



REQUEST FOR PROJECT TEAM MEMBER APPLICATIONS FOR CONDUCTING CLINICAL TRIALS USING CBX-12 (NSC# TBD)

The National Cancer Institute (NCI) Cancer Therapy Evaluation Program (CTEP) is accepting Project Team Member Applications for a project using CBX-12, a pH-sensitive peptide-drug conjugate of the topoisomerase inhibitor exatecan, which is being developed by CTEP as an anticancer agent in collaboration with Cybrexa Therapeutics (Gayle *et al.*, 2021). CBX-12 is a peptide-drug conjugate (PDC) that selectively targets tumor cells in an antigen-independent manner based on low pH. CBX-12 contains a pH-low insertion peptide that senses the acidic tumor cell surface found in all cancers. In acidic conditions, the pH-low insertion peptide forms a transmembrane alpha helix, which delivers the therapeutic agent exatecan across the cancer cell membrane where it is then released into the cell via glutathione reduction of the linker (Gayle *et al.*, 2021). CBX-12 exhibits antitumor activity across multiple xenograft models.

At the present time, the preliminary CTEP drug development plan has included preclinical xenograft studies that have been completed by NCI investigators and will be followed by clinical trials that may include phase 1 studies, combination studies, and topoisomerase-1 inhibitor biomarker correlation studies for the treatment of multiple types of cancer. The alphalex™ platform of the Cybrexa agent employs a “payload” drug conjugated to a pH-sensitive peptide via a self-immolating linker. The advantages of this approach include antigen-independence for tumor selection, allowing for penetration of solid tumors and metastases despite antigenic heterogeneity. CBX-12 includes the topoisomerase-1 inhibitor exatecan as the therapeutic payload. In mice, it showed dose-dependent reductions in tumor volume in colorectal carcinoma and TNBC models with no detectable bone marrow or gastrointestinal toxicity, which represents an improved safety profile compared to the free compound. The limited clinical use of exatecan has been related in many instances to excessive toxicity of the drug. The role of the project team is to evaluate all available evidence to modify and refine this initial plan.

It is anticipated that CTEP will activate 4 different single agent or combination trials with CBX-12. The project team will include:

1. **Clinician Scientists** with expertise in phase 1 and 2 studies and with an interest (although not exclusively) in ovarian, colorectal, gastric/esophageal and small cell lung cancers (fill out **Part A** of the attached Application; Clinician Scientists must belong to a qualifying NCI grant funded institution as defined at the end of this letter);
2. **Translational scientists** with an interest in biomarker development in topoisomerase I inhibition in tumor cells with an evaluation with apoptosis and cell necrosis (fill out **Part B** of the attached Application and see the submission instructions at the end of this letter); and
3. **Basic scientists** with expertise in topoisomerase I inhibition (fill out **Part C** of the attached Application and see the submission instructions at the end of this letter).

Prospective team members may apply for multiple roles using a single application form by completing all the appropriate Parts. The project team will be recruited nationally and will prioritize the research questions regarding CBX-12 in single agent or combination, including prioritization of biomarker studies. It is anticipated that the clinicians on the drug project team will be tasked with writing the Letters of Intent describing the study design, based upon the team’s recommendations, 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 12 weeks or less.

Background/Rationale

CBX-12 is a pH-sensitive peptide-drug conjugate (PDC) consisting of exatecan conjugated to a pH-low insertion peptide (pHLIP) variant via a self-immolating, glutathione-sensitive linker (Gayle *et al.*, 2021). CBX-12 was developed to overcome the following challenges of unconjugated exatecan therapy and the antibody-drug conjugate (ADC) delivery system: the dose-limiting toxicities that occur as a result of exatecan monotherapy, the limitations of ADCs to a small subset of cancers with specific antigens, and the side-effects of unstable ADC linkers and antigen expression in healthy tissues, which leads to dose-limiting toxicities in the clinic.

Mechanism of Action

In acidic conditions, pHLIP variants undergo a conformational change into transmembrane alpha helices that directly insert into cancer cell membranes to deliver the agent at the C-terminal end of the peptide into the cell (Gayle *et al.*, 2021). CBX-12 is conjugated to the topoisomerase-1 inhibitor, exatecan.

Nonclinical Studies of CBX-12

In vitro plasma stability assays found a CBX-12 peptide/linker conjugation conformation that displayed robust linker stability and undetectable warhead release after 8 hours, and minimal warhead release after 24 hours, in mouse, rat, dog and human plasma samples (Gayle *et al.*, 2021). *In vivo*, CBX-12 showed robust anti-tumor activity and efficacy in multiple *in vivo* xenograft mouse models, including HER2, HCT116 flank, MDA-MB-231 breast cancer, JIMT-1 breast cancer and MKN45 gastric cancer xenograft models.

Pharmacokinetics (PK) studies in mouse plasma, HCT116 flank xenografts, and mouse bone marrow showed that after a single 10 mg/kg injection, CBX-12 maintained stability in plasma, and accumulated in mouse tumor tissue, but not in mouse bone marrow. CBX-12 stability was also similar in pharmacokinetics studies in rat plasma after a single 5 mg/kg injection, in comparison to unconjugated exatecan. Mice treated with 2.6 µmol/kg of CBX-12 did not show any significant signs of toxicity in comparison to the control (unconjugated exatecan). Duodenum from mice treated with 2.6 and 5.2 µmol/kg of CBX-12 did not show any significant signs of GI toxicity.

Clinical Studies of CBX-12

Clinical studies are in progress, as Cybrexa has dosed their first patient in a phase 1/2 clinical trial of CBX-12 in advanced or metastatic refractory solid tumors (Cybrexa Therapeutics, 2021). This is a first-in-human, Phase 1/2 open-label, multicenter, dose-escalation, safety, PK, and biomarker study of CBX-12 in subjects with advanced or metastatic refractory solid tumors (ClinicalTrials.gov, 2021). The ClinicalTrials.gov identifier is [NCT04902872](https://clinicaltrials.gov/ct2/show/study/NCT04902872). Phase 1 is currently open for accrual. Initially, subjects were treated with CBX-12 on a daily x 5 every 3 weeks schedule (Part A). Currently, subjects are being treated with CBX-12 on a daily x 3 every 3 weeks schedule (Part B), and on a once weekly schedule (Part C). Once the recommended phase 2 doses (RP2D) have been established in both Part B and Part C, Phase 2 expansion cohorts may open.

CTEP's Plans for CBX-12 Development

CTEP will form a CBX-12 project team to define a clinical trials program that complements the company's development program and that will identify the highest priority trials for initial CTEP development. CTEP envisions sponsoring 3-4 trials in the initial development plan, which may include randomized phase 2 trials that compare single agent CBX-12 to a Standard of Care (SOC) topoisomerase 1 inhibitor monotherapy in an appropriate clinical context (e.g. small cell lung cancer, ovarian cancer) and/or Phase 1 combination trials that determine the RP2D of CBX-12 in a multi-drug regimen where a SOC topoisomerase is currently part of the approved combination regimen (e.g., irinotecan in CRC). The intention would be to follow any Phase 1 combination study with a randomized Phase 2 trial of the combination. Consideration of additional Phase 1 and Phase 2 studies in other tumor histologies (e.g. pediatric tumors, CNS tumors, rare cancers) will be considered, based on preclinical and/or clinical data suggesting anti-tumor activity of CBX-12 in these tumors.

Correlative Studies of Interest to CTEP

Incorporation of pharmacodynamic endpoints into the project team studies is a high priority for CTEP. These endpoints may include, but are not limited to:

- Determination of exatecan concentration in tumors whether delivered by CBX-12 or as free drug and the effects of dosing modification on altering tissue concentrations.
- Effective activity of other combinations including check-point inhibitors with CBX-12 (based on preclinical data).
- Identification of tumor characteristics that predict sensitivity to CBX-12 with a focus on pH of the tumor microenvironment (TME), including defining the appropriate techniques for measurement of tumor pH, identifying tumors that can or cannot modulate pH of the TME, and establishing whether modulation of the pH of certain tumors (*e.g.* with mitochondrial inhibitors) will enhance tumor sensitivity for certain tumors.
- Measure topo-1 levels, apoptosis (*e.g.*, cleaved caspases), DNA damage repair (DDR) panels, and immune cell phenotypes.

CBX-12 Project Team Selection, Composition, and Tasks

The CBX-12 drug project team will meet regularly by WebEx to review available evidence and determine promising strategies, identify appropriate biomarkers, prioritize initial strategies, and develop efficient clinical trial designs to test these strategies. Non-prioritized trial concepts may be further developed and independently submitted to CTEP by clinicians following the completion of the project team, as appropriate. The project team will be composed of intramural and extramural members. The extramural members will include clinician-scientists with experience in phase 1 and 2 studies in ovarian, cervical, colorectal, gastric, bladder, rare genitourinary, and small cell and non-small cell lung cancers; translational scientists with expertise in biomarker development; and basic scientists with expertise in topoisomerase I inhibition. Since the clinician scientists selected for the project team will be expected to lead the clinical trials that emerge from this process, the evaluation criteria for the clinician scientists will include not only clinical trial expertise but also their documented record of accrual to specific tumor types with approved indications for topo-1 inhibitors, such as ovarian, colorectal, gastric, bladder, rare genitourinary, and small cell and non-small cell lung cancer early phase studies.

Questions regarding this request for applications may be addressed John J. Wright, M.D., Ph.D., Medical Officer, Investigational Drug Branch, CTEP, DCTD, NCI (phone: 240-276-6105; FAX: 240-276-7894; e-mail: wrightj@ctep.nci.nih.gov).

CTEP recognizes the importance of encouraging and supporting young investigators as they embark upon a clinical cancer research career. CTEP highly encourages Career Development Applications (CrDAs) from these investigators and their mentors to participate as Project Team members and to develop Career Development Letters of Intent (CrDLs) after conclusion of Project Team activities.

Project Team Member Applications (PTMAs) should contain a clear indication of the applicant's desired role on the CBX-12 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), March 28, 2022**. The most recent version of the PTMA form, which has been distributed with this communication, must be used. PTMAs should be submitted electronically to:

PIO, CTEP/DCTD/NCI

E-mail: CTEPPTMASubmissions@mail.nih.gov

Please note that Clinician Scientists may only participate through association with the ETCTN, an NCTN Group, or a consortium (see below), and will need to submit the PTMA through their ETCTN

LAO's Coordinating Center or the Group/Consortium Operations office, as applicable. That organization will then need to submit the Clinician's application to PIO on your behalf to confirm that they are in support of the proposal. All submissions funded by NCTN organizations should include a Letter of Support from the Group Chair. Please allow sufficient time for your organization's review. Qualifying clinical institutions include:

- ETCTN Participating Institution (under UM1 grant)
- NCTN Group member institution (under U10 grant; Alliance, COG, ECOG-ACRIN, NRG Oncology, or SWOG)
- Institutional affiliation with the Pediatric Brain Tumor Consortium (PBTC)

Basic and Translational Scientists who belong to a participating ETCTN institution (Lead Academic Organization [LAO] or Affiliated Organization [AO]) **must** submit applications through your LAO's Coordinating Center. Please allow sufficient time for your organization's review. Basic and Translational Scientists from non-ETCTN-affiliated institutions may submit their applications directly to PIO.

Bibliography

Cybrexa Therapeutics. (2021). Cybrexa Therapeutics announces first patient dosed in Phase 1/2 clinical trial of cbx-12 (alphalex™-exatecan) in advanced or metastatic refractory solid tumors. September 15, 2021.

ClinicalTrials.gov. (2021). Study of CBX-12 in subjects with advanced or metastatic refractory solid tumors. September 15, 2021.

Gayle, S., R. Aiello, N. Leelatian, *et al.* (2021). Tumor-selective, antigen-independent delivery of a pH sensitive peptide-topoisomerase inhibitor conjugate suppresses tumor growth without systemic toxicity. *NAR Cancer*. 3(2):zcab021.