REQUEST FOR PROJECT TEAM MEMBER APPLICATIONS FOR DESIGNING CLINICAL TRIALS USING AZD9291 (CAS#: 1421373-65-0)

The Cancer Therapy Evaluation Program (CTEP) is accepting Project Team Member Applications for a project to develop AZD9291, an inhibitor of the tyrosine kinase activity of the epidermal growth factor receptor (EGFR), which is under development by CTEP as an anticancer agent in collaboration with AstraZeneca. AZD9291 is a third generation, irreversible selective inhibitor of EGFR with activity against activating (*e.g.,* L858R) and resistance (T790M) mutations, but with marginal activity against wild-type EGFR. In AstraZeneca's first phase 1 study of AZD9291 in patients with non-small cell lung cancer (NSCLC) who have activating EGFR mutations and have progressed on first generation EGFR inhibitors (AURA1), to date there have been 44 partial responses (PR) (confirmed and unconfirmed) and 31 stable disease (SD) out of 92 evaluable patients. The recommended phase 2 dose (RP2D) is 80 mg/day. AstraZeneca is currently planning a phase 2 study and a randomized phase 3 study, both testing single agent AZD9291 in NSCLC with both activating and T790M resistance mutations.

At the present time, CTEP is interested in phase 1/2 novel-novel combination studies that include AZD9291 with other agents known to play a role in EGFR inhibitor resistance. The rationale for using AZD9291 as a platform for testing combination strategies in mutant EGFR NSCLC is that the relative inactivity of AZD9291 against wild-type EGFR will help to minimize toxicities associated with EGFR-pathway inhibition in combination therapy regimens. The initial CTEP plan proposed to combine AZD9291 with cetuximab, with a mitogen activated protein kinase kinase (MEK) inhibitor, and with an Aurora kinase inhibitor in NSCLC patients with activating mutations of EGFR who have progressed on, or are refractory to, first generation (*e.g.,* erlotinib or gefitinib) or second generation (*e.g.,* afatinib) therapy. The role of the project team is to evaluate all available evidence to refine this initial plan.

It is anticipated that CTEP will activate three different combination trials with AZD9291. The project team will include clinician-scientists with expertise in phase 1 and/or phase 2 studies and with an interest in NSCLC, translational scientists with an interest in biomarker development as it relates to response to EGFR-directed therapy, and basic scientists with expertise in mechanisms of EGFR inhibitor resistance. The project team will be recruited nationally and will prioritize the research questions regarding AZD9291 in combination trials, 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 8-12 weeks.

Background/Rationale

Activation of the EGFR tyrosine kinase triggers a cascade of intracellular downstream signaling events affecting cell proliferation, survival, angiogenesis and, potentially, metastases (Investigator's Brochure, 2014). Mutations in EGFR are identified in 10%-30% of patients with NSCLC (Gainor and Shaw, 2013). The most common oncogenic mutations are small, in-frame deletions in exon 19 and a point mutation that results in a substitution of a leucine with an arginine (L858R) in EGFR (EGFRm+) (Lynch *et al*., 2004). These mutations are thought to lead to constitutive activation of the EGFR kinase, resulting in cellular proliferation, but also confer sensitivity to EGFR tyrosine kinase inhibitors (TKIs) such as gefitinib and erlotinib (Lynch *et al*., 2004; Paez *et al.*, 2004; Pao *et al.*, 2004). Patients with EGFRm+ tumors have a response rate (RR) of approximately 75% to EGFR-TKIs as compared to less than 10% for patients with wild-type EGFR (Zhang and Chang, 2007). However, the beneficial effects are often of limited duration due to the emergence of drug resistance. A second EGFR mutation, the substitution of threonine 790 with methionine (T790M), is a major mechanism of resistance that has been observed in approximately 50% of patient tumors following disease progression (Balak

et al., 2006; Yun *et al.*, 2008). Introduction of the T790M mutation to EGFRm+ increases its affinity for adenosine triphosphate (ATP) by more than an order of magnitude, leading to loss of TKI activity.

AZD9291 shows less inhibitory activity against wild-type EGFR when compared to other EGFR-TKIs, giving it a wider therapeutic index and potential for use in combination studies. Combined inhibition of EGFR and other targets may confer longer and deeper responses than an EGFRm+ inhibitor alone by delaying the onset of resistance. While previous combinations of non-selective EGFR inhibitors required dose modifications due to toxicity associated with the inhibition of wild-type EGFR, phase 1 data of AZD9291 demonstrates that plasma target levels can be exceeded with minimal toxicity.

Mechanism of Action

AZD9291 is a third generation, irreversible, selective inhibitor of EGFR with both activating (EGFRm+) and resistance (T790M) mutations with a margin of selectivity against wild-type EGFR (apparent half-maximal inhibitory concentrations [IC_{50}s] <12 nM for mutant *vs.* 184 nM for wild-type EGFR) (Investigator's Brochure, 2014).

Nonclinical Studies of AZD9291

In a counter-screen against 277 other protein kinases, AZD9291 only showed significant activity (>75% inhibition at 1 μM) against a limited number of other kinases (*e.g.*, ACK1, BLK, ErbB4, BRK, MLK1, MNK2), indicating high selectivity towards EGFR family (Investigator's Brochure, 2014). AZD9291 has two pharmacologically active metabolites, AZ5104 and AZ7550, which showed similar pharmacological selectivity profiles.

In *in vitro* cellular EGFR phosphorylation assays, AZD9291 demonstrated potent phospho-EGFR (pEGFR) inhibition across various EGFRm+ and T790M cells lines (apparent IC_{50}: 6, 13, 15, 17, and 54 nM in PC9 VanR, H1650, H1975, PC9, and H3255, respectively), while demonstrating weaker inhibition toward wild-type EGFR cell lines (apparent IC_{50}: 480, 1684, and 1865 nM in LOVO, A431, and H2073, respectively) (Investigator's Brochure, 2014). AZD9291 similarly demonstrated significant *in vitro* anti-proliferative activity in EGFRm+ and T790M cells lines (IC_{50}: 8, 11, and 40 nM in PC9, H1975, and PC9VanR, respectively), and less activity in wild-type EGFR cell lines (IC_{50}: 461, 650, and 4089 nM in H2073, CALU3, and CALU6, respectively).

Oral treatment of mice bearing EGFRm+ and T790M xenograft tumors (H1975, PC9VanR, PC9, and H3255) with low 5 mg/kg doses daily of AZD9291 leads to profound tumor growth regression (Investigator's Brochure, 2014). In contrast, higher doses of AZD9291 (25 mg/kg) were required to achieve significant tumor growth inhibition in wild-type EGFR xenograft models (A431 and LOVO), consistent with *in vitro* selectivity margins. Long-term daily oral dosing of 25 mg/kg AZD9291 resulted in complete macroscopic response in all 8 PC9 and all 9 H1975 tumor groups, with no visible tumors after approximately 90 days (PC9) and 20 days (H1975) and with no evidence of disease progression for 160 days (PC9; ongoing study) and 200 days (H1975; study stopped). Lower 5 mg/kg/day AZD9291 treatment caused a durable complete response in 5/8 PC9 tumor groups and 10/11 H1975 tumor groups. Xenograft growth regression with AZD9291 was also accompanied by dose and time-dependent pharmacodynamic inhibition of pEGFR together with key downstream biomarkers phospho-protein kinase B (pAKT) and phospho-extracellular signal-regulated kinase (pERK) across mutant and wild-type EGFR disease models *in vivo*.

Clinical Studies of AZD9291

There are currently two studies of AZD9291: D5160C00001 and D5160C00005 (Investigator's Brochure, 2014). Clinical data are derived primarily from D5160C00001, which is the ongoing 2-part, open-label, multi-center, dose-escalation phase 1 study of AZD9291 in patients with advanced NSCLC who have progressed on prior therapy with an EGFR-TKI (NCT 01802632). Part A of this study was designed to assess the safety, tolerability, pharmacodynamics, pharmacokinetics (PK), efficacy, and maximum tolerated dose (MTD); Part B

was designed to further explore the efficacy, safety, tolerability, PK, and biological activity in additional expansion cohorts in patients selected according to the T790M status of their advanced NSCLC tumor.

As of November 19, 2013, 174 patients have received at least one dose of AZD9291 in D5160C00001 (Investigator's Brochure, 2014). In the dose escalation part of the study, each patient received a single dose of AZD9291, followed 1 week later by continuous once daily dosing, until the patient withdrew from the study. In the dose expansion cohort of the study, patients commenced once daily dosing with AZD9291 with no washout period (Investigator's Brochure, 2014). Expansion cohorts have enrolled a total of 139 patients.

D5160C00005 is an open-label, single-center, sequential design phase 1 study in healthy volunteers to determine the relative bioavailability of different oral formulations and the effect of food (NCT 01951599) (Investigator's Brochure, 2014). As of November 19, 2013, 16 healthy volunteers had received at least one dose of AZD9291.

Pharmacokinetics

As of November 19, 2013, preliminary AZD9291 plasma PK parameters are available from 64 patients; 27 patients from the escalation cohort and 37 from the expansion cohorts of D5160C00001 (Investigator's Brochure, 2014). Across the 20-240 mg/day dose range, the median time to reach maximum plasma concentration (t_{max}) is 7-8 hours, and the median half-life ($T_{1/2}$) is between 41.09-60.78 hours. The exposure increases approximately dose proportionally, with AUC_{1-72h} between 1615-12680 nM•h across the dose range. Two active metabolites have been identified, AZ5104 and AZ7550, both of which retain selectivity of mutant EGFR over wild-type EGFR. The metabolite exposures are approximately 10-fold lower after multiple dosing than the AZD9291 geometric mean exposures at each dose.

Efficacy

Of the 174 patients enrolled, 92 patients had a baseline scan and at least one follow up assessment of which 44 PR (confirmed and unconfirmed) were indicated (Investigator's Brochure, 2014). In the 39 T790M+ patients, this includes 21 PR (5 confirmed), 14 SD, 1 progressive disease (PD), and 3 non-evaluable (NE). In the 19 T790M- patients, this includes 3 PR (1 confirmed), 8 SD, 6 PD, and 2 NE. Of 18 confirmed responses, all are still ongoing with the longest duration of response to date being 6 months. Responses were observed regardless of prior EGFR-TKI use immediately before study entry and across the range of dose levels.

Safety

As of November 19, 2013, AZD9291 exposure durations range from 0 to 245 days (mean 63 days) with 17% of patients having reached 3 months of dosing and 3% reached 6 months of dosing (Investigator's Brochure, 2014). Of the 174 patients dosed, 105/174 (60%) reported an adverse event (AE) during the study, with the majority (>55%) of the AEs being Grade 1. The most common AEs have been rash, diarrhea, pruritus, and nausea. There were 4 patients (2%) who discontinued study treatment due to AEs (2 at 20 mg, 1 at 40 mg, 1 at 80 mg), one dose reduction (160 mg reduced to 80 mg due to Grade 2 diarrhea), and 15 dose interruptions due to AEs (14 patients; 0, 3, 5, 5 and 1 on 20, 40, 80, 160 and 240 mg doses, respectively).

Twenty-two patients (13%) experienced 32 serious AEs (SAEs) on study treatment (Investigator's Brochure, 2014). Only SAEs of pneumonia, pleural effusion, and diarrhea have been reported in more than one patient. Four patients died after receiving at least one dose of AZD9291; the primary causes of death are reported as pneumonia, septic shock, hypercalcemia, and disease progression. There have been five reports of unconfirmed interstitial lung disease (ILD)-like events identified in D5160C00001. Of these, one occurred prior to commencing treatment with AZD9291, and one event had the initial diagnosis changed from "pneumonitis" to "disease progression" by the investigator, following discussion with a radiologist. Of the remaining three events reported, two were SAEs, and one was a non-serious adverse event. Following discontinuation of AZD9291 and steroid therapy, all three cases recovered with no sequelae. Assessment of causality is

complicated by the diagnosis of advanced NSCLC and prior treatments; an association between AZD9291 and drug-induced ILD has not been established.

No significant treatment-related changes in liver function parameters have been reported; platelet count changes have been seen at the 160 mg/day and 240 mg/day dose levels followed by stabilization (Investigator's Brochure, 2014). No clinically significant ocular surface effects (corneal ulceration) have been reported. The R2PD has been set by AstraZeneca for its phase 2 and 3 studies at 80 mg/day.

Pharmaceutical Information:
AZD9291 mesylate is a crystalline powder (Investigator's Brochure, 2014). The solubility of AZD9291 free base has been measured as 7.2 mg/mL in Simulated Gastric Fluid (pH 1.5) and 0.2 mg/mL in Fasted State Simulated Intestinal Fluid (pH 6.5). It has two measured logarithmic acid dissociation constants (pK_a's) of 4.9 and 10.0. The AZD9291 drug product for oral administration will be provided as beige film-coated tablets containing AZD9291 mesylate, mannitol, microcrystalline cellulose, low-substituted hydroxypropyl cellulose and sodium stearyl fumarate.

CTEP's Plans for AZD9291 Development

CTEP is interested in developing AZD9291 in novel-novel combinations with other agents, including CTEP-held agents, in EGFRm+ NSCLC patients. AZD9291 not only inhibits both the activating and resistance mutations in EGFR, but also shows less inhibitory activity against wild-type EGFR when compared to other EGFR-TKIs, giving it a wider therapeutic index and potential for use in combination studies.

CTEP is requesting investigators to assist in the development of phase 2 combination trials with AZD9291, possibly with phase 1 components or safety lead-in phases. The combinations of interest include, but are not limited to:

- AZD9291 in combination with AZD6244 (selumetinib), a potent, selective, allosteric inhibitor of MEK.
- AZD9291 in combination with cetuximab, a chimeric human/mouse monoclonal antibody (MAb) of the immunoglobulin (IgG1) subclass that targets EGFR.
- AZD9291 in combination with MLN8237, a selective small molecule inhibitor of Aurora A kinase.

Correlative Studies of Interest to CTEP
Combination therapy with AZD9291 creates the opportunity to study, in a coordinated way, the biological effects of different clinical strategies of EGFR pathway inhibition. Therefore, biomarker studies that examine EGFR pathway activity, alternative signaling through the MAP/MEK/ERK pathway, and alternative signaling through PI3K/AKT/mTOR pathways will be of interest. Common biomarker assays and platforms for AZD9291 combination studies would allow comparison of biomarker activation and inhibition across the trials.

AZD9291 Project Team selection, composition and tasks

The AZD9291 drug project team will meet regularly by WebEx to 1) review the available evidence regarding mechanism of resistance to EGFR inhibition; 2) determine the most promising strategies of improving response and extending the duration of response to EGFR-targeted agents; 3) identify the most appropriate biomarkers for evaluating these strategies and select the optimal technologies for measuring these biomarkers in clinical specimens; and 4) evaluate and select the clinical trial design to test the therapeutic strategies. It is expected that the AZD9291 Drug Project Team will be in existence for only 8-12 weeks, and must complete its work no later than October 3, 2014. 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 and phase 2 studies in NSCLC; translational scientists with expertise in biomarker development, especially in the area of EGFR-directed therapeutics in NSCLC; and

basic scientists with expertise in mechanisms of EGFR resistance. 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 NSCLC early phase studies. 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 Jeff Moscow, M.D., Senior Clinical Investigator, Investigational Drug Branch, CTEP, Division of Cancer Treatment and Diagnosis (DCTD), National Cancer Institute (NCI) (phone: 240-276-6101; FAX: 240-276-7894; e-mail: jeffrey.moscow@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.

Project Team Member Applications (PTMAs) should contain a clear indication of the desired role on the AZD9291 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), June 24, 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
Phone: (240) 276-6535

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