requiring a delay of > 2 weeks should be discussed with the PI. Patients may continue therapy if receiving benefit. Patients with a grade 4 toxicity requiring a delay of > 2 weeks, study should be discontinued.** Patients requiring > two dose reductions should be discussed with PI. Patients may continue therapy if receiving benefit.</p> <p>*** M6620 (VX-970) dose is given on day 2; however, the platelet count from day 1 will be used to determine the day 2 dose</p>			

Dose Modifications for Thrombocytopenia at Time of Treatment on Day 8 of Any Cycle

<u>Thrombocytopenia</u>	Management/ Next Dose for M6620 (VX- 970)**	Management/ Next Dose for Gemcitabine	Management/ Next Dose for Carboplatin***
≤ Grade 1	No change in dose	No change in dose	No change in dose
Grade 2	No change in dose	No change in dose	No change in dose
Grade 3	Omit current cycle day 8 dose. Resume at one dose lower, at dose level 3 or 4 and if indicated* If at dose level 2 or lower, continue at same dose.	Omit current cycle day 8 dose. Resume at one dose lower, if indicated.* If at dose level 3 or 4, reduce gemcitabine to dose level 1 at first occurrence and dose level -1 at second occurrence.	No change in dose
Grade 4	Omit current cycle day 8 dose. Resume at one dose lower, at dose level 3 or 4 and if indicated* If at dose level 2 or lower, continue at same dose.	Omit current cycle day 8 dose. Resume at one dose lower, if indicated.* If at dose level 3 or 4, reduce gemcitabine to dose level 1 at first occurrence and dose level -1 at second occurrence.	No change in dose
<p>* Patients with a grade 3 toxicity requiring a delay of > 2 weeks should be discussed with the PI. Patients may continue therapy if receiving benefit. Patients with a grade 4 toxicity requiring a delay of > 2 weeks, study should be discontinued..</p> <p>** M6620 (VX-970) dose is given on day 9; however, the platelet count from day 8 will be used to determine the day 9 dose</p> <p>*** No carboplatin is given on day 8</p>			

Dose Modifications for Other Non-Hematological Toxicities at Time of Treatment on Days 1 or 8*

<u>Event</u>	Management/ Next Dose for M6620 (VX- 970)****	Management/ Next Dose for Gemcitabine	Management/ Next Dose for Carboplatin
≤ Grade 1	No change in dose	No change in dose	No change in dose
Grade 2	No change in dose	No change in dose	No change in dose
Grade 3 or 4	Hold** until ≤ Grade 2. Resume at one dose level lower, at dose level 3 or 4 and if indicated.*** If at dose level 2 or lower, continue at same dose.	Hold** until ≤ Grade 2. Resume at one dose level lower, if indicated.*** If at dose level 3 or 4, reduce gemcitabine to dose level 1 at first occurrence, keep at dose level 1 for second occurrence, reduce to dose level -1 at third occurrence, and keep at dose level -1 at fourth occurrence.	Hold** until ≤ Grade 2. Resume at same dose at first occurrence, at AUC 3 at second occurrence, at AUC3 at third recurrence if indicated.**
<p>* Does not include alopecia and fatigue</p> <p>** Patients with a grade 3 toxicity requiring a delay of > 2 weeks should be discussed with the PI. Patients may continue therapy if receiving benefit. Patients with a grade 4 toxicity requiring a delay of > 2 weeks, study should be discontinued.</p> <p>*** Patients requiring > two dose reductions should be discussed with PI. Patients may continue therapy if receiving benefit.</p> <p>**** M6620 (VX-970) dose is on day 2, 9, but dose can be determined based on toxicity assessment performed on day 1 and 8 as well as on days 2 and 9.</p>			

Dose Modifications Based on Interim Toxicity

<u>Event</u>	Management/ Next Dose for M6620 (VX- 970)	Management/ Next Dose for Gemcitabine	Management/ Next Dose for Carboplatin
≤ Grade 1	No change in dose	No change in dose	No change in dose
Grade 2	No change in dose	No change in dose	No change in dose
Grade 3 or 4	Hold* until ≤ Grade 2. Resume at one dose level lower, at dose level 3 or 4 and if indicated.** If at dose level 2 or lower, continue at same dose.	Hold* until ≤ Grade 2. Resume at one dose level lower, if indicated.** If at dose level 3 or 4, reduce gemcitabine to dose level 1 at first occurrence, keep at dose level 1 for second occurrence, reduce to dose level -1 at third occurrence, and keep at dose level -1 at fourth occurrence.	Hold* until ≤ Grade 2. Resume at same dose at first occurrence, reduce to AUC 3 at second occurrence, maintain at AUC3 at third recurrence and reduce to AUC 2 at fourth occurrence if indicated.**
<p>* Patients with a grade 3 toxicity requiring a delay of > 2 weeks should be discussed with the PI. Patients may continue therapy if receiving benefit. Patients with a grade 4 toxicity requiring a delay of > 2 weeks, study should be discontinued.</p> <p>** Patients requiring > two dose reductions should be discussed with PI. Patients may continue therapy if receiving benefit.</p>			

6.2 Dose Modification Tables for Expansion Cohort

Three patients were enrolled at dose level 1 (carboplatin AUC 4, gemcitabine, 640 mg/m², M6620 90 mg/m²) and no DLTs were identified. Dose level 2 (carboplatin AUC4, gemcitabine 800 mg/m² and M6620 90 mg/m²) was opened to three patients and one of the three developed grade 4 thrombocytopenia. Therefore, two additional patients were added at dose level 2. Of these, one additional patient also developed a DLT, namely grade 4 thrombocytopenia. Thus, dose level 2 was determined to have exceeded the MTD. Therefore, an additional three patients were enrolled at dose level 1. One of these three patients developed a DLT (grade 4 thrombocytopenia), bringing the total DLTs at dose level 1 to 1 of 6 patients. Therefore, the MTD was deemed to be dose level 1, carboplatin AUC 4 day 1, gemcitabine 640mg/m² day 1, 8 and M6620 90 mg/m² day 2,9.

Dose Modification Table

Dose Level	M6620 (VX-970)	Gemcitabine	Carboplatin
MTD	90 mg/m ² day 2, 9*	640 mg/m ² day 1, 8*	AUC 4, day 1
-1	90 mg/m ² day 2, 9	560mg/m ² day 1, 8	AUC 3, day 1
-2	90 mg/m ² day 2, 9	480 mg/m ² day 1, 8	AUC 2, day 1

At Time of Treatment

<u>Nausea/ Vomiting*</u>	Management/ Next Dose for M6620 (VX-970)	Management/ Next Dose for Gemcitabine	Management/ Next Dose for Carboplatin
≤ Grade 1	No change in dose	No change in dose	No change in dose
Grade 2	No change in dose	No change in dose	No change in dose
Grade 3	Hold** until ≤ Grade 2. Resume at same dose.	Hold** until ≤ Grade 2. Resume at one dose level lower, if indicated.***	Hold** until ≤ Grade 2. Resume at the same dose for the first occurrence and reduce by one dose level at second recurrence, if indicated. ***
Grade 4	Hold** until ≤ Grade 2. Resume at same dose.	Hold** until ≤ Grade 2. Resume at one dose level lower, if indicated.***	Hold** until ≤ Grade 2. Resume at the same dose for the first occurrence and reduce by one dose level at second recurrence, if indicated. ***

* Nausea and vomiting after maximal therapy (see premedication suggestions and institutional guidelines for optimal management of nausea and vomiting).

** Patients with a grade 3 toxicity requiring a delay of > 2 weeks should be discussed with the PI. Patients may continue therapy if receiving benefit. Patients with a grade 4 toxicity requiring a delay of > 2 weeks, study should be discontinued..

*** Patients requiring > two dose reductions should be discussed with PI. Patients may continue therapy if receiving benefit.

Recommended management: antiemetics.

At Time of Treatment

<u>Diarrhea</u>	Management/ Next Dose for M6620 (VX- 970)	Management/ Next Dose for Gemcitabine	Management/ Next Dose for Carboplatin
≤ Grade 1	No change in dose	No change in dose	No change in dose
Grade 2	No change in dose	No change in dose	No change in dose
Grade 3	Hold* until ≤ Grade 2. Resume at same dose.	Hold* until ≤ Grade 2. Reduce by one dose level for the first occurrence and resume at that same dose level for the second occurrence, if indicated.**	Hold* until ≤ Grade 2. Resume at the same dose at first occurrence and reduce by one dose level for second occurrence, if indicated.**
Grade 4	Hold* until ≤ Grade 2. Resume at same dose.	Hold* until ≤ Grade 2. Reduce by one dose level for the first occurrence and resume at that same dose level for the second occurrence, if indicated.**	Hold* until ≤ Grade 2. Resume at the same dose at first occurrence and reduce by one dose level for second occurrence, if indicated.**
<p>* Patients with a grade 3 toxicity requiring a delay of > 2 weeks should be discussed with the PI. Patients may continue therapy if receiving benefit. Patients with a grade 4 toxicity requiring a delay of > 2 weeks, study should be discontinued.</p> <p>** Patients requiring > two dose reductions should be discussed with PI. Patients may continue therapy if receiving benefit.</p> <p>Recommended management: Loperamide antidiarrheal therapy Dosage schedule: 4 mg at first onset, followed by 2 mg with each loose motion until diarrhea-free for 12 hours (maximum dosage: 16 mg/24 hours) Adjunct anti-diarrheal therapy is permitted and should be recorded when used.</p>			

Dose Modifications for Neutropenia on Day 1 at Time of Treatment of Any Cycle

<u>Neutropenia</u>	Management/ Next Dose for M6620 (VX-970)***	Management/ Next Dose for Gemcitabine	Management/ Next Dose for Carboplatin
≤ Grade 1	No change in dose	No change in dose	No change in dose

Grade 2	Hold until \leq Grade 1. Resume at same dose level.	Hold until \leq Grade 1. Resume at same dose level.	Hold until \leq Grade 1. Resume at same dose level.
Grade 3	Hold* until \leq Grade 1. Resume at same dose.**	Hold* until \leq Grade 1. Resume at same dose for the first occurrence and reduce by one dose level for second occurrence, if indicated.**	Hold* until \leq Grade 1. Resume at same dose for first occurrence and reduce by one dose level at second occurrence.
Grade 4	Hold* until \leq Grade 1. Resume at same dose.**	Hold* until \leq Grade 1. Reduce by one dose level for first occurrence, resume at the same dose for second occurrence, if indicated.**	Hold* until \leq Grade 1. Resume at same dose for first occurrence and reduce by one dose level at second occurrence.
<p>* * Patients with a grade 3 toxicity requiring a delay of > 2 weeks should be discussed with the PI. Patients may continue therapy if receiving benefit. Patients with a grade 4 toxicity requiring a delay of > 2 weeks, study should be discontinued.</p> <p>** Patients requiring > 2 dose reductions should be discussed with PI. Patients may continue therapy if receiving benefit.</p> <p>*** Although M6620 (VX-970) is given on day 2, neutrophil count from day 1 will be used to determine the day 2 dose.</p>			
<p>Recommended management: If dose delay occurs due to grade 2 or 3 neutropenia, growth factor (G-CSF or GM-CSF) can be used at the discretion of the treating physician with subsequent cycles.</p>			

Dose Modifications for Neutropenia on Day 8 at Time of Treatment of Any Cycle

<u>Neutropenia</u>	Management/ Next Dose for M6620 (VX-970)*	Management/ Next Dose for Gemcitabine
\leq Grade 1	No change in dose	No change in dose
Grade 2	No change in dose	No change in dose
Grade 3	Omit dose	Omit dose
Grade 4	Omit dose	Omit dose
<p>* Although M6620 (VX-970) is given on day 9, neutrophil count from day 8 will be used to determine the day 9 dose.</p>		
<p>Recommended management: If dose delay occurs due to grade 2 or 3 neutropenia, growth factor (G-CSF or GM-CSF) can be used at the discretion of the treating physician with subsequent cycles.</p>		

Dose Modifications for Thrombocytopenia on Day 1 at Time of Treatment of Any Cycle

<u>Thrombocytopenia</u>	Management/ Next Dose for M6620 (VX- 970)***	Management/ Next Dose for Gemcitabine	Management/ Next Dose for Carboplatin
≤ Grade 1	No change in dose	No change in dose	No change in dose
Grade 2	Hold until ≤ Grade 1. Resume at same dose level.	Hold until ≤ Grade 1. Resume at same dose level.	Hold until ≤ Grade 1. Resume at same dose level.
Grade 3	Hold* until ≤ Grade 1. Resume at same dose.**	Hold* until ≤ Grade 1. Resume at the same dose for the first occurrence, resume at one dose lower for second occurrence if indicated. **	Hold* until ≤ Grade 1. Resume at one lower dose if indicated. **
Grade 4	Hold* until ≤ Grade 1. Resume at same dose.**	Hold* until ≤ Grade 1. Resume at one lower dose if indicated. **	Hold* until ≤ Grade 1. Resume at one lower dose if indicated. **
<p>* * Patients with a grade 3 toxicity requiring a delay of > 2 weeks should be discussed with the PI. Patients may continue therapy if receiving benefit. Patients with a grade 4 toxicity requiring a delay of > 2 weeks, study should be discontinued</p> <p>** Patients requiring > two dose reductions should be discussed with PI. Patients may continue therapy if receiving benefit.</p> <p>*** Platelet count from day 1 will be used to determine the day 2 dose of M6620 (VX-970)</p>			

Dose Modifications for Thrombocytopenia on Day 8 at Time of Treatment of Any Cycle**

<u>Thrombocytopenia</u>	Management/ Next Dose for M6620 (VX- 970) (day 9)	Management/ Next Dose for Gemcitabine
≤ Grade 1	No change in dose	No change in dose
Grade 2	No change in dose	No change in dose
Grade 3	Omit dose. Resume same dose*	Omit dose. Resume at one dose lower, if indicated*
Grade 4	Omit dose. Resume at same dose*	Omit dose. Resume at one dose lower, if indicated*
<p>** Patients with a grade 3 toxicity requiring a delay of > 2 weeks should be discussed with the PI. Patients may continue therapy if receiving benefit. Patients with a grade 4 toxicity requiring a delay of > 2 weeks, study should be discontinued ** Platelet count from day 8 will be used to determine the day 9 dose of M6620 (VX-970)</p>		

Dose Modifications for Other Non-Hematological Toxicities At Time of Retreatment on Days 1, 2, 8 or 9*

<u>Event</u>	Management/ Next Dose for M6620 (VX-	Management/ Next Dose for Gemcitabine	Management/ Next Dose for Carboplatin
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	970)****		
≤ Grade 1	No change in dose	No change in dose	No change in dose
Grade 2	No change in dose	No change in dose	No change in dose
Grade 3*	Day 1: Hold** until ≤ Grade 2. Resume at same dose.***	Day 1: Hold** until ≤ Grade 2. Resume at one dose level lower. Day 8: Omit dose.	Day 1: Hold** until ≤ Grade 2. Resume at same dose for first occurrence; reduce one dose level for second occurrence, if indicated. ***
Grade 4*	Day 1: Hold** until ≤ Grade 2. Resume at same dose.***	Day 1: Hold** until ≤ Grade 2. Resume at one dose level lower.*** Day 8: Omit dose	Day 1: Hold** until ≤ Grade 2. Resume at same dose for first occurrence; reduce one dose level for second occurrence, if indicated. ***
<p>* Excludes alopecia and fatigue</p> <p>*** Patients with a grade 3 toxicity requiring a delay of > 2 weeks should be discussed with the PI. Patients may continue therapy if receiving benefit. Patients with a grade 4 toxicity requiring a delay of > 2 weeks, study should be discontinued.</p> <p>*** Patients requiring > two dose reductions should be discussed with PI. Patients may continue therapy if receiving benefit.</p> <p>**** M6620 (VX-970) should not be given if the day 1 or 8 chemotherapy was held or omitted. M6620 (VX-970) should only be given if it was preceded by chemotherapy the day prior.</p>			

Dose Modifications Based on Interim Toxicity

<u>Event</u>	Management/ Next Dose for M6620 (VX- 970)	Management/ Next Dose for Gemcitabine	Management/ Next Dose for Carboplatin
≤ Grade 1	No change in dose	No change in dose	No change in dose
Grade 2	No change in dose	No change in dose	No change in dose
Grade 3	Hold* until ≤ Grade 2. Resume at same dose.	Hold* until ≤ Grade 2. Resume at one dose level lower, if indicated.**	Hold* until ≤ Grade 2. Resume at one dose level lower, if indicated.**
Grade 4	Hold* until ≤ Grade 2. Resume at same dose, if indicated.**	Hold* until ≤ Grade 2. Resume at one dose level lower and is indicated.**	Hold* until ≤ Grade 2. Resume at one dose level lower and is indicated.**

* Patients with a grade 3 toxicity requiring a delay of > 2 weeks should be discussed with the PI. Patients may continue therapy if receiving benefit. Patients with a grade 4 toxicity requiring a delay of > 2 weeks, study should be discontinued ** Patients requiring > two dose reductions should be discussed with PI. Patients may continue therapy if receiving benefit.

Carboplatin Hypersensitivity Reaction: In the case of a carboplatin hypersensitivity reaction in any treatment group or cycle, omit remainder of carboplatin for the current cycle and attempt carboplatin desensitization day 1 of next cycle per institutional protocol. In the acute setting, please treat per institutional guidelines with IV corticosteroids, fluids, antihistamines and oxygen as needed. Rechallenge should be restricted to patients with mild to moderate reactions that do not include features of anaphylaxis. Carboplatin desensitization can be done per institutional protocol. Desensitization at Mayo Clinic includes medication with dexamethasone, diphenhydramine, famotidine, and hydrocortisone as well as beginning with low doses of carboplatin (0.0001 mg/dose in 100 mL 0.9% NaCl over 15 minutes) and titrating that upwards (0.0001 mg/dose, 0.001 mg/dose, 0.01 mg/dose, 0.1 mg/dose over 15 minutes) to 24.9 mg/dose over 3 hours. If this is tolerating the remaining carboplatin is infused in 250 mL 0.9% NaCl over 4 hours. Diphenhydramine and hydrocortisone is given every 4 hours during the desensitization. An anaphylaxis tray should be available at the bedside and the patient is staffed 1:1 during the first 8 hours of the infusion. If unable to rechallenge or patient refusal, patient will discontinue all protocol therapy.

M6620 (VX-970) Acute Hypersensitivity Reaction: Infusion reactions are common with intravenous administration of drugs used to treat cancer and have been seen with M6620 (VX-970) administration. These reactions occur during or shortly after administration of the drug and are diverse. They may include pruritus, flushing, chills/rigors, urticaria/rash, headache, bronchospasm/dyspnea, and hypotension or hypertension, among others. If any subject develops pruritus, flushing, or any other symptom suggestive of an infusion reaction, standard measures may be employed to manage these symptoms (e.g., antihistamines and/or steroids not prohibited by the protocol). To prevent recurrence of these symptoms in subsequent administrations, appropriate desensitizing measures prior to the administration of the study drugs may be employed (e.g., premedication with 200 mg hydrocortisone approximately 60 minutes before infusion, and 10 mg of chlorphenamine approximately 30 minutes before infusion; alternative antihistamine and steroid combinations may be considered). If standard procedures to limit symptoms of an infusion reaction are insufficient, then the infusion time may be extended beyond 60 minutes but no more than 90 minutes. Management of an infusion reaction may occur pre-emptively, if symptoms of an infusion reaction have been observed in subjects at a particular dose level of M6620 (VX-970).

Serious acute hypersensitivity reactions have occurred in a few subjects receiving M6620 (VX-970) as well. These reactions occurred within minutes of re-exposure to M6620 (VX-970), and in cases reported to date, they have occurred during the second infusion. They may include hypotension and mental status changes. All subjects have fully recovered with standard treatment for this reaction, including immediate discontinuation of the inciting infusion and administration of IV corticosteroid. If the reaction included significant hypotension and mental status changes, the patient cannot be rechallenged. The patient will no longer be able to continue on study; however, continued treatment with carboplatin and gemcitabine should be considered if a benefit is noted, per the discretion of the treating physician.

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of AEs (Section 7.1) and the characteristics of an observed AE (Section 7.2) will determine whether the event requires expedited reporting via the CTEP Adverse Event Reporting System (CTEP-AERS) **in addition** to routine reporting.

7.1 Comprehensive Adverse Events and Potential Risks List (CAEPR)

The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with ***bold*** and ***italicized*** text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements'

http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification. The CAEPR does not provide frequency data; refer to the Investigator's Brochure for this information. Below is the CAEPR for M6620 (VX-970).

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

7.1.1 CAEPR for M6620 (VX-970)

Comprehensive Adverse Events and Potential Risks list (CAEPR) for M6620 (VX-970) (NSC 780162)

The Comprehensive Adverse Events and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with bold and italicized text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements'

http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification. The CAEPR does not provide frequency data; refer to the Investigator's Brochure for this information. Below is the CAEPR for M6620 (VX-970).

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

Adverse Events with Possible Relationship to M6620 (VX-970) (CTCAE 5.0 Term)	Specific Protocol Exceptions to Expedited Reporting (SPEER)
BLOOD AND LYMPHATIC SYSTEM DISORDERS	
Anemia	<i>Anemia (Gr 3)</i>
GASTROINTESTINAL DISORDERS	
Diarrhea	<i>Diarrhea (Gr 2)</i>
Nausea	<i>Nausea (Gr 2)</i>
Vomiting	<i>Vomiting (Gr 2)</i>
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS	
Fatigue	<i>Fatigue (Gr 2)</i>
IMMUNE SYSTEM DISORDERS	
Anaphylaxis	
INFECTIONS AND INFESTATIONS	
Urinary tract infection	
INJURY, POISONING AND PROCEDURAL COMPLICATIONS	
Infusion related reaction	<i>Infusion related reaction (Gr 2)</i>
INVESTIGATIONS	
Alanine aminotransferase increased	<i>Alanine aminotransferase increased (Gr 2)</i>
Aspartate aminotransferase increased	<i>Aspartate aminotransferase increased (Gr 2)</i>
Blood bilirubin increased	
Creatinine increased	
Lymphocyte count decreased	<i>Lymphocyte count decreased (Gr 2)</i>
Neutrophil count decreased	
Platelet count decreased	
White blood cell decreased	
METABOLISM AND NUTRITION DISORDERS	
Hyperglycemia	
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS)	
Tumor pain	
NERVOUS SYSTEM DISORDERS	
Dizziness	
Headache	<i>Headache (Gr 2)</i>
SKIN AND SUBCUTANEOUS TISSUE DISORDERS	
Pruritus	
Rash maculo-papular	
VASCULAR DISORDERS	
Flushing	

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

Adverse events reported on M6620 (VX-970, NSC 780162) trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that M6620 (VX-970, NSC 780162) caused the adverse event:

CARDIAC DISORDERS - Palpitations

GASTROINTESTINAL DISORDERS - Abdominal pain; Ascites; Colonic obstruction; Mucositis oral

GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Edema limbs; Fever

IMMUNE SYSTEM DISORDERS - Allergic reaction

INFECTIONS AND INFESTATIONS - Infections and infestations - Other (lower respiratory tract infection); Otitis externa; Sepsis; Soft tissue infection

INVESTIGATIONS - GGT increased; Hemoglobin increased; Weight loss

METABOLISM AND NUTRITION DISORDERS - Anorexia; Dehydration; Hypophosphatemia

MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Generalized muscle weakness

NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS) - Neoplasms benign, malignant and unspecified (incl cysts and polyps) - Other (malignant neoplasm progression)

NERVOUS SYSTEM DISORDERS - Lethargy; Spinal cord compression; Syncope

PSYCHIATRIC DISORDERS - Confusion

RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Atelectasis; Dyspnea

VASCULAR DISORDERS - Hypertension; Hypotension; Thromboembolic event

Note: M6620 (VX-970) in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

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

7.1.2.1 Gemcitabine

Consult the package insert for the most current and complete information.

Common known potential toxicities, > 10%:

Cardiovascular: Peripheral edema, edema

Central nervous system: Pain, fever, somnolence

Dermatologic: Rash, alopecia, pruritus

Gastrointestinal: Nausea/vomiting, constipation, diarrhea, stomatitis

Hematologic: Anemia, leukopenia, thrombocytopenia, neutropenia, hemorrhage, myelosuppression is the dose-limiting toxicity

Hepatic: Transaminases increased, alkaline phosphatase increased, bilirubin increased

Renal: Proteinuria, hematuria, BUN increased

Respiratory: Dyspnea

Miscellaneous: Flu-like syndrome, infection

Less common known potential toxicities, 1% - 10%:

Local: Injection site reactions

Neuromuscular & skeletal: Paresthesia

Renal: Creatinine increased

Respiratory: Bronchospasm

Rare known potential toxicities, <1% (Limited to important or life-threatening):

Adult respiratory distress syndrome, anaphylactoid reaction, anorexia, arrhythmias, bullous skin eruptions, cellulitis, cerebrovascular accident, CHF, chills, cough, desquamation, diaphoresis, gangrene, GGT increased, headache, hemolytic uremic syndrome (HUS),

hepatotoxic reaction, hypertension, insomnia, interstitial pneumonitis, liver failure, malaise, MI, peripheral vasculitis, Petechiae, pulmonary edema, pulmonary fibrosis, radiation recall, renal failure, respiratory failure, rhinitis, sepsis, supraventricular arrhythmia, weakness

7.1.2.2 Carboplatin

Consult the package insert for the most current and complete information. Percentages reported with single-agent therapy. **Note:** Myelosuppression is dose related, schedule related, and infusion-rate dependent (increased incidences with higher doses, more frequent doses, and longer infusion times) and, in general, rapidly reversible upon discontinuation.

Common known potential toxicities, > 10%:

Central nervous system: Pain

Endocrine & metabolic: Hyponatremia, hypomagnesemia, hypocalcemia, hypokalemia

Gastrointestinal: Vomiting, abdominal pain, nausea

Hematologic: Myelosuppression (dose related and dose limiting; nadir at ~21 days; recovery by ~28 days), leukopenia, anemia, neutropenia, thrombocytopenia

Hypersensitivity: Hypersensitivity

Neuromuscular & skeletal: Weakness

Renal: Creatinine clearance decreased, BUN increased

Less common known potential toxicities, 1% - 10%:

Central nervous system: Peripheral neuropathy, neurotoxicity

Dermatologic: Alopecia

Gastrointestinal: Constipation, diarrhea, stomatitis/mucositis, taste dysgeusia

Hematologic: Hemorrhagic/bleeding complications

Hepatic: Bilirubin increased, increased serum alkaline phosphatase, increased serum AST

Infection: Infection

Local: Pain at the injection site

Neuromuscular & skeletal: Peripheral neuropathy

Ocular: Visual disturbance

Otic: Ototoxicity

Renal: Creatinine increased

Rare known potential toxicities, <1% (Limited to important or life-threatening):

Anaphylaxis, anorexia, bronchospasm, cardiac failure, cerebrovascular accident, dehydration, embolism, erythema, febrile neutropenia, hemolytic anemia (acute), hemolytic uremic syndrome, hyper-/hypotension, injection site reaction (pain, redness, swelling), limb ischemia (acute), malaise, metastases, pruritus, skin rash, tissue necrosis (associated with extravasation), urticaria, vision loss.

7.2 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.
- **For expedited reporting purposes only:**
 - AEs for the agent that are ***bold and italicized*** in the CAEPR (*i.e.*, those listed in the SPEER column, Section 7.1.1) should be reported through CTEP-AERS only if the grade is above the grade provided in the SPEER.
 - Other AEs for the protocol that do not require expedited reporting are outlined in section 7.3.4.
- **Attribution of the AE:**
 - Definite – The AE *is clearly related* to the study treatment.
 - Probable – The AE *is likely related* to the study treatment.
 - Possible – The AE *may be related* to the study treatment.
 - Unlikely – The AE *is doubtfully related* to the study treatment.
 - Unrelated – The AE *is clearly NOT related* to the study treatment.

7.3 Expedited Adverse Event Reporting

- 7.3.1 Expedited AE reporting for this study must use CTEP-AERS (CTEP Adverse Event Reporting System), accessed via the CTEP Web site (<https://eapps-ctep.nci.nih.gov/ctepaers>). The reporting procedures to be followed are presented in the “NCI Guidelines for Investigators: Adverse Event Reporting Requirements for DCTD (CTEP and CIP) and DCP INDs and IDEs” which can be downloaded from the CTEP Web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/adverse_events.htm). These requirements are briefly outlined in the tables below (Section 7.3.3).

In the rare occurrence when Internet connectivity is lost, a 24-hour notification is to be made to CTEP by telephone at 301-897-7497. Once Internet connectivity is restored, the 24-hour notification phoned in must be entered electronically into CTEP-AERS by the original submitter at the site.

7.3.2 Distribution of Adverse Event Reports

CTEP-AERS is programmed for automatic electronic distribution of reports to the following individuals: Principal Investigator and Adverse Event Coordinator(s) (if applicable) of the Corresponding Organization or Lead Organization, the local treating physician, and the Reporter and Submitter. CTEP-AERS provides a copy feature for other e-mail recipients.

7.3.3 Expedited Reporting Guidelines

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

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

Death due to progressive disease should be reported as **Grade 5 “Disease Progression”** in the system organ class (SOC) “General disorders and administration site conditions.” Evidence that the death was a manifestation of underlying disease (*e.g.*, radiological changes suggesting tumor growth or progression; clinical deterioration associated with a disease process) should be submitted.

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

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)

NOTE: Investigators **MUST** immediately report to the sponsor (NCI) **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)

An adverse event is considered serious if it results in **ANY** of the following outcomes:

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

ALL SERIOUS adverse events that meet the above criteria **MUST** be immediately reported to the NCI via electronic submission within the timeframes detailed in the table below.

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

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

Expedited AE reporting timelines are defined as:

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

¹Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:
Expedited 24-hour notification followed by complete report within 5 calendar days for:

- All Grade 3, 4, and Grade 5 AEs

Expedited 10 calendar day reports for:

- Grade 2 AEs resulting in hospitalization or prolongation of hospitalization

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

Effective Date: May 5, 2011

7.3.4 Additional Protocol-Specific Expedited Adverse Event Reporting Exclusions

For this protocol only, the AEs/grades listed below do not require expedited reporting via CTEP-AERS. However, they still must be reported through the routine reporting mechanism (Section 7.4):

CTCAE SOC	Adverse Event	Grade
Skin and subcutaneous disorders	Alopecia	Grade 2
Investigations	Neutropenia	Grade 3
Investigations	Thrombocytopenia	Grade 3

Due to the expected high frequency of the above events, these do not require expedited reporting but will be reported through the routine reporting mechanism.

7.4 Routine Adverse Event Reporting

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

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of patients enrolled in the studies as well as those who will enroll in future studies using similar agents. AEs are reported in a routine manner at scheduled times during the trial using Medidata Rave. For this trial the Adverse Event CRF is used for routine AE reporting in Rave.

7.5 Secondary Malignancy

A *secondary malignancy* is a cancer caused by treatment for a previous malignancy (e.g., treatment with investigational agent/intervention, radiation or chemotherapy). A secondary malignancy is not considered a metastasis of the initial neoplasm.

CTEP requires all secondary malignancies that occur following treatment with an agent under an NCI IND/IDE be reported expeditiously via CTEP-AERS. Three options are available to describe the event:

- Leukemia secondary to oncology chemotherapy (e.g., acute myelocytic leukemia [AML])

- Myelodysplastic syndrome (MDS)
- Treatment-related secondary malignancy

Any malignancy possibly related to cancer treatment (including AML/MDS) should also be reported via the routine reporting mechanisms outlined in each protocol.

7.6 Second Malignancy

A second malignancy is one unrelated to the treatment of a prior malignancy (and is **NOT** a metastasis from the initial malignancy). Second malignancies require **ONLY** routine AE reporting unless otherwise specified.

8. PHARMACEUTICAL INFORMATION

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

8.1 CTEP IND Agent(s)

8.1.1 M6620

Other Names: VRT-0768079, MSC2527093A, VX-970

Chemical Name: 5-(4-(isopropylsulfonyl)phenyl)-3-(3-(4-((methylamino)methyl)phenyl)isoxazol-5-yl)pyrazin-2- amine

Classification: ATR inhibitor

CAS Registry Number: 1232416-25-9

Molecular Formula: C₂₄H₂₅N₅O₃S

M.W.: 463.55 Da

Mode of Action: Ataxia telangiectasia mutated and Rad3-related (ATR) kinase is an apical regulator of checkpoint pathways triggered by DNA damage. The DNA damage response (DDR) is regulated by ATR kinase and ataxia telangiectasia mutated (ATM) kinase, which are recruited to distinct DNA damage structures. M6620 (VX-970) disrupts ATR-mediated DNA damage response signaling and leads to sustained accumulation of DNA damage in cancer cells co-treated with DNA-damaging agents.

Description: The drug substance for M6620 (VX-970) is the free base.

How Supplied: M6620 (VX-970) is supplied by Merck KGaA/EMD Serono, Inc. and distributed by the Pharmaceutical Management Branch, CTEP/DCTD/NCI as single-use 200 mg vials containing a sterile solution (20 mg/mL). M6620 (VX-970) solution for injection is a yellow liquid formulated in 20% betadex sulfobutyl ether sodium (w/v) and 86 mM acetate buffer, 10 mL total volume, supplied in clear glass vials in cardboard boxes with foam inserts.

Preparation: M6620 (VX-970) solution for injection must be diluted with 5% dextrose in water solution prior to administration. Do not use 0.9% Sodium Chloride due to incompatibility with

M6620 (VX-970). To prepare the infusion solution add the dose volume of M6620 (VX-970) to a non-polyvinyl chloride (non-PVC), di(2-ethylhexyl) phthalate (DEHP)-free EVA infusion bag containing 5% dextrose in water. Gently invert the IV bag 5-10 times to mix the solution. Confirm the solution is clear and free of precipitates and/or particulates. The final concentration must be between 0.075 mg/mL to 1 mg/mL. Place the IV bag into an opaque cover to protect from light.

Storage: Store intact vials protected from light inside cardboard boxes at room temperature, 25°C (77°F), with excursions allowed between 15 and 30°C (59 and 86°F).

If a storage temperature excursion is identified, promptly return M6620 (VX-970) to between 15 and 30°C and quarantine the supplies. Provide a detailed report of the excursion (including documentation of temperature monitoring and duration of the excursion) to PMBAAfterHours@mail.nih.gov for determination of suitability.

Stability: Stability testing of the intact vials is on-going. Prepared solutions must be protected from light and used within 4 hours from time of preparation if stored at room temperature or 24 hours if stored refrigerated (2-8°C).

Route of Administration: Intravenous (IV) infusion.

Method of Administration: Prior to administration the solution should be given one hour at ambient temperature to warm up if stored refrigerated following preparation. Infuse over 60 minutes using an infusion set containing low-sorption or non-PVC, DEHP-free tubing and an in-line 0.2 micron filter. 5% dextrose in water solution must be used for IV line priming and flushing. M6620 (VX-970) should not come in contact with 0.9% Sodium Chloride due to incompatibility. The infusion time may be extended beyond 60 minutes (as tolerated) but no more than 90 minutes if standard procedures to limit symptoms of an infusion reaction are insufficient or if the total volume of the infusion exceeds 600 mL. To minimize the possibility of phlebitis, M6620 (VX-970) should be administered through a large bore catheter into a large caliber peripheral vein or central venous access.

Patient Care Implications: Monitor for infusion site reactions, irritation, and phlebitis. M6620 (VX-970) absorbs in the UV-visible radiation spectrum and is widely distributed including skin, so patients receiving M6620 (VX-970) should take protective measures to minimize sun exposure.

Women of childbearing potential and men should use appropriate contraception while on study drug and for 6 months after discontinuation of M6620 (VX-970).

Potential Drug Interactions: M6620 (VX-970) is primarily metabolized by CYP3A4. M6620 (VX-970) has a low potential to inhibit CYP1A2, 2C9, 2C19, 2D6, and 3A4, and a moderate potential to reversibly inhibit CYP2E1. The potential for M6620 (VX-970) to induce CYP450 enzymes is low. Concomitant administration with strong inhibitors or inducers of CYP3A4 should be avoided.

M6620 (VX-970) is a weak/moderate inhibitor of UGT1A1, UGT1A14, UGT1A9, UGT2B15, and UGT2B17. UGT2B7, UGT1A3, and UGT1A6 were weakly or not inhibited. M6620 (VX-970) is predicted to not inhibit significantly the metabolic clearance of SN-38 (active metabolite of irinotecan) at therapeutic exposures.

M6620 (VX-970) is a moderate inhibitor of P-gp and BCRP. Use caution when administered with substrates of P-gp and BCRP transporters.

8.1.2 Agent Ordering and Agent Accountability

- 8.1.2.1 NCI-supplied agents may be requested by eligible participating Investigators (or their authorized designee) at each participating institution. The CTEP-assigned protocol number must be used for ordering all CTEP-supplied investigational agents. The eligible participating investigators at each participating institution must be registered with CTEP, DCTD through an annual submission of FDA Form 1572 (Statement of Investigator), NCI Biosketch, Agent Shipment Form, and Financial Disclosure Form (FDF). If there are several participating investigators at one institution, CTEP-supplied investigational agents for the study should be ordered under the name of one lead participating investigator at that institution.

In general, sites may order initial agent supplies when a subject is being screened for enrollment onto the study.

Submit agent requests through the PMB Online Agent Order Processing (OAOP) application. Access to OAOP requires the establishment of a CTEP Identity and Access Management (IAM) account and the maintenance of an “active” account status, a “current” password, and active person registration status. For questions about drug orders, transfers, returns, or accountability, call or email PMB any time. Refer to the PMB’s website for specific policies and guidelines related to agent management.

- 8.1.2.2 Agent Inventory Records – The investigator, or a responsible party designated by the investigator, must maintain a careful record of the receipt, dispensing and final disposition of all agents received from the PMB using the appropriate NCI Investigational Agent (Drug) Accountability Record (DARF) available on the CTEP forms page. Store and maintain separate NCI Investigational Agent Accountability Records for each agent, strength, formulation and ordering investigator on this protocol.

- 8.1.2.3 Investigator Brochure Availability
The current versions of the IBs for the agents will be accessible to site investigators and research staff through the PMB Online Agent Order Processing (OAOP) application. Access to OAOP requires the establishment of a CTEP Identity and Access Management (IAM) account and the maintenance of an “active” account status and a “current” password and an active person registration status. Questions about IB access may be directed to the PMB IB coordinator via email.

- 8.1.2.4 Useful links and Contacts
CTEP Forms, Templates, Documents: <http://ctep.cancer.gov/forms/>
NCI CTEP Investigator Registration: RCRHelpDesk@nih.gov
PMB policies and guidelines: http://ctep.cancer.gov/branches/pmb/agent_management.htm
PMB Online Agent Order Processing (OAOP) application: <https://ctepcore.nci.nih.gov/OAOP>
CTEP Identity and Access Management (IAM) account: <https://ctepcore.nci.nih.gov/iam/>
CTEP Associate Registration and IAM account help:

ctepreghelp@ctep.nci.nih.gov **PMB email:** PMBAfterHours@mail.nih.gov
PMB phone and hours of service: (240) 276-6575 Monday through Friday between
8:30 am and 4:30 pm (ET)
IB Coordinator: IBCoordinator@mail.nih.gov

8.2 Commercial Agent(s)

8.2.1 Gemcitabine (Gemzar®)

Background: Gemcitabine is a pyrimidine antimetabolite that inhibits DNA synthesis by inhibition of DNA polymerase and ribonucleotide reductase, specific for the S-phase of the cell cycle. Gemcitabine is phosphorylated intracellularly by deoxycytidine kinase to gemcitabine monophosphate, which is further phosphorylated to active metabolites gemcitabine diphosphate and gemcitabine triphosphate. Gemcitabine diphosphate inhibits DNA synthesis by inhibiting ribonucleotide reductase; gemcitabine triphosphate incorporates into DNA and inhibits DNA polymerase.

Formulation: Commercially available for injection: Powder for reconstitution: 200 mg and 1 gram vials. Solution for injection: 38 mg/mL 200 mg, 1 gm, and 2 gm vials. **MUST BE DILUTED BEFORE USE.**

Preparation, Storage, and Stability:

Powder for reconstitution:

Store intact vials at room temperature. Reconstitute the 200 mg vial with preservative free 0.9% NaCl 5 mL or the 1000 mg vial with preservative free 0.9% NaCl 25 mL. Resulting solution is 38 mg/mL. Dilute with 50-500 mL 0.9% NaCl or D₅W to concentrations as low as 0.1 mg/mL. Reconstituted vials are stable for up to 35 days and infusion solutions diluted in 0.9% NaCl are stable up to 7 days at 23°C when protected from light; however, the manufacturer recommends use within 24 hours for both reconstituted vials and infusion solutions. Do not refrigerate.

Solution for injection:

Store intact vials at refrigeration temperature between 2° to 8°C (36° to 46°F). Do not freeze. Each vial contains a gemcitabine concentration of 38 mg/mL. The appropriate amount of drug should be further diluted with 50-500 mL 0.9% NaCl or D₅W to concentrations as low as 0.1 mg/mL. When prepared as directed, diluted gemcitabine solutions are stable for 24 hours at controlled room temperature.

Administration: Refer to the drug treatment section of the protocol for specific administration directions and infusion rates. Gemcitabine is normally infused IV over 30 minutes. **Note:** Prolongation of the infusion time > 60 minutes has been shown to increase toxicity. Gemcitabine is being investigated in clinical trials for fixed dose rate infusion administration at doses from 1000 mg/m² to 2200 mg/m² at a rate of 10 mg/m²/minute. Prolonged infusion times increase the accumulation of the active metabolite, gemcitabine triphosphate. Patients who receive gemcitabine fixed dose rate infusions experience more grade three and four hematologic toxicities.

Pharmacokinetic information:

Distribution: Infusions <70 minutes: 50 L/m²; Long infusion times: 370 L/m²

Protein binding: Low

Metabolism: Metabolized intracellularly by nucleoside kinases to the active diphosphate (dFdCDP) and triphosphate (dFdCTP) nucleoside metabolites

Half-life elimination:

Gemcitabine: Infusion time ≤ hour: 32-94 minutes; infusion time 3-4 hours: 4-10.5 hours

Metabolite (gemcitabine triphosphate), terminal phase: 1.7-19.4 hours

Time to peak, plasma: 30 minutes after completion of infusion

Excretion: Urine (92% to 98%; primarily as inactive uridine metabolite); feces (<1%)

Potential Drug Interactions:

Increased Effect/Toxicity: Gemcitabine may increase the levels/effects of fluorouracil. Gemcitabine may enhance the adverse pulmonary effects of bleomycin.

Ethanol/Nutrition/Herb Interactions Ethanol: Avoid ethanol (due to GI irritation).

Drug procurement: Commercial supplies. Pharmacies or clinics shall obtain supplies from normal commercial supply chain or wholesaler.

8.2.2 Carboplatin (Paraplatin[®], CBDCA)

Background: Carboplatin is an alkylating agent which covalently binds to DNA; interferes with the function of DNA by producing interstrand DNA cross-links.

Product description: Commercially available for injection as:

Solution Reconstituted: 150 mg; Solution 10 mg/mL (5 mL, 15 mL, 45 mL, 60 mL)

Solution preparation: Refer to package insert for complete preparation and dispensing instructions. Store intact vials at room temperature at 25°C (77°F); excursions permitted to 15°C to 30°C (59°F to 86°F). Protect from light. Further dilution to a concentration as low as 0.5 mg/mL is stable at room temperature (25°C) for 8 hours in 0.9% NaCl or D5W; Stability has also been demonstrated for dilutions in D5W in PVC bags at room temperature for 8 days; however the manufacturer states to use within 8 hours due to lack of preservative.

Pharmacokinetic information:

Distribution: V_d: 16 L; into liver, kidney, skin, and tumor tissue.

Protein binding: 0%; however the platinum from carboplatin becomes irreversibly bound to plasma proteins.

Metabolism: Minimally hepatic to aquated and hydroxylated compounds.

Half-life elimination: CrCl > 60 mL/min: Carboplatin: 2.6-5.9 hours (based on dose of 300-500 mg/m²); Platinum (from carboplatin): ≥ 5 days.

Excretion: Urine (~70% as carboplatin within 24 hours; 3% to 5% as platinum within 1-4 days).

Potential Drug Interactions:

Increased Effect/Toxicity: Aminoglycosides increase risk of ototoxicity and/or nephrotoxicity. When administered as sequential infusions, observational studies indicate a potential for increased toxicity when platinum derivatives (carboplatin, cisplatin) are administered before taxane derivatives (docetaxel, paclitaxel).

Herb/Nutraceutical Interactions: Avoid black cohosh, dong quai in estrogen-dependent tumors.

Route of administration: Refer to the treatment section for specific administration instructions. When administered as a part of a combination chemotherapy regimen, sequence of administration may vary by regimen; refer to specific protocol for sequence recommendation. Needles or IV administration sets that contain aluminum should not be used in the preparation or administration of carboplatin; aluminum can react with carboplatin resulting in precipitate formation and loss of potency.

Agent Ordering: Commercial supplies. Pharmacies or clinics shall obtain supplies from normal commercial supply chain or wholesaler.

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

Summary of Required Specimens

All Groups: Phase 1 and Expansion Cohort

Study	Required Sample	Collection Timepoints	Handling and Shipping/ Protocol Section
Germline mutation testing by BROCA	20 mL whole blood	After consent and before Cycle 1 Day 2	Collect in EDTA (purple top) tubes. Samples should be refrigerated and shipped with cold packs on the day of collection. See Section 9.2.3.

Expansion Cohort

Study	Required Sample	Collection Timepoints	Handling and Shipping/ Protocol Section
Multiplex Quantitative IFA Assay and Mass Spectrometry Assay	Tumor tissue	Pretreatment: Before C1D1 On-Treatment: C1D2 C2D2	Tissue cores will be transferred into a 1.5 mL pre-chilled cryovial and then flash frozen in liquid nitrogen per DCTD SOP340507 (see Appendix D). Immediately snap-freeze the biopsy. Biopsies are stored in liquid nitrogen until shipped. See Section 9.2.2.

Expansion Cohort:

Study	Required Sample	Collection Timepoints	Handling and Shipping/ Protocol Section
Tumor BROCA Testing	Tumor tissue	Pre-treatment biopsy	Tissue cores will be transferred into a 1.5 mL pre-chilled cryovial and then flash frozen in liquid nitrogen per DCTD SOP340507 (see Appendix D). Immediately snap-freeze the biopsy. Biopsies are stored in liquid nitrogen until shipped. See Section 9.2.3.

9.1 Integrated Biomarker Studies

9.1.1 Multiplex Quantitative IFA Assay (Integrated biomarker, expansion cohort only)

9.1.1.1 Hypothesis and Rationale

Hypothesis: We hypothesize that i) exposure to carboplatin + gemcitabine will activate the ATR/CHK1 pathway as manifested by increased ATR autophosphorylation as well as increased H2AX phosphorylation in samples harvested 1 day after carboplatin + gemcitabine compared to pretreatment biopsies and ii) treatment with M6620 (VX-970) will diminish the increase in ATR autophosphorylation but will increase the DNA damage as manifested by H2AX and NBS1 phosphorylation.

Rationale: The purpose of this multiplex assay is to provide proof of mechanism for i) the activation of ATR by the carboplatin/gemcitabine combination at the doses administered in ovarian cancer in the clinical setting and ii) diminished ATR activation accompanied by enhanced DNA damage in tumors exposed to M6620 (VX-970) 24 hours after carboplatin + gemcitabine. A pretreatment biopsy is needed to assess the baseline level of pATR and H2AX. A second biopsy is required either at 24 hours after the gemcitabine infusion to assess the level of pATR and H2AX after the cytotoxic chemotherapy or 4-6 hours after the infusion of M6620 (VX-970) in order to assess the effects of M6620 (VX-970). Ideally, all three samples would be taken from the same patient during the first cycle, in order to assess the effect of M6620 (VX-970). However, obtaining two invasive tumor biopsies within 4-6 hours is not feasible. Therefore, patients will undergo one post treatment biopsy in cycle 1 and one post treatment biopsy in cycle 2.

9.1.1.2 Assay Validity and Appropriateness for Study

All participating sites will ship the tumor specimens to Mayo Clinic for storage. One

core from each time point will be stored in the Mayo Biospecimen Repository for repeat analysis if necessary and use for further biomarkers if they become available.

In batches, one core from each of the tumor specimens will be shipped to Dr. Ralph Parchment's laboratory for evaluation of these markers. His laboratory is fully equipped to carry out these investigations and has previously developed this assay and is serving as a central resource laboratory for the tumor specimen correlates for some of the ongoing NCI approved ETCTN M6620 (VX-970) clinical trials.

9.1.1.3 Performance Site

The pATR and Rad51 IFA assays will be performed in the laboratory of Dr. Parchment at PADIS, Frederick National Laboratory for Cancer Research (FNLCR) under the guidance of Dr. Deborah Wilsker. γ H2AX, pNBS1 IFA with β CATN segmentation assay will be performed in the NCLN PD Assay Laboratory at MD Anderson under the guidance of Dr. Kate Ferry-Galow.

9.1.1.4 Justification of the Number of Patients and Specimens

The purpose of the assay is to provide proof of mechanism that the carboplatin/gemcitabine therapy is indeed activating the pathway of interest and M6620 (VX-970) is decreasing that activation. This will be tested by obtaining information regarding the levels of DNA damage markers after exposure to cytotoxic therapy or after cytotoxic therapy and M6620 (VX-970). The number of samples will allow for analysis only in an exploratory fashion.

9.1.1.5 Description of Biological Samples Required for Assay

Tumor biopsies will be collected and processed using validated SOPs that will ensure specimen quality and patient confidentiality pursuant to informed consent provisions. The tissue cores will be transferred into 1.5-mL pre-chilled cryovials and then flash frozen in liquid nitrogen per DCTD SOP340507

(http://dctd.cancer.gov/ResearchResources/biomarkers/docs/par/SOP340507_Biopsy_Frozen.pdf). Detailed instructions on biopsy collection and handling as well as sample shipping manifests are included in SOP340507 (Appendix D). Samples will be first submitted to Dr. Ralph Parchment's laboratory at PADIS, Frederick National Laboratory for Cancer Research (FNLCR), then routed to IFA assay performance sites as described in Section 9.1.1.3. The frozen biopsy specimens are transferred to PADIS on dry ice, where the core biopsy samples are stored at -80°C , or colder and subsequently processed for analysis as directed by the Principal Investigator according to SOP340522

(http://dctd.cancer.gov/ResearchResources/biomarkers/docs/gH2AXIFA_Biopsy/SOP340522_Biopsy_Section.pdf and Appendix E). Biopsy samples will be analyzed for γ H2AX, Rad51, pNbs1, and pATR as described above; any additional cores will be returned to Mayo.

9.1.2 Multiplexed Mass Spectrometry Based Phospho-signaling Assay (Exploratory biomarker, expansion cohort only)

9.1.2.1 Hypothesis and Rationale

Hypothesis: We hypothesize that i) exposure to carboplatin + gemcitabine will activate the ATR/CHK1 pathway as manifested by increased ATR autophosphorylation as well as increased H2AX phosphorylation in samples

harvested 1 day after carboplatin + gemcitabine compared to pretreatment biopsies and ii) treatment with M6620 (VX-970) will diminish the increase in ATR autophosphorylation but will increase the DNA damage as manifested by phosphorylation of substrates of ATM, for example phospho-RAD50.

Rationale: The purpose of this multiplex mass spectrometry based assay is to provide proof of mechanism for i) the activation of ATR by the carboplatin/gemcitabine combination at the doses administered in ovarian cancer in the clinical setting and ii) diminished ATR activation accompanied by enhanced DNA damage in tumors exposed to M6620 (VX-970) 24 hours after carboplatin + gemcitabine. A pretreatment biopsy is needed to assess the baseline level of phospho-Ser1989-ATR (pATR) and other DNA damage response proteins. A second biopsy is required either at 24 hours after the gemcitabine infusion to assess the level of pATR and after the cytotoxic chemotherapy or 4-6 hours after the infusion of M6620 (VX-970) in order to assess the effects of M6620 (VX-970) and ATR activation. Ideally, all three samples would be taken from the same patient during the first cycle, in order to assess the effect of M6620 (VX-970). However, obtaining two invasive tumor biopsies within 4-6 hours is not feasible. Therefore, patients will undergo one post treatment biopsy in cycle 1 and one post treatment biopsy in cycle 2.

9.1.2.2 Assay Validity and Appropriateness for Study All participating sites will ship the tumor specimens to Mayo Clinic for storage. One core from each time point will be stored in the Mayo Biospecimen Repository for repeat analysis if necessary and use for further biomarkers if they become available.

In batches, one core from each of the tumor specimens will be shipped to Dr. Paulovich's laboratory for evaluation of these markers. Her laboratory is fully equipped to carry out these investigations and has previously developed this assay (<https://doi.org/10.1667/RR14963.1>).

9.1.2.3 Performance Site

The assay will be performed in the laboratory of Dr. Paulovich at the Fred Hutchinson Cancer Research Center. This laboratory has extensive experience with developing, validating and implementing assays assessing drug response.

9.1.2.4 Justification of the Number of Patients and Specimens

The purpose of the assay is to provide proof of mechanism that the carboplatin/gemcitabine therapy is indeed activating the pathway of interest and M6620 (VX-970) is decreasing that activation. This will be tested by obtaining information regarding the levels of DNA damage markers after exposure to cytotoxic therapy or after cytotoxic therapy and M6620 (VX-970). Because this is an expansion cohort, the number of samples will allow for analysis only in an exploratory fashion.

9.1.2.5 Description of Biological Samples Required for Assay

Tumor biopsies will be collected and processed using validated SOPs that will ensure specimen quality and patient confidentiality pursuant to informed consent provisions. The tissue cores will be transferred into 1.5-mL pre-chilled cryovials and then flash frozen in liquid nitrogen per DCTD SOP340507 (http://dctd.cancer.gov/ResearchResources/biomarkers/docs/par/SOP340507_Biopsy_Frozen.pdf). Detailed instructions on biopsy collection and handling as well as sample shipping manifests are included in SOP340507 (Appendix D). Once we are

assured that the integrated biomarker has been successfully assayed, samples will be submitted to Dr. Paulovich's laboratory for evaluation of phosphorylation of DNA damage markers. The frozen biopsy specimens are transferred and shipped on dry ice, where the core biopsy samples are stored at -80°C, or colder and subsequently processed for analysis as directed by the Principal Investigator according to SOP340522

(http://dctd.cancer.gov/ResearchResources/biomarkers/docs/gH2AXIFA_Biopsy/SOP340522_Biopsy_Section.pdf and Appendix E).

9.1.3 Germline mutation testing by BROCA (exploratory biomarker)

9.1.3.1 Hypothesis and Rationale

Hypothesis: Ovarian cancers in patients with germline *BRCA1* or *BRCA2* mutations will have a higher response rate to carboplatin/gemcitabine/M6620 (VX-970) than ovarian cancers in patients without germline *BRCA1* or *BRCA2* mutations.

Rationale: Preclinical studies have shown that ovarian cancer cell lines are sensitized by ATR inhibitors to platinum compounds and gemcitabine independent of whether the cell lines have HR defects or not. However, those cell lines with HR defects such as *BRCA1* loss are particularly sensitive to the combination [32]. This germline mutation testing will allow us to determine whether the correlation between inactivating mutations of HR genes and cancer hypersensitivity to this combination is also observed in the clinic.

9.1.3.2 Assay Validity and Appropriateness for Study

BRCA1 and *BRCA2* are part of the BROCA panel (see below), which is being run at the University of Washington in a CLIA-certified laboratory. This assay involves the targeted capture of select DNA repair genes (including *BRCA1* and *BRCA2*) followed by massively parallel sequencing to achieve substantial read depth.

9.1.3.3 Performance Site

This BROCA analysis will be performed at the University of Washington in collaboration with Dr. Elizabeth Swisher.

9.1.3.4 Data Supporting the Degree of Biomarker “Fit for Purpose”

Sequencing is performed in the University of Washington CLIA-certified Department of Laboratory Medicine sequencing laboratory. This panel has been used by Elizabeth Swisher and Mary Claire King to determine the frequency of mutations in *BRCA1*, *BRCA2*, and a number of additional genes encoding DNA repair proteins in patients with ovarian cancer [36].

9.1.3.5 Justification of the Number of Patients and Specimens

One sample of DNA from peripheral blood cells is needed for this analysis of germline DNA. The Swisher laboratory prefers to have 1 microgram of total DNA for these assays.

This assay will be performed on both the control and experimental treatment groups. This will allow us to i) determine whether the two treatment groups of the randomized trial are evenly balanced for patients with germline deleterious mutations

in HR genes and ii) assess, in an exploratory manner, the relationship between germline HR gene mutations and response to the carboplatin/gemcitabine regimen in this setting.

9.1.3.6 Description of Biological Samples Required for Assay

Assuming a normal leukocyte count, 6 cc of peripheral blood is sufficient for isolation of leukocyte DNA for this analysis.

9.1.4 Tumor BROCA testing (Exploratory biomarker, expansion cohort)

9.1.4.1 Hypothesis and Rationale

Hypothesis: Ovarian cancers with germline or somatic mutations in HR pathway genes will have a better response to the carboplatin/gemcitabine/M6620 (VX-970) combination.

Rationale: Preclinical studies have shown that ovarian cancer cell lines are sensitized by ATR inhibitors to platinum compounds and gemcitabine independent of whether the cell lines have HR defects or not. However, those cell lines with HR defects such as BRCA1 loss are particularly sensitive to the combination [32]. This tumor sample mutation testing will allow us to determine which tumors have deleterious germline or somatic mutations in HR repair pathway genes and then assess whether these deleterious HR pathway mutations are associated with an improved outcome in either the control or experimental treatment group.

9.1.4.2 Assay Validity and Appropriateness for Study

The BROCA assay performed in the Swisher laboratory at the University of Washington involves custom capture and massively parallel sequencing of approximately 40 genes encoding proteins known to be involved in HR, non-homologous end-joining, alternative end-joining and the Fanconi Anemia pathway. This sequencing is performed at the University of Washington in a CLIA-certified laboratory. The assay has been validated as described in ref. 35 and references therein.

9.1.4.3 Performance Site

This BROCA analysis will be performed at the University of Washington in collaboration with Dr. Elizabeth Swisher.

9.1.4.4 Data Supporting the Degree of Biomarker “Fit for Purpose”

Sequencing is performed in the University of Washington CLIA-certified Department of Laboratory Medicine sequencing laboratory. This panel has been used by Elizabeth Swisher and Mary Claire King to determine the frequency of mutations in *BRCA1*, *BRCA2*, and a number of additional genes encoding DNA repair proteins in patients with ovarian cancer [36]. More recently, this same methodology has been utilized to show that patients with somatic vs. germline mutations in these genes have similar disease-free survival after platinum-based chemotherapy [34]. Preliminary studies using samples from the Mayo Clinic Biospecimens Repository for Ovarian Cancer Research have demonstrated that 16.8% of HGSOCS seen at Mayo have deleterious germline mutations in HR genes (10% in *BRCA1* or *BRCA2* and 6.8% in

other HR genes); and an additional 9.9% have somatic mutations in these same genes (5.9% in *BRCA1* or *BRCA2* and 4.0% in other HR genes).

9.1.4.5 Justification of the Number of Patients and Specimens

One sample of tumor DNA is needed for this analysis. To test the hypothesis that tumor genotype correlates with response to the carboplatin/gemcitabine/M6620 (VX-970) combination, we will need a tumor DNA specimen from each patient in the expansion cohort.

9.1.4.6 Description of Biological Samples Required for Assay

Tumor biopsy samples that are formaldehyde fixed and paraffin embedded, snap frozen or embedded in OTC medium are sufficient for extraction of DNA. We prefer FFPE samples for this analysis because of the possibility of performing laser capture microdissection to isolate DNA from tumor cells if the percentage of tumor in the biopsy is small.

9.1.5 Prioritization of Tissue-Based Integrated Biomarkers

Tumor biopsies are being obtained from patients enrolled in the expansion cohort, in an attempt to confirm the mechanism of action of M6620 (VX-970) when combined with carboplatin and gemcitabine. Accordingly, when tissue obtained at biopsy is limiting, the highest priority will be the Multiplex Quantitative IFA Assay (Section 9.1.1), the second highest will be the Multiplexed Mass Spectrometry Based Phospho-signaling Assay (Section 9.1.2) and the third highest will be tumor sequencing for DNA repair genes (Section 9.1.4).

9.2 Laboratory Correlative Studies

9.2.1 Multiplex Quantitative IFA Assay (Integrated biomarker, expansion cohort)

9.2.1.1 Collection of Specimens

In all patients enrolled in the expansion cohort, three tumor biopsies will be obtained. All patients will undergo a pretreatment biopsy which will be obtained after registration and before day 1 of therapy. Patients will then be randomized to the timing of the next two biopsies. Group A: Cycle 1, day 2, 18-24 hours after carboplatin/gemcitabine and Cycle 2, day 2 4-6 hours after M6620 inhibitor. Group B: Cycle 1, day 2 4-6 hours after M6620 and Cycle 2, day 2, 18-24 hours after gemcitabine/carboplatin infusion. Biopsies should be obtained by interventional radiology using four (4) passes of an 18 gauge needle under ultrasound or CT guidance.

9.2.1.2 Handling of Specimens

Tissue cores will be transferred into a 1.5 mL pre-chilled cryovial and then flash frozen in liquid nitrogen per DCTD SOP340507 (see Appendix D). The preferred method of collection is for the interventional radiologist to eject the biopsy directly into the pre-chilled tube. Immediately snap-freeze the biopsy by placing the tube into liquid nitrogen or dry ice/ethanol bath. Biopsies are to be stored in liquid nitrogen until shipped.

9.2.1.3 Shipping of Specimens

All samples should be shipped via overnight express courier in insulated containers with enough dry ice to maintain samples at -80 °C for at least 72 hours. All specimens are to be shipped on Monday, Tuesday, or Wednesday to:

Mayo Clinic Attn:
Jill Wagner
Gonda 19-205B
221 4th Avenue SW
Rochester, MN 55905

Wagner.Jill@mayo.edu

Once monthly, one core from each time point will be sent to NCI from Mayo Clinic per directions below:

FedEx return shipment labels will be provided to Mayo Clinic, which will be sending all frozen shipments for the study to PADIS, Frederick National Laboratory for Cancer Research (FNLCR) Specimen Central Receiving. All samples should be shipped via overnight express courier in insulated containers with enough dry ice to maintain samples at -80 °C for at least 72 hours. All specimens are to be shipped on Monday, Tuesday, or Wednesday.

Just prior to shipping, place specimen tubes into an 81-place freezer box and then in a shipping container with sufficient dry ice to maintain the samples at -80 °C for at least 72 hours. All weekly processing specimens are recommended to ship out via FedEx on the following Monday afternoon for delivery by 10 a.m. overnight (FedEx First Overnight). PADIS, Frederick National Laboratory for Cancer Research (FNLCR) Specimen Central Receiving (NCI_PD_Support@mail.nih.gov) a shipment notification. Further details attached in Appendix D)

9.2.1.4 Site Performing Correlative Study.

The pATR and RAD51 IFA assays will be performed at PADIS, Frederick National Laboratory for Cancer Research (FNLCR). The γH2AX, pNBS1 IFA with βCATN segmentation assay will be performed at the NCLN PD Assay Laboratory at MD Anderson..

9.2.2 Multiplexed Mass Spectrometry Based Phospho-signaling Assay

9.2.2.1 Collection of Specimens:

In all patients enrolled in the expansion cohort, three tumor biopsies will be obtained. All patients will undergo a pretreatment biopsy, which will be obtained after registration and before Day 1 of therapy. Patients will then be randomized to the timing of the next two biopsies. Group A: Cycle 1, Day 2, 18-24 hours after carboplatin/gemcitabine and Cycle 2, Day 2 4-6 hours after M6620 inhibitor. Group B: Cycle 1, Day 2 4-6 hours after M6620 and Cycle 2, Day 2, 18-24 hours after gemcitabine/carboplatin infusion. Biopsies should be obtained by interventional radiology using two passes of an 18 gauge needle under ultrasound or CT guidance.

9.2.2.2 Handling of Specimens

Tissue cores will be transferred into a 1.5 mL pre-chilled cryovials and then flash frozen in liquid nitrogen per DCTD SOP340507 (see Appendix D). The preferred method of collection is for the interventional radiologist to eject the biopsy directly into the pre-chilled tube. Immediately snap-freeze the biopsy by placing the tube into liquid nitrogen or dry ice/ethanol bath. Biopsies are to be stored in liquid nitrogen until shipped.

9.2.2.3 Shipping of Specimens

All samples should be shipped via overnight express courier in insulated containers with enough dry ice to maintain samples at -80 °C for at least 72 hours. All specimens are to be shipped on Monday, Tuesday, or Wednesday to:

Mayo Clinic Attn:
Jill Wagner
Gonda 19-205B
221 4th Avenue SW
Rochester, MN 55905
Wagner.Jill@mayo.edu

Once the trial has completed accrual, one core from each timepoint will be sent to the Fred Hutchinson Cancer Center from Mayo Clinic per directions below:

Just prior to shipping, place specimen tubes into an 81-place freezer box and then in a shipping container with sufficient dry ice to maintain the samples at -80 °C for at least 72 hours. All weekly processing specimens are recommended to ship out via FedEx on the following Monday afternoon for delivery by 10 a.m. overnight (FedEx First Overnight). Emailtlorentz@fredhutch.org.

ATTN: Travis Lorentzen
1100 Fairview Ave North
Paulovich Lab, Room E2-423
Seattle, WA 98109
(206) 667-5107

9.2.2.1 Site Performing Correlative Study.

The Multiplex Mass Spectrometry Assay will be performed at the Fred Hutchinson Cancer Research Center in Seattle, WA.

The additional tissue core will be stored at the Mayo Clinic Biospecimens Repository.

9.2.3 BROCA Analysis of germline DNA

9.2.3.1 Collection of Specimens

All patients enrolled in the study will undergo a blood draw after signed consent. Two 10mL EDTA (purple top) whole blood specimens will be collected prior to treatment. This should be done on a Monday through Wednesday and can be drawn any time after consent has been signed or on day 1 or 2 of cycle 1.

9.2.3.2 Handling of Specimens

If possible, these specimens should be refrigerated and shipped with cold packs on the

same day (if necessary, tubes can be stored for up to 5 days before shipping if properly refrigerated).

9.2.3.3 Shipping of Specimens

All samples should be shipped via overnight express courier in insulated containers with enough cold packs to maintain samples around 4 °C for at least 72 hours. All specimens are to be shipped on Monday, Tuesday, or Wednesday to:

Mayo Clinic
Biospecimen Accessioning and Processing Shared Resource
Attn: BAP Supervisor
2915 Valleyhigh Drive NW
2915 Building Dock 200
Rochester, MN 55901

Shipment of samples must comply with appropriate regulations as specified by the carrier. At a minimum, all samples must be packaged within two containers with absorbent material between containers to control any spill or leakage. The outer container must be puncture-resistant. A biohazard sticker must be affixed to both the inner and outer containers.

9.2.3.4 Site Performing Correlative Study

The BROCA analysis will be performed batchwise in the laboratory of Dr. Elizabeth Swisher at the University of Washington.

Elizabeth Swisher MD
University of Washington
1959 NE Pacific Street
HSB, K154
Seattle, WA 98195
(206)616-4296
swishere@u.washington.edu

9.2.4 BROCA Analysis of Tumor Sample (Expansion Cohort)

9.2.4.1 Collection of Specimens

All patients enrolled in the expansion cohort of the study will undergo a pretreatment biopsy, which will be obtained after registration and before Day 1 of therapy. Biopsies should be obtained by interventional radiology using two passes of an 18 gauge needle under ultrasound or CT guidance (one core for this correlative and one core for the integrated multiplex immunofluorescence assay described in Section 9.2.2). This tumor sample for BROCA analysis is only required at the time of the pretreatment biopsy. No additional tissue needed on the additional biopsies unless there was inadequate tissue collection on Day 1.

In the Event of an Inadequate Pretreatment Biopsy in the Expansion Cohort
If the pretreatment biopsy in the experimental treatment group is not sufficient to provide material for BROCA analysis, two unstained 5 micron slides are required for the genomic analysis from an archival tumor sample. An adjacent slide H&E slide

(or an electronic version of the image) is also required to confirm the presence of tumor.

9.2.4.2 Handling of Specimens

Expansion Cohort

Tissue cores will be cut in half, transferred into two 1.5 mL pre-chilled cryovials and then flash frozen in liquid nitrogen by placing the tube into liquid nitrogen or dry ice/ethanol bath.

Expansion Cohort with Inadequate Pretreatment Biopsy Specimen

Slides should be labeled with the clinical trial ID number and clinical trial patient ID number. Slides will not be returned to the original institution.

9.2.4.3 Shipping of Specimens

All biopsy samples should be shipped via overnight express courier in insulated containers with enough dry ice maintain samples at -80 °C for at least 72 hours. All specimens are to be shipped on Monday, Tuesday, or Wednesday to:

Mayo Clinic Attn:
Jill Wagner
Gonda 19-205B
221 4th Avenue SW
Rochester, MN 55905
Wagner.Jill@mayo.edu

Slides with archival tissue specimens should also be sent to the address above. If electronic images of immediately adjacent slides are provided to confirm tumor content, those should be sent by e-mail to the study PI.

9.2.4.4 Site Performing Correlative Study

BROCA analysis will be performed batchwise in the laboratory of Dr. Elizabeth Swisher at the University of Washington.

University of Washington
Elizabeth Swisher MD
1959 NE Pacific Street
HSB, K154
Seattle, WA 98195
(206)616-4296
swishere@u.washington.edu

- | | |
|----|---|
| a: | Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, phosphorus, potassium, total protein, SGOT [AST], SGPT [ALT], sodium. Labs should be drawn on days 1 (or -1) and days 7 or 8 |
| b: | Serum pregnancy test for women of childbearing potential only. |
| c: | Pretreatment tumor biopsy in the expansion cohort only (gemcitabine, carboplatin and M6620 (VX-970)) |
| d: | Expansion Cohort only: Cycle 1 Day 2 and Cycle 2 Day 2 tumor biopsies (randomized to either 18-24 hours after gemcitabine infusion or 4-6 hours after M6620 (VX-970) infusion on day 2 of cycle 1 and day 2 of cycle 2) |
| e: | All women enrolled in the study will undergo a blood draw to assess for BRCA mutation status. This can be done any time after signed consent and up to day 2 of cycle 1. |
| f: | Tumor biopsies and BROCA panel do not apply to the phase 1 dose escalation, they are performed only in expansion cohort, only |
| g: | CBC with differential to be performed on days -1 or 1 and days 7 or 8 of every cycle |

h. Once therapy is discontinued (unacceptable adverse events, patient decision or disease progression) they will be followed for 3 years after removal from study or until death, whichever occurs first (see Section 5.4)
R: Research funded

11. MEASUREMENT OF EFFECT

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) guidelines (version 1.1)³⁶. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the short axis measurements in the case of lymph nodes are used in the RECIST guideline.

11.1 Schedule of Evaluations

For the purposes of this study, patients should be reevaluated every 6 weeks. In addition to a baseline scan, confirmatory scans should also be obtained at least 4 weeks following initial documentation of objective response.

11.2 Definitions of Measurable and Non-Measurable Disease

11.2.1 Measurable Disease

- 11.2.1.1 A non-nodal lesion is considered measurable if its longest diameter can be accurately measured as ≥ 2.0 cm with chest x-ray, or as ≥ 1.0 cm with CT or MRI scan.
- 11.2.1.2 A superficial non-nodal lesion is measurable if its longest diameter is ≥ 1.0 cm in diameter as assessed using calipers (e.g. skin nodules) or imaging. In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.
- 11.2.1.3 A malignant lymph node is considered measurable if its short axis is ≥ 1.5 cm when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm).

NOTE: *Tumor lesions in a previously irradiated area are not considered measurable disease.*

11.2.2 Non-Measurable Disease

- 11.2.2.1 All other lesions (or sites of disease) are considered non-measurable disease, including pathological nodes (those with a short axis ≥ 1.0 to < 1.5 cm). Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable as well.

Note: '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. In addition, lymph nodes that have a short axis < 1.0 cm are

considered non-pathological (i.e., normal) and should not be recorded or followed.

11.3 Guidelines for Evaluation of Measurable Disease

11.3.1 Measurement Methods

- ☐ All measurements should be recorded in metric notation (i.e., decimal fractions of centimeters) using a ruler or calipers.
- ☐ The same method of assessment and the same technique must be used to characterize each identified and reported lesion at baseline and during follow-up. For patients having only lesions measuring at least 1 cm to less than 2 cm must use CT imaging for both pre- and post-treatment tumor assessments.
- ☐ Imaging-based evaluation is preferred to evaluation by clinical examination when both methods have been used at the same evaluation to assess the antitumor effect of a treatment.

11.3.2 Acceptable Modalities for Measurable Disease

- ☐ 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.
- ☐ 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. The lesions should be measured on the same pulse sequence. 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.
- ☐ Chest X-ray: Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT scans are preferable.
- ☐ Physical Examination: For superficial non-nodal lesions, physical examination is acceptable, but imaging is preferable, if both can be done. In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

11.3.3 Measurement at Follow-up Evaluation

- ☐ A subsequent scan must be obtained at least 4 weeks following initial documentation of an objective status of either complete response (CR) or partial response (PR).
- ☐ In the case of stable disease (SD), follow-up measurements must have met the SD criteria at least once after study entry at a minimum interval of 6 weeks (see Section 11.44).
- ☐ The cytological confirmation of the neoplastic origin of any effusion that appears

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

- ☐ Cytologic and histologic techniques can be used to differentiate between PR and CR in rare cases (e.g., residual lesions in tumor types such as germ cell tumors, where known residual benign tumors can remain.)

11.4 Measurement of Effect

11.4.1 Target Lesions & Target Lymph Nodes

- Measurable lesions (as defined in Section 11.21) up to a maximum of 5 lesions, representative of all involved organs, should be identified as “Target Lesions” and recorded and measured at baseline. These lesions can be non-nodal or nodal (as defined in 11.21), where no more than 2 lesions are from the same organ and no more than 2 malignant nodal lesions are selected.

Note: If fewer than 5 target lesions and target lymph nodes are identified (as there often will be), there is no reason to perform additional studies beyond those specified in the protocol to discover new lesions.

- ☐ Target lesions and target lymph nodes should be selected on the basis of their size, be representative of all involved sites of disease, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion (or malignant lymph node) does not lend itself to reproducible measurements in which circumstance the next largest lesion (or malignant lymph node) which can be measured reproducibly should be selected.
- ☐ Baseline Sum of Dimensions (BSD): A sum of the longest diameter for all target lesions plus the sum of the short axis of all the target lymph nodes will be calculated and reported as the baseline sum of dimensions (BSD). The BSD will be used as reference to further characterize any objective tumor response in the measurable dimension of the disease.
- ☐ Post-Baseline Sum of the Dimensions (PBSD): A sum of the longest diameter for all target lesions plus the sum of the short axis of all the target lymph nodes will be calculated and reported as the post-baseline sum of dimensions (PBSD). If the radiologist is able to provide an actual measure for the target lesion (or target lymph node), that should be recorded, even if it is below 0.5 cm. If the target lesion (or target lymph node) is believed to be present and is faintly seen but too small to measure, a default value of 0.5 cm should be assigned. If it is the opinion of the radiologist that the target lesion or target lymph node has likely disappeared, the measurement should be recorded as 0 cm. The minimum sum of the dimensions (MSD) is the minimum of the BSD and the PBSD.

11.4.2 Non-Target Lesions & Non-Target Lymph Nodes

Non-measurable sites of disease (Section 11.22) are classified as non-target lesions or non-target lymph nodes and should also be recorded at baseline. These

lesions and lymph nodes should be followed in accord with 11.4.3.3.

11.4.3 Response Criteria

- 11.4.3.1 All target lesions and target lymph nodes followed by CT/MRI/PET-CT/Chest X-ray/physical examination must be measured on re-evaluation at evaluation times specified in Section 11.1. Specifically, a change in objective status to either a PR or CR cannot be done without re-measuring target lesions and target lymph nodes.

Note: Non-target lesions and non-target lymph nodes should be evaluated at each assessment, especially in the case of first response or confirmation of response. In selected circumstances, certain non-target organs may be evaluated less frequently. For example, bone scans may need to be repeated only when complete response is identified in target disease or when progression in bone is suspected.

11.4.3.2 Evaluation of Target Lesions

Complete Response (CR): All of the following must be true:

- a. Disappearance of all target lesions.
- b. Each target lymph node must have reduction in short axis to <1.0 cm.

Partial Response (PR): At least a 30% decrease in PBS (sum of the longest diameter for all target lesions plus the sum of the short axis of all the target lymph nodes at current evaluation) taking as reference the BSD (*see* Section 11.41).

Progression (PD): At least one of the following must be true:

- a. At least one new malignant lesion, which also includes any lymph node that was normal at baseline (< 1.0 cm short axis) and increased to ≥ 1.0 cm short axis during follow-up.
- b. At least a 20% increase in PBS (sum of the longest diameter for all target lesions plus the sum of the short axis of all the target lymph nodes at current evaluation) taking as reference the MSD (Section 11.41). In addition, the PBS must also demonstrate an absolute increase of at least 0.5 cm from the MSD.
- c. See Section 11.32 for details in regards to the requirements for PD via FDG-PET imaging.

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR, nor sufficient increase to qualify for PD taking as reference the MSD.

11.4.3.3 Evaluation of Non-Target Lesions & Non-target Lymph Nodes

Complete Response (CR): All of the following must be true:

- a. Disappearance of all non-target lesions.

- b. Each non-target lymph node must have a reduction in short axis to <1.0 cm.

Non-CR/Non-PD: Persistence of one or more non-target lesions or non-target lymph nodes.

Progression (PD): At least one of the following must be true:

- At least one new malignant lesion, which also includes any lymph node that was normal at baseline (< 1.0 cm short axis) and increased to ≥ 1.0 cm short axis during follow-up.
- Unequivocal progression of existing non-target lesions and non-target lymph nodes. (NOTE: Unequivocal progression should not normally trump target lesion and target lymph node status. It must be representative of overall disease status change.)
- See Section 11.32 for details in regards to the requirements for PD via FDG-PET imaging.

11.4.4 Overall Objective Status

The overall objective status for an evaluation is determined by combining the patient's status on target lesions, target lymph nodes, non-target lesions, non-target lymph nodes, and new disease as defined in the following table:

Target Lesions and Target Lymph Nodes	Non-Target Lesions and Non-Target Lymph Nodes	New Sites of Disease	Overall Objective Status
CR	CR	No	CR
CR	Non-CR/Non-PD	No	PR
PR	CR Non-CR/Non-PD	No	PR
CR/PR	Not All Evaluated*	No	PR
SD	CR Non-CR/Non-PD Not All Evaluated*	No	SD
Not all Evaluated	CR Non-CR/Non-PD Not All Evaluated*	No	Not Evaluated (NE)
PD	Unequivocal PD CR Non-CR/Non-PD Not All Evaluated*	Yes or No	PD
CR/PR/SD/PD/Not all Evaluated	Unequivocal PD	Yes or No	PD
CR/PR/SD/PD/Not all Evaluated	CR Non-CR/Non-PD Not All Evaluated*	Yes	PD

*See Section 11.431

11.4.5 Symptomatic Deterioration

Patients with global deterioration of health status that is not attributable to reversible drug side effects requiring discontinuation of treatment without objective evidence of disease progression at that time, and not either related to study treatment or other medical conditions, should be reported as PD due to “symptomatic deterioration.” Every effort should be made to document the objective progression even after discontinuation of treatment due to symptomatic deterioration. A patient is classified as having PD due to “symptomatic deterioration” if any of the following occur that are not either related to study treatment or other medical conditions:

- Weight loss >10% of body weight.
- Worsening of tumor-related symptoms.
- Decline in performance status of >1 level on ECOG scale.

12. DATA REPORTING / REGULATORY REQUIREMENTS

Study Oversight

This protocol is monitored at several levels, as described in this section. The Protocol Principal Investigator is responsible for monitoring the conduct and progress of the clinical trial, including the ongoing review of accrual, patient-specific clinical and laboratory data, and routine and serious adverse events; reporting of expedited adverse events; and accumulation of reported adverse events from other trials testing the same drug(s). The Protocol Principal Investigator and statistician have access to the data at all times through the CTMS web-based reporting portal.

For the Phase 1 portion of this study, all decisions regarding dose escalation/expansion/de-escalation require sign-off by the Protocol Principal Investigator through the CTMS/IWRS. In addition, for the Phase 1 portion, the Protocol Principal Investigator will have at least monthly, or more frequently, conference calls with the Study Investigators and the CTEP Medical Officer(s) to review accrual, progress, and adverse events and unanticipated problems.

For a Phase 1/2 trial, enrollment to the Phase 2 portion of the trial will not begin until a protocol amendment has been submitted which summarizes the Phase 1 results, the recommended Phase 2 dose, and the rationale for selecting it. The amendment must be reviewed and approved by CTEP before enrollment to the Phase 2 portion can begin.

During the Phase 2 portion of the study, the Protocol Principal Investigator will have, at a minimum, quarterly conference calls with the Study Investigators and the CTEP Medical Officer(s) to review accrual, progress, and pharmacovigilance. Decisions to proceed to the second stage of a Phase 2 trial will require sign-off by the Protocol Principal Investigator and the Protocol Statistician.

All Study Investigators at participating sites who register/enroll patients on a given protocol are responsible for timely submission of data via Medidata Rave and timely reporting of adverse events for that particular study. This includes timely review of data collected on the electronic CRFs submitted via Medidata Rave.

All studies are also reviewed in accordance with the enrolling institution's data safety monitoring plan.

12.1 Data Reporting

Data collection for this study will be done exclusively through Medidata Rave. Access to the trial in Rave is granted through the iMedidata application to all persons with the appropriate roles assigned in the Regulatory Support System (RSS). To access Rave via iMedidata, the site user must have an active CTEP IAM account (check at <https://ctepcore.nci.nih.gov/iam>) and the appropriate Rave role (Rave CRA, Read-Only, RAVE CRA (Lab Admin), SLA, or Site Investigator) (<https://eapps-ctep.nci.nih.gov/iam>) and the appropriate Rave role (Rave CRA, Read-Only, or Site Investigator) on either the Corresponding Organization or Participating Organization roster at the enrolling site. To hold Rave CRA role or RAVE CRA (Lab Admin) role, the user must hold a minimum of an AP registration type. To hold the Rave Investigator role, the individual must be registered as an NPIVR or IVR. Associates can hold read-only roles in Rave.

Upon initial site registration approval for the study in RSS, all persons with Rave roles assigned on the appropriate roster will be sent a study invitation e-mail from iMedidata. To accept the invitation, site users must log into the Select Login (<https://login.imedidata.com/selectlogin>) using their CTEP-IAM user name and password, and click on the "accept" link in the upper right -corner of the iMedidata page. Please note, site users will not be able to access the study in Rave until all required Medidata and study specific trainings are completed. Trainings will be in the form of electronic learnings (eLearnings), and can be accessed by clicking on the link in the upper right pane of the iMedidata screen.

Users that have not previously activated their iMedidata/Rave account at the time of initial site registration approval for the study in RSS will also receive a separate invitation from iMedidata to activate their account. Account activation instructions are located on the CTSU website, Rave tab under the Rave resource materials (Medidata Account Activation and Study Invitation Acceptance). Additional information on iMedidata/Rave is available on the CTSU members' website under the Rave tab or by contacting the CTSU Help Desk at 1-888-823-5923 or by e-mail at ctsucontact@westat.com.

12.1.1 Method

This study will be monitored by the Clinical Trials Monitoring Service (CTMS). Data will be submitted to CTMS at least once every two weeks via Medidata Rave (or other modality if approved by CTEP). Information on CTMS reporting is available at <http://www.theradex.com/clinicalTechnologies/?National-Cancer-Institute-NCI-11>. On-site audits will be conducted on an 18-36 month basis as part of routine cancer center site visits. More frequent audits may be conducted if warranted by accrual or due to concerns regarding data quality or timely submission. For CTMS monitored studies, after users have activated their accounts, please contact the Theradex Help Desk at (609) 799-7580 or by email at CTMSSupport@theradex.com for additional support with Rave and completion of CRFs.

12.1.2 Responsibility for Data Submission

For ETCTN trials, it is the responsibility of the PI(s) at the site to ensure that all

investigators at the ETCTN Sites understand the procedures for data submission for each ETCTN protocol and that protocol specified data are submitted accurately and in a timely manner to the CTMS via the electronic data capture system, Medidata Rave.

Data are to be submitted via Medidata Rave to CTMS on a real-time basis, but no less than once every 2 weeks. The timeliness of data submissions and timeliness in resolving data queries will be tracked by CTMS. Metrics for timeliness will be followed and assessed on a quarterly basis. For the purpose of Institutional Performance Monitoring, data will be considered delinquent if it is greater than 4 weeks past due.

Data from Medidata Rave and CTEP-AERS is reviewed by the CTMS on an ongoing basis as data is received. Queries will be issued by CTMS directly within Rave. The queries will appear on the Task Summary Tab within Rave for the CRA at the ETCTN to resolve. Monthly web-based reports are posted for review by the Drug Monitors in the IDB, CTEP. Onsite audits will be conducted by the CTMS to ensure compliance with regulatory requirements, GCP, and NCI policies and procedures with the overarching goal of ensuring the integrity of data generated from NCI-sponsored clinical trials, as described in the ETCTN Program Guidelines, which may be found on the CTEP (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/adverse_events.htm) and CTSU websites.

An End of Study CRF is to be completed by the PI, and is to include a summary of study endpoints not otherwise captured in the database, such as (for phase 1 trials) the recommended phase 2 dose (RP2D), and a description of any dose-limiting toxicities (DLTs). CTMS will utilize a core set of eCRFs that are Cancer Data Standards Registry and Repository (caDSR) compliant (<http://cbiit.nci.nih.gov/ncip/biomedical-informatics-resources/interoperability-and-semantics/metadata-and-models>). Customized eCRFs will be included when appropriate to meet unique study requirements. The PI is encouraged to review the eCRFs, working closely with CTMS to ensure prospectively that all required items are appropriately captured in the eCRFs prior to study activation. CTMS will prepare the eCRFs with built-in edit checks to the extent possible to promote data integrity.

Further information on data submission procedures can be found in the ETCTN Program Guidelines (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/adverse_events.htm).

12.2 Collaborative Agreements Language

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

(http://ctep.cancer.gov/industryCollaborations2/intellectual_property.htm) contained within the terms of award, apply to the use of the Agent(s) in this study:

1. Agent(s) may not be used for any purpose outside the scope of this protocol, nor can Agent(s) be transferred or licensed to any party not participating in the clinical study. Collaborator(s) data for Agent(s) are confidential and proprietary to Collaborator(s) and shall be maintained as such by the investigators. The protocol documents for studies utilizing Agents contain confidential information and should not be shared or distributed without the permission of the NCI. If a copy of this protocol is requested by a patient or patient's family member participating on the study, the individual should sign a confidentiality agreement. A suitable model agreement can be downloaded from: <http://ctep.cancer.gov>.
2. For a clinical protocol where there is an investigational Agent used in combination with (an)other Agent(s), each the subject of different Collaborative Agreements, the access to and use of data by each Collaborator shall be as follows (data pertaining to such combination use shall hereinafter be referred to as "Multi-Party Data"):
 - a. NCI will provide all Collaborators with prior written notice regarding the existence and nature of any agreements governing their collaboration with NCI, the design of the proposed combination protocol, and the existence of any obligations that would tend to restrict NCI's participation in the proposed combination protocol.
 - b. Each Collaborator shall agree to permit use of the Multi-Party Data from the clinical trial by any other Collaborator solely to the extent necessary to allow said other Collaborator to develop, obtain regulatory approval or commercialize its own Agent.
 - c. Any Collaborator having the right to use the Multi-Party Data from these trials must agree in writing prior to the commencement of the trials that it will use the Multi-Party Data solely for development, regulatory approval, and commercialization of its own Agent.
3. Clinical Trial Data and Results and Raw Data developed under a Collaborative Agreement will be made available to Collaborator(s), the NCI, and the FDA, as appropriate and unless additional disclosure is required by law or court order as described in the IP Option to Collaborator (http://ctep.cancer.gov/industryCollaborations2/intellectual_property.htm). Additionally, all Clinical Data and Results and Raw Data will be collected, used and disclosed consistent with all applicable federal statutes and regulations for the protection of human subjects, including, if applicable, the *Standards for Privacy of Individually Identifiable Health Information* set forth in 45 C.F.R. Part 164.
4. When a Collaborator wishes to initiate a data request, the request should first be sent to the NCI, who will then notify the appropriate investigators (Group Chair for Cooperative Group studies, or PI for other studies) of Collaborator's wish to contact them.
5. Any data provided to Collaborator(s) for Phase 3 studies must be in accordance with the guidelines and policies of the responsible Data Monitoring Committee (DMC), if there is a

DMC for this clinical trial.

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

Email: ncicteppubs@mail.nih.gov

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

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Primary Endpoint

13.1.1 Phase 1 Dose Escalation

The safety analysis will enroll in cohorts of 3 patients each where the study will be temporarily suspended after the enrollment of each cohort of 3 patients. For this protocol, dose-limiting toxicity (DLT) will be defined as an adverse event attributed (definitely, probably, or possibly) to the study treatment for cycle 1 only and meeting the following criteria:

- DLT will be defined as grade 4 absolute neutrophil count for ≥ 5 days, grade 4 anemia, platelet count $< 25,000$, or other non-hematologic events \geq grade 3 as per NCI Common Terminology Criteria for Adverse Events Version 4.0 (except fatigue, alopecia and anorexia).

Maximum Tolerated Dose (MTD) Determination

The MTD in this study will be defined as the highest safely tolerated dose, up to a maximum of dose level 4, where at most 1 out of six patients experience a DLT with the next higher dose having at least 2 DLTs in 3 or more patients. A cohort of 3

patients will be enrolled and observed for 6 weeks (i.e., 2 cycles) to assess toxicity before new patients are treated. If ≥ 2 of 3 patients at a dose level experience a DLT, then the MTD has been exceeded, and the previous dose must be considered as MTD for the randomized phase II portion, as long as 6 patients were treated at that dose level. If dose level 1 is found too toxic, we will consider adding additional lower dose levels to assess safety further.

A total of 6 patients treated at the MTD will be sufficient to identify common toxicities at the MTD. For instance, those toxicities with an incidence of at least 25% will be observed with a probability of at least 82% ($1-(1-0.25)^6$).

If 1 of 3 patients at a dose level experiences DLT, then 3 additional patients will be treated at the same dose level, expanding that dose cohort to 6 patients. Upon dose cohort expansion to 6 patients, if ≥ 2 out of 6 patients at a dose level experiences a DLT, then the MTD has been exceeded, and the previous dose must be considered as the MTD, as long as 6 patients were treated at that dose level. If only 1 of 6 patients experience a DLT at a given dose level, the next higher dose level will be assessed up to the maximum dose level, dose level 4. If 0 of 3 patients at a dose level experience a DLT, then 3 additional patients will be treated at the next higher dose level up to a maximum of dose level 3.

From this phase, treatment data, adverse events, and the MTD will be reported descriptively. The maximum grade for each type of adverse event will be summarized using CTCAE version 5.0. The frequency and percentage of grade 3+ adverse events will be reported.

Evaluable Patients

If a patient fails to complete the first cycle for reasons other than toxicity, the patient will be regarded as inevaluable and will be replaced in the phase I portion of this study.

13.1.2 Expansion Cohort

The expansion cohort will enroll an additional 20 patients at the MTD, for 26 total patients treated at the MTD.

Primary Endpoint of expansion cohort:

The primary endpoint of the expansion cohort is to confirm the safety at the MTD of the addition of M6620 (VX-970) to carboplatin and gemcitabine in first or second recurrence of platinum sensitive high grade serous or endometrioid ovarian, primary peritoneal or fallopian tube carcinoma. From this phase, adverse events will be reported descriptively. The maximum grade for each type of adverse event will be summarized using CTCAE version 5.0. The frequency and percentage of grade 3+ adverse events will be reported. We will ensure that the treatment combination is safe enough for future studies in this disease population by comparing the AE data from our trial to historical data in this disease. If we feel the treatment combination is safe enough at the MTD to proceed to larger studies, we will make that recommendation in our summary of conclusions in future publications.

13.2 Sample Size/Accrual Rate

Total Sample Size

Including the phase I dose escalation phase and the expansion cohort, we anticipate enrolling 31 patients total (11 during the dose escalation phase + 20 during expansion phase).

Expected Accrual and Accrual Duration

The expected accrual rate is about 2 patients per month across the ETCTN sites. With this accrual rate, we expect to finish accrual within about 16 months, assuming we accrue 31 total patients.

Anticipated Time to Study Completion

We anticipate that the study will take approximately 2 years to complete. This allows a 6-month follow-up for the final patient enrolled, along with data entry, data clean-up, and analysis.

PLANNED ENROLLMENT REPORT

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/ Alaska Native	3	0	1	0	4
Asian	2	0	0	0	2
Native Hawaiian or Other Pacific Islander	1	0	1	0	2
Black or African American	3	0	1	0	4
White	15	0	4	0	19
More Than One Race	0	0	0	0	0
Total	24	0	7	0	31

13.3 Stratification Factors

Phase 1 Dose Escalation/Expansion Cohort

- No stratification factors for the trial

13.4 Analysis of Secondary Endpoints

The following secondary endpoints will be assessed for patients treated at the MTD: confirmed response rate, overall survival, duration of response, and progression free survival.

- 13.4.1 The key secondary endpoint is to determine if the MTD for the combination of carboplatin, gemcitabine and M6620 (VX-970) improves the confirmed response rate in adult women with platinum sensitive recurrent high grade serous or high grade endometrioid ovarian, primary peritoneal or fallopian tube cancer. Any confirmed response that occurs during the first 8 cycles of treatment will count as a success (24 weeks). Per RECIST 1.1, responses need to be confirmed (2 consecutive responses at least 4 weeks apart) to count as a response (see Section 11). All patients who meet the eligibility criteria, sign the consent form, and start treatment will be considered evaluable for this endpoint.

[REDACTED]

- 13.4.2 Overall Survival

Overall survival (OS) is defined as the time from registration to death from any cause. OS will be estimated using the Kaplan-Meier method. All patients who meet the eligibility criteria, sign the consent form, and start treatment will be considered evaluable for this endpoint.

- 13.4.3 Duration of Response: The duration of confirmed responses will be assessed using the Kaplan-Meier method, where the duration of confirmed response will be defined as the time from the first documented date of confirmed response (CR or PR) to the date at which progression is first documented.

- 13.4.4 Progression Free Survival

PFS is defined as the time from registration to the first of either disease progression or death from any cause, where disease progression will be determined based on RECIST 1.1 criteria. All patients who meet the eligibility criteria, sign the consent form, and start treatment will be considered evaluable for this endpoint. PFS will be estimated using the Kaplan-Meier method.

13.4.5 [REDACTED]

13.4.6 Exploratory Endpoints

Due to the limited sample size in this study, the proposed translational studies are considered exploratory and hypothesis generating. We will correlate the DNA damage markers and mutation data with clinical endpoints (i.e. response, PFS, OS). Statistical and graphical techniques will be used to explore these relationships. For time-to-event endpoints, we will use Cox proportional hazards models, and for response data we will use Logistic regression models. In addition, we will use Fisher's exact tests to test the association between categorical marker data and response. All patients who meet the eligibility criteria, sign the consent form, start treatment and have translational data will be considered evaluable for these endpoints.

13.5 Reporting and Exclusions

13.5.1 Evaluation of Toxicity

All patients will be evaluable for toxicity from the time they start treatment.

13.5.2 Evaluation of Response

All patients who meet the eligibility criteria, sign the consent form, and start treatment will be considered evaluable for efficacy endpoints. This analysis will not be an intent-to-treat analysis. All conclusions should be based on all eligible patients.

Sub analyses may then be performed on the basis of a subset of patients, excluding those for whom major protocol deviations have been identified (*e.g.*, early death due to other reasons, early discontinuation of treatment, major protocol violations, etc.). However, these sub analyses may not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding patients from the analysis should be clearly reported. The 95% confidence intervals will also be provided.

13.6 Adverse Event Stopping Rule

13.6.1 Monitoring

The principal investigator and the study statistician will review the study periodically (every 4-6 months, or more frequently if clinically indicated) to identify accrual and any endpoint problems that might be developing. For toxicity monitoring for the phase 1 portion of the study, the study team will review toxicity data after every 3 patients and after the occurrence of each DLT by conference calls, as well as during the Phase I DOG meetings on a bi-weekly basis. For the expansion cohort, the study team will review toxicity data every 4 months.

13.6.2 Adverse Event Stopping Rule

In this study, we will suspend accrual to allow for a full review of the data, if any of the following occur during the expansion cohort portion of the trial:

- (1) If at any time, 2 of the initial 10 patients (or 20% of all patients when accrual is greater than 10) have experienced any grade 4 adverse event (at least possibly related to the study medication).
- (2) If at any time, 1 of the initial 10 patients (or 10% of all patients when accrual is greater than 10) have experienced any grade 5 adverse event (at least possibly related to the study medication). Of course, we will also closely monitor each grade 5 event on a case-by-case basis, and may immediately suspend accrual after one grade 5 event if we feel it is best for patient safety.

After consideration by the study team (ie, Study Chair and statisticians) and consultation with representatives at the Cancer Therapy Evaluation Program (CTEP) and the Central Institutional Review Board (CIRB), a decision will be made as to whether and how the study will proceed.

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APPENDIX A: PERFORMANCE STATUS CRITERIA

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

APPENDIX B: PATIENT DRUG INFORMATION HANDOUT AND WALLET CARD

Information for Patients, Their Caregivers and Non-Study Healthcare Team on Possible Interactions with Other Drugs and Herbal Supplements

Patient
Name:

Diagnosis:

Trial #:

Study
Doctor:

Study Doctor
Phone #:

Study
Drug(s):

Please show this paper to all your healthcare providers (doctors, physician assistants, nurse practitioners, pharmacists), and tell them you are taking part in a clinical trial sponsored by the National Cancer Institute.

These are the things that your healthcare providers need to know:

M6620 (VX-970) interacts with specific enzymes in the liver or other tissues like the gut and certain transport proteins that help move drugs in and out of the cell.

Explanation	
CYP isoenzymes	The enzyme in question is CYP3A4 . M6620 (VX-970) is metabolized by CYP3A4 and may be affected by other drugs that inhibit or induce this enzyme.
Protein transporters	The proteins in questions are P-gp and BCRP . M6620 (VX-970) is a moderate inhibitor of these proteins and may affect drugs that are moved in and out of cells/organs by these transport proteins.

These are the things that you need to know:

The study drug M6620 (VX-970), may interact with other drugs which can cause side effects. For this reason, it is very important to tell your doctors about all your medicines, including: (a) medicines you are taking before this clinical trial, (b) medicines you start or stop taking during this study, (c) medicines you buy without a prescription (over-the-counter remedy), (d) herbals or supplements (e.g. St. John's Wort). It is helpful to bring your medication bottles or an updated medication list with you.



Before you enroll onto the clinical trial, your study doctor will work with your regular health care providers to review any medicines and herbal supplements that are considered strong inhibitors or inducers of CYP3A4 and substrates of P-gp and BCRP.

- Please be very careful! Over-the-counter drugs (including herbal supplements) may contain ingredients that could interact with your study drug. Speak to your doctors or pharmacist to determine if there could be any side effects.
- Make sure your doctor knows to avoid certain prescription medications.
- Your regular health care provider should check a frequently updated medical reference or call your study doctor before prescribing any new medicine or discontinuing any medicine.

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(Next page: Patient Drug Interaction Wallet Card)

PATIENT DRUG INTERACTION WALLET CARD

 NATIONAL CANCER INSTITUTE EMERGENCY INFORMATION		 NATIONAL CANCER INSTITUTE DRUG INTERACTIONS	
Show this card to all of your healthcare providers. Keep it with you in case you go to the emergency room.		Carry this card with you at all times M6620 (VX-970) interacts with specific enzymes in your liver or other tissues like the gut and transport proteins that help move drugs in and out of cells and must be used very carefully with other medicines.	
Patient Name: Diagnosis: Study Doctor: Study Doctor Phone #: NCI Trial #: Study Drug(S):		Use caution and avoid the following drugs if possible: Your healthcare providers should be aware of any medicines that are strong inhibitors or inducers of CYP3A4, and substrates of P-gp and BCRP. <ul style="list-style-type: none"> • Strong inhibitors or inducers of CYP3A4 should be avoided. • Substrates of P-gp and BCRP should be used with caution. Before prescribing new medicines , your health care provider should check a frequently-updated medical reference for a list of drugs to avoid or contact your study doctor.	
For more information: 1-800-4-CANCER cancer.gov clinicaltrials.gov		Version Feb/2019	
For more information: 1-800-4-CANCER cancer.gov clinicaltrials.gov		For more information: 1-800-4-CANCER cancer.gov clinicaltrials.gov	

APPENDIX C: BROCA GENE LIST

University of Washington –Department of Laboratory Medicine

BROCA-Cancer Risk Panel

http://tests.labmed.washington.edu/BROCA#BROCA_Gene_List

Gene	Function/Path way	Heterozygote Cancer risk*	Associated syndrome	References (PMID)
<i>AKT1</i>	AKT signaling	Breast, Thyroid	Cowden-like	23246288
<i>APC</i>	WNT signaling	Colon	Familial adenomatous polyposis	20301519
<i>ATM</i>	Double stranded break repair	Breast, Pancreatic	Ataxia telangiectasia (recessive)	16832357,19781682,22585167
<i>ATR</i>	Double stranded break repair	Oropharyngeal	Seckel (recessive)	22341969
<i>NEW AXIN2</i>		Colon	Oligodontia-colorectal cancer syndrome	15042511
<i>BAP1</i>	BRCA1-associated protein complex	Uveal Melanoma, Mesothelioma		21874000,21874003
<i>BARD1</i>	BRCA1-associated protein complex	Breast, Ovarian		21344236
<i>BMPRIA</i>	TGF-beta signaling	Colon	Juvenile polyposis	20301642
<i>BRCA1</i>	BRCA1-associated protein complex	Breast, Ovarian	Hereditary breast and ovarian cancer	22006311,2270482,7545954
<i>BRCA2</i>	Fanconi/BRCA	Breast, Ovarian	Hereditary breast and ovarian cancer, Fanconi anaemia FA-D1 (recessive)	22006311,8524414
<i>BRIP1</i>	Fanconi/BRCA	Breast, Ovarian	Fanconi anaemia FA-J (recessive)	22006311,17033622,21964575
<i>CDH1</i>	Cell adhesion	Breast, Gastric	Hereditary	20301318

			diffuse gastric cancer	
<i>CDK4</i>	Cell cycle	Melanoma		19585149
<i>CDKN2A</i>	Cell cycle	Pancreatic, Melanoma		19585149
<i>CHEK1</i>	Double stranded break repair	Unknown		11479205
<i>CHEK2</i>	Double stranded break repair	Breast		11967536
<i>CTNNA1</i>	Beta-catenin, e-cadherin complex	Gastric	Hereditary diffuse gastric cancer	23208944
<i>FAM175A/Abra xas</i>	Double stranded break repair	Breast		22357538
<i>NEW FH</i>		Renal	Hereditary leiomyomatosis and renal cell cancer	25018647,11865300,25004247
<i>NEW FLCN</i>		Renal	Birt-Hogg-Dube syndrome	12204536,20301695
<i>GALNT12</i>	O-glycosylation	Colon		19617566,22461326
<i>GEN1</i>	Double stranded break repair	Breast		2052659
<i>GREM1</i>	BMP antagonist	Colon	Hereditary mixed polyposis syndrome	22561515
<i>HOXB13</i>	transcription factor	Prostate		22236224
<i>MEN1</i>	Gene expression regulation	Endocrine	Multiple endocrine neoplasia type 1	9215689
<i>MLH1</i>	Mismatch DNA repair	Colon, Ovarian, Endometrial	Lynch syndrome	20301390
<i>MRE11A</i>	Double stranded break repair	Breast	Ataxia-telangiectasia-like disorder (recessive)	10612394,19383352
<i>MSH2 (+EPCAM)</i>	Mismatch DNA repair	Colon, Ovarian, Endometrial	Lynch syndrome	20301390
<i>MSH6</i>	Mismatch DNA	Colon,	Lynch syndrome	20301390

	repair	Endometrial		
<i>MUTYH</i>	DNA repair	Colon (homozygotes)	MUTYH-associated polyposis	20301519,21952991
<i>NBN</i>	Double stranded break repair	Breast	Nijmegen breakage syndrome (recessive)	15185344,9590180
<i>NEW NF1</i>		Optic Glioma, Peripheral Nerve Sheath, Breast	Neurofibromatosis	2114220,23165953,20301288
<i>PALB2</i>	Fanconi/BRCA	Breast, Pancreatic	Fanconi anaemia FA-N (recessive)	17200668,17200671,25099575
<i>NEW PALLD</i>		Pancreatic	Familial pancreatic cancer	17194196
<i>PIK3CA</i>	AKT signaling	Breast, Thyroid	Cowden-like	22729224,23246288
<i>PMS2</i>	Mismatch DNA repair	Colon, Endometrial	Lynch syndrome	20301390
<i>POLD1</i>	DNA Polymerase	Colon, Endometrial	Familial polyposis, colorectal cancer	23263490,23770608
<i>POLE</i>	DNA Polymerase	Colon	Familial polyposis, colorectal cancer	23263490
<i>NEW POT1</i>		Brain, Melanoma	Familial melanoma and brain cancer	25482530,24686849
<i>NEW PRKAR1A</i>		Endocrine	Carney complex (recessive)	4010501
<i>PRSS1</i>	Digestion (Trypsin 1)	Pancreatic	Pancreatitis	22379635
<i>NEW PTCH1</i>		Basal cell carcinoma, PNET	Nevoid basal cell-carcinoma syndrome	8681379,8658145,20301330
<i>PTEN</i>	PI3K/MAPK Signaling	Breast	Cowden syndrome	20301661
<i>RAD51B</i>	Double stranded	Unknown		24139550

	break repair			
<i>RAD51C</i>	Fanconi/BRCA	Ovarian, Breast	Fanconi anaemia FA-O (recessive)	22006311,22538716
<i>RAD51D</i>	Fanconi/BRCA	Ovarian, Breast	Fanconi anaemia (recessive)	21822267,22415235
<i>NEW RB1</i>		Retinoblastoma, Sarcoma, Melanoma	Hereditary retinoblastoma	25621664,22355046,20301625
<i>RET</i>	Receptor Tyrosine Kinase	Endocrine	Multiple endocrine neoplasia type 2	20301434
<i>NEW RINT1</i>		Breast, Colon		25050558
<i>NEW RPS20</i>		Colon		24941021
<i>SDHB</i>	Succinate dehydrogenase complex	Pheochromocytoma, Paraganglioma	Hereditary paraganglioma-pheochromocytoma	11404820
<i>SDHC</i>	Succinate dehydrogenase complex	Pheochromocytoma, Paraganglioma	Hereditary paraganglioma-pheochromocytoma	11062460
<i>SDHD</i>	Succinate dehydrogenase complex	Pheochromocytoma, Paraganglioma	Hereditary paraganglioma-pheochromocytoma	10657297
<i>SLX4</i>	Fanconi/BRCA	Unknown	Fanconi anaemia (recessive)	23840564
<i>SMAD4</i>	TGF-beta signaling	Colon	Juvenile polyposis	20301642
<i>NEW SMARCA4</i>		Ovarian		24658002
<i>STK11</i>	Cell Cycle/p53 regulation	Breast, Pancreatic	Peutz-Jeghers syndrome	20301443
<i>TP53</i>	Cell growth	Breast, Ovarian	Li-Fraumeni syndrome	22006311,20301488
<i>VHL</i>	p53 regulation	Kidney, Neuroendocrine	von Hippel-Lindau syndrome	20301636

<i>XRCC2</i>	Double stranded break repair	Breast	Fanconi anaemia (recessive)	22464251,22232082
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*Only the most commonly associated cancer types are listed. A more detailed description of cancer risk for some BROCA genes can be found at [GeneReviews](#).

NCI Protocol #: 9948

Local Protocol #: MC1563

NCI Version Date: July 23, 2020

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APPENDIX D: SOP340507 FOR TUMOR FROZEN NEEDLE BIOPSY SPECIMEN COLLECTION AND HANDLING

DCTD Standard Operating Procedures (SOP)

Title:	Tumor Frozen Needle Biopsy Specimen Collection and Handling			Page 1 of 11
Doc. #:	SOP340507	Revision:	F	Effective Date: 2/11/2015

Laboratory of Human Toxicology & Pharmacology

Applied/Developmental Research Directorate, Leidos Biomedical Research, Inc.

Frederick National Laboratory for Cancer Research

Technical Reviewer:	<u>Yvonne A. Evrard</u>	Date:	<u>Feb 11, 2015</u>
NCTVL Reviewer:	<u>Jiuping Ji</u>	Date:	<u>Feb 11, 2015</u>
IQC Approval:	<u>Katherine V. Ferry-Galow</u>	Date:	<u>2/11/15</u>
LHTP Approval:	<u>Ralph E. Parchment</u>	Date:	<u>03/23/2015</u>
DCTD OD Approval:	<u>Joseph E. Tomaszewski</u>	Date:	<u>03/23/2015</u>

Ralph E. Parchment
 -S (Affiliate)

Change History

Revision	Approval Date	Description	Originator	Approval
F	2/11/2015	Updated contact shipping address and process for advance notification of shipments.	KFG	REP
E	7/3/2013	Updated tube-type to 1.5-mL conical bottom screw cap tubes to allow for broader use in DCTD assays and minimize the need to transfer biopsies during sample extraction steps. Decreased maximum time from biopsy collection to freezing to 2 minutes.	YAE	REP
D	1/8/2013	Update handling in surgical suite including details on halving of biopsy. Record total time elapsed from biopsy collection to freezing.	YAE, MM	JJ
C	12/29/2010	Update sample snap freeze to dry ice/ethanol bath or liquid nitrogen.	YAE	JJ
B	7/24/2009	Updated SOP format and prepared for publication to the DCTD Biomarkers Web site	YAE	JJ
A	10/13/2006	Revision with New Shipping Address	YZ	JJ
--	8/25/2006	New Document	YZ	JJ

Please check for revision status of the SOP at

<http://dctd.cancer.gov/ResearchResources/ResearchResources-biomarkers.htm>

and be sure to use the current version.



DCTD Standard Operating Procedures (SOP)

Title:	Tumor Frozen Needle Biopsy Specimen Collection and Handling			Page 2 of 11
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DCTD Standard Operating Procedures (SOP)

Title:	Tumor Frozen Needle Biopsy Specimen Collection and Handling			Page 3 of 11
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1.0 PURPOSE

Standardize the method for collecting and handling frozen needle tumor biopsies to enable specimen use for measurement of pharmacodynamic (PD) markers following treatment with anticancer agents.

2.0 SCOPE

This procedure applies to all personnel involved in the collection and handling of frozen needle tumor biopsies for use in PD marker assays during clinical trials. The goal of this SOP and associated training is to ensure consistency in tumor needle biopsy collection and handling between clinical sites.

3.0 ABBREVIATIONS

DCTD	=	Division of Cancer Treatment and Diagnosis
ELISA	=	Enzyme-Linked ImmunoSorbent Assay
FNLCR	=	Frederick National Laboratory for Cancer Research
ID	=	Identification / Identifier
IQC	=	Internal Quality Control
LHTP	=	Laboratory of Human Toxicology and Pharmacology
NCTVL	=	National Clinical Target Validation Laboratory
PADIS	=	Pharmacodynamics Assay Development & Implementation Section
PD	=	Pharmacodynamic
SOP	=	Standard Operating Procedure

4.0 INTRODUCTION

Specimen handling, shipping, and storage procedures (pre-analytical variables) can have a significant impact on the reliability of biomarker measurements in the laboratory. Following detailed steps for sample collection and handling procedures and recording any deviations from this procedure allows retrospective identification of artifactual changes in biomarker readout and increases the reliability of the data and validity of the analytical results.

5.0 ROLES AND RESPONSIBILITIES

Laboratory Director/Supervisor The Laboratory Director/Supervisor, directs laboratory operations, supervises technical personnel and reporting of findings, and is responsible for the proper performance of all laboratory procedures. Oversees the personnel who follow the SOPs in the laboratory and is responsible for ensuring the personnel are certified and have sufficient experience to handle clinical samples.

Certified Assay Operator and/or PK/PD Support Lab Personnel

A Certified Assay Operator and/or PK/PD Support Lab personnel may be a Laboratory Technician/ Technologist, Research Associate, or Laboratory Scientist who has been certified through DCTD training on this SOP. Work under the guidance of the Laboratory Director/Supervisor. This person performs laboratory procedures and examinations in accordance with the current SOP(s), as well as any other procedures conducted by a laboratory, including maintaining equipment and records and performing quality assurance activities related to performance.

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- 5.1 It is the responsibility of the Laboratory Director/Supervisor to ensure that all personnel have documented training and qualification on this SOP prior to the actual handling and processing of samples from clinical trial patients. The Laboratory Director/Supervisor is responsible for ensuring the Certified Assay Operator running the SOP has sufficient experience to handle and analyze clinical samples.
- 5.2 It is the responsibility of the Certified Assay Operator and/or PK/PD Support Lab personnel to confirm scheduled specimen collection time points, pre-print all labels and data collection sheets in advance, check documentation for accuracy, and verify that the required collection tubes, supplies, and equipment are available for successful collection and handling of biopsy samples.
- 5.3 The Certified Assay Operator and/or PK/PD Support Lab personnel responsible for conducting the specimen collection and handling procedures are to follow this SOP and complete the required tasks and associated documentation. The Batch Record ([Appendix 1](#)) must be completed in *real-time* for each experimental run, with each page *dated and initialed*, and placed with the clinical sample information.
- 5.4 The responsible personnel are to check the DCTD Biomarkers Web site (<http://dctd.cancer.gov/ResearchResources/ResearchResources-biomarkers.htm>) to verify that the latest SOP version is being followed.

6.0 MATERIALS AND EQUIPMENT REQUIRED

- 6.1 Stop watch, total time in minutes and seconds required
- 6.2 1.5-mL Sarstedt o-ring screw cap, conical bottomed tubes (Sarstedt, Cat#: 72.703.416)
- 6.3 Disposable, fine-tipped tweezers (e.g., VWR, Cat#: 83009-010). Tweezer tips need to easily fit to bottom of a 1.5-mL Sarstedt tube
- 6.4 Printable microcentrifuge tube labels or BSI labeling system
- 6.5 81-place freezer boxes (e.g., Fisher Scientific, Cat#: 12-565-182)
- 6.6 Thermoflask cooler or polystyrene foam container
- 6.7 Ice bucket
- 6.8 Liquid nitrogen or dry ice/ethanol bath
- 6.9 Wet ice
- 6.10 -80°C freezer (or lower)

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7.0 OPERATING PROCEDURES

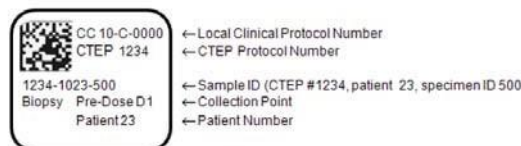
- 7.1 Record the name and certification number of the Certified Assay Operator and/or PK/PD Support Lab personnel performing the SOP, the facility/clinic collecting the specimens, the Patient/Sample ID, and the clinical protocol number in the Batch Record ([Appendix 1](#)).

- The Batch Record for this SOP is sufficient for collection of a **single** patient's biopsy samples; if collecting biopsy samples for more than one patient, prepare a separate Batch Record for each patient.

- 7.2 Prepare enough pre-printed specimen labels for each whole or halved biopsy sample to be collected and frozen as defined in the Pharmacodynamic/Correlative Study section of the Clinical Protocol; be sure to coordinate with the clinical center if they prepare the labels for sample collection.

If two passes are collected from one tumor, the labels would be identical except that the specimen ID would be followed by a lower case a/b to designate pass number. The specimen ID includes the CTEP protocol number followed by a unique patient identifier and a specimen series ID.

NCI tumor biopsy specimen IDs for PD sampling are series 500 with consecutive numbers identifying the collection time point as defined in the Clinical Protocol. Sample pre-printed label:



- 7.3 Of the pre-printed labels prepared for each sample, one label will go on each 1.5-mL Sarstedt tube, one on the Batch Record ([Appendix 1](#)), and the last will be given to the research nurse to place into the patient record sheet.

7.4 Tumor Needle Biopsy Collection and Handling

- 7.4.1 The research nurse is to notify the laboratory of scheduled PD sample collections, preferably giving at least 24-h notice. Arrive at the biopsy collection site early enough to allow sufficient time to set up laboratory supplies, collect relevant clinical information, and ensure rapid transport of specimens to the laboratory for placement at -80°C (or lower) after collection.

- 7.4.2 Bring all necessary lab supplies including: disposable tweezers, a minimum of two 1.5-mL Sarstedt tubes (one for each whole biopsy core) pre-cooled on liquid nitrogen or dry ice/ethanol in an insulated bucket, and one pre-printed specimen label to give to the research nurse for the patient record.

Note: Pre-chill additional 1.5-mL Sarstedt tubes for specimen collection in case the interventional radiologist collects additional passes, or one of the other tubes is compromised prior to collection.

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7.4.3 The total time elapsed between biopsy collection and placement into the pre-chilled tube is of **key importance** to biomarker analysis; biopsies should be frozen within **2 min** of collection. The interventional radiologist will eject the biopsy onto a sterile slide (for optimal analyte recovery the slide should be pre-chilled). Start a stop watch (or note the time) at this point (Appendix 1, Section 1) and immediately walk the slide to the sample preparation table.

Note: The preferred method of collection, when whole biopsies are collected, is for the interventional radiologist to eject the biopsy directly into the pre-chilled tube (next step). This minimizes the time between collection and fixation of analytes.

7.4.4 Indicate if a full or halved biopsy, as defined in the Pharmacodynamic/Correlative Study section of the Clinical Protocol, is prepared in the Batch Record (Appendix 1, Section 1).

7.4.4.1 For whole biopsies: Uncap an empty, prechilled 1.5-mL Sarstedt tube and using disposable tweezers, pick up the freshly collected needle biopsy with the tweezers at one end, and touch the opposite end of the biopsy to the inner surface of the prechilled 1.5-mL Sarstedt tube. This should attach the tissue to the tube, allowing it to be dropped into the tube while releasing the tissue from the tweezers without sticking. Dispose of the tweezers in the appropriate biohazardous waste container(s).

7.4.4.2 For halved biopsies: Use 1-2 disposable tweezers and cut/shear the biopsy in half cross-wise while it is on the slide (do not pull or stretch the biopsy longitudinally). Use the tweezers to transfer the halved biopsies to sterile pre-chilled tubes as indicated above.

7.4.5 Immediately snap freeze the biopsy by placing the tube in liquid nitrogen or a dry ice/ethanol bath.

7.4.6 Calculate the total time elapsed from biopsy collection to biopsy freezing and record the total number of **minutes and seconds** elapsed in the Batch Record (Appendix 1, Section 1).

7.5 If biopsy procedure details can be obtained from the interventional radiologist or research nurse, record them in the Batch Record (Appendix 1, Section 2.). Some information may not be available until a later time from the clinical staff.

During **first-in-human** PD sample collection studies, information such as type of anesthesia and time-lag between biopsy needle withdrawal and sample freezing need to be tracked in order to determine the optimal sample collection procedure for the clinical community.

7.6 Return to the sample processing laboratory and transfer the frozen biopsy specimen(s) to -80°C (or lower) for storage until shipment to the PD processing laboratory. Record the date and time specimens are placed at -80°C (or lower; Appendix 1, Section 3).

7.7 Review and finalize the Batch Record and document ANY and ALL deviations from this SOP in the Batch Record (Appendix 1, Section 5).

7.8 The Laboratory Director/Supervisor should review the Batch Record and sign to affirm the data contained within are correct (Appendix 1, Section 6).

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8.0 SHIP TO FNLCR FOR ANALYSIS (OPTIONAL)

If shipping to a location other than FNLCR, use the following steps as a guide.

- 8.1 FedEx return shipment labels will be provided to each approved site sending frozen shipments to FNLCR PD Specimen Central Receiving.
 - 8.1.1 To request return shipment labels send an e-mail to NCI_PD_Support@mail.nih.gov and state "Protocol Name Shipment Labels Requested" in the subject line. Specify the address to which the shipment labels should be provided and the number of shipment labels requested. Shipment labels will be provided within 6 business days.
- 8.2 Once a tumor biopsy has been collected from a patient and placed at -80°C (or lower), FNLCR PD Specimen Central Receiving should be notified that the specimens are ready for shipment.
- 8.3 Send an e-mail to FNLCR PD Specimen Central Receiving (NCI_PD_Support@mail.nih.gov) to advise that biopsy samples are being prepared for shipment. State "Protocol Name PD Specimens Ready for Shipment" in the subject line. Request a confirmation e-mail that personnel will be available on the expected delivery date and time. Personnel are generally available to receive frozen shipments Tuesday through Friday, exclusive of government holidays. If needed, FNLCR PD Central Receiving can be contacted directly at 240-344-5697.
- 8.4 Use the PD Sample Shipping Manifest template in [Appendix 2](#) to generate a shipping list containing pertinent sample information and FNLCR PD Specimen Central Receiving shipping address.

Attention: Dan Danner
 NCI-F/FNLCR
 1073 Beasley Street, Building 1073
 Fort Detrick
 Frederick, MD 21701
 Phone: 301-846-5748
- 8.5 Make a copy of the Shipping Manifest and specimen Batch Records so one copy can be sent to FNLCR with the biopsy samples and one can be maintained at the collection site for internal records.
- 8.6 Day of Shipment
 - 8.6.1 Just prior to shipment, place specimen tubes into an 81-place freezer box and then in a shipping container with sufficient dry ice to maintain the samples at -20°C for at least 72 h. All weekly processing specimens are recommended to ship out via FedEx on the following Monday afternoon for delivery by 10 AM Tuesday (FedEx First Overnight).
 - 8.6.2 Verify the contents of the package match the Shipping Manifest and sign and date the bottom of both copies of the Shipping Manifest. Place one copy of the Shipping Manifest inside the shipping box along with copies of the completed Batch Records for all specimens.
 - 8.6.3 Seal the box and print and attach the shipping address onto the outside of the shipping container; be sure the container is labeled as containing biohazardous specimens.
 - 8.6.4 Record the shipping date, time, tracking number, and shipping information in the Batch Record (Appendix 1, Section 4).

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- 8.6.5 E-mail FNLCR PD Specimen Central Receiving (NCI_PD_Support@mail.nih.gov) a shipment notification. State "*Protocol Name* PD Specimen Shipment" in the subject line and reference the tracking number in the e-mail.
- 8.6.6 Once specimens arrive at the receiving laboratory, they should be immediately placed at -80°C (or lower) pending delivery to the processing laboratory for protein extraction.

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APPENDIX 1: BATCH RECORD

A separate Batch Record should be started for each patient sample.

Note: A pre-dose and post-dose sample from the same patient would have the same Patient ID, but different Specimen ID numbers.

Note: Record times using **military time** (24-h designation); for example, specify 16:15 to indicate 4:15 PM.

Place
PD Specimen
Label Here

Certified Assay Operator: _____

Certification Number: _____

☐ Check here if PK/PD Support Lab Personnel

Facility/Clinic Collecting Specimens: _____

Clinical Protocol Number: _____

Patient ID: _____

1. Biopsy Collection

	1 st Pass	2 nd Pass	3 rd Pass	4 th Pass
Specimen ID				
Biopsy size prepared for PD or histological analysis:	<input type="checkbox"/> Full <input type="checkbox"/> Halved	<input type="checkbox"/> Full <input type="checkbox"/> Halved	<input type="checkbox"/> Full <input type="checkbox"/> Halved	<input type="checkbox"/> Full <input type="checkbox"/> Halved
Required: Time elapsed from collection to placement in tube	min sec	min sec	min sec	min sec
Time biopsy collected (opt)	:	:	:	:
Time biopsy placed in tube (opt)	:	:	:	:

BATCHRECORD

INITIALS: _____

DATE: _____

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2. Biopsy Procedure Details

Specimen ID	
Time local anesthesia administered	:
Dose of local anesthetic	mg
Name of local anesthetic used (from Research Nurse)	
Time of skin incision	:
Needle Type (e.g., Temno)	
Needle diameter	gauge
Needle Length	cm
Time guide needle introduced	:
Time guide needle placement confirmed	:
Time biopsy needle introduced	:

3. Biopsy Storage

Date/time biopsy specimen(s) placed at
 -80°C (or lower) / / ; °C

4. Shipping to FNLCR (optional)

Date and time samples shipped :
 Tracking information

****Attach copy of Shipping Manifest**

5. Notes, including any deviations from the SOP:

6. Laboratory Director/Supervisor Review of Batch Record

Laboratory Director/Supervisor: _____ (PRINT)

_____ (SIGN)

Date: / /

BATCHRECORD

INITIALS: _____

DATE: _____

DCTD Standard Operating Procedures (SOP)					
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APPENDIX 2: PD SAMPLE SHIPPING MANIFEST

From:		PD Sample Shipping Manifest					
Phone:							
E-mail:							
In Package	Item No	Patient/Specimen ID	Clinical Protocol	Description	Time Point Scheduled	Collection Date	Collection Time
	Example	1234-1023-500	12-C-0000	Full biopsy	Pre-dose D1	06/12/12	08:50
	Example	1234-1023-501	12-C-0000	Half biopsy	Cycle 1, D8	06/20/12	16:05
<input type="checkbox"/>	1					/ /	
<input type="checkbox"/>	2					/ /	
<input type="checkbox"/>	3					/ /	
<input type="checkbox"/>	4					/ /	
<input type="checkbox"/>	5					/ /	
<input type="checkbox"/>	6					/ /	
<input type="checkbox"/>	7					/ /	
<input type="checkbox"/>	8					/ /	
<input type="checkbox"/>	9					/ /	
<input type="checkbox"/>	10					/ /	

Verification of Contents	Signature	Date
Contents Verified Collection Laboratory	_____	/ /
Contents Verified FNLCR PD Central Receiving	_____	/ /



NCI Protocol #: 9948

Local Protocol #: MC1563

NCI Version Date: July 23, 2020

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APPENDIX E: SOP340522 TUMOR FROZEN NEEDLE BIOPSY PREPARATION FOR THE GH2AX IFA

DCTD Standard Operating Procedures (SOP)

Title:	Tumor Frozen Needle Biopsy Preparation for the γ H2AX IFA			Page 1 of 25
Doc. #:	SOP340522	Revision:	D	Effective Date: 1/13/2015

National Clinical Target Validation Laboratory

Applied/Developmental Research Directorate, Leidos Biomedical Research, Inc.

Frederick National Laboratory for Cancer Research

Technical Reviewer:	<u>Donna Butcher</u>	Date:	<u>1/22/15</u>
NCTVL Approval:	<u>Jiuping Ji</u>	Date:	<u>1/26/15</u>
IQC Approval:	<u>Katherine V. Ferry-Galow</u>	Date:	<u>1/29/15</u>
LHTP Approval:	<u>Ralph E. Parchment</u>	Date:	<u>Ralph E. Parchment -S (Affiliate)</u>
DCTD OD Approval:	<u>Joseph E. Tomaszewski</u>	Date:	<u>03/23/2015</u>

Please check for revision status of the SOP at

<http://dctd.cancer.gov/ResearchResources/ResearchResources-biomarkers.htm>

and be sure to use the current version.

DCTD Standard Operating Procedures (SOP)

Title:	Tumor Frozen Needle Biopsy Preparation for the γ H2AX IFA			Page 2 of 25
Doc. #:	SOP340522	Revision:	D	Effective Date: 1/13/2015

Change History

Revision	Approval Date	Description	Originator	Approval
D	1/13/2015	Modified method for fixing and embedding tissue to ensure maximal tissue area will be available per section following microtomy. Clinical slide preparation and H&E slide quality control criteria updated. Added visual inspection of slides with operator-specified range of 18 or 35 slides for IFA analyses. Modified slide designations. Paraffin dipping of some backup slides added to preserve analytes.	KFG, YAE	KFG
C	9/22/2013	Update SOP flow diagram and relabel biopsy samples to specify location of pre- and post-dose biopsies. H&E QC criteria clarified and presence of neoplastic tissue added. Water bath temperature adjusted.	YAE	JJ
B	12/29/2010	Update SOP following in-house assay runs of patient samples.	WHY	JJ
A	2/01/2010	Format SOP, add Appendices 1 and 2, remove references to biopsy collection procedures, remove references to calibrator/control slide preparation, and define biopsy sectioning procedure by slide and use	YAE	JJ
--	10/22/2008	New document	WHY	JJ

DCTD Standard Operating Procedures (SOP)

Title:	Tumor Frozen Needle Biopsy Preparation for the γ H2AX IFA			Page 3 of 25
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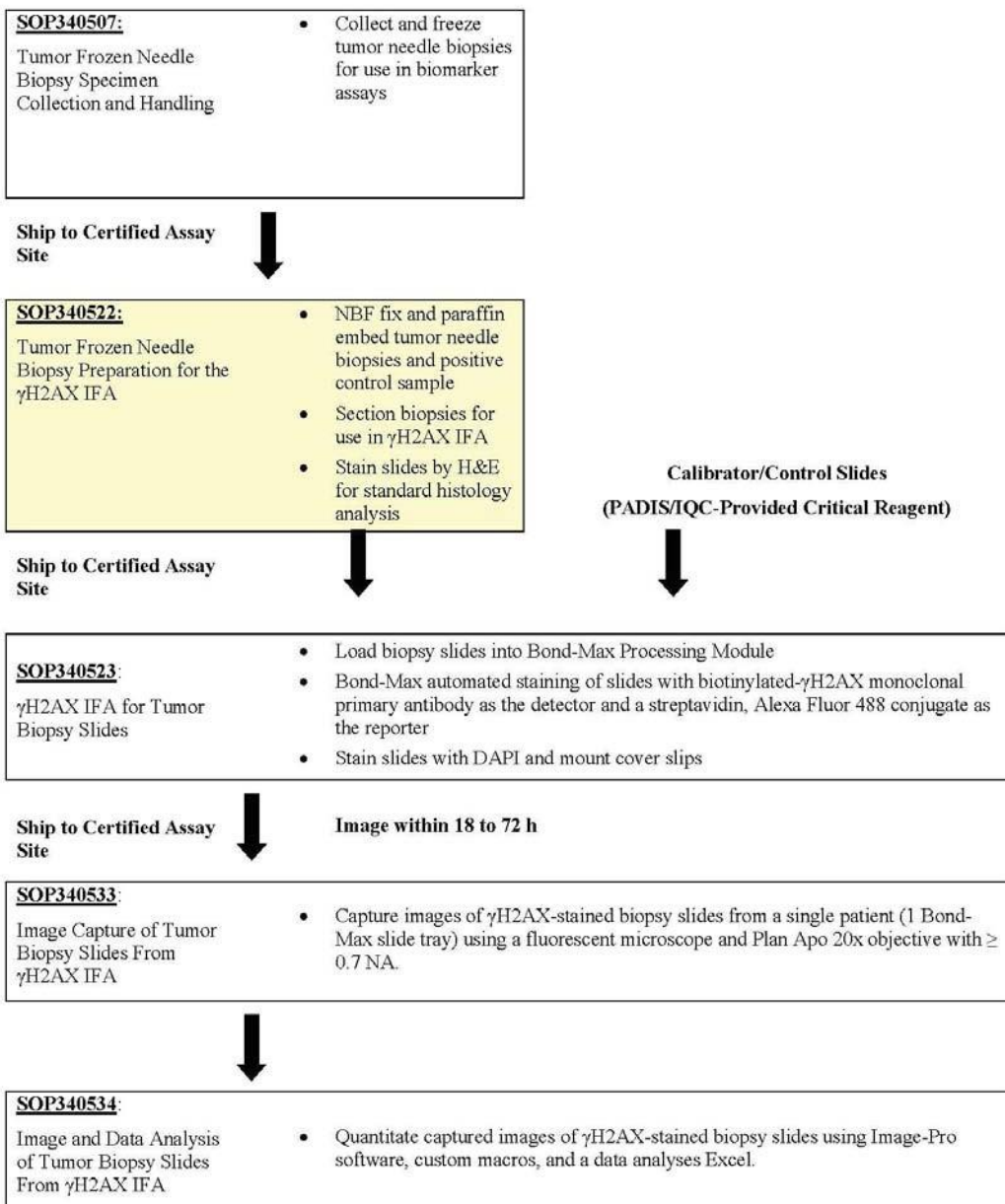
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2.0 SCOPE	5
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DCTD Standard Operating Procedures (SOP)

Title:	Tumor Frozen Needle Biopsy Preparation for the γ H2AX IFA			Page 4 of 25
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OVERVIEW OF IMMUNOFLUORESCENCE ASSAY FOR BIOPSIES



DCTD Standard Operating Procedures (SOP)

Title:	Tumor Frozen Needle Biopsy Preparation for the γ H2AX IFA			Page 5 of 25
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				1/13/2015

1.0 PURPOSE

Standardize the method for fixing, embedding, and sectioning pre- and post-dose clinical biopsies along with control tissue for the immunohistochemical detection and quantification of histone H2AX phosphorylated at serine 139 (γ H2AX) for pharmacodynamic studies of chemotherapeutic DNA-damaging agents.

2.0 SCOPE

This procedure applies to all personnel involved in processing clinical trial biopsy samples for the preparation of slides for quantitation of γ H2AX using the γ H2AX Immunofluorescence Assay (IFA) for Tumor Biopsy Slides (SOP340523). This SOP includes the procedures for specimen preparation by fixation, dehydration and paraffin-embedding for microtomy, and for slide preparation of sectioned tissues samples. The goal of the SOP and associated training is to ensure consistency of γ H2AX measurement between operators and clinical sites.

3.0 ABBREVIATIONS

Cal	=	Calibrator
DAPI	=	4',6-Diamidino-2-Phenylindole
DCTD	=	Division of Cancer Treatment and Diagnosis
DI	=	Deionized
γ H2AX	=	Histone H2AX Phosphorylated at Serine 139
H&E	=	Hematoxylin and Eosin
ID	=	Identification/Identifier
IFA	=	Immunofluorescence Assay
LHTP	=	Laboratory of Human Toxicology & Pharmacology
NA	=	Numerical Aperture
NBF	=	Neutral Buffered Formalin
NCTVL	=	National Clinical Trial Validation Laboratory
QC	=	Quality Control
RT	=	Room Temperature
SOP	=	Standard Operating Procedure

4.0 INTRODUCTION

The γ H2AX IFA is an immunohistochemistry-based staining assay developed to quantify the nuclear DNA damage marker, histone γ H2AX. The assay uses a biotinylated- γ H2AX monoclonal antibody as the detector and an Alexa Fluor 488-streptavidin conjugate (Strp488) as the reporter for immunostaining.

DCTD Standard Operating Procedures (SOP)

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5.0 ROLES AND RESPONSIBILITIES

Laboratory Director/Supervisor The Laboratory Director/Supervisor directs laboratory operations, supervises technical personnel and reporting of findings, and is responsible for the proper performance of all laboratory procedures. The Laboratory Director/Supervisor oversees the personnel who follow the SOPs in the laboratory and is responsible for ensuring the personnel are certified and have sufficient experience to handle clinical samples.

Certified Assay Operator A Certified Assay Operator may be a Laboratory Technician/Technologist, Research Associate, or Laboratory Scientist who has been certified through DCTD training on this SOP. The Certified Assay Operator works under the guidance of the Laboratory Director/Supervisor. This person performs laboratory procedures and examinations in accordance with the current SOP(s), as well as any other procedures conducted by a laboratory, including maintaining equipment and records and performing quality assurance activities related to performance.

- 5.1 It is the responsibility of the Laboratory Director/Supervisor to ensure that all personnel have documented DCTD training and qualification on this SOP prior to the actual handling and processing of samples from clinical trial patients. The Laboratory Director/Supervisor is responsible for ensuring the Certified Assay Operator running the SOP has sufficient experience to handle and analyze clinical samples.
- 5.2 The Certified Assay Operator for this SOP should be well versed and comfortable with tissue embedding and sectioning techniques.
- 5.3 Digital versions of the Slide Preparation Table in the Batch Record (Appendix 1, Sections 4) can be created for logging sample information as long as all column information exactly matches the tables in the Batch Record. A copy of the completed, digital sample tables must be printed and attached to the Batch Record in order to maintain a complete audit trail.
- 5.4 The Certified Assay Operator responsible for conducting the assay is to follow this SOP and complete the required tasks and associated documentation. The Batch Record ([Appendix 1](#)) must be completed in *real-time* for each experimental run, with each page *dated and initialed*, and placed with the clinical sample information.
- 5.5 All responsible personnel are to check the DCTD Biomarkers Web site (<http://dctd.cancer.gov/ResearchResources/ResearchResources-biomarkers.htm>) to verify that the most recent version of the SOP for the assay is being used.

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6.0 MATERIALS AND EQUIPMENT REQUIRED

6.1 PADIS/IQC-Supplied Critical Reagents

6.1.1 Fresh-frozen murine testes halves (commercial sources are available but have not been validated for this SOP)

- 6.2** 20-mL borosilicate glass scintillation vials (e.g., Fisher Scientific, Cat#: 03-337-15)
- 6.3** Scintillation vial caps with cone-shaped plastic liner (e.g., Fisher Scientific, Cat#: 03-337-7)
- 6.4** Transfer pipettes
- 6.5** Forceps
- 6.6** Tissue embedding cassettes and molds
- 6.7** Tissue/biopsy processing cassettes
- 6.8** Small petri dish (e.g., Falcon, Cat#: 351007, 60 x 15mm)
- 6.9** Metric ruler
- 6.10** Black paper
- 6.11** Digital camera
- 6.12** Laboratory utility wipe (e.g., Kimberly Clark, WYPALL-L 10 Utility Wipes, Cat#: 05322). Wipe needs to be thick enough to prevent biopsy from curling during fixation process
- 6.13** Lens paper (e.g., Cat#: VWR Scientific, Cat#: 52846-001)
- 6.14** Containers for graded ethanol and xylene washes of tissue embedding cassettes
- 6.15** Superfrost plus slides (e.g., Fisher Scientific, Cat#: 12-550-15)
- 6.16** Accu-Edge low-profile microtome blades (e.g., Sakura Finetek, Cat#: 4689 or Fisher Scientific, Cat#: NC9292148)
- 6.17** Slide box (e.g., Fisher Scientific, Cat#: 03-448-10)
- 6.18** Dry ice
- 6.19** Sterile-filtered, molecular biology grade deionized (DI) water (e.g., Invitrogen, Cat#: 10977-015) or Milli-Q ultra-pure water
- 6.20** Paraffin (e.g., Paraplast)
- 6.21** 10% neutral buffered formalin (NBF; e.g., Fisher Scientific, Cat#: 22-050-105)
- 6.22** Anhydrous ethanol, histology grade (e.g., Fisher Scientific, Cat#: A405-20 [Filtered using 0.22 μ m pore size before use.]) ACS/USP Grade can be purchased and used without filtration (Pharmco-AAPER, Cat#: 111000200PL05)
- 6.23** Xylenes – histology grade
- 6.24** H&E staining solutions, histology grade (standard methods)
- 6.25** Tissue embedding station (should include paraffin dispenser with heated work block and a second cooling block). *Alternate:* 60°C incubator, 60°C heated work block, and cooling block (approx. -5°C)
- 6.26** Low-profile water bath, set to 50°C
- 6.27** Microtome (e.g., Leica RM2255 Automated Microtome, Leica Microsystems)
- 6.28** -80°C freezer
- 6.29** Liquid nitrogen storage system
- 6.30** 37°C incubator
- 6.31** Frozen needle biopsies processed following SOP340507

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7.0 OPERATING PROCEDURES

NOTE: A separate Batch Record (Appendix 1) should be started for each set of biopsy samples from a single patient.

7.1 Clinical specimens for this assay will be frozen needle biopsies collected and stored according to SOP340507. After clinical biopsy collection, the specimens are snap-frozen and stored at -80°C . Biopsies can be stored at -80°C for up to 8 d after collection. After 8 d, the biopsies should be moved to liquid nitrogen storage. Biopsy pairs (pre- and post-dose biopsy) should always be stored together and processed as a pair.

7.2 If samples were shipped from a separate site, save the clinical shipping manifest for the laboratories' record and attach a copy to this Batch Record.

7.3 Record the name and certification number of the Certified Assay Operator, the facility running the SOP, the Patient/Sample ID and the clinical protocol number in the Batch Record ([Appendix 1](#)).

7.4 Critical Reagent

7.4.1 Record the date of receipt, lot number, and expiration date for the Critical Reagent in the Batch Record (Appendix 1, Section 1).

7.4.2 Store the reagent as indicated below. Label reagent with the date of receipt and store under the specified conditions for no longer than the recommended duration.

- **Fresh-frozen murine testes:** Positive control sample, murine testes halves. Store at -80°C for up to 4 mo.

7.5 For the pre- and post-dose biopsy samples, record the date of receipt, Patient/Sample ID, and number of passes received in the Batch Record (Appendix 1, Section 2).

Note: A pre- and post-dose biopsy from the same patient would have the same Patient ID but different Sample IDs. The Patient/Sample ID should include the CTEP protocol number followed by a unique patient identifier and a sequential specimen ID (NCI tumor biopsies for PD sampling are series 500).

7.5.1 For a single biopsy time point, multiple passes through the tumor may have been collected. A **single pass** of the **pre- and post-dose biopsy** samples should be used for embedding. Additional passes should be stored in liquid nitrogen until needed. If data are acquired from the first pass, the remaining biopsy passes can be used per institutional guidelines.

7.5.2 For each patient, two paraffin tissue blocks will be prepared in parallel. One paraffin tissue block will contain the patient's pre-dose biopsy sample and a fresh-frozen murine testis specimen. A second paraffin block will contain the patient's post-dose biopsy sample. Parallel processing of all 3 tissues should be done to ensure minimal sample handling and processing variability.

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7.6 Protocol for Specimen Fixation

7.6.1 Remove a single pass of a pre- and post-dose biopsy sample for one patient, as well as the testis positive control Critical Reagent vial from -80°C/liquid nitrogen storage; immediately place on dry ice. Record information for each specimen to be embedded in the Batch Record (Appendix 1, Section 3).

7.6.2 Biopsy Samples:

- 7.6.2.1 Fill two (2) scintillation vials with 20 mL 10% NBF each and label one vial each for the pre- and post-dose biopsy with appropriate Sample ID.
- 7.6.2.2 One biopsy sample at a time, warm the microtube containing the frozen biopsy slightly by gently rolling between palms of hands for 10 sec. Using a transfer pipette, transfer 0.5-1 mL 10% NBF from the scintillation vial to the corresponding biopsy tube. Let sit for 2-5 min at RT.
- 7.6.2.3 Carefully pour the NBF and clinical sample into a small petri dish containing 7 mL NBF. If necessary, flush the microtube with 10% NBF from the scintillation vial until the entire sample is in the petri dish.
- 7.6.2.4 Optional: A digital photograph may be taken to document the tissue appearance at this point. Recommended photography steps:
 - Place a black piece of paper under the petri dish and a metric ruler alongside the dish. A sample ID should also be visible in the photograph so that the correct identity of the sample is captured. The original sample vial label may be used or a pre-prepared label for the biopsy block cassette may also be used.
 - Take a digital photograph of the sample at close range while ensuring the camera is focused. Most standard digital cameras have a mode for close-up photographs.
 - Save the image to a secure location using the specimen ID in the file name.
- 7.6.2.5 Using forceps transfer the partially fixed tissue to a small piece of laboratory wipe orienting the tissue to prevent it from folding or curling during the fixation process. The laboratory wipe will be removed prior to embedding.
- 7.6.2.6 Transfer the tissue, adhered to the laboratory wipe, and 10% NBF into the correctly labeled 20-mL scintillation vial and discard the residual NBF from the petri dish.
- 7.6.2.7 Be sure each specimen is completely immersed in NBF in an individually labeled scintillation vial.

7.6.3 Testis Positive Control Sample:

- 7.6.3.1 Label a 20-mL scintillation vial as "positive control" and fill with 20 mL 10% NBF.
- 7.6.3.2 Warm the microtube containing the frozen testis half slightly by gently rolling between palms of hands for 10 sec. Using a transfer pipette, transfer 0.5-1 mL 10% NBF from the scintillation vial to the corresponding biopsy tube. Let sit for at least 1 min at RT.

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- 7.6.3.3 Carefully pour the NBF and testis into the correctly labeled 20-mL scintillation vial. If necessary, flush the microtube with 10% NBF from the scintillation vial until the entire sample has been transferred to the vial.
- 7.6.3.4 Be sure specimen is completely immersed in NBF in an individually labeled scintillation vial.
- 7.6.4 Allow the tissue to fix for 16 to 24 h at RT (optimal fixation time is 20 h). Do not let fixation proceed for longer than 96 h. Record the start and stop dates and times for fixation in the Batch Record (Appendix 1, Section 3).
- 7.7 **Protocol for Paraffin-Embedding of Specimens**
 - 7.7.1 Prepare the tissue embedding station by pre-warming the paraffin and a heat block to 60°C and pre-cooling a cooling block to -5°C.
 - 7.7.2 Prepare containers containing the graded-ethanol series (made with DI water and filtered) and xylenes as outlined in SOP Step 7.7.4.
 - 7.7.3 For a single patient, pre-label 3 tissue processing cassettes.
 - 7.7.3.1 For the pre-dose biopsy sample, label a processing cassette with the **Patient/Sample ID**; repeat for the post-dose biopsy sample. Each clinical biopsy processing cassette should be assigned a unique **Block Number** for tracking unsectioned samples.
 - 7.7.3.2 Record the Block Number for each clinical cassette in the Batch Record (Appendix 1, Section 3). These processing cassettes will be placed on the embedding molds in SOP Step 7.7.6.
 - 7.7.3.3 The third processing cassette should be labeled as the **Testis**; this tissue will be embedded together with the pre-dose biopsy specimen and therefore does not need a separate Block Number assigned.
 - 7.7.4 Using clean forceps gently remove the specimens adhered to the laboratory wipe from the NBF scintillation vials and gently detach the tissue from the laboratory wipe.
 - 7.7.4.1 Carefully orient the tissue for full-face presentation onto a small piece of lens paper pre-moistened with NBF.
 - 7.7.4.2 Fold the lens paper over the biopsy to secure the tissue in the correct orientation and place within the pre-labeled tissue processing cassettes. The lens paper will help prevent the biopsy from curling up during the dehydration process; it will not be embedded with the tissue.
 - 7.7.4.3 Place the cassettes into 70% ethanol and begin the paraffin-embedding sequence. Be sure to process a single patient's pre- and post-dose biopsy samples as well as the testis control tissue in parallel.

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7.7.5 Paraffin-embedding sequence:

Step	Solution	Time	Temperature
1	70% Ethanol	30 min	RT
2	80% Ethanol	30 min	RT
3	80% Ethanol	30 min	RT
4	95% Ethanol	30 min	RT
5	95% Ethanol	30 min	RT
6	100% Ethanol	30 min	RT
7	100% Ethanol	30 min	RT
8	100% Ethanol	30 min	RT
9	100% Xylenes	30 min	RT
10	100% Xylenes	30 min	RT
11	Paraffin	45 min	60°C
12	Paraffin	45 min	60°C
13	Paraffin	45 min	60°C
14	Paraffin	30 min	60°C

7.7.6 Place a small amount of melted paraffin in the bottom of an embedding mold.

- 7.7.6.1 Using a clean preheated forceps, carefully transfer the testis positive control and pre-dose biopsy from their processing cassettes into the embedding mold. For the pre-dose biopsy, carefully remove the tissue from the lens paper and use heated forceps to orient the biopsy within the mold to allow longitudinal sectioning of the biopsy.
- 7.7.6.2 The section orientation will match that in SOP Step 7.8.5. This embedding procedure and orientation ensures that the pre- and post-dose biopsy molds and sections are easily distinguishable.
- 7.7.6.3 Briefly transfer the mold onto a cooling block; the paraffin will partially solidify into a thin layer and hold the tissue pieces in position.
- 7.7.6.4 Immediately place the correctly labeled clinical biopsy processing cassette on top of the mold, then fill the combined mold and cassette with paraffin and return it to the cold plate to finish solidifying. Record the date the samples were embedded in the Batch Record (Appendix 1, Section 3).

7.7.7 Repeat Step 7.7.6 for the post-dose biopsy. The post-dose biopsy is embedded alone, without a testis specimen.

7.7.8 Immediately proceed to microtomy. For temporary storage of blocks, store at 2°C to 8°C away from volatile chemicals.

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7.8 Protocol for Microtomy and Clinical Slide Preparation

7.8.1 Ensure a low-profile water bath filled with ddH₂O is preheated to 50°C.

7.8.2 Select a paired set of clinical sample blocks (a testis/pre-dose biopsy block and a post-dose biopsy block) for a single patient.

7.8.2.1 A maximum of 50 slides will be made.

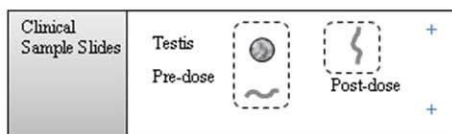
7.8.3 Pre-label 50 slides with sequential slide numbers (#1-50; may not use all slides), the Patient/Sample IDs for both the pre- and post-dose biopsies.

Sections for each biopsy **MUST BE CONSECUTIVE CUTS, PLACED IN ORDER** on the slides. Section #1 (Slide #1) for each biopsy should be the first section from each block that has tissue pieces at least 2 mm².

7.8.4 Section paraffin blocks in 5-micron sections. Each section placed on slides should have tissue pieces at least 2 mm². Record the date blocks are sectioned in the Batch Record (Appendix 1, Section 4).

7.8.5 Carefully float each section from each block on water in a 50°C water bath.

7.8.5.1 Collect paired specimen sections such that one section of each of the two sections represented below (dashed lines) are placed onto each of the pre-labeled slides **in the following orientation:**



7.8.6 If any section is skipped or placed on a slide out of order, or if a slide is removed due to issues associated with placement of the paraffin section on the slide, make a notation of the deviation(s) for the slide(s) affected in the Batch Record (Appendix 1, Section 4).

7.8.7 Verify that all slides are labeled correctly and are generated with the appropriate tissues and orientation by placing a checkmark in the appropriate column of the Slide Preparation Table in the Batch Record (Appendix 1, Section 4).

7.8.8 Dry the slides overnight in a 37°C incubator.

7.9 Visual Inspection of Slides, Determination of Slide Range for Analysis and Paraffin Dipping of Slides

7.9.1 The operator will visually inspect the entire set of up to 50 slides using a microscope. A range of 35 consecutive slides should be designated as optimal for H&E and subsequent IFA analysis.

- Selection of the 35-slide range designated for analysis is based on visual inspection of the slides and comparison of the relative area of the biopsy sections across the slide set.
- The designated range should have pre- and post-dose biopsy sections optimally at, or near, full-face longitudinal view and contain sufficient control tissue for the IFA analysis.

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- 7.9.2** The 1st, 18th and 35th slide designated in the optimal 35-slide range will be used for H&E analysis. For example if cut slides 5 – 39 are designated as the slides that should proceed for further analysis, slides 5, 22 and 39 will be designated for H&E analysis. Selection of which slides in the 35-slide range are used for the primary IFA analysis and as backup slides are made after the H&E analysis of the slides is complete (SOP Section 7.10).
- 7.9.3** If < 35 slides were prepared during microtomy due to one of the tissues being exhausted the following guidelines should be followed:
- 7.9.3.1** A range of 18 consecutive slides should be designated from the available slide set as optimal for the H&E and subsequent IFA analysis following the guidelines in SOP Step 7.9.1.
- 7.9.3.2** The 1st and 18th slide in the optimal 18-slide range will be used for H&E analysis. For example, if a total of 24 slides are prepared during microtomy and slides 5 – 22 are designated as the slides that should proceed further for analysis, slides 5 and 22 will be designated for H&E analysis.
- 7.9.4** A minimum of 18 slides are needed to proceed with further analysis.
- 7.9.4.1** If < 18 slides were prepared during microtomy due to one of the tissues being exhausted, and a second pass of the biopsy is available for analysis, repeat the SOP and embed a second pass of both the pre- and post-dose biopsy, starting a new Batch Record.
- 7.9.4.2** If no second pass biopsy is available, or if a subsequent biopsy block also yields < 18 slides, no further analysis should be performed on the tissues. The tissues will be designated as “TQ” and not reportable due to insufficient or poor tissue quality on the Clinical Sample Data Report in SOP340534.
- 7.9.5** All slides except those designated for H&E analysis should be dipped in paraffin to prolong stability. The slides should be dipped in paraffin within 24 hours of the completion of microtomy.
- 7.9.5.1** Any additional slides prepared during microtomy that were determined to fall out of the optimal range (35 or 18) by visual inspection should be considered unanalyzable (UA).
- 7.10 H&E Slide Quality Control (QC)**
- 7.10.1** The slides designated for H&E should be stained according to standard methods. The H&E-stained slides should be analyzed by a staff pathologist. Depending on the institution, a whole-slide digital scan of both H&E slides can be performed or the slides themselves can be provided to use for analyses.
- 7.10.2** H&E slides must meet the following QC criteria:
- Presence of neoplastic tissue
 - Morphology of each section should indicate acceptable nuclear and cellular definition,
 - Sufficient cellularity should be present in each section so that at least one (1) 20x field (~0.45 mm²) can be analyzed, and
 - There should be sufficiently low necrotic areas in each section so that at least one (1) 20x field with ≥ 80% viable tissue can be analyzed.

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7.10.3 Pass/Fail QC Decisions and Designation of Slides for IFA Analysis

7.10.3.1 If the H&E-stained slides meet any of the following criteria the slides **Pass QC**:

- If the 1st, 18th and 35th slide were stained and each slide passes H&E Slide QC, the slide set **Passes QC**.
- If the 1st, 18th and 35th slide were stained and two consecutive slides (either the 1st and the 18th or the 18th and the 35th) pass H&E Slide QC and the third slide fails H&E Slide QC, the slide set **Passes QC**.
- If only the 1st and 18th slide were stained and each slide passes H&E slide QC, the slide set **Passes QC**.
- Proceed to SOP Step 7.11.

7.10.3.2 If the H&E-stained slides meet any of the following criteria the slides **Fail QC**:

- If the 1st, 18th and 35th slide were stained and no two consecutive slides pass QC, the slide set **Fails QC**.
- If only the 1st and 18th slide were stained and either or both slides fail H&E QC, the slide set **Fails QC**.

7.10.3.3 If the H&E-stained slides Fail QC and a second pass of the biopsies was collected, repeat the SOP and embed the second pass, starting a new Batch Record.

- If no second pass biopsy is available, or if a subsequent biopsy block also fails H&E slide QC, the tissues will be designated as "TQ" as not reportable due to insufficient or poor tissue quality on the Clinical Sample Data Report in SOP340534, Appendix 5.

7.10.4 If the H&E-stained slide set **Failed QC**, record the specific reason for QC failure (e.g., insufficient tumor tissue or cellularity in the biopsy) in the deviations section of the Batch Record (Appendix 1, Section 6) and proceed to SOP Step 7.12.

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7.11 Slide Designations for IFA Analyses

7.11.1 Based on the optimal slide range and H&E Slide QC pass/fail outcome, assign slides to the following slide-use designations in the Batch Record (Appendix 1, Section 5).

7.11.1.1 Any additional slides prepared during microtomy that were determined to fall out of the optimal range (35 or 18) by visual inspection should be considered unanalyzable (UA).

7.11.2 If the optimal slide range was 35 slides and the 1st, 18th and 35th slide passed H&E slide QC, the slide designations are as follows:

Slide Designations:				
Slide numbers refer to <u>order</u> in the optimal 35-slide range based on visual inspection				
H&E	IFA	Backup-1	Backup-2	Backup-3
1	2	3	4	5
	6	7	8	9
	10	11	12	13
	14	15	16	17
18	19	20	21	22
	23	24	25	26
	27	28	29	30
	31	32	33	34
35				

7.11.3 If the optimal slide range was 35 slides and the 1st and 18th slide passed H&E slide QC (but slide 35 failed) OR if the optimal slide range was 18 and the 1st and 18th slide passed H&E slide QC, the slide designations are as follows:

Slide Designations:		
Slide numbers refer to <u>order</u> in the optimal 35-slide range based on visual inspection		
H&E	IFA	Backup-1
1	2	3
	4	5
	6	7
	8	9
	10	11
	12	13
	14	15
	16	17
18		

- If the optimal slide range was 35, slides 19 – 35 should be designated at UA.

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7.11.4 If the optimal slide range was 35 slides and the 18th and 35th slide passed H&E QC (but slide 1 failed), the slide designations are as follows:

Slide Designations:		
Slide numbers refer to <u>order</u> in the optimal 35-slide range based on visual inspection		
H&E	IFA	Backup-1
18	19	20
	21	22
	23	24
	25	26
	27	28
	29	30
	31	32
	33	34
35		

- Slides 1 – 17 should be designated at UA.

- 7.12** If the 35- or 18-slide set failed H&E QC (SOP Step 7.10), all slides in the optimal slide range and any additional slides prepared during microtomy should be designated as UA.
- 7.13** Place the H&E and paraffin-dipped slides, grouped by label (i.e. H&E, IFA, Backup 1, Backup 2, Backup 3 or UA), in a slide box. The slides should be stored in a desiccator at 2°C to 8°C away from volatile chemicals until use. If there is tissue remaining in the paraffin block, store with the Backup slides.
- 7.14** Once IFA data are acquired for a patient, any remaining Backup slides and the paraffin block can be used per institutional guidelines.
- 7.15** Review and finalize the Batch Record and document ANY and ALL deviations from this SOP in the Batch Record (Appendix 1, Section 6).
- 7.16** The Laboratory Director/Supervisor should review the Batch Record and sample reports and sign the Batch Record affirming the data contained within the reports are correct (Appendix 1, Section 8).

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8.0 OPTIONAL: SHIP TO CERTIFIED ASSAY SITE FOR ANALYSIS

If the IFA will be performed at a separate certified assay site, ship the slides as follows:

IMPORTANT: Include a copy of the Batch Record for all samples being shipped with the Shipping Manifest.

- 8.1 Send an e-mail to the certified assay site prior to shipping to advise recipient of scheduled shipping time. Be sure to request and receive a confirmation e-mail prior to shipping.
- 8.2 Generate a shipping list containing all the specimen records using the Shipping Manifest template as shown in [Appendix 2](#). In the Batch Record, verify that all slides in the slide box are from a single patient and indicate if a paraffin block is included (Appendix 1, Section 7).
 - 8.2.1 A Shipping Manifest may include more than one patient's samples, but a single patient's slide box should contain only a single patient's slides and be clearly labeled.
- 8.3 Verify that the contents of the package match the Shipping Manifest.
- 8.4 Print and attach the shipping address onto the outside of the shipping container.
- 8.5 Record the shipping date, time, tracking number, and shipping information in the Batch Record (Appendix 1, Section 7).
- 8.6 Ship the specimens **with a copy of** the Shipping Manifest and copies of the completed Batch Records for all patient specimens. Retain copies of the completed Shipping Manifest and Batch Records in your records.
- 8.7 E-mail the certified assay site shipment notification. State "*Protocol Name* PD Specimen Shipment" in the subject line and reference the tracking number and shipping information in the e-mail.

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APPENDIX 1: BATCH RECORD

A separate Batch Record should be started for **each** patient.

NOTE: Record times using **military time** (24-h designation); for example, specify 16:15 to indicate 4:15 PM.

Certified Assay Operator: _____

Certification Number: _____

Facility Preparing Paraffin Blocks and Sections: _____

Patient ID: _____

Clinical Protocol Number: _____

Pre-dose
Specimen Label
Here

Post-dose
Specimen Label
Here

1. Critical Reagents

Reagent Name	Date Received	Lot Number	Expiration Date
Fresh-frozen murine testis	/ /		/ /

2. Patient Samples Received From Clinical Site

	*Date Received	*Patient/Sample ID	No. of Passes Received
Pre-dose Biopsy:	/ /		
Post-dose Biopsy:	/ /		

3. Sample Information

	Pass Number	Fixation Start (Date/Time)	Fixation Stop (Date/Time)	Date Embedded	*Paraffin Block Number
Pre-dose Biopsy:		/ / :	/ / :	/ /	
Positive Control (testis):	N/A	/ / :	/ / :		
Post-dose Biopsy:		/ / :	/ / :	/ /	

*Required information

BATCH RECORD: INITIALS _____ DATE: _____

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4. Slide Preparation and Visual Inspection

Date Blocks Sectioned: _____

Verify that each slide contains the following CONSECUTIVE sections in the appropriate orientation.

Note: Slides should contain tissue pieces at least 2 mm².

Slide No.	Optimal 35 or 18 slide range based on Visual Inspection.	Notes or Deviations During Microtomy
1	<input type="checkbox"/>	
2	<input type="checkbox"/>	
3	<input type="checkbox"/>	
4	<input type="checkbox"/>	
5	<input type="checkbox"/>	
6	<input type="checkbox"/>	
7	<input type="checkbox"/>	
8	<input type="checkbox"/>	
9	<input type="checkbox"/>	
10	<input type="checkbox"/>	
11	<input type="checkbox"/>	
12	<input type="checkbox"/>	
13	<input type="checkbox"/>	
14	<input type="checkbox"/>	
15	<input type="checkbox"/>	
16	<input type="checkbox"/>	
17	<input type="checkbox"/>	
18	<input type="checkbox"/>	
19	<input type="checkbox"/>	
20	<input type="checkbox"/>	
21	<input type="checkbox"/>	
22	<input type="checkbox"/>	
23	<input type="checkbox"/>	
24	<input type="checkbox"/>	
25	<input type="checkbox"/>	

BATCH RECORD:

INITIALS _____

DATE: _____

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(Slide Preparation and Visual Inspection, Continued)

Slide No.	Optimal 35 or 18 slide range based on Visual Inspection.	Notes or Deviations During Microtomy
26	<input type="checkbox"/>	
27	<input type="checkbox"/>	
28	<input type="checkbox"/>	
29	<input type="checkbox"/>	
30	<input type="checkbox"/>	
31	<input type="checkbox"/>	
32	<input type="checkbox"/>	
33	<input type="checkbox"/>	
34	<input type="checkbox"/>	
35	<input type="checkbox"/>	
36	<input type="checkbox"/>	
37	<input type="checkbox"/>	
38	<input type="checkbox"/>	
39	<input type="checkbox"/>	
40	<input type="checkbox"/>	
41	<input type="checkbox"/>	
42	<input type="checkbox"/>	
43	<input type="checkbox"/>	
44	<input type="checkbox"/>	
45	<input type="checkbox"/>	
46	<input type="checkbox"/>	
47	<input type="checkbox"/>	
48	<input type="checkbox"/>	
49	<input type="checkbox"/>	
50	<input type="checkbox"/>	

BATCH RECORD:

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5. H&E Slide QC and Slide-Use Designation

Order	Slide No. in Optimal Slide Range	H&E	IFA	Backup-1	Backup-2	Backup-3	UA
1		<input type="checkbox"/>					
2			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
3			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
4			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
5			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
6			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
7			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
8			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
9			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
10			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
11			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
12			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
13			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
14			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
15			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
16			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
17			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
18		<input type="checkbox"/>					
19			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
20			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
21			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
22			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
23			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
24			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
25			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
26			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
27			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
28			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
29			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
30			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
31			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
32			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
33			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
34			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
35		<input type="checkbox"/>					

BATCH RECORD: INITIALS _____ DATE: _____

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6. Notes, including any deviations from the SOP:

7. Shipping to Certified Assay Site

Verify a single patient's slides are in slide box: ☐ Yes ☐ No

Verify H&E stained slide included: ☐ Yes ☐ No

Paraffin block included: ☐ Yes ☐ No

Date and time samples shipped: _____

Tracking information: _____

Attach copy of Shipping Manifest

8. Laboratory Director/Supervisor Review of Batch Record

Laboratory Director/Supervisor: _____ (PRINT)

_____ (SIGN)

Date: _____

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APPENDIX 2: SAMPLE SHIPPING MANIFEST

Ship From: Contact Name: Tel: E-mail:		Shipping Manifest		Ship To: Attn: Tel: E-mail:	
Shipping Date:		Carrier:			
In Package	Item No.	Patient/Sample ID	Clinical Protocol/CTEP#	Item/Description	
<input checked="" type="checkbox"/>	Example	1234-1025-500 and -501	12-C-0000/ 1234	Patient slide set, H&E slides, and paraffin block	
<input type="checkbox"/>	1				
<input type="checkbox"/>	2				
<input type="checkbox"/>	3				
<input type="checkbox"/>	4				
<input type="checkbox"/>	5				
<input type="checkbox"/>	6				
<input type="checkbox"/>	7				
<input type="checkbox"/>	8				
<input type="checkbox"/>	9				
<input type="checkbox"/>	10				

APPENDIX F: PREPARATION OF THU FOR PHARMACOKINETIC STUDIES

Prepare a stock solution of tetrahydrouridine (THU) at 10 mg/mL (may be frozen).

THU can be added to Vacutainer tubes up to several days in advance without causing significant loss of vacuum. Using a 3/10 cc insulin syringe or other similar sized syringe with a fine needle, draw up 10 μ L of the THU solution for each mL of blood to be drawn and transfer it to a heparinized Vacutainer tube (cat # 366667) by piercing the stopper. Do not draw up THU solution for more than one tube at a time. You will not be able to control the volume of THU solution that leaves the needle, as it is sucked out by the vacuum. Because of the fine needle, you will not lose the vacuum (apart from the volume added) in the collection tube.