The Selective Class I PI3K Inhibitor CH5132799 Targets Human Cancers Harboring Oncogenic PIK3CA Mutations

Hiroshi Tanaka1, Miyuki Yoshida1, Hiromi Tanimura1, Toshihiko Fujii1, Kiyoaki Sakata1, Yukako Tachibana1, Jun Ohwada1, Hiroshi Tanaka2, Hirosato Ebiike2, Shino Kuramoto3, Keiichi Morita3, Yasushi Yoshimura4, Toshikazu Yamazaki1, Nobuya Ishii1, Osamu Kondoh1, and Yuko Aoki1

Abstract

Purpose: The phosphatidylinositol 3-kinase (PI3K) pathway plays a central role in cell proliferation and survival in human cancer. PIK3CA mutations, which are found in many cancer patients, activate the PI3K pathway, resulting in cancer development and progression. We previously identified CH5132799 as a novel PI3K inhibitor. Thus, this study aimed to clarify the biochemical and antitumor activity of CH5132799 and elucidate the correlation between CH5132799 response and genetic alterations in the PI3K pathway.

Experimental Design: Kinase inhibitory activity was profiled in cell-free assays. A large panel of human breast, ovarian, prostate, and endometrial cancer cell lines, as well as xenograft models, were used to evaluate the antitumor activity of CH5132799, followed by analysis for genetic alterations. Effects on Akt phosphorylation induced by mTORC1 inhibition were tested with CH5132799 and compared with mTORC1 and PI3K/mTOR inhibitors.

Results: CH5132799 selectively inhibited class I PI3Ks and PI3Kα mutations in in vitro kinase assays. Tumors harboring PIK3CA mutations were significantly sensitive to CH5132799 in vitro and were remarkably regressed by CH5132799 in vivo mouse xenograft models. In combination with trastuzumab, tumors disappeared in the trastuzumab-insensitive breast cancer model with the PIK3CA mutation. Moreover, CH5132799 did not reverse a negative feedback loop of PI3K/Akt/mTOR signaling and induced regression against tumors regrown after long-term mTORC1 inhibitor treatment.

Conclusions: CH5132799 is a selective class I PI3K inhibitor with potent antitumor activity against tumors harboring the PIK3CA mutations. Prediction of CH5132799 response on the basis of PIK3CA mutations could enable patient stratification in clinical settings. Clin Cancer Res; 17(10); 3272–81. ©2011 AACR.

Introduction

The phosphatidylinositol 3-kinase (PI3K)/Akt/mTOR pathway regulates various cellular processes such as proliferation, growth, apoptosis, and cytoskeletal rearrangement (1). PI3Ks are categorized into 3 classes: I, II, and III. Closely related protein kinases, including mTOR, are called class IV PI3Ks or PI3K-related protein kinases (PIKK). Class I PI3Ks form heterodimers consisting of regulatory and p110 catalytic subunits; they are activated by signals from growth factor receptors and G-protein coupled receptors. There are 4 p110 isoforms (α, β, γ, and δ), and among them, p110α (PI3Kα) is known to be frequently mutated at the E542, E545, or H1047 positions (hot spots) in various human cancers such as breast, ovarian, endometrial, and colorectal cancer (2–5). These oncogenic mutations result in constitutive activation of PI3K (6). E542 and E545 mutations are thought to abolish negative regulation by interaction with a regulatory subunit p85 (7, 8). The H1047 mutation most likely changes interaction with substrates (9). Activation of the PI3K pathway is also known to be caused by amplification of PIK3CA, the gene for PI3Kα, and genetic inactivation of PTEN, a tumor suppressive negative regulator of the pathway (10, 11). Alternatively, amplification and mutation of molecules in the PI3K pathway, including Akt and receptor tyrosine kinases (RTK) such as HER2, are observed in a wide variety of cancers (3, 12). These tumors are considered to be addicted to the PI3K pathway.
pathway. Therefore, inhibition of PI3K signaling seems to be a promising strategy for the treatment of PI3K pathway–addicted cancers. In fact, several PI3K pathway inhibitors that target PI3K, Akt, and mTORC1 are under early clinical development (13–16).

mTOR has 2 functionally distinct multiprotein complexes: mTOR complex 1 (mTORC1) and mTORC2 (10). mTORC1 regulates protein translation through phosphorylation of p70 ribosomal S6 kinase (S6K) and eukaryotic translation initiation factor binding protein (4E-BP). The other complex, mTORC2, phosphorylates and activates Akt. The mTORC1 inhibitors temsirolimus and everolimus have been approved for the therapy of advanced renal cell carcinoma. However, the use of mTORC1 inhibitors risks the possible activation of Akt by disruption of a negative feedback loop, and patients treated with these drugs eventually become refractory (17, 18). The negative feedback, which is dependent on insulin-like growth factor 1 receptor (IGF1R)/insulin receptor substrate 1, involves the S6K-mediated suppression of upstream signaling; mTORC1 inhibitors abrogate this feedback suppression, resulting in Akt activation. On the other hand, PI3K inhibitors are expected to block mTORC1 inhibition-induced Akt activation because PI3K acts upstream of Akt. We have previously identified the novel PI3K inhibitor CH5132799 (19).

In this study, we investigated the pharmacologic action and antitumor activity of CH5132799 against tumors with PI3K pathway activation, including PIK3CA mutations, using a large panel of cell lines. Moreover, the differences between CH5132799 and mTORC1 and PI3K/mTOR inhibitors were examined in terms of PI3K pathway inhibition and efficacy in vivo.

Materials and Methods

Cell culture

Cell lines were obtained from the American Type Culture Collection, the Deutsche Sammlung von Mikroorganismen und Zellkulturen, Health Science Research Resources Bank, Asterand Inc., and the Health Protection Agency Culture Collections. The breast cancer cell line MCF-7 was kindly provided by Dr. Junichi Kurebayashi (Kawasaki Medical School, Kurashiki, Japan; ref. 20). The androgen-independent prostate cancer cell lines LNCaP-C8 and LNCaP-C2 were previously established (21, 22). The ovarian cancer cell line 2008 and IGROV1 were generous gifts from Dr. Aikou Okamoto (The Jikei University School of Medicine, Tokyo, Japan) and Dr. Marc Maliepaard (The Netherlands Cancer Institute, Amsterdam, The Netherlands), respectively. Gastric cancer cell line AX797 was kindly provided from Dr. Heinz H. Fiebig (Freiburg University, Freiburg, Germany). All cell lines were cultured according to suppliers’ instructions.

Reagents

CH5132799 (patent: WO 2008018426) and BEZ235 (13) were chemically synthesized. Trastuzumab was obtained from Chugai Pharmaceutical/F. Hoffmann-La Roche. Everolimus was purchased from Sequoia Research Products and Hangzhou APIChem Technology.

Cell proliferation assay

The cell lines were added to the wells of 96-well plates containing 0.076 to 10,000 nmol/L CH5132799 and incubated at 37°C. After 4 days of incubation, Cell Counting Kit-8 solution (Dojindo Laboratories) was added and, after incubation for several more hours, absorbance at 450 nm was measured with Microplate-Reader iMark (Bio-Rad Laboratories). The antiproliferative activity was calculated by the formula $(1 - T/C) \times 100$ (%), in which $T$ and $C$ represent absorbance at 450 nm of the cells treated with drugs ($T$) and that of untreated control cells ($C$). The IC$_{50}$ values were calculated by using Microsoft Excel 2007.

Mouse xenograft study

Female BALB-nu/nu mice (CAnN.Cg-Foxn1 < CrCrlj nu/nu) were obtained from Charles River Laboratories Japan and kept under specified pathogen-free conditions. A total of $4 \times 10^7$ to $1.2 \times 10^7$ cells were suspended in 100 to 200 μL serum-free culture medium and injected subcutaneously into the right flank of the mice. Tumor size was measured by using a gauge twice per week, and tumor volume (TV) was calculated by using the following formula: $TV = \frac{a \times b^2}{2}$, in which $a$ is the length of the tumor and $b$ is the width. Once the tumors reached a volume of approximately 200 to 300 mm$^3$, animals were randomized into groups ($n = 4$ or 5 in each group) and treatment was initiated. CH5132799 and everolimus were orally administered once a day and trastuzumab was intravenously injected once a week.
Results

CH5132799 selectively inhibited class I PI3K and PI3Kα mutants in vitro and the PI3K pathway in cells

We previously identified the aminopyrimidine derivative CH5132799 as a PI3K inhibitor (Supplementary Fig. S1; ref. 19). The inhibitory effects of CH5132799 on PI3K kinase activity were examined in cell-free systems (Table 1). CH5132799 inhibited class I PI3Ks, particularly PI3Kα, with an IC50 of 14 nmol/L. IC50 values against class II PI3Ks (C2α and C2β), a class III PI3K (Vps34), and a class IV PI3K (mTOR) were more than 100-fold higher than that against PI3Kα. Interestingly, slightly lower IC50 values were observed against PI3Kα with oncogenic mutations E542K, E545K, and H1047R than against wild-type (WT) PI3Kα (Table 1 and Supplementary Fig. S2). In an analysis of cocrystal structure with PI3Kα (PDB ID: 3APC), CH5132799 was shown to interact with ATP-binding sites of the enzyme, suggesting an ATP competitive mode of inhibition. No significant inhibitory activities of CH5132799 were observed against a representative panel of 26 protein kinases, including RTKs, nonreceptor tyrosine kinases, and serine/threonine kinases. These data indicate that CH5132799 is a selective class I PI3K inhibitor, especially against PI3Kα and its mutants.

Next, we tested the effect of CH5132799 on cells. When breast cancer KPL-4 cells harboring the PIK3CA mutation were treated with CH5132799, phosphorylation of Akt and its direct substrates, PRAS40 and FoxO1/3a, and phosphorylation of downstream factors, including S6K, S6, and 4E-BP1, were effectively suppressed, indicating complete suppression of the pathway (Fig. 1). Similar results were obtained by using BT-474 cells (Supplementary Fig. S3). Furthermore, CH5132799 induced cell-cycle arrest in G1 phase and increased the sub-G1 population in KPL-4 cells (Supplementary Fig. S4A). Caspase 3/7 activation was also observed in CH5132799-treated cells (Supplementary Fig. S4B). Therefore, class I PI3K inhibition by CH5132799 suppressed signaling in the PI3K pathway in cells, leading to G1 arrest and apoptosis induction.

Cancer cell lines harboring PIK3CA mutations were significantly sensitive to CH5132799

On the basis of the potent inhibitory activity of CH5132799 against PI3Kα and its oncogenic mutants, as shown in Table 1, we expected CH5132799 to show antitumor activity against tumors with PI3K pathway activation, including those with PIK3CA mutations. The spectrum of antiproliferative activity was analyzed by using a large panel of various cancer cell lines belonging to 4 types of cancer—breast, ovarian, prostate, and endometrial cancer—in which the PIK3CA mutation and PTEN deficiency are frequently found and in which RAS/RAF is rarely mutated (Fig. 2A and Supplementary Table S2; refs. 10, 23). CH5132799 showed superior antiproliferative activity across the 4 tumor types, with 75% (45/60) of lines having an IC50 below 1 µmol/L and 38% (23/60) of lines having an IC50 below 0.3 µmol/L. As shown in Figure 2A, we annotated the cell lines with their genetic status; mutations of PIK3CA, PTEN, KRAS, HRAS, NRAS, and BRAF genes; genetic inactivation of PTEN; and amplification of the HER2 gene (24–27). Most of the cell lines that were highly sensitive to CH5132799 (left side in the graphs) had PIK3CA mutations. A similar tendency was observed in HER2 amplification. On the other hand, cell lines with RAS or RAF mutations tended to be less sensitive to CH5132799 (right side in the graphs). PTEN-deficient cell lines exhibited both sensitivity and
insensitivity to CH5132799, suggesting additional determinant(s) of antitumor activity.

Further precise analysis was then carried out based on the positions of the mutations within the PIK3CA gene (Fig. 2B). When the PIK3CA mutations were divided into 3 groups (H1047, E542/545, and non–hot spot mutations), as expected, each group was significantly more sensitive than the cell lines without PIK3CA mutations (P < 0.0001, P = 0.0004, and P = 0.0018, respectively; Tukey’s test; Fig. 2B). Among the 3 groups with PIK3CA mutations, no significant difference was observed (H1047 vs. E542/545: P = 0.97, H1047 vs. non–hot spot: P = 0.73, and E542/545 vs. non–hot spot: P = 0.23).
When all the PIK3CA mutations were combined, the cell lines with all PIK3CA mutations showed highly more significant sensitivity to CH5132799 than cell lines without the mutations \( (P = 7.4 \times 10^{-5}; \text{Student's } t \text{ test}) \). However, in contrast, we did not find a significant association between PTEN deficiency and response to CH5132799 \( (P = 0.11) \).

Cell lines were also classified in terms of HER2 amplification and RAS or RAF mutations (Fig. 2C). Among cell lines without PIK3CA mutations, HER2-amplified or RAS/RAF mutant cell lines showed lower or higher IC50s to CH5132799, respectively, but not significantly \( (P = 0.43 \) or \( P = 0.68; \text{Tukey's test}) \). HER2-amplified cell lines with PIK3CA mutations were as sensitive as cell lines harboring only PIK3CA mutations \( (P = 0.91) \). Coexistence of the PIK3CA and RAS/RAF mutations seemed to result in higher sensitivity to CH5132799 than only RAS/RAF mutations \( (P = 0.015) \).

CH5132799 showed potent in vivo antitumor activity in xenograft models with PIK3CA mutations

We next sought to confirm our in vitro finding that the PIK3CA mutation determines the response to CH5132799, by conducting in vivo studies using xenograft models. Daily oral administration of CH5132799 showed remarkable antitumor activity in the xenograft model of breast cancer KPL-4 cells harboring the PIK3CA H1047R mutation (Fig. 3A). Particularly, high-dose administration resulted in rapid tumor regression. Tumor regression was also observed in breast cancer BT-474 cells with the PIK3CA K111N mutation. Inhibition of the PI3K pathway was confirmed in CH5132799-treated xenograft tumors (Fig. 3B). In both the KPL-4 and BT-474 models, effective suppression of Akt phosphorylation was observed in a time-dependent manner. Consistent with in vitro observations, proliferation was reduced and apoptosis was induced in CH5132799-treated tumors (Supplementary Fig. S5).

**Figure 3.** Antitumor activity in mouse xenograft models of cell lines harboring genetic alterations, including PIK3CA mutations. A, mice bearing subcutaneously implanted tumors with PIK3CA mutations were orally administered the indicated doses of CH5132799 on a daily basis. Mean tumor volumes are shown (left: KPL-4, \( n = 5 \); right: BT-474, \( n = 4 \)). B, mice bearing tumors were orally administered 12.5 mg/kg of CH5132799. At the indicated times after administration, tumors were resected, lysed, and analyzed by Western blotting. C, efficacy of CH5132799 in xenograft models with various genetic alterations, including PIK3CA mutations (SK-OV-3, \( n = 5 \); MFE-280, \( n = 4 \)), PTEN deletions (GXF97, \( n = 4 \); PC-3, \( n = 5 \)), and KRAS/BRAF mutations (MDA-MB-231, \( n = 4 \)).
Potent antitumor activity against tumors with PIK3CA mutations was also evident in the ovarian cancer SK-OV-3 and endometrial cancer MFE-280 models (Fig. 3C). In addition, in the PTEN-deficient model, gastric cancer GXF97 tumors in which the PTEN gene was deleted were shrunk remarkably by CH5132799 administration, and tumor growth was inhibited in a PC-3 prostate cancer model. Furthermore, CH5132799 was less effective in the MDA-MB-231 breast cancer model with KRAS and BRAF mutations, but colorectal cancer HCT116, harboring both PIK3CA and KRAS mutations, showed strong tumor growth inhibition by CH5132799, consistent with our in vitro observations. Therefore, we concluded that in vivo xenograft studies reproduced our in vitro finding that CH5132799 has potent antitumor activity against cancer harboring the PIK3CA mutation. In all in vivo studies, the doses of CH5132799 tested were well tolerated, with no gross toxicity observed in the treated animals (data not shown).

We further explored antitumor activity in rational combination investigations. HER2-amplified cell lines with PIK3CA mutations, including KPL-4, are reportedly insensitive to the HER2-targeted antibody trastuzumab because of HER2-independent activation of the PI3K pathway (28). We therefore investigated the ability of CH5132799 to overcome trastuzumab insensitivity. Indeed, KPL-4 tumors continued to grow after trastuzumab administration in the xenograft model (Fig. 4).

However, combining CH5132799 with trastuzumab induced remarkable antitumor activity, resulting in the disappearance of the tumors. Surprisingly, the disappearance was maintained through the follow-up period—more than 1 month—without any additional administration, indicating that CH5132799 overcomes trastuzumab insensitivity in tumors with PIK3CA mutation through PI3K inhibition.

Selective inhibition of class I PI3K by CH5132799 showed an advantage over mTORC1 and PI3K/mTOR inhibitors

S6K inhibition by mTORC1 inhibitors has been reported to abrogate the negative feedback loop, resulting in activation of Akt and downstream effectors (17, 18). We examined the effect of the selective class I PI3K inhibitor CH5132799 on the feedback loop compared with the mTORC1 inhibitor everolimus and the PI3K/mTOR dual inhibitor BEZ235 (Fig. 5A). Everolimus enhanced Akt phosphorylation in BT-474 cells accompanied by inhibition of S6K phosphorylation. Furthermore, at a low dose (0.01 μmol/L), BEZ235 also enhanced Akt phosphorylation, as previously reported, although it inhibits Akt phosphorylation at high doses (29, 30). On the other hand, CH5132799 did not induce Akt phosphorylation through inhibition of S6K phosphorylation at any dose. Moreover, 4E-BP1 phosphorylation was inhibited by both CH5132799 and BEZ235, though everolimus had little effect on 4E-BP1 phosphorylation, as previously reported (16, 29). The same effects were observed in SK-OV-3, MDA-MB-453, and HCT116 cells (Supplementary Fig. S6). These results suggest that a selective class I PI3K inhibitor has an advantage over mTORC1 and PI3K/mTOR inhibitors in that it overcomes mTORC1 inhibition-mediated Akt activation.

To mimic clinical refractoriness to mTORC1 inhibitors, mice bearing BT-474 xenograft tumors were continuously treated with everolimus for 1 month until the tumors regrew (Fig. 5B). Then, we switched from everolimus to CH5132799 administration, leading to a remarkable regression in a dose-dependent manner of the tumors regrown after the long-term everolimus treatment. The regrowth did not come from unexpected reduction of everolimus exposure, because we did not find apparent differences between the exposure of the first dose and that after the long-term treatment (data not shown). The tumors were resected at the end of treatment and analyzed by Western blotting with respect to PI3K pathway inhibition. CH5132799 suppressed various effectors in the PI3K pathway, including Akt, FoxO1, S6K, and S6, and 4E-BP1, whereas everolimus inhibited only phosphorylation of S6K and S6, both downstream effectors of mTORC1 (Fig. 5C). Consistent with our in vitro observations, everolimus also induced Akt activation in vivo, resulting in upregulation of FoxO1 phosphorylation. In particular, 4E-BP1 phosphorylation, which has been reported to be important in cell proliferation and survival as a key downstream effector of mTORC1 (31, 32), was inhibited by CH5132799.
administration, but not by everolimus. Moreover, everolimus administration also induced phosphorylation of ERK, as previously reported (33), though CH5132799 had little effect on the induction of phosphorylation. Collectively, CH5132799 suppressed PI3K/mTOR signaling without activating Akt and showed potent antitumor activity against tumors regrown after long-term everolimus treatment with robust inhibition of the PI3K pathway.

Discussion

The PI3K/Akt pathway is one of the most frequently deregulated pathways in common human cancers (10, 12, 34). Specifically, in breast cancer, PIK3CA mutation (~27%), HER2 amplification (~15%-30%), and PTEN deficiency (~25%) are frequently reported to activate the PI3K pathway, leading to cell proliferation and survival. In ovarian (~8%) and endometrial (~24%) cancer, PIK3CA is also frequently mutated. LOH and mutation of PTEN often occur in prostate (~30%) and endometrial (~38%) cancer. It is known that activation of the mitogen-activated protein kinase pathway, another key signaling cascade including KRAS and BRAF mutations, is rare in these 4 tumor types except for low-grade ovarian cancer (23). In high-grade ovarian cancer, KRAS and BRAF mutations are rarely found (35). Therefore, these types of cancer are possibly addicted to the PI3K pathway and could be potential target segments for PI3K inhibitors.

Our in vitro experiments showed that CH5132799 was a selective class I PI3K inhibitor, with potent inhibitory activity against PI3Kε and its mutants (Table 1). Analyses by using a large panel of cancer cell lines revealed that
CH5132799 had overall strong antiproliferative activity against breast, ovarian, prostate, and endometrial cancer cells in which the PI3K pathway is often activated, as previously mentioned (Fig. 2). In particular, cell lines harboring PIK3CA mutations showed significantly higher sensitivity in vitro and in vivo to CH5132799 than those without mutations, indicating that PIK3CA mutation is a CH5132799-sensitive factor (Figs. 2 and 3). Many groups have reported that clinically observed PIK3CA mutations promote cell proliferation and survival in vitro and in vivo (2, 6). In addition, oncogenic PIK3CA mutant–transfected MCF10A cells were sensitive to the classical PI3K inhibitor LY294002 as compared with WT PIK3CA transfectants, suggesting that the PIK3CA mutation causes PI3K addiction in cells (36). Therefore, higher sensitivity to CH5132799 in cancer cell lines harboring PIK3CA mutations can be explained by their addiction to PI3K. Amplification of HER2, which acts as an upstream regulator of PI3K, also seemed to be a factor that was sensitive to CH5132799 regardless of its coexistence with or without PIK3CA mutations. In contrast, KRAS mutant cell lines were relatively insensitive to CH5132799 in a manner similar to that of other PI3K inhibitors, whereas cell lines harboring both PIK3CA and KRAS mutations were sensitive. On the basis of these results, we theorize that the PIK3CA mutation is the primary predictive marker of CH5132799 sensitivity; moreover, the PIK3CA mutation could be a stratification marker that can predict which patients will benefit from CH5132799 therapy in clinical settings. Although several PI3K inhibitors, including PX-866 (37) and BEZ235 (38), which are in early-phase clinical trials and, more recently, the pan-PI3K inhibitor GDC-0941 (27, 39) have been reported to exhibit significant antiproliferative activity against cell lines harboring PIK3CA mutations, CH5132799 is the first PI3K inhibitor reported to possess statistically significant antitumor activity against cancer cells harboring PIK3CA mutations in large numbers of cancer cell lines spanning multiple tumor types. More recently, in addition to PI3Kα, the role of PI3Kβ in cancer has been studied by using genetically engineered mice, short-hairpin RNA, and chemical inhibitors (40–42). These studies suggest that tumors driven by PTEN deficiency are dependent on PI3Kβ rather than PI3Kα. Remarkably, CH5132799 also has PI3Kβ inhibitory activity, with an IC50 of 0.12 μmol/L (Table 1) and is therefore expected to exhibit antitumor activity against PTEN-deficient cancer. In fact, CH5132799 has shown growth inhibitory effects on some PTEN-deficient cancer cell lines in vitro antiproliferative assays and strong tumor regression in a PTEN-deleted model in vivo (Figs. 2 and 3).

About 20% of HER2-positive breast cancer patients have a PIK3CA mutation, which has resulted in a trend toward shorter progression-free survival with trastuzumab-based therapy (43, 44). This insensitivity of HER2-positive breast cancer harboring the PIK3CA mutation can be explained by its resistance to trastuzumab-induced PI3K pathway inhibition because of constitutive PI3K activity (28). When we combined CH5132799 with trastuzumab in the xenograft model harboring both HER2 amplification and the PIK3CA mutation, CH5132799 not only overcame trastuzumab insensitivity but also showed prominent synergistic anti- tumor activity leading to disappearance of the tumor (Fig. 4). Thus, combining CH5132799 with trastuzumab-based therapy is a promising strategy for providing clinical benefits to trastuzumab-insensitive patients.

Precise regulation of intracellular signaling pathways by feedback loops is an emerging topic in cell biology and has been vigorously studied (17). The S6K-mediated negative feedback loop in the PI3K pathway is one of the well-characterized feedback systems (18). As previously reported, the mTORC1 inhibitor everolimus enhanced Akt phosphorylation accompanied by S6K inhibition, both in vitro and in vivo (Fig. 5). Moreover, the PI3K/mTOR dual inhibitor BEZ235 also activated Akt at a low dose but inhibited Akt at high doses, consistent with similar observations by other groups (29, 30). At dosage levels that activated Akt, S6K phosphorylation was completely inhibited. These data suggest that BEZ235 has more potent mTOR inhibitory activity than PI3K inhibitory activity, resulting in S6K inhibition–mediated Akt activation at particular doses. In contrast, the selective class I PI3K inhibitor CH5132799 did not enhance Akt phosphorylation at any dose that inhibited S6K phosphorylation. As shown in Figure 5C, CH5132799 inhibited a wide variety of effectors in the pathway compared with everolimus because PI3K is located upstream of mTORC1. In addition, we showed that phosphorylation of 4E-BP1, which is another key substrate of mTOR, was efficiently inhibited by CH5132799 but not by everolimus treatment in vitro and in vivo. Consistently, rapamycin-insensitive mTORC1 has been suggested to be responsible for 4E-BP1 phosphorylation by several studies using mTORC1/2 inhibitors (16, 45). Dowling and colleagues reported that, among the downstream effectors of the PI3K/mTOR pathway, 4E-BP is important in cell proliferation, and S6K is critical for cell size regulation but not for cell proliferation in mammalian cells (31). 4E-BP is also known to function as a tumor suppressor gene (46). Therefore, CH5132799 has advantages over mTORC1 inhibitors in the robust inhibition of PI3K/Akt/mTOR signaling in 2 ways: (i) avoiding S6K inhibition–mediated Akt activation and (ii) suppressing 4E-BP1 phosphorylation. These characteristics could lead to remarkable regression of tumors that have regrown after long-term everolimus treatment.

Recent developments in detection of genetic alterations enable patient stratification in clinical trials by utilizing predictive biomarkers based on preclinical evidence (47). Our preclinical study indicates that CH5132799 is an effective therapy against human cancer cells harboring PIK3CA mutations. Clinical trials designed to clarify the value of the PIK3CA mutation for predicting clinical efficacy of CH5132799 are eagerly awaited.

Disclosure of Potential Conflicts of Interest

All of the authors are employees of Chugai Pharmaceutical Co., Ltd.
Acknowledgments

We thank Dr. Neal Rosen (Memorial Sloan-Kettering Cancer Center, NY) and Dr. José Baselga (Vall d’Hebron University Hospital, Barcelona, Spain) for helpful discussion. We also thank Maiko Izawa, Kurisu Honda, Masato Moriduki, Sachia Yamamoto, Yumiko Hashimoto, Onoda Hideyuki, Takeshi Kuge, Eriko Yasutomi, and Natsuko Furuya for their technical support. We are grateful to Dr. Junichi Kurebayashi for providing KPL-4 cells.

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 October 28, 2010; revised December 21, 2010; accepted December 23, 2010; published OnlineFirst May 10, 2011.

References


Clin Cancer Res; 17(10) May 15, 2011


The Selective Class I PI3K Inhibitor CH5132799 Targets Human Cancers Harboring Oncogenic PIK3CA Mutations

Hiroshi Tanaka, Miyuki Yoshida, Hiromi Tanimura, et al.

Clin Cancer Res 2011;17:3272-3281. Published OnlineFirst May 10, 2011.

Updated version
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-10-2882

Supplementary Material
Access the most recent supplemental material at:
http://clincancerres.aacrjournals.org/content/suppl/2011/05/18/1078-0432.CCR-10-2882.DC1

Cited articles
This article cites 47 articles, 26 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/17/10/3272.full#ref-list-1

Citing articles
This article has been cited by 15 HighWire-hosted articles. Access the articles at:
http://clincancerres.aacrjournals.org/content/17/10/3272.full#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.