CTEP
Rapid Communication

SOLICITATION FOR LETTERS OF INTENT
Clinical trials
Preclinical experiments

E7389, Halichondrin B analog (NSC 707389)

CTEP is soliciting for phase 1 combination and phase 2 single-agent clinical trials of E7389, a halichondrin B analogue tubulin polymerization inhibitor. E7389 is being developed by CTEP in collaboration with Eisai Research Institute (Eisai). E7389 is also available for nonclinical studies via the NCI through a cooperative agreement with Eisai. CTEP is specifically interested in experiments that will generate data useful for prioritization of clinical combination studies, mechanism of action studies, and studies to evaluate radiation sensitization.

E7389 is a structurally simplified synthetic macrocyclic ketone analog of halichondrin B, an antimitotic agent that exhibits potent anticancer effects in both *in vitro* and *in vivo* models of cancer. E7389 encompasses the biologically active portion of halichondrin B and exhibits similar or identical anticancer properties in preclinical models. Xenograft studies in mice (ovary, breast, colon, melanoma) demonstrate tumor regressions, remissions, and increased lifespans at dosing levels below the maximum tolerated dose (MTD) (Towle *et al.*, 2001). Preclinical models suggest that E7389 may have a wide therapeutic window relative to other cytotoxic anticancer agents. In one DCTD, NCI-sponsored phase 1 study of E7389 in patients with advanced solid tumors and four ongoing company-sponsored studies of E7389 (two phase 1 and two phase 2 trials in patients with breast and lung cancers), the drug has been well tolerated and has demonstrated clinical activity in breast, non-small cell lung cancer (NSCLC), urothelial/bladder, and thyroid cancers.

MECHANISM OF ACTION

The mechanism of action of halichondrin B is distinct from all other known classes of tubulin-based agents. While overall patterns of cytotoxicity of halichondrin B in the NCI cell line screen were similar to those of other antitubulin drugs such as vincristine, vinblastine, paclitaxel, colchicine, combretastatin A-4, dolastatin 10, rhizoxin, and maytansine, halichondrin B showed a unique profile among these agents with respect to the biochemistry of its interaction with tubulin (Bai *et al.*, 1991; Hamel, 1992; Luduena *et al.*, 1993; Fodstad *et al.*, 1996). For instance, halichondrin B is a non-competitive inhibitor of vinblastine binding to tubulin, has no effect on iodoacetamide alkylation of tubulin sulfhydryl groups, stimulates BisANS binding, yet does not stabilize colchicine binding to tubulin. Thus, halichondrin B's interactions with tubulin are unique among known antitubulin drugs. This unique mechanism may be associated with distinct antitumor characteristics in the clinic. Studies with E7389 indicate that its mechanism is similar

or identical to that of halichondrin B (Bai *et al.*, 1991; Towle *et al.*, 2001). E7389 is a tubulin-binding agent that suppresses microtubule dynamics and inhibits microtubule polymerization. Pre clinical data show that sub- to low-nanomolar levels of E7389 inhibit cancer cell proliferation via induction of dose- and time-dependent cell cycle block at G_2/M, disruption of mitotic spindle formation, and initiation of apoptosis.

PRECLINICAL EFFICACY STUDIES

In Vitro Studies

In vitro, E7389 inhibits the growth of COLO 205 and DLD-1 colon cancer cells, HL-60 promyelocytic leukemia, U937 histiocytic lymphoma, LNCaP and DU 145 prostate cancer, LOX human melanoma, and MDA-MB-435 human breast cancer cells with a mean IC_{50} (concentration producing a 50% decrease in total cells) value of 1.8 nM (range, 0.09-9.5 nM), while overall mean IC_{50} values for vinblastine and paclitaxel in the same experiments were 3.2 ± 0.7 nM and 7.3 ± 1.9 nM, respectively (Towle *et al.*, 2001). Cytotoxic effects of E7389 were not observed in quiescent human fibroblasts at concentrations up to 1 μM, suggesting that the effects of this agent are specific for proliferating cells. Performance of this agent in the NCI 60-cell line screen was virtually identical to that of natural halichondrin B.

In addition, E7389 showed p53-independent anticancer activity in the NSCLC cell lines A549 (wild type p53) and Calu-1 (p53-null) in the 0.5 pM range, consistent with levels clinically achievable (Kimura *et al.*, 2003). Twenty-four-hour treatment with E7389 resulted in M-phase arrest, dose-dependent induction of the Cdk inhibitor p27, and phosphorylation of Bcl2 in both cell lines.

E7389 induces G_2/M cell cycle arrest at 1-10 nM concentrations without affecting progression through the G_1 or S phases or the G_1/S transition point (Towle *et al.*, 2001). Similar concentrations induce profound disruption of mitotic spindles. E7389 inhibits tubulin polymerization *in vitro* with IC_{50} values of 6.9 μM and 6.0 μM for rate and extent of inhibition, respectively, similar to the 7.2 μM rate inhibition value reported for halichondrin B (Bai *et al.*, 1991; Towle *et al.*, 2001).

In Vivo Studies

E7389 demonstrates significant antitumor activity, including complete regressions, in a spectrum of nonclinical tumor models, including models considered chemorefractory. Intermittent dosing schedules were associated with the greatest efficacy.

The initial *in vivo* activity studies of halichondrin B showed inhibited tumor growth and increased host survival in the murine B16 melanoma and P388 and L1210 leukemia models at extremely low dose levels (2.5-100 μg/kg) (Hirata and Uemura, 1986). Subsequent studies using 0.1-1 mg/kg E7389 given as an intraperitoneal (IP) or intravenous (IV) infusion to treat LOX human melanoma, MDA-MB-435 human breast, COLO 205 human colon, and NIH:OVCAR-3 human ovarian cancer xenograft models have shown increased survival rates, tumor growth

delays, and reductions in size and number of metastases (Fodstad *et al.*, 1996; Alley *et al.*, 1998; Towle *et al.*, 2001). Responses ranged from tumor growth inhibition to tumor regression and eradication of tumors. On a per dose basis, E7389 activity surpasses that of paclitaxel by 10- to 40-fold. Moreover, E7389 showed a wide therapeutic window relative to other cytotoxic anticancer agents (Towle *et al.*, 2001). For instance, in the human MDA-MB-435 breast cancer xenograft model >95% tumor suppression occurred over the 4-fold dosing range of 0.25-1.0 mg/kg, with no evidence of toxicity based on body weight losses or decreased water consumption. In contrast, the therapeutic window for paclitaxel in this model is 1.7-fold, with complete tumor suppression seen only at its MTD of 25 mg/kg. Similarly wide therapeutic windows for E7389 were seen in the LOX melanoma and NCI-H522 models. In terms of efficacy, studies in the MDA-MB-435 breast, COLO 205 colon, and LOX melanoma models have shown clear superiority of E7389 compared to paclitaxel at its MTD in terms of duration of regrowth suppression following cessation of treatment.

Towle and colleagues extended their *in vivo* studies by testing different E7389 dosing schedules and by assessing E7389 effects in xenograft models under monotherapy and combination therapy conditions (Towle *et al.*, 2003). E7389 schedule dependency was tested in the MDA-MB-435 breast cancer xenograft model by comparing qd×5, q2d×3 [×3] (3 weekly cycles), q4d×3, and q7d×3 IV schedules. Low and high dose levels were used for each schedule so that total drug given over all injections was 4.5 mg/kg or 9 mg/kg, respectively. While the high dose level was lethal for all groups, the low dose level was lethal only on the qd×5 schedule. The best antitumor efficacies were seen with intermittent schedules: q2d×3 [×3] . q4d×3 > q7d×3 >> qd×5 (lethal). Intermittent schedules were also the least toxic: q2d×3 [×3] . q7d×3 < q4d×3 << qd×5 (lethal).

E7389 as a single agent was tested in the HT-1080 fibrosarcoma and PANC-1 pancreatic cancer xenograft models using q4d×3 IV dosing (Towle *et al.*, 2003). In HT-1080, 1.3-4 mg/kg E7389 led to long-lasting tumor regression at all doses. At 1.7 mg/kg (MTD), 10 of 10 mice were tumor-free by day 38, while at 1.3 mg/kg, 9 of 10 mice were tumor-free by day 42. In PANC-1, all doses from 0.4 to 4 mg/kg also led to long-lasting tumor regression.

The ability of E7389 to synergize with existing chemotherapy drugs was tested in combination studies with gemcitabine, doxorubicin, docetaxel, and carboplatin (Towle *et al*., 2003; Littlefield, 2005). The combination of 0.1-0.4 mg/kg E7389 on the q4d×3 schedule (which causes only limited tumor inhibition alone) with maximally effective gemcitabine doses of 120-270 mg/kg on the q3d×4 schedule (which alone cause only tumor stasis) induced clear regression in H522 NSCLC xenografts (Towle *et al.*, 2003). No synergy was observed between E7389 and doxorubicin in the MDA-MB-435 breast cancer xenograft model. *In vitro* combination of E7389 and docetaxel in several breast carcinoma cell lines demonstrated additive activity, while E7389 in combination with carboplatin in several NSCLC cell lines demonstrated antagonism in some cases and additivity in others (Littlefield, 2005). Additional preclinical combination studies are ongoing.

PRECLINICAL PHARMACOKINETICS AND METABOLISM

Rat

In rats, E7389 exhibited tri-exponential plasma distribution following an IV dose of 1.5 mg/kg (9 mg/m^2) with short α- (0.03 hours) and β- (0.36 hours) half-lives ($t_{1/2}$) and a $t_{1/2}\gamma$ of 11.4 hours resulting in sustained but low plasma levels. Area under the plasma concentration-time curve (AUC) and total body clearance (CL) were 826 ng×hour/mL and 1816 mL/hour/kg, respectively. A steady state volume of distribution (Vd_{ss}) of 18.6 L/kg (109.7 L/m^2 in the Rhie *et al*., 2002 report) indicated extensive tissue distribution. The oral bioavailability of E7389 given alone at 2.5 mg/kg (9 mg/m^2) via oral gavage was negligible. The oral bioavailability of E7389 in rats pre-treated with the p-glycoprotein inhibitor cyclosporine was 18.1%.

Dog

In dogs given a single IV dose of 0.03 mg/kg or 0.075 mg/kg (0.6 mg/m^2 or 1.5 mg/m^2) as a 1-hour infusion, the AUC and the maximum plasma concentration (C_{max}) increased in proportion to dose, and plasma profiles were best fit to a two-compartment model. The Vd was 1.91 L/kg (38.2 L/m^2) and 4.1 L/kg (38.2 L/m^2) for the 0.03 mg/kg (0.6 mg/m^2) and 0.075 mg/kg (1.5 mg/m^2) doses, respectively. A multiple dose PK study was also conducted in dogs at dose levels of 0.004 mg/kg/day, 0.03 mg/kg/day, and 0.04 mg/kg/day (0.08 mg/m^2/day, 0.6 mg/m^2/day, and 0.8 mg/m^2/day) (n=4/dose level) administered via a 1-hour IV infusion on a q4d×3 schedule. E7389 was not detected in any pre-dose samples and post-infusion levels were proportional to dose. At the 0.004 mg/kg/day (0.08 mg/m^2/day) level, E7389 was detectable in only the post-infusion plasma samples and ranged from 4.4 ng/mL to 7.6 ng/mL (5.85-7.18 nM in the Rhie *et al*., 2002 report). The post-infusion levels for the 0.03 mg/kg/day (0.6 mg/m^2/day) dose on all days ranged from 14.6 ng/mL to 28.1 ng/mL (17.7-34 nM). The high-dose group (0.04 mg/kg/day; 0.8 mg/m^2/day) exhibited a similar profile with post-infusion levels ranging from 25 ng/mL to 52.2 ng/mL (30.3-63.2 nM). The $t_{1/2}\beta$ for the 0.03 mg/kg (0.6 mg/m^2/day) and 0.04 mg/kg/day (0.8 mg/m^2/day) dose levels ranged from 11 hours to 45 hours, CL ranged from 71 mL/m^2/kg to 132 mL/m^2/kg, and Vd_{ss} ranged from 85 L/m^2 to 187 L/m^2 (Rhie *et al.*, 2002). Drug accumulation and changes in clearance between the multiple doses were not evident.

In summary, E7389 exhibits rapid and extensive tissue distribution in both rats and dogs with an extended terminal $t_{1/2}$.

Protein Binding and Metabolism

A species difference in protein binding of E7389 was observed *in vitro*. Between 100 ng/mL and 1000 ng/mL of E7389, the plasma protein binding of E7389 appeared to be the lowest in dog plasma (15.41-26.37%) and the highest in human plasma (48.92-65.07%). The binding percentages were similar between mouse (28.48-35.94%) and rat (23.01-34.09%) plasma. With the exception of human plasma, the percent binding appeared to be concentration independent. In human plasma, there was a statistically significant difference among the three concentration groups. In summary, E7389 was not strongly bound to mouse, rat, dog, or human plasma

protein. The relatively low plasma protein binding suggests that patient variability in either albumin or α1-acid glycoprotein will not significantly affect the pharmacokinetics of E7389.

CYP3A4 appears to be the major enzyme responsible for the human hepatic metabolism of E7389 *in vitro*, based on the CYP3A4- and NADPH-dependent metabolism, inhibitory effects observed from CYP3A4-specific inhibitors, and the significant correlations between the capability of E7389 metabolism and CYP3A4-specific activity using individual human liver microsomes.

Drug Interactions

Although E7389 has been shown to inhibit CYP3A4-mediated hydroxylation of R-warfarin and testosterone, and dehydration of nifedipine, the concentrations required to inhibit CYP3A4 are unlikely to be achieved clinically. The apparent K*i* values ranged from 3 μM to 17 μM, and the inhibition was demonstrated to be reversible and competitive.

PRECLINICAL TOXICOLOGY

E7389 administered IV once a day on days 1, 5, and 9 produced dose-limiting bone marrow depression in both rats and dogs, intestinal toxicity in dogs, and liver toxicity in rats. The MTD in rats was less than 0.25 mg/kg/day (1.5 mg/m²/day) on this schedule. In dogs, E7389 was lethal at doses of 0.075 mg/kg/day (1.5 mg/m²/day) administered as a 1-hour IV infusion on days 1 and 5. Reversible myelosuppression occurred in dogs given 0.03 mg/kg/day or 0.04 mg/kg/day (0.6 mg/m²/day or 0.8 mg/m²/day) E7389 on days 1, 5, and 9. Other toxicities that were considered to be drug-related occurred in the lymphoid tissue, testes, and skeletal muscle. All observed toxicities, except testicular toxicity, were reversible in both dogs and rats.

In a study designed to test the efficacy and toxicity of E7389 in mice bearing MDA-MB-435 breast cancer xenografts, four different IV schedules of administration were compared: qd×5, q2d×3 [×3] (3 weekly cycles), q4d×3, and q7d×3 IV (Towle *et al.*, 2003). Low and high dose levels were used for each schedule so that total drug given over all injections was 4.5 or 9 mg/kg, respectively. While the high dose level was lethal for all groups, the low dose level was lethal only on the qd×5 schedule. Intermittent schedules were the least toxic: q2d×3 [×3] . q7d×3 < q4d×3 << qd×5 (lethal).

In vitro bone marrow assays did not demonstrate significant species difference between human, dog, and mouse CFU-G/M cell sensitivity to E7389. Based on the ratios of human to murine and human to canine IC_{90} values (concentration producing a 90% decrease in total cells) and *in vivo* MTDs, the calculated human MTD is projected to be between 0.9 mg/m^2/day (based on canine data) and 3.1 mg/m^2/day (based on murine data). In *in vitro* genotoxicity studies, E7389 was negative in the Ames test with or without S9, but was weakly positive in the L5178Y/TK+/- mouse lymphoma mutagenesis assay.

In summary, doses of 0.08 mg/m^2/day of E7389 administered on days 1, 5, and 9 produced no toxicity in either rats or dogs, while doses of 0.6 mg/m^2/day or 0.8 mg/m^2/day produced reversible bone marrow toxicity in both species (Tosca *et al.*, 2002). The recommended starting dose for phase 1 clinical trials is 0.12 mg/m^2/day. The *in vitro* bone marrow data suggest that rodent, dog, and human bone marrow cells are not significantly different in their response to the toxic effects of E7389. Based on the *in vivo* data in dogs, the highest non-severely toxic dose was 0.8 mg/m^2/day and 1/6 of this dose is 0.13 mg/m^2/day. The MTD in rats was between 1.2-1.5 mg/m^2/day, and <1/10 of this dose is 0.12 mg/m^2/day. Therefore, a starting dose of 0.12 mg/m^2/day is predicted to be safe.

CLINICAL EXPERIENCE

Safety

One DCTD, NCI-sponsored phase 1 study of E7389 in patients with advanced solid tumors and four Eisai-sponsored studies (two phase 1 and two phase 2) are ongoing. E7389 has been well tolerated and has demonstrated clinical activity in breast, NSCLC, urothelial/bladder, and thyroid cancers.

The CTEP sponsored phase 1 study is evaluating E7389 administered as an IV bolus over 1-2 minutes once a week for 3 weeks, every 28 days (Synold *et al.*, 2003). This study is being conducted by the California Cancer Consortium. The starting dose is 0.125 mg/m^2 (<1/10 of the MTD in rats and <1/3 of the toxic dose low in dogs). The trial is using a two-part accelerated dose escalation. Since this is the first study of E7389 in humans, correlative pharmacokinetic studies are performed in all patients and in real-time to provide timely feedback to guide ongoing dosage escalations. To date, 40 patients with refractory or advanced solid tumors have been treated (Synold *et al.*, 2005). Three patients were enrolled during the accelerated phase of the study, for a total of nine doses with PK (Synold *et al.*, 2003). The first patient received two cycles (0.125 mg/m^2 and 0.25 mg/m^2), and the second patient received two cycles (0.25 mg/m^2 and 0.5 mg/m^2) of E7389. The third patient experienced dose-limiting toxicity (DLT; grade 3 alkaline phosphatase elevation) following one dose of 0.5 mg/m^2, ending the accelerated phase of the study (Synold *et al.*, 2003). The second escalation phase ended at 2.0 mg/m^2 with two DLTs, one grade 3 febrile neutropenia and one grade 4 neutropenia (Synold *et al.*, 2005). Other serious non-hematologic adverse events (AEs) included hypoglycemia, hypophosphatemia, and fatigue. The MTD of E7389 was 1.4 mg/m^2 (Synold *et al.*, 2005). A radiation recall reaction was seen in one patient. Two partial responses (PRs; NSCLC, bladder) and three minor responses (MR; NSCLC, breast, and thyroid) occurred in 38 evaluable patients (Synold *et al.*, 2005). Stable disease (SD) as best response was seen in 12 patients lasting a median of 4 months (range, 2-14 months).

Two Eisai-sponsored phase 1 trials are designed to determine the MTD, AEs, and pharmacokinetic parameters of E7389 administered as a 1-hour IV infusion either on days 1, 8, and 15 of a 28-day cycle, or on day 1 of a 21-day cycle. On a weekly × 3, every-28-day schedule (Eisai study # E7389-A001-101), eight patients have been enrolled. The doses evaluated are 0.25 mg/m^2 and 0.5 mg/m^2. A patient experienced grade 3 neutropenia (ANC=900) on day 15 after receiving 0.5 mg/m^2 E7389. A second patient experienced

progression of pre-study fatigue from grade 1 to grade 3 (a DLT) upon receiving the second dose of E7389 during cycle 1. This patient discontinued his participation in the trial. Accrual at the 0.5 mg/m^2 dose level is ongoing. Once the MTD is reached, an additional regimen (days 1 and 8 of a 21-day cycle) will be studied. On an every-3-week schedule (Eisai study # E7389-A001-102), 13 patients have been treated at 0.25 -4 mg/m^2 (Elsayed *et al.*, 2004). Three out of three patients at 4 mg/m^2 developed a dose-limiting febrile neutropenia, accompanied by grade 2 mucositis in one. The first patient at the next lower dose (2.8 mg/m^2) experienced febrile neutropenia. A grade 3 neutropenia attributed to extensive prior radiation occurred in every cycle (6) in one patient treated at 1 mg/m^2. The other drug-related AEs were grade 1/2 and included anorexia, fatigue, nausea, anemia and thrombocytopenia (grade 1), increased alkaline phosphatase, increased ALT, and hyperkalemia. The median number of cycles received was two. Four patients had SD for four cycles or more. Accrual at the 4 mg/m^2 dose level is ongoing. No PRs or complete responses (CRs) have been observed in these studies to date.

Two Eisai-sponsored phase 2 trials of E7389 are evaluating 1.4 mg/m^2 E7389 administered as an IV bolus on days 1, 8, and 15, every 28 days. Eisai study # 201 includes patients with refractory breast cancer who progressed on prior taxane therapy (2-3 prior chemotherapies). Eisai study # 202 is enrolling patients with refractory lung cancer (following doublet therapy containing a platinum agent), stratified by prior taxane therapy.

Pharmacokinetics

Pharmacokinetic results from the initial, DCTD-sponsored phase 1 trial of E7389 administered as a bolus IV injection weekly × 3 demonstrated tri-phasic elimination, with a rapid distributive phase followed by prolonged elimination from plasma (Synold *et al*., 2005; Synold, 2005). The median peak plasma concentration of E7389 following a bolus dose at the MTD (1.4 mg/m^2) was 1.1 μM (range, 0.6-2.2 μM). Following rapid tissue distribution (median $t_{1/2}$ of 11.2 minutes), E7389 was slowly eliminated from plasma with a median terminal $t_{1/2}$ of 39 hours (range, 13-77 hours). The median plasma concentration of E7389 in patients treated with 1.4 mg/m^2 measured 1 week after a dose was 0.8 nM (range, 0.2-2.5 nM), which are well above the levels required for cytotoxicity *in vitro*. The median systemic CL (CL_{sys}) of E7389 in man is 2.2 L/hr (range, 1.1-7.0 L/hr), and the median Vd is 81.6 L (range, 25-620 L). E7389 CL in man is dose-independent over the range of doses tested, and is unrelated to either body surface area or total body weight. Despite an apparent wide inter-patient variability in CL_{sys}, the intra-patient variation in CL is quite low. Recovery of unchanged E7389 in the urine during the first 48 hours after dose administration is <10% of the total drug administered. Although several hydroxylated metabolites could be identified by *in vitro* experimentation, circulating metabolites were not detected in patients.

Preliminary pharmacokinetics (10 patients) from the Eisai-sponsored phase 1 trial E7389 administered as a 1-hour IV infusion once every 3 weeks were best described by a two-compartment model, in which there was rapid distribution, slow clearance, and prolonged elimination with a small fraction excreted unchanged into the urine (Elsayed *et al.*, 2004). Average Vd_{ss}, CL, and mean residence time (MRT) ranged from 53.2 L to 218.5 L, 1.4 L/hr to 4.4 L/hr, 28.1 hours to 50.1 hours, respectively, at the dose levels tested. The C_{max} and AUC increased in a dose-dependent manner between 0.25 mg/m^2 and 1 mg/m^2. Higher dose levels are

being studied. The ratios of C_{max} to $C_{trough,\ 96\ hr}$ ranging from 0.33% to 1.5%, as well as the calculated accumulation factor of 1 for all 10 subjects, indicated that despite the prolonged elimination phase, repeated dosing is unlikely to cause drug accumulation with the regimen studied.

Pharmacodynamics

For molecular target validation studies, serial tumor biopsies were obtained from 13 patients treated at 1.4 mg/m^2 (the MTD) on the CTEP-sponsored study (Synold *et al*., 2005; Synold, 2005). Biopsies were obtained both before and after (1 hour and 24 hours) the first dose of E7389. Fluorescent immunohistochemical (FIHC) analyses demonstrate that E7389 disrupts microtubule structure in tumors *in vivo*. These data indicate that E7389 is reaching the site of the tumor and hitting its presumed target.

CTEP CLINICAL TRIALS SOLICITATION

CTEP is soliciting for phase 1 combination and phase 2 single-agent clinical trials of IV E7389, a novel tubulin polymerase inhibitor.

CTEP is interested in phase 1 studies of E7389 in combination with conventional chemotherapeutics. The schedule CTEP is most interested in is a weekly × 3 every-28-days schedule of E7389. Please contact Dr. Colevas at CTEP prior to submission of LOIs to obtain up to date information on preclinical combination data.

CTEP is also interested in single-agent phase 2 studies to explore for evidence of activity in several common cancers. CTEP will consider proposals from cooperative groups or consortia of phase 2 sites to perform a single trial with a screening design whereby there would be parallel enrollment for several cancer types in two-stage designs. CTEP will also consider the traditional single-disease phase 2 trial proposals in appropriate populations of patients with cervical, prostate, bladder, pancreatic, sarcoma, NSCLC, ovarian, head and neck, thyroid, and breast cancers on a weekly × 3 every-28-days schedule. The planned starting dose for this schedule is 1.4 mg/m^2/week as a bolus IV.

CTEP has not established a set of correlative studies essential to the development of E7389. Therefore, we will evaluate proposals for correlative studies included in the LOIs, but such proposals are not necessary for LOI approval. Translational Research Initiative (TRI) support may be available for some of the proposed correlative laboratory work that will be part of the clinical trials, but TRI support is not available for independent preclinical studies.

Evaluation criteria for proposals include scientific rationale, accrual potential, past performance in accrual to phase 1 or phase 2 studies, and adequacy of trial design. If an investigator intends to apply for TRI support for laboratory correlative studies, this must be noted in the LOI, and the LOI must include an estimated budget and justification, using the cost estimate worksheet.

A protocol must be submitted within 30 days (within 60 days for cooperative groups) of final

approval of the LOI. Prompt submission of a protocol is important, and any delay will result in withdrawal of the LOI approval. CTEP will provide some materials for the protocol to facilitate rapid turnaround.

Questions regarding this solicitation may be addressed to A. Dimitrios Colevas, M.D., Senior Clinical Investigator, Investigational Drug Branch, CTEP, DCTD, NCI (phone: 301-496-1196; Fax: 301-402-0428; E-mail: colevasa@ctep.nci.nih.gov).

Letters of Intent should be e-mailed to the LOI coordinator at the e-mail address below by **5:00 PM (EST) May 18, 2005.** The most recent version of the LOI form available on the CTEP Website (http://ctep.info.nih.gov) must be used. **Electronic submission of LOIs is required.**

E-mail address: pio@ctep.nci.nih.gov
Fax: (301) 496-9384
CTEP Website: http://ctep.info.nih.gov/
Questions: LOI Coordinator, phone (301) 496-1367
E-mail: pio@ctep.nci.nih.gov

CTEP PRECLINICAL EXPERIMENTS SOLICITATION

E7389 is also available for nonclinical studies via the NCI through a cooperative agreement with Eisai. CTEP is interested in studies that would support additional clinical development such as mechanism of action studies, combination studies with conventional and other investigational agents, and studies to evaluate radiation sensitization.

Requests for E7389 for preclinical/nonclinical studies may be sent via e-mail to Dr Colevas. **The clinical trial LOI application form should not be used for preclinical requests.** The proposal should include the background, rationale, and experimental design for the studies, as well as the quantity of E7389 required for these investigations. Proposals will be evaluated by CTEP and Eisai for scientific merit. If approved, the Regulatory Affairs Branch at CTEP will execute a Materials Transfer Agreement to cover this collaborative research. Translational Research Initiative (TRI) funding **will not** be available for these studies. CTEP will be providing E7389 only, and will not provide other agents or any funding. If the request is for a combination study, please specify the source of the other agent(s). Requests should be submitted to:

A. Dimitrios Colevas, M.D.,
Senior Clinical Investigator,
Investigational Drug Branch, CTEP, DCTD, NCI
Phone: (301) 496-1196; Fax: (301) 402-0428
E-mail: colevasa@ctep.nci.nih.gov

REFERENCES

Alley, M., D. Dykes, W. Waud, and A. Et. (1998). Efficacy evaluations of halichondrin B in selected xenografts. *Proc Am Assoc Cancer Res*. 39:A1545.

Bai, R.L., K.D. Paull, C.L. Herald, *et al.* (1991). Halichondrin B and homohalichondrin B, marine natural products binding in the vinca domain of tubulin. Discovery of tubulin-based mechanism of action by analysis of differential cytotoxicity data. *J Biol Chem*. 266:15882-15889.

Elsayed, Y.A., L. Rosen, V. Rajeev, *et al.* (2004). Phase I study of a new Halichondrin B analog, E7389, administered by 1 hour IV infusion every 21 days. *Eur J Cancer*. 2 (Suppl.):163.

Fodstad, O., K. Breistol, G.R. Pettit, *et al.* (1996). Comparative antitumor activities of halichondrins and vinblastine against human tumor xenografts. *J Exp Ther Oncol*. 1:119-125.

Hamel, E. (1992). Natural products which interact with tubulin in the vinca domain: maytansine, rhizoxin, phomopsin A, dolastatins 10 and 15 and halichondrin B. *Pharmacol Ther*. 55:31-51.

Hirata, Y., and D. Uemura. (1986). Halichondrins: antitumor polyether macrolides from a marine sponge. *Pure Appl Chem*. 58:701-710.

Kimura, T., T. Synold, C.M. Mahaffey, *et al.* (2003). E7389, a novel antimicrotubule agent with potent p53-independent induction of p27, Bcl2 phosphorylation and cytotoxicity in non-small cell lung cancer (NSCLC). *Proc Am Soc Clin Oncol*. 22:697 (A2804).

Littlefield, B. A. (2005). Personal communication.

Luduena, R.F., M.C. Roach, V. Prasad, and G.R. Pettit. (1993). Interaction of halichondrin B and homohalichondrin B with bovine brain tubulin. *Biochem Pharmacol*. 45:421-427.

Rhie, J.K., T.-H. Lin, J.D. Johnson, *et al.* (2002). Preclinical pharmacokinetics of halichondrin macrocyclic ketone analog E7389 (NSC-707389) in rats and dogs. *Proc Am Assoc Cancer Res*. 43:A1066.

Synold, T.W., J. Lawrence, B. Xi, *et al.* (2003). Human pharmacokinetics of E7389 (Halichondrin B analog), a novel anti-microtubule agent undergoing phase I investigation in the California Cancer Consortium (CCC). *Proc Am Soc Clin Oncol*. 22:144 (A575).

Synold, T.W., R.J. Morgan, E.M. Newman, *et al.* (2005). A phase I pharmacokinetic and target validation study of the novel anti-tubulin agent E7389: A California Cancer Consortium Trial. *Proc Am Soc Clin Oncol*. 24:Submitted for publication.

Synold, T.W. (2005). Personal communication.

Tosca, P., L.H. Bollinger, J.W. Merrill, *et al.* (2002). Preclinical toxicology studies for

halichondrin b macrocylic ketone analog E7389 (NSC-707389) in beagle dogs and rats. *Proc Am Assoc Cancer Res*. 43:A5422.

Towle, M.J., K.A. Salvato, J. Budrow, *et al.* (2001). *In vitro* and *in vivo* anticancer activities of synthetic macrocyclic ketone analogues of halichondrin B. *Cancer Res*. 61:1013-1021.

Towle, M.J., S. Agoulnik, G. Kuznetsov, *et al.* (2003). *In vivo* efficacy of E7389, a synthetic analog of the marine sponge antitubulin agent halichondrin B, against human tumor xenografts under monotherapy and combination therapy conditions. *Proc Am Assoc Cancer Res*. 44:A2749.