REQUEST FOR PROJECT TEAM MEMBER APPLICATIONS FOR DESIGNING CLINICAL TRIALS USING VX-970 (NSC 780162)

The Cancer Therapy Evaluation Program (CTEP) is accepting Project Team Member Applications (PTMAs) for a project with VX-970, a potent inhibitor of ataxia telangiectasia mutated and Rad3-related (ATR) kinase, being developed by CTEP as an anticancer agent in collaboration with Vertex Pharmaceuticals Incorporated (Vertex), Boston, MA. VX-970 is anti-proliferative in all cells tested. As a single agent, it shows potent cytotoxicity towards certain cancer cells, but is only weakly toxic to normal cells. VX-970 sensitizes many cancer cell lines and primary human tumor cells, but not normal cell lines, to the lethal effects of a range of DNA-damaging drugs and ionizing radiation (IR). Currently, VX-970 is being tested as a single agent and in various combinations with the DNA-damaging agents gemcitabine, gemcitabine/cisplatin, cisplatin, cisplatin/etoposide, and carboplatin in phase 1 clinical studies sponsored by Vertex.

After discussions with Vertex, Division of Cancer Treatment and Diagnosis (DCTD)/CTEP is interested in studies in the following areas:

- Phase 1 trials of VX-970 in combination with:
  - IR in patients with central nervous system (CNS) metastasis
  - topoisomerase I inhibitors
  - cisplatin and IR in locally advanced recurrent head and neck cancer (post-surgery).
- One or two phase 2 randomized trial(s) possibly in, but not limited to, ovarian, gastrointestinal (GI), or genitourinary (GU) cancers to show proof of principle using one of the combinations currently in phase 1 trials (gemcitabine, gemcitabine/cisplatin, carboplatin, cisplatin/etoposide).

The role of the project team is to evaluate all available evidence to refine this initial plan and develop actual trial designs that will address important questions that are not covered in the company's development plan.

Strong preclinical evidence exists for the enhancement of synthetic lethality of tumor cells through simultaneous inhibition of multiple DNA repair pathways. Disabling ATR disrupts homologous recombination (HR) repair, a pathway that protects cells from the DNA-damaging agents like cisplatin, topotecan, and veliparib, and further sensitizes cells with disabled HR to these agents (Huntoon *et al.*, 2013). VX-970 enhances, in a dose-dependent manner, the antitumor effects of IR and multiple DNA-damaging drugs, including cisplatin, gemcitabine, and irinotecan in mouse xenograft models derived from human cancer cell lines or primary human tumors (Hall *et al.*, 2014; Investigator's Brochure, 2013).

The project team will include clinician-scientists with expertise in phase 1/2 studies and/or experience in radiation combination trials, translational scientists with an interest and expertise in biomarker development in DNA damage response (DDR), and basic scientists with expertise in DDR. The team will also include disease-specific experts with interest in developing this agent with gemcitabine- or platinum-based regimens to design trials to test the benefits of this agent. The project team will be recruited nationally and will prioritize the research questions regarding VX-970 in combination trials, including prioritization of biomarker studies. Also, the project team will advise on selected phase 2 trials using the current tested combinations for proof of principle. Based upon the team's recommendations, it is anticipated that the clinicians on the project team will be tasked with writing the Letters of Intent (LOIs) describing the study design for CTEP approval, and that these clinicians will ultimately lead the clinical studies. It is also anticipated that other extramural members of the drug project team will stay involved in the subsequent design and execution of the proposed trials. It is anticipated that the project team will complete its work in 8 to12 weeks.

Background/Rationale

ATR is a member of the phosphoinositide 3-kinase (PI3K)-related protein kinases (PIKKs) that regulate DDR to maintain genome integrity (Cimprich and Cortez, 2008). Although ATR is activated in response to many different types of DNA damage, including double-strand DNA (dsDNA) breaks, base adducts, crosslinks and replication stress, a single-stranded DNA (ssDNA) with a 5′ double-stranded primer junction is responsible, in most instances, for ATR activation. ATR signals to regulate DNA replication, cell cycle transitions, and DNA repair through the phosphorylation of hundreds of substrates, including the checkpoint kinase 1 (Chk1). Activated Chk1 contributes to DDR by effecting S-phase and G2/M phase arrest through its interaction with cell division cycle 25 (cdc25) phosphatase, giving the cells the necessary time to repair its genome, recover DNA replication, and enter into mitosis with the genome fully and accurately duplicated. Additionally, Chk1 coordinates Rad51-mediated HR involved with dsDNA break repair and is important for the maintenance of viable replication structures after DNA polymerase stalling (McNeely *et al.*, 2010).

ATR is essential for the survival of most replicating cells perhaps due to the ubiquitous presence of DNA lesions and replication stress (Toledo *et al.*, 2011). Many tumors appear to have a critical reliance on ATR for survival from DNA damage based on the expression of certain oncogenes that drive high replicative stress, frequent defects in other DDR or DNA repair pathways, and the common use of DNA-damaging agents in cancer treatment. Thus, inhibition of ATR has the potential to sensitize many tumors to the cytotoxic effects of a wide range of DNA-damaging drugs and IR. ATR inhibitor VE-821 was found to be highly synergistic with topoisomerase 1 inhibitors under conditions where it is not cytotoxic alone (Reaper *et al.*, 2011). In contrast, normal cells and tissues are able to tolerate ATR inhibition because of low replicative stress levels and the presence of compensatory DNA repair pathways, including the pathway mediated by the related kinase ataxia telangiectasia mutated kinase (ATM). ATM and ATR have overlapping but non-redundant functions in DDR. Crosstalk between these pathways often occurs as a consequence of inter-conversion of the activating DNA lesions (Conti *et al.*, 2007).

Mechanism of Action

VX-970 is an adenosine triphosphate (ATP)-competitive, highly potent, tightly binding inhibitor of ATR with an inhibition constant (Ki) <300 pM (range <0.1 to 0.3 nM) that blocks ATR activity in cells, with a concentration associated with 50% inhibition (IC_{50}) of 20 nM (Investigator's Brochure, 2013). VX-970 is very selective in inhibiting ATR; it showed >100-fold selectivity against the closely related DDR proteins, ATM (Ki 38 ± 30 nM [mean ± SD]) and DNA-dependent protein kinase (Ki >4000 nM). ATR and ATM have overlapping functions in the cellular response to dsDNA breaks and replication stress. Accordingly, cells with defects in ATM signaling, for example from loss of p53, have been shown to be especially sensitive to ATR inhibition by VX-970. Minimal inhibitory activity was observed against a large panel of unrelated protein kinases, with 278 of 291 kinases tested having a measured IC_{50} of >400 nM and a measured Ki >200 nM, corresponding to >500-fold selectivity for ATR. For 12 of the remaining 13 kinases, the Ki was ≥15 nM (>50-fold selectivity for ATR) and ≥8 nM for one of the 13 kinases, Flt4 kinase (>25-fold selectivity for ATR).

Nonclinical Studies of VX-970

In Vitro and *In Vivo* Activity

VX-970 blocked proliferation of all the cells tested with IC_{50} values ranging from 35 to 1100 nM (Investigator's Brochure, 2013). As a single agent, VX-970 was weakly cytotoxic in all non-cancer cell lines tested: median effective dose (ED_{50}) values ranged from 1.0 to 5.0 μM. Potent cytotoxicity was observed for one of three cancer cell lines tested (HCT116, colorectal carcinoma cells, with an ED_{50}=60 nM).

The impact of VX-970 on the cellular response to DNA-damaging drugs (including cross-linking agent [cisplatin], antimetabolite [gemcitabine], topoisomerase I inhibitor [irinotecan/SN38], and topoisomerase II inhibitor [etoposide]) was assessed, *in vitro* (Investigator's Brochure, 2013). VX-970 synergized with the

DNA-damaging agents in HCT116 cells; a dramatic 20-fold reduction in the IC_{50} for cisplatin was observed when VX-970 was added. The effects of VX-970 in combination with a range of DNA-damaging drugs were further tested against a large panel of 35 lung and 15 pancreatic cancer cell lines. Most lung cancer cell lines responded well to VX-970 in combination with cisplatin or gemcitabine: 84% and 76% of cell lines, respectively, showed a 3-fold or greater shift in the IC_{50} of the DNA-damaging drug when VX-970 was added. Enhanced sensitivity was also observed with etoposide (53% of cell lines), irinotecan (49% of cell lines), and oxaliplatin (39% of cell lines). Most pancreatic cancer lines also responded well to combinations of VX-970 and cisplatin or gemcitabine: over 70% of cell lines showed a 3-fold or greater shift in IC_{50} for the DNA-damaging drugs when VX-970 was added. VX-970 also synergized with cisplatin in 7 of 9 primary human lung tumors tested. In contrast, no synergy was seen in a non-cancer cell line, HFL1 (lung fibroblasts), for any agents except for gemcitabine, where weak synergy was observed (maximum 2-fold shift in gemcitabine IC_{50}). Normal cells (lung and skin fibroblasts, and mammary epithelial cells) treated with DNA-damaging agents tolerated ATR inhibition with just a reversible increase in growth arrest.

The impact of VX-970 on radiosensitivity was assessed by clonogenic assay (10 to 15 days) in cells treated with VX-970 and a range of IR doses (Investigator's Brochure, 2013). VX-970 significantly radiosensitized two human pancreatic cancer cell lines (MiaPaCa-2 and PSN1), but not a non-cancer cell line (MRC5, lung fibroblast) when treated with IR over the range 2 to 6 Gy (P <0.05) (Fokas *et al*., 2012).

The *in vivo* activity of VX-970 was tested in multiple mouse xenograft models derived from human cancer cell lines and primary human tumor cells (Investigator's Brochure, 2013). VX-970 sensitized tumors to cisplatin, gemcitabine, irinotecan, and IR in a dose- and schedule-dependent manner. VX-970 was generally well tolerated at efficacious doses; optimal efficacy with VX-970 was achieved by dosing intravenously (IV) at 20 mg/kg/week (either as a single injection or as two 10 mg/kg injections 3 days apart). VX-970 administered before gemcitabine, or 48 hours after gemcitabine, provided no benefit over gemcitabine treatment alone. Marked tumor regression was observed with VX-970 in combination with irinotecan, cisplatin, gemcitabine, or IR. Triple combinations of VX-970, IR, and gemcitabine were assessed in the human pancreatic cancer PSN1 xenograft model. VX-970 (60 mg/kg, given on Days 1, 3, and 5), when combined with gemcitabine (a single dose of 100 mg/kg on Day 0) and a single dose of IR (6 Gy, 2 hours after VX-970 on Day 1), led to profound tumor regression and significant (P <0.01) tumor growth delay when compared with all treatment doublets or single agents.

Biomarkers

The impact of ATM pathway defects on cell sensitivity to VX-970 in combination with cisplatin, was assessed in matched cell pairs with defects in either ATM (161BR primary skin fibroblasts compared with ATM null AT1BR skin fibroblasts, obtained from a patient with ataxia telangiectasia) or p53 (p53 wild-type A549 lung cancer cell line compared with A549 cells stably expressing short hairpin RNA [shRNA] to p53) (Investigator's Brochure, 2013). VX-970 synergized with cisplatin in ATM null fibroblasts, in contrast to ATM wild-type cells. Synergy was observed between VX-970 and cisplatin in p53 wild-type A549 cells; however, it was markedly enhanced in p53-null matched cells.

Pharmacokinetics

VX-970 is well distributed into tissues after oral or IV administration to rats, with maximum concentrations reached after 3 hours of infusion for all tissues except kidney and thymus, which peaked at 6 hours (Investigator's Brochure, 2013). The tumor:plasma ratio of area under the concentration-time curve (AUC) from the IV administered mouse tumor pharmacokinetic (PK) study was 24.7, indicating extensive distribution into tumors. VX-970 demonstrated extensive blood to plasma partitioning; the volume of distribution (V_d) for VX-970 was determined to be 20, 21, 10, and 6 L/kg for mouse, rat, dog, and monkey, respectively. The unbound fraction of VX-970 in blood was low in all species. VX-970 had high plasma protein binding and the

free fraction in human blood was 2.1%. VX-970 appears to be mainly metabolized by cytochrome P450 (CYP) 3A4 in human liver microsomes. VX-970 was primarily eliminated by oxidative metabolism, with CYP3A4 as the principle isozyme responsible. 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 (CL) values of VX-970 following IV administration were determined to be 82, 26, 13, and 29 mL/min/kg in the mouse, rat, dog, and monkey, respectively.

Safety Pharmacology

A number of *in vitro* and *in vivo* safety pharmacology studies designed to evaluate effects of VX-970 against multiple protein targets and the cardiovascular (CV) system did not demonstrate any toxicologically significant effects at exposures or concentrations that significantly exceed the targeted maximum circulating concentration of VX-970 in humans (Investigator's Brochure, 2013). An in-house manual patch-clamp human ether-a-go-go-related gene (hERG) assay demonstrated moderate inhibition of the hERG channel; however, a subsequent dedicated dog CV study, with telemetry evaluation, did not demonstrate any CV effects *in vivo* at exposures exceeding the projected human efficacious exposure. VX-970 was non-genotoxic in a 2-strain Ames (mutagenicity) assay. VX-970 absorbs in the UV-visible radiation spectrum and is widely distributed, including to the skin.

Toxicology

VX-970 was administered both orally (PO) (every 2 days) and IV (dosed twice per week) to rats and dogs for up to 28 days (Investigator's Brochure, 2013). The rat severely toxic dose in 10% of rodents (STD10) was considered to be an IV dose of 30 mg/kg/day, and the dog highest non-severely toxic dose (HNSTD) was considered to be an IV dose of 20 mg/kg/day. Target organs of toxicity in the rat included the testes and peripheral blood cell populations (red cell mass, eosinophils, and platelets), and VX-970 produced mild irritation at the infusion site. Target organs in the dog included the liver (oral administration only), testes, and peripheral blood cell populations (red cell mass and eosinophils). The starting dose determined for the first-in-human study of VX-970 IV is 18 mg/m^2.

Clinical Study of VX-970

Vertex (Investigator's Brochure, 2013) is conducting two phase 1 trials of VX-970. The first-in-human phase 1 clinical study (VX12-970-001 [Study 001]) evaluates VX-970 in combination with gemcitabine +/- cisplatin, and cisplatin +/- etoposide, and a second study VX13-970-002 [Study 002]) evaluates VX-970 as a single agent, and in combination with carboplatin. A dose escalation strategy is being employed to determine the maximum tolerated dose (MTD) of VX-970 when given with either gemcitabine or cisplatin and combination of cisplatin with gemcitabine or etoposide (Study 001) or as a single agent or in combination with carboplatin (Study 002) in subjects with advanced solid tumors. So far, 16 subjects had received VX-970 (18, 36, 60, and 72 mg/m^2) through at least 1 cycle (21 days) with gemcitabine at 875 mg/m^2; 6 subjects had received VX-970 (90 mg/m^2) with gemcitabine at 500 mg/m^2 through 1 cycle; 1 subject had received VX-970 (90 mg/m^2) with cisplatin 40 mg/m^2 through 1 cycle; 1 subject had received VX-970 (60 mg/m^2) as single agent through 1 cycle (Vertex Pharmaceuticals Inc., Personal Communication, 2014). Further escalation of VX-970 is ongoing with gemcitabine at 500 mg/m^2 or cisplatin 40 mg/m^2 and as single agent.

Pharmacokinetics

Study 001 evaluated systemic pharmacokinetics (PK) in both whole blood and plasma matrices (Vertex Pharmaceuticals Inc., Personal Communication, 2014). Across all dose levels (18, 36, 60, 72, and 90 mg/m^2) in the monotherapy period, mean CL was similar in both matrices (40.0 to 82.4 L/h in whole blood and 62.5 to 98.2 L/h in plasma) and median terminal elimination half-life ($t_{1/2}$) was consistent between both matrices at approximately 15 hours post end of infusion. VX-970 was extensively distributed to the tissues; however, V_d at steady state (V_{ss}) was greater in plasma than in the whole blood matrix (794 to 1155 L in whole blood and

1041 to 1602 L in plasma). Exposure parameters tended to be higher in the whole blood matrix (mean maximum observed concentration [C_{max}] between 105 and 778 ng/mL and AUC_{inf} between 425 and 3574 h*ng/mL) versus plasma (mean C_{max} between 72.0 and 536 ng/mL and AUC_{inf} between 351 and 2275 h*ng/mL); however, both matrices proved to adequately characterize systemic PK. The VX-970 RBC penetration was ~33% based upon overall exposure (AUC_{inf}) of study drug. Exposure (C_{max} and AUC) tended to be somewhat linear across the studied doses and accumulation of study drug was not observed during the once weekly dosing regimen. After the evaluation of one subject in Study 002, PK results were similar (within standard error) to those seen in Study 001.

Preliminary Efficacy

One subject with nasopharyngeal cancer treated with gemcitabine and VX-970 had shown partial response (PR) through 4 cycles (Vertex Pharmaceuticals Inc., Personal Communication, 2014). Also among subjects receiving a combination with gemcitabine, stable disease (SD) has been noted in 1 subject with NSCLC through 10 cycles (still ongoing), 1 subject with gastrointestinal stromal tumor (GIST) through 6 cycles (still ongoing), 2 subjects with NSCLC through 6 cycles, and in 1 subject with colorectal cancer (CRC) through 5 cycles. Additionally, one subject with CRC receiving single agent VX-970 weekly at 60 mg/m^2 in 21-day cycles had SD through 4 cycles (still ongoing).

Preliminary Safety

No dose-limiting toxicities (DLTs) or safety trends were observed during the 2-week single agent lead-in period that comprised two monotherapy doses of VX-970 for subjects who subsequently received combination therapy with gemcitabine in Study 001 (Vertex Pharmaceuticals Inc., Personal Communication, 2014). No DLT or safety trend has been noted in a single subject receiving VX-970 monotherapy at 60 mg/m2 in Study 002 through 4 cycles. Doses of VX-970 18 mg/m^2, 36 mg/m^2, and 60 mg/m^2 were tolerated with gemcitabine dosed at 875 mg/m^2. Two DLTs (transient grade 3 alanine transaminase/aspartate aminotransferase [ALT/AST] elevation and grade 4 thrombocytopenia, requiring transfusion, in a subject with prior platelet decline with a previous gemcitabine-containing regimen) were observed, each in one subject, out of 7 subjects at 72 mg/m^2 when given in combination with gemcitabine at 875 mg/m^2. One DLT was observed in a single subject out of 6 subjects (transient grade 3 ALT elevation, accompanied by mild nausea, vomiting and low grade fever) receiving 90 mg/m^2 of VX-970 with gemcitabine 500 mg/m^2; this subject remained on study, and has since tolerated 6 cycles of VX-970 (17 additional doses) and gemcitabine at an unchanged dose.

Pharmaceutical Information

VX-970 is supplied as a 5 mg/mL sterile solution (Investigator's Brochure, 2013). Single-use sterile, light-protected vials of VX-970 should be stored at room temperature (15°C to 30°C). Diluted IV solution (IV bags) should be covered to protect from light and stored in the dark for a maximum of 24 hours.

CTEP's Plans for VX-970 Development

As stated above, CTEP is interested in:

- Phase 1 trials of VX-970 in combination with:
  - IR in patients with CNS metastasis;
  - topoisomerase I inhibitors;
  - cisplatin and IR in locally advanced recurrent head and neck cancer (post-surgery).
- One or two phase 2 randomized trial(s) possibly but not limited to ovarian, GI, or GU cancers to show proof of principle using one of the combinations currently in phase 1 trial (gemcitabine, gemcitabine/cisplatin, carboplatin, cisplatin/etoposide).

VX-970 Project Team Selection, Composition, and Tasks

The VX-970 drug project team will meet regularly by WebEx to review available evidence and determine promising strategies, identify biomarkers to evaluate these strategies, and evaluate clinical trial designs to test these strategies within the proposed areas. Also, the project team will make recommendations for phase 2 trials exploring the combinations to be tested. It is expected that the VX-970 Drug Project Team will be in existence for only 8-12 weeks, and must complete its work no later than January 05, 2015. In order for the process to be successful, Drug Project Team members must be willing to adapt their current commitments to be able to attend the virtual team meetings.

The project team will be composed of intramural and extramural members. The extramural members will include clinician scientists with experience in phase 1 studies, translational scientists with expertise in biomarker development in DDR, and basic scientists with expertise in DDR. The team will also include disease-specific experts with interest in developing this agent with gemcitabine- or platinum-based regimens to design trials to test the benefits of this agent. Since the clinician scientists selected for the project team will be expected to lead the clinical trials that come out of this process, the evaluation criteria for the clinician scientists will include not only clinical trial expertise but also their documented record of accrual to phase 1 early phase studies or phase 2 disease-specific trials. Translational scientists are especially encouraged to apply, and may be asked to collaborate with clinicians on the clinical trials that come out of the process.

Applicants selected for the drug project team will be required to sign a standard Conflict of Interest form and a confidentiality agreement that will cover the deliberations of the team.

Questions regarding this request for applications may be addressed to Alice Chen, M.D., Senior Investigator, Investigational Drug Branch, CTEP, DCTD, National Cancer Institute (NCI) (phone: 240-276-6565; FAX: 240-276-7894; e-mail: chenali@mail.nih.gov). All applicants must have an active CTEP Identity and Access Management (IAM) account before the submission deadline. To create a CTEP-IAM account, go to https://eapps-ctep.nci.nih.gov/iam/index.jsp and click the "Request New Account" link at the right. For questions about CTEP-IAM account creation, please contact the CTEP Registration Help Desk: ctepreghelp@ctep.nci.nih.gov.

PTMAs should contain a clear indication of the desired role on the VX-970 Project Team (clinician scientist, translational scientist, or basic scientist). The PTMA should also be accompanied by an NIH Biosketch containing a personal statement customized to this project. The PTMAs should be sent to the Protocol and Information Office (PIO) at the address below by **5:00 PM Eastern Time (ET), October 28, 2014**. The most recent version of the PTMA form, available on the CTEP Website (http://ctep.cancer.gov), must be used. PTMAs should be submitted electronically to:

PIO, CTEP/DCTD/NCI
E-mail: pio@ctep.nci.nih.gov

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