First-in-Human Pharmacokinetic and Pharmacodynamic Study of the Dual m-TORC 1/2 Inhibitor AZD2014

Bristi Basu1, Emma Dean2, Martina Puglisi1, Alastair Greystoke2, Michael Ong1, Wendy Burke3, Maria Cavallin1, Graham Bigley9, Christopher Womack3, Elizabeth A. Harrington3, Stephen Green3, Elisabeth Oelmann3, Johann S. de Bono1, Malcolm Ranson2, and Udai Banerji1

Abstract

Purpose: AZD2014 is a novel, oral, m-TORC 1/2 inhibitor that has shown in vitro and in vivo efficacy across a range of preclinical human cancer models.

Experimental Design: A rolling six-dose escalation was performed to define an MTD (part A), and at MTD a further cohort of patients was treated to further characterize toxicities and perform pre- and posttreatment biopsies (part B). AZD2014 was administered orally twice a day continuously. Flow cytometry, ELISA, and immunohistochemistry were used to quantify pharmacodynamic biomarkers. Pharmacokinetic analysis was carried out by mass spectrometry.

Results: A total of 56 patients were treated across a dose range of 25 to 100 mg. The MTD was 50 mg twice daily. The dose-limiting toxicities were fatigue and mucositis. At the MTD, the most common adverse events (AE) were fatigue (78%), nausea (51%), and mucositis (49%), but these were equal to or greater than grade 3 in only 5% of patients. Drug levels achieved at the MTD (AUC0–24 6686 ng·h/mL, Cmax 1,664 ng/mL) were consistent with activity in preclinical models. A reduction in p-S6 levels and Ki67 staining was observed in 8 of 8 and 5 of 9 evaluable paired biopsy samples. Partial responses were seen in a patient with pancreatic cancer and a patient with breast cancer, who were found to have a PDGFR and ERBB2 mutation, respectively.

Conclusions: The recommended phase II dose for further evaluation of AZD2014 is 50 mg twice daily, and at this dose it has been possible to demonstrate pharmacologically relevant plasma concentrations, target inhibition in tumor, and clinical responses. Clin Cancer Res; 21(15); 3412–9. ©2015 AACR.

Introduction

The PI3K pathway is deregulated in more than 50% of all cancers. Mechanisms of deregulation include activating mutations in PIK3CA and AKT and loss of function of tumor suppressor genes such as PTEN (1). m-TOR consists of two essential complexes, TORC1 and TORC2 and is a crucial node in the PI3K signaling network (2). Inhibition of TORC1 could lead to inhibition of cell growth and metabolism via inactivation of downstream targets such as p-S6, p-4EBP1, and p-GSK3B (3). In addition, m-TORC2 is critical to AKT signaling (4).

1The Institute of Cancer Research and The Royal Marsden, London, United Kingdom. 2University of Manchester and The Christie NHS Foundation Trust, Manchester, United Kingdom. 3AstraZeneca, Macclesfield, United Kingdom.

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

B. Basu and E. Dean share first authorship of this article.

Previous presentation of data: This work was presented in part at the 2012 ASCO Annual Meeting (abstract 3004), Chicago, Illinois, June 1-5.

Corresponding Author: Udai Banerji, The Institute of Cancer Research and The Royal Marsden, Downs Road, London SM2 5PT, United Kingdom. Phone: 44-20-8661-3993; Fax: 44-20-8642-7979; E-mail: udai.banerji@icr.ac.uk

doi: 10.1158/1078-0432.CCR-14-2422

©2015 American Association for Cancer Research.
Translational Relevance

The m-TOR consists of two essential complexes, m-TORC1 and m-TORC2. Conventional, allosteric m-TOR inhibitors inhibit m-TORC1 but not m-TORC2 function. This may lead to feedback loops activating PI3K via IRS1 and continued phosphorylation of AKT at Ser473 by uninhibited m-TORC2. AZD2014 is a dual m-TORC1 and m-TORC2 kinase inhibitor. This study identified the maximally tolerated dose and schedule for AZD2014 as 50 mg twice daily. It was possible to demonstrate m-TORC1 inhibition in normal tissue and tumor as evidenced by reduction in the phosphorylation of 4EBP1 and S6, respectively, and importantly, m-TORC2 inhibition (reduction in p-AKT) in normal tissue and tumor. Furthermore, there was evidence of reduced metabolism (reduction in SUV-max in FDG PET scans). Other key findings included reduction in proliferation (Ki67 reduction in posttreatment biopsies and two partial responses). The dual m-TORC1/2 inhibitor AZD2014 should be investigated further.

Materials and Methods

This study used a rolling six dose-escalation design during the dose-escalation phase. At the recommended phase II dose, an expansion cohort to further characterize tolerability, PK and PD profiles, including pre- and posttreatment biopsies in a subset of patients, was instituted. Patients were recruited at the Royal Marsden and Christie Hospital NHS Foundation Trust in the UK following ethics committee approvals. Patients with advanced solid tumors who had already received standard-of-care treatment and had adequate organ function were eligible for the study. There was no difference between the eligibility criteria between dose-escalation and -expansion cohorts of the study. Following informed, written consent, patients received a single run-in dose of AZD2014 in a liquid (solution) formulation. During the clinical trial, AZD2014 was administered orally, with patients fasting (except for water) for 2 hours before dosing and 1 hour after dosing. Dose-limiting toxicities (DLT) were evaluated during the run-in dose and the first 21 days of continuous dosing.

Common toxicity criteria (CTC) grade 4 hematologic toxicity or any grade 3 or grade 4 non-hematologic toxicity was considered a DLT, with the exception of alopecia, inadequately treated grade 3 or 4 nausea and vomiting or isolated laboratory change without any clinical significance. Concomitant exposure to potent and moderate inhibitors and inducers of CYP3A4/5 and CYP2C8 were not permitted. Details of drugs and washout periods are mentioned in the Supplementary Data. Blood was drawn for PK and PD analysis over 24 hours. The PD samples for the run-in single dose were drawn at pre dose, 2 hours, 6 to 8 hours and 24 hours after dose. The patient then commenced continuous dosing of AZD2014 twice a day, 3 to 7 days later. The length of a cycle was 28 days. Adverse events were recorded using National Cancer Institute Common Toxicity Criteria 3.1. On days 15, 21, and 28, blood was collected over 12 hours to assess steady state PK profiles. Tumor biopsies were carried out between days 8 to 15. Patients were seen every week to assess safety. CT scans were carried out at baseline and every 8 weeks to assess disease response using RECIST 1.1. The drug concentrations in plasma were assessed using mass spectrometry (see Supplementary Data for details). PK analysis was carried out using Phoenix-WinNonLin v6 for NCA. The PK assay included p-4EBP1 in peripheral blood mononuclear cells (PBMC) using flow cytometry, p-AKT in platelet-rich plasma (PRP), which was performed using an immunoassay from Meso Scale Discovery.

Results

A total of 56 patients across a dose range of 25 to 100 mg twice daily were treated on this phase 1 study (n = 23 in part A and n = 33 in part B). Nineteen male and 37 female patients...
were treated, and the median age was 59 (range, 33–76). The most common tumor type recruited to the study was breast cancer (15/56). Details of the demographic profile, ECOG performance status, and tumor types treated are listed in Supplementary Table S1.

### Dose escalation

The starting dose was 50 mg twice daily and, as no DLTs were seen in 6 patients, the dose was doubled to 100 mg twice daily. As there were four of four DLTs, an intermediate dose of 70 mg twice daily was explored. A total of 5 patients were dosed in this cohort, but one patient withdrew consent before becoming evaluable for the dose decision. A further two of four evaluable patients had a DLT at 70 mg twice daily, and thus the remaining two patients who had consented to this cohort received 50 mg twice daily. Thus, 50 mg twice daily was considered the maximally tolerated dose. Fifty mg twice daily was both the starting dose and the MTD and this dose showed PD activity in normal tissue. It is possible that future combination studies would use lower doses of AZD2014, and thus a further cohort of 6 patients were treated at 25 mg twice daily to further characterize PK and PD profiles over a range of doses of AZD2014 that could be used in the future. Subsequently, 50 mg twice daily was declared the recommended phase II dose (RP2D) and a further 33 patients were treated to further evaluate toxicity, PK and PD.

### Dose-limiting toxicities

At the dose of 100 mg twice daily, DLTs were seen in four of four patients. In all instances these were CTC grade 2 or 3 fatigue, occurring within the first week of treatment. The fatigue was reversible on discontinuation of the drug. At a dose of 70 mg twice daily, DLTs were seen in two of four evaluable patients treated, with grade 3 fatigue and grade 3 mucositis in one patient and grade 3 fatigue in another. All events were reversible on cessation of AZD2014.

### Adverse events

The adverse events (AE) that occurred in >15% of patients at the RP2D of 50 mg twice daily are listed in Table 1. All AEs reported in this paragraph pertain to patients treated at 50 mg twice daily on parts A and B (Table 1). Fatigue was the most commonly seen AE in 32 of 41 (78%) patients; however, only 2 of 41 (5%) were grade 3 or higher and this was reversible on interruption of dosing. Gastrointestinal symptoms such as nausea, mucositis, diarrhea, and vomiting were seen in 21 of 41 (51%), 20 of 41 (49%), 17 of 41 (42%), and 12 of 41 (29%), respectively; however, less than 5% of these AEs were grade 3 or 4. Nausea and vomiting were well controlled with antiemetics, such as domperidone, if necessary, and the grade 1 and 2 diarrhea did not consistently require treatment. Mucositis was grade 1 and 2 in most instances and patients responded to mouth-washes and did not require dose interruptions due to it. A predominantly maculopapular rash was observed in 17 of 41 (42%) patients and less than 5% of these were grade 3 or higher. Lower respiratory tract infections were seen in 7 of 41 (17%), and it was grade 3 in only 1 of 41 (2%). Interestingly, no patients were diagnosed with pneumonitis. The most common laboratory abnormality was anemia, with 7 of 41 (17%) having recorded grade 1 and 2 anemia; given the advanced cancer and degrees of comorbidities in these patients, it was difficult to
attribute this specifically to AZD2014. Hyperglycemia was seen in 5 of 41 (12%), 0 of 5 (0%), and 2 of 4 (50%) of patients treated at 50, 70, and 100 mg twice daily, respectively. At the RP2D of 50 mg twice daily, no grade 3 or 4 hyperglycemia was seen. Furthermore, no grade 3 or above hypercholesterolemia or triglyceridemia was seen in this cohort. ECG monitoring revealed an increase in QT corrected Bazett’s formula (QTCB) and a reduction in QTCB by 30 to 60 msec in 3 of 41 (7%) and 5 of 41 (12%), respectively, thus showing no defined trend for increase or decrease in QTCB. Because of the modest number of patients treated across different dose levels and the standard timing of ECG done, a formally powered testing of the relationship of plasma concentrations to QTCB has not been done. At the RP2D of 50 mg twice daily, 10 of 41 (24.4%) had an interruption and 1 of 41 (2.4%) had a dose reduction due to an AE.

Pharmacokinetics

Following oral administration, AZD2014 was rapidly absorbed, with median time to peak following a single dose between 0.5 and 1 hour across the 25 to 100 mg dose range. Terminal elimination half-life was approximately 3 hours. Geomean exposure (AUC and $C_{\text{max}}$) increased greater than proportionally with increasing dose and interpatient variability was seen with exposures overlapping across the dose range (Fig. 1A and B and Table 2). At dose of 50 mg twice daily ($n = 27$), the $C_{\text{mean}}$
Cmin was 169.5 ng/mL, with a coefficient of variation (CV) of 163.6% and a range of 0 to 2,120 ng/mL.

Pharmacodynamics

In samples of patients treated at 50 mg, phosphorylation of 4E-BP1 was reduced in PBMCs to approximately −45% and −41% of baseline at 2 and 6 to 8 hours following a single dose of AZD2014, respectively. Phosphorylation of AKT (S473) in PRP was reduced to −62% and −37% of baseline at 2 and 6 to 8 hrs, respectively. These data indicate inhibition of m-TORC1 and m-TORC2 (Fig. 2A and B). Changes in phosphorylation of 4EBP1 and AKT were seen at dose levels of 50 to 70 mg and, importantly, changes in phosphorylation of AKT were seen at doses below the MTD (25 mg cohort; Fig. 2C and D). POM PD biomarkers such as p-S6, p-4EBP1 (m-TORC1), and p-AKT (m-TORC2), and POC biomarkers (Ki67) were assessed in pre- and posttreatment biopsies and showed evidence of target inhibition (Fig. 3A–E). Of note, 11 patients in part B (50 mg twice daily) had paired FDG PET scans, 8 of 11 patients showed a reduction of SUVmax with 3 of 11 attaining a PR (30% reduction in SUVmax; Fig. 3F).

Efficacy

There were two PRs in the study. The first was a patient with acinar pancreatic cancer who had previously responded to two lines of gemcitabine-based treatment. The patient had a maintained PR and was on treatment for six cycles. The patient was found to have KRAS, PDGFRA, APC, ERBB4, KIT, and FBXW7 mutation. The second patient to respond was a patient with estrogen receptor (ER)–positive breast cancer who had six prior lines of chemotherapy and one line of hormonal treatment for her metastatic breast cancer. She received AZD2014 treatment for four cycles. Her tumor had a mutation in H Ras, NRAS, TP53, and ERBB2 (Fig. 4). In addition, 2 patients, one each with ovarian and
endometrial cancer, had prolonged stable disease and remained on treatment for more than 1 year.

Discussion

The toxicity profile of AZD2014 had similarities with other TORC 1/2 inhibitors and more generally with allosteric m-TOR inhibitors and these were rash, mucositis, and fatigue (17, 18, 20, 22–25). At 50-mg twice-daily continuous dosing AZD2014 was well tolerated. Interestingly, in this present study there were no instances of grade 3 and 4 hyperglycemia that had been seen in clinical trials of m-TOR inhibitors (23–25). Patients with diabetes were excluded and all patients had to have a fasting glucose of less than 126 mg/dL (7 mmol/L). Given previous experience of hyperglycemia seen with m-TOR inhibitors, it is not known how AZD2014 would affect glycemic control of patients with type I or type II diabetes. Renal (20), hepatic (22), and left ventricular dysfunction (20) seen with other m-TOR 1/2 inhibitors were not seen in patients treated with AZD2014. Of note, there were no instances of pneumonitis, seen more generally across m-TOR inhibitors (7, 9, 26) in this study. The tolerability of once a day AZD2014 given continuously and twice a day dosing given intermittently (2 days every week) has been subsequently studied and the results will be presented separately when the studies are complete.

The pharmacokinetic profile of AZD2014 showed rapid absorption. Although the elimination half-life of AZD2014 was approximately 3 hours and is shorter than allosteric m-TOR inhibitors such as everolimus, temsirolimus, or ridaforolimus, which have half-lives of approximately 24 hours or longer (23–25), it allows twice-daily dosing and the possibility of more flexible intermittent dosing when used in combination with other anticancer drugs such as cytotoxic chemotherapy or other targeted agents. There are multiple reasons that could give rise to the interindividual pharmacokinetic variability. Preliminary analysis of some of the potential reasons have been investigated and include differences in the percentage of drug bound to protein between patients, but a preliminary investigation using measured AZD2014-free plasma concentrations instead of the usual way of using total plasma concentrations to determine PK parameters.

Figure 3. PD profile of AZD2014 in tumor tissue. A, the percentage of reduction in mean H-score of p-S6 seen in posttreatment samples. Eight of eight samples showed a reduction of p-S6 levels. This is a POM PD biomarker of m-TORC1 inhibition. B, the percentage of reduction in mean H-score of p-4EBP1 seen in posttreatment samples. Three of seven samples showed a reduction of p-4EBP1 levels. This is a POM PD biomarker of m-TORC1 inhibition. C, the percentage of reduction in mean H-score of p-AKT seen in posttreatment samples. Three of four samples showed a reduction of p-AKT levels. This is a POM PD biomarker of m-TORC2 inhibition. D, the percentage of reduction in Ki67 seen in posttreatment samples. Five of nine samples showed reduction in Ki67 expression. This is a POC distal biomarker that reflects proliferation. E, representative data from a patient who had a PR showing reduction in p-S6, p-AKT levels, and the percentage of cells stained for Ki67. F, maximal reduction in SUVmax in FDG PET scans in 11 patients treated at 50 mg twice daily who had evaluable paired pre- and posttreatment FDG PET scans. Eight of 11 patients showed a reduction in SUVmax. This is a distal POC biomarker that reflects metabolism.
found that they were equally variable and the relationships with PD no better. It is also not likely to be formulation performance because the drug is a solution, and therefore does not require a dissolution step before being absorbed. It could be hypothesized that differences in CYP metabolizing enzymes and/or transporter enzymes may affect ability to clear and/or distribute the drug in and out of tissue and affect PK variability. The data in this publication relate to the liquid formulation of AZD2014. Further studies have been conducted to evaluate the PK of the tablet formulation and the effect of food on exposure to AZD2014 and dissolution step before being absorbed. It could be hypothesized that differences in CYP metabolizing enzymes and/or transporter enzymes may affect ability to clear and/or distribute the drug in and out of tissue and affect PK variability. The data in this publication relate to the liquid formulation of AZD2014. Further studies have been conducted to evaluate the PK of the tablet formulation and the effect of food on exposure to AZD2014 and will be submitted for publication once complete. AZD2014 produces >70% total growth inhibition (TGI) at 4,500 nmol/L/h free weekly AUC, and ≥100% TGI at 17,000 nmol/L/h in MCF7 xenograft models and human PK achieved in this trial was in the range that would be efficacious in this model (on average the 50-mg twice-daily solution dose achieved a free weekly AUC of 11,050 nmol/L-h, with the vast majority of patients achieving more than 4,500 nmol/L-h). To provide the comparison of the clinical exposure with the preclinical data, the measured clinical exposure of AZD2014 reported in ng/ml of total (bound and unbound) drug was converted to nmol/L-h of free (unbound) drug using the molecular weight of AZD2014 (462.56 g/mol) and the measured human plasma protein binding of 5.47% free (unbound) drug.

The PD profile of AZD2014 showed target engagement in normal tissue. POM biomarkers of m-TORC1 and m-TORC2 inhibition, that is, reduction in levels of p-4EBP1 in PBMCs and p-AKT in PRP, respectively, were seen at 2 to 8 hours but recovered at 24 hours following a single dose of AZD2014, supporting a twice a day schedule. Importantly, reduction in p-AKT was seen at the 25 mg cohort, suggesting PD activity at this dose if dose reductions are necessary in future trials. Substantial interindividual variability was seen in the PK profile and this is likely to influence the PD profile seen in this study. Further studies evaluating PK, PD, and PK-PD profiles of the tablet formulation that will be taken forward into later phase clinical trials are warranted. Crucially, it was possible to demonstrate reduction of p-S6 (m-TORC1 inhibition) in all evaluable posttreatment biopsies. Reduction in p-AKT levels was seen in three of four assessable posttreatment biopsies, and no instances of induction of phosphorylation of AKT were seen in these samples. This is in contrast with increase in phosphorylation of AKT due to a feedback loop via IRS1, which is a possible mechanism of resistance seen when tumor tissue is exposed to allosteric m-TOR inhibitors (13, 14). In addition to the POM PD biomarkers discussed above, more distal, POC biomarkers such as reduction of proliferation (Ki67) and reduction in metabolism (FDG-PET scans) also support evidence of target inhibition in tumor. Overall PD biomarkers have been informative to the trial; normal tissue has shown a PD engagement at 25 to 100 mg twice daily, which will be taken into consideration while designing intermittent schedules and combination studies with dosing a higher or lower doses of AZD2014. At the MTD of 50 mg twice daily pre- and postbiopsies and FDG PET scans have confirmed target engagement in tumor tissue.

Figure 4. Patients on study who achieved confirmed PRs. A, a patient with acinar pancreatic cancer who was previously treated with a Whipple operation and two lines of gemcitabine-based chemotherapy for pancreatic cancer. He received six cycles of treatment. Arrows denote a mediastinal metastasis. B, a patient with ER-positive metastatic breast cancer who had six lines of chemotherapy and one line of hormonal therapy for metastatic breast cancer before entry in the clinical trial. She received four cycles of treatment. The arrow denotes hepatic metastasis.
AZD2014 as a single agent and in combination with other anticancer agents should be explored in clinical trials.

Disclosures of Conflicts of Interest
M. Puglisi is an employee of MSD. W. Burke holds ownership interest (including patents) in AstraZeneca. J.S. de Bono is a consultant/advisory board member for AstraZeneca. M. Ranson reports receiving a commercial research grant from and is a consultant/advisory board member for AstraZeneca. U. Banerji reports receiving a commercial research grant from AstraZeneca. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions
Conception and design: S. Green, J.S. de Bono, M. Ranson, U. Banerji
Development of methodology: E. Dean, M. Puglisi, A. Greystoke, G. Bigley, C. WOMACK, J.S. de Bono
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): B. Basu, E. Dean, M. Puglisi, A. Greystoke, M. Ong, M. Cavallin, C. WOMACK, E.A. Harrington, J.S. de Bono, M. Ranson, U. Banerji
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): B. Basu, E. Dean, M. Ong, W. Burke, M. Cavallin, C. WOMACK, E.A. Harrington, J.S. de Bono, M. Ranson

References

Writing, review, and/or revision of the manuscript: E. Dean, M. Puglisi, A. Greystoke, M. Ong, W. Burke, M. Cavallin, G. Bigley, C. WOMACK, E.A. Harrington, E. Oelmann, J.S. de Bono, M. Ranson, U. Banerji
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): E. Dean, M. Puglisi, G. Bigley, J.S. de Bono, M. Ranson
Study supervision: B. Basu, E. Dean, J.S. de Bono, M. Ranson, U. Banerji
Other (project leader; AstraZeneca): S. Green

Grant Support
This study was funded by AstraZeneca. Infrastructure support was provided by a Cancer Research UK Joint Phase I Clinical Core Grant (grant number CS/ A6883) to The Institute of Cancer Research and The Royal Marsden. The recruiting sites acknowledge infrastructural funding from the Experimental Cancer Medicine Centres and NIBR Biomedical Research Centre initiatives.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received September 18, 2014; revised March 9, 2015; accepted March 11, 2015, published OnlineFirst March 24, 2015.
First-in-Human Pharmacokinetic and Pharmacodynamic Study of the Dual m-TORC 1/2 Inhibitor AZD2014

Bristi Basu, Emma Dean, Martina Puglisi, et al.


Updated version
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-14-2422

Supplementary Material
Access the most recent supplemental material at:
http://clincancerres.aacrjournals.org/content/suppl/2015/03/25/1078-0432.CCR-14-2422.DC1

Cited articles
This article cites 23 articles, 9 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/21/15/3412.full.html#ref-list-1

Citing articles
This article has been cited by 1 HighWire-hosted articles. Access the articles at:
/content/21/15/3412.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.