REQUEST FOR PROJECT TEAM MEMBER APPLICATION FOR CONDUCTING CLINICAL TRIALS USING AMG 232

The Cancer Therapy Evaluation Program (CTEP) is accepting Project Team Member applications for a project with AMG 232, being developed by CTEP as an anticancer agent in collaboration with Amgen, Inc. AMG 232, a potent, selective, orally (PO) available, piperidinone small molecule inhibitor, can inhibit the interaction between the tumor suppressor protein p53 and the human homolog of murine double minute 2 (Mdm2) (Rew and Sun, 2014; Sun *et al.*, 2014).

At the present time, CTEP is proposing three phase 1b trials of AMG 232 in combination with

1. radiation in soft tissue sarcomas,
2. decitabine or cytarabine in relapsed or refractory acute myeloid leukemia (AML), and
3. lenalidomide in multiple myeloma.

The role of the project team will be to evaluate all available evidence to modify and refine this preliminary clinical plan as well as identify appropriate biomarkers for the studies and maximize the scientific information from these trials.

The project team will include:

1. **Clinician-scientists** with expertise in phase 1 studies and with an interest in soft tissue sarcomas, AML, or multiple myeloma (fill out **Part A** of the attached Application);
2. **Translational scientists** with an interest in biomarker development for agents like AMG 232 in soft tissue sarcomas, AML, or multiple myeloma (fill out **Part B** of the attached Application); and
3. **Basic scientists** with expertise in cell-cycle arrest, senescence, apoptosis, and autophagy (fill out **Part C** of the attached Application).

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 AMG 232 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 (LOIs) 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 two and a half months or less.

Background/Rationale

In undisturbed normal cells, p53, a potent cell growth and tumor suppressor, exists at very low levels in the nucleus. In response to signals from a wide range of physiologic stress (lack of nucleotide precursors, UV or ionizing radiation, oncogene signaling, hypoxia, or blocked transcription), p53 can initiate a variety of responses, including cell-cycle arrest, senescence, apoptosis, and autophagy. Upon activation, p53 induces transcription of a large number of target genes, including itself. Among these genes is *Mdm2*. Once synthesized, Mdm2 proteins bind to p53 proteins and suppress further p53 expression, while they also trigger rapid p53 and Mdm2 protein turnover via ubiquitylation, and subsequent export from nucleus to cytoplasm for degradation in the proteasome. In cells with wild-type (wt) p53, Mdm2 is the primary negative p53 regulator. This negative feedback loop ensures that p53 levels eventually drop back to a low level.

Approximately 50% of human tumors have p53 mutations resulting in the loss of its function. In tumors with wt p53, other mechanisms can block high levels of p53 activation, such as aberrant over-expression of Mdm2, p53 kinases, or genes like $p14^{ARF}$. All of these interfere with the negative feedback loop and result in unchecked tumor cell growth. AMG 232 potently inhibits the interaction between p53 and Mdm2 proteins, resulting in prolonged p53 activation, limiting tumor cell growth.

Mechanism of Action

AMG 232 specifically inhibits human Mdm2-p53 interaction *in vitro* (IC_{50}=0.6 nM) (Rew and Sun, 2014; Sun *et al.*, 2014). It binds human Mdm2 *in vitro* (K_D=0.045 nM), but does not affect other Mdm family members at concentrations up to 10 µM. In cultured SJSA-1 osteosarcoma tumor cells, which contain amplified *Mdm2*, AMG 232 substantially suppresses growth (IC_{50}=9.1 nM). It similarly inhibits proliferation of non-*Mdm2* amplified HCT116 colorectal cancer cells (IC_{50}=10 nM). Growth inhibition requires functional p53; in p53-deficient HCT116 cells, AMG 232, at concentration as high as 10 µM, could not inhibit proliferation.

Nonclinical Studies of AMG 232

In vivo efficacy studies of AMG 232, as a single agent, were performed in murine xenograft models using human cancer cell lines containing wt p53 (Rew and Sun, 2014; Sun *et al.*, 2014). In the *Mdm2*-amplified SJSA-1 osteosarcoma model, AMG 232 robustly inhibited tumor growth (ED_{50} of 9.1 mg/kg QD). Tumor regression was observed with doses of 30 and 60 mg/kg, and 60 mg/kg caused complete tumor regression in 10 of 12 mice. Robust induction of p21 was observed, with a peak induction of 30-fold occurring at 4 hours after treatment. AMG 232 was also evaluated in the non-*Mdm2*-amplified HCT-116 colorectal carcinoma xenograft model. Treatment twice daily (BID) caused dose-dependent tumor growth inhibition (ED_{50} of 16 mg/kg BID). The highest dose of 100 mg/kg resulted in tumor stasis.

AMG 232 augments radiation response across a variety of tumor types in xenograft models, including lung (A549, H460, H226, and H1299), melanoma (A375), breast (MCF7), colon (HCT116), and osteosarcoma (SJSA-1) (Werner *et al.*, 2015b). All transplanted cell lines harbor wt p53 except H1299, which is p53 null. In A375 melanoma and SJSA-1 osteosarcoma, the combined treatment of AMG 232 and radiation induced a more profound increase in p21 and Mdm2 expression than with either treatment alone. AMG 232 and radiation resulted in a significant cell cycle arrest in both G1 and G2/M phases. The addition of AMG 232 to radiation treatment significantly reduced clonogenic survival in all cell lines except p53-null H1299 cells. Flow cytometric analysis of γH2AX, a marker of double-stranded DNA breaks (DSB), revealed that radiation alone induced a dramatic increase in the number of γH2AX-positive cells, especially in late S and G2/M phases. This peaked 1 hour after treatment and subsequently declined. However, in tumors exposed to AMG 232 and radiation, γH2AX-positive cells increased again at 24 and 48 hours post-treatment. Similar results were seen in other xenograft cell lines, including H460, MCF7, A549, and H226. Notably, AMG 232 alone did not induce accumulation of γH2AX at 48 hours. After cessation of treatment, tumors in the combination treatment groups remained cytostatic and failed to regrow. Because a lethally irradiated cell with unrepaired DSB may continue to divide and result in the reproduction of γH2AX in daughter cells, these findings suggests that AMG 232 enhances radiosensitivity by blocking DSB repair, resulting in the accumulation of dying or senescent cells with unrepaired lethal DNA damage.

In addition to blocking tumor cell responses to DNA damage, AMG 232 combined with radiation also inhibited both proliferation and angiogenesis-related tube formation of human endothelial cells (Werner *et al.*, 2015a). In lung (H460) and osteosarcoma (SJSA-1) tumor xenografts, combined treatment reduced both cellular proliferation and vascular density. Furthermore, under hypoxic conditions that lead to radiation resistance, AMG 232 still significantly augmented the radiation response in tumor cells.

Combination studies of AMG 232 with DNA damaging agents cisplatin, carboplatin, doxorubicin, and irinotecan showed similar results in models of osteosarcoma (SJSA-1), lung cancer (H460), melanoma (A375), and colorectal cancer (HCT116) (Canon *et al.*, 2015). Studies of these combinations showed induced expression of markers of p53 activation (p53, p21, Mdm2, and PUMA) and inhibited tumor growth in

xenografts *in vivo*. AMG 232 combined with cisplatin or carboplatin was synergistic, and AMG 232 was also effective when combined with doxorubicin causing tumor regression *in vivo*.

Pharmacokinetics & Toxicology

Preclinical pharmacokinetic (PK) parameters of AMG 232 were studied in mouse, rat, beagle dog, and cynomolgus monkey following single-dose intravenous (IV) or PO administration (Ye *et al.*, 2015). AMG 232 had a low clearance (CL) and good PO bioavailability in mice, rats, and cynomolgus monkeys, but high CL and low PO bioavailability in beagle dog. AMG 232 is predicted to have a low CL of 0.15 L/h/kg and a long half-life ($t_{½}$) of 23 hours in humans.

Species	n	Dose mg/kg	CL L/h/kg	V_{SS} L/kg	$t_{½}$ h	$AUC_{0-\infty}$ µM·h
IV						
Mouse	14[a]	1.0	1.33[b]	1.10[b]	1.32[b]	1.32[b]
Rat	2	0.5	0.771, 0.543[c]	1.26, 0.977[c]	2.34, 3.693[c]	1.114, 1.62[c]
Dog	3	0.5	1.435 ± 0.35	1.53 ± 0.34	2.60 ± 0.94	0.643 ± 0.172
Monkey	3	0.5	0.507 ± 0.017	4.67 ± 2.13	12.2 ± 4.05	1.74 ± 0.061
		Dose mg/kg	**C_{max} µM**	**T_{max} h**	**$AUC_{0-\infty}$ µM·h**	**F %**
PO						
Mouse	12[a]	5	2.18[b]	0.333[b]	2.74[b]	41.5[b]
Rat	3	2	0.851 ± 0.157	0.667 ± 0.289	4.24 ± 0.54	76.8 ± 0.97
Dog	3	2	0.772 ± 0.713	0.250 ± 0	0.459 ± 0.362	18.6 ± 14.7
Monkey	3	2	0.188 ± 0.076	4.33 ± 2.89	3.52 ± 1.86	50.7 ± 26.8

[a] Two animals per time point were used for mouse studies.
[b] PK parameters were calculated from mean composite values.
[c] Two rats were used in rat IV study; data shows individual values for both animals.
Other data represent mean ± standard deviation.

AMG 232 specifically and selectively inhibits Mdm2 (Rew and Sun, 2014). It does not specifically inhibit other enzymes, nor does it bind other receptors. Among 392 non-mutated kinases tested, AMG 232 could weakly inhibit only protein kinase D2 (69% inhibition at 10 µM). Toxicology studies of AMG 232 indicate no major safety findings, such as cardiovascular liability, when tested at exposure levels significantly higher than pharmacologically active exposures and concentrations.

Drug metabolism, studied in rat, dog, monkey and human hepatocytes *in vitro*, and *in vivo* in rats (Rew and Sun, 2014; Ye *et al.*, 2015) showed that hepatic glucuronidation produced the primary metabolite in all species.

AMG 232 is a weak competitive inhibitor of CYP2C8 *in vitro* (IC_{50}=8.5 µM) and a weaker inhibitor of CYP1A2, 2C9, 2C19, 2D6, 2E1, or 3A4 (IC_{50}>30 µM), indicating low potential to affect clearance of co-administrated drugs by competitive inhibition of human CYP isoforms(Rew and Sun, 2014).

Cytochrome P_{450} (CYP) induction assays were carried out with human hepatocytes. Minor changes in the mRNA level and activity of CYP3A4, CYP2B6, and CYP1A2 were detected at 1 µM AMG 232 (Rew and Sun, 2014). The potential for AMG 232 to increase clearance of CYP3A4 substrates is expected to be low because of the relatively low exposure at the projected clinically effective dose.

Clinical trials of other inhibitors of Mdm2-p53 interaction have revealed thrombocytopenia as an on-target effect, causing delayed DLT in some patients (Ray-Coquard *et al.*, 2012). The agent RG7112 promoted apoptosis of megakaryocyte progenitor cells, resulting in a reduction of their numbers, impairing platelet production (Iancu-Rubin *et al.*, 2014). It is believed that AMG 232 may act similarly.

Clinical Studies of AMG 232

Amgen, Inc. is presently sponsoring three clinical trials of AMG 232.

Phase	Sponsor	Title	ID Number	Status
1	Amgen	A Phase 1 First-in-Human Study Evaluating the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of AMG 232 in Adult Subjects With Advanced Solid Tumors or Multiple Myeloma	NCT01723020	Active 12/2012
1b	Amgen	A Phase 1b Study Evaluating the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of AMG 232 Alone and in Combination With Trametinib in Adult Subjects With Relapsed/Refractory Acute Myeloid Leukemia	NCT02016729	Active 4/2014
1b/2a	Amgen	A Phase 1b/2a Study Evaluating the Safety, Tolerability, Pharmacokinetics, Pharmacodynamics and Efficacy of AMG 232 Combined With Trametinib and Dabrafenib or Trametinib in Adult Subjects With Metastatic Cutaneous Melanoma	NCT02110355	Active 12/2014

These ongoing clinical studies clearly demonstrate that AMG 232 treatment leads to prolonged p53 activation. Increased plasma concentration of the pharmacodynamic biomarker macrophage inhibitory cytokine-1 (MIC-1) was observed at the lowest dose evaluated. Promising hematological responses have been observed in a population of relapsed/refractory AML patients treated with AMG 232 as a monotherapy (study NCT02016729). Treatment resulted in an acceptable safety profile, consisting mainly of gastrointestinal and hematopoietic toxicities.

CTEP's Plans for AMG 232 Development

At the present time, CTEP is proposing a development program with three phase 1b combination trials of AMG 232:

1. AMG 232 combined with radiation in patients with soft tissue sarcomas.
2. AMG combined with either decitabine or cytarabine in patients with relapsed or refractory acute myeloid leukemia (AML).
3. AMG 232 combined with lenalidomide in patients with multiple myeloma

Correlative Studies of Tentative Interest to CTEP

These studies may include, but are not limited to, assays for AMG 232-treatment increased expression of markers of activated p53 (expression of p53, p21, Mdm2, PUMA) in tumor cells with both normal and amplified *Mdm2*. In plasma, AMG 232-treatment increased MIC-1 although it is not considered a tumor marker. Affected tumor tissues may also be assayed for markers of DNA damage (γH2AX), senescence (SA-β-Gal), decreased proliferative transcription factors (FoxM1), increased expression of autophagy-related genes (UNC-51-like autophagy activating kinase 1 [ULK1] and damage-regulated autophagy modulator [DRAM1]), as well as decreased markers of cell proliferation (Ki67).

AMG 232 Project Team Selection, Composition, and Tasks

The AMG 232 drug project team will meet regularly by WebEx to review available evidence and determine promising strategies, to identify biomarkers to evaluate these strategies, and to evaluate clinical trial designs to test these strategies. The project team will be composed of intramural and extramural members. The extramural members will include clinician-scientists with experience in phase 1 studies in soft tissue sarcomas, AML and multiple myeloma; translational scientists with expertise in biomarker development; and basic scientists with expertise in p53 activation, apoptosis, senescence, and DNA damage responses. Since the clinician scientists selected for the project team will ultimately 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 early phase studies of the diseases mentioned above.

Questions regarding this request for applications may be addressed to Naoko Takebe, M.D., Ph.D., Senior Clinical Investigator, Investigational Drug Branch, CTEP, DCTD, NCI (phone: 240-276-6121; FAX: 240-276-7894; e-mail: takeben@mail.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 as Project Team members to develop Career Development LOIs (CrDLs).

Project Team Member Applications (PTMAs) should contain a clear indication of the applicant's desired role on the AMG 232 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), January 5, 2016**. 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: CTEPPTMASubmissions@mail.nih.gov

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