Clinical Cancer Research

Cancer Therapy: Clinical
See commentary by Huang, p. 2124

The Clinical Effect of the Dual-Targeting Strategy Involving PI3K/AKT/mTOR and RAS/MEK/ERK Pathways in Patients with Advanced Cancer

Toshio Shimizu[1], Anthony W. Tolcher[1], Kyriakos P. Papadopoulos[1], Muralidhar Beeram[2], Drew W. Rasco[1], Lon S. Smith[2], Shelly Gunn[3], Leslie Smetzer[1], Theresa A. Mays[1], Brianne Kaiser[1], Michael J. Wick[1], Cathy Alvarez[1], Aracely Cavazos[1], Gina L. Mangold[1], and Amita Patnaik[1]

Abstract

Purpose: This study evaluated the clinical relevance of the dual-targeting strategy involving PI3K/AKT/mTOR and RAF/MEK/ERK pathways.

Experimental Design: We investigated safety, efficacy, and correlations between tumor genetic alterations and clinical benefit in 236 patients with advanced cancers treated with phase I study drugs targeting phosphoinositide 3-kinase (PI3K) and/or mitogen-activated protein kinase (MAPK) pathways in our Phase I Clinical Trials Program.

Results: Seventy-six (32.2%) patients received a PI3K pathway inhibitor in combination with a MAPK pathway inhibitor (D), whereas 124 (52.5%) and 36 (15.3%), respectively, received an inhibitor of either the PI3K or MAPK pathways (S). The rates of drug-related grade >III adverse events were 18.1% for (S) and 53.9% for (D; $P < 0.001$); the rates of dose-limiting toxicities were 9.4% for (S) and 18.4% for (D; $P = 0.06$). The most frequent grade >III adverse events were transaminase elevations, skin rash, and mucositis. In our comprehensive tumor genomic analysis, of 9 patients who harbored coactivation of both pathways (colorectal cancer, $n = 7$; melanoma, $n = 2$), all 5 patients treated with (D) had tumor regression ranging from 2% to 64%.

Conclusions: These results suggest that dual inhibition of both pathways may potentially exhibit favorable efficacy compared with inhibition of either pathway, at the expense of greater toxicity. Furthermore, this parallel pathway targeting strategy may be especially important in patients with coexisting PI3K pathway genetic alterations and *KRAS* or *BRAF* mutations and suggests that molecular profiling and matching patients with combinations of these targeted drugs will need to be investigated in depth. *Clin Cancer Res; 18(8); 2316–25. ©2012 AACR.*

Introduction

The PI3K/AKT and RAS/RAF/MEK/ERK pathways are among the most frequently deregulated pathways in cancer thereby suggesting a key role in carcinogenesis. Aberrant activation of the PI3K/AKT pathway occurs in virtually every type of human malignancy through either activating mutations in *PIK3CA*, which encodes the catalytic p110α kinase subunit, or loss-of-function mutations, deletions, or promoter methylation silencing of *PTEN* (1), a negative regulator of phosphoinositide 3-kinase (PI3K). More rarely, an activating mutation of *AKT*1 or *AKT*3 leads to *PI3K*/*PTEN*-independent pathway activation (2, 3). Mutated oncogenic forms of *RAS* are found in approximately 15% of all cancers (4), and deregulation of the RAF/MEK/ERK pathway by extracellular signal—regulated kinase (ERK) hyperactivation is seen in approximately 30% of all cancers (5). In addition, activating *BRAF* mutations have been identified at a high frequency in melanoma (30%–60%), thyroid cancer (30%–50%), colorectal cancer (CRC; 5%–20%), and ovarian cancer (~30%; refs. 4, 6). Although activating mutations in mitogen—activated protein (MAP)/ERK kinase (MEK) itself have not been identified in human cancers, MEK is thought to be an important drug target for treating human cancer because of its central role in the ERK pathway.

The PI3K/AKT and RAS/RAF/MEK/ERK pathways also interact extensively. Not only do these pathways share common inputs but also they can both be activated by

Authors' Affiliations: [1]START (South Texas Accelerated Research Therapeutics); [2]STOH (South Texas Oncology and Hematology); and [3]Genome Consult Division, START Center, San Antonio, Texas

Note: Presented in part of Clinical Science Symposium at the 47th Annual Meeting of the American Society of Clinical Oncology (ASCO) with Merit Award, June 3–7, 2011, Chicago, IL.

Corresponding Author: Amita Patnaik, START (South Texas Accelerated Research Therapeutics), 4383 Medical Drive, San Antonio, TX 78229. Phone: 210-593-5250; Fax: 210-615-1121; E-mail: amita.patnaik@start.stoh.com

doi: 10.1158/1078-0432.CCR-11-2381

Translational Relevance

The dual-targeting strategy involving PI3K/AKT/mTOR and RAF/MEK/ERK pathways in cancer treatment is particularly intriguing as there is evidence that both pathways are extensively interconnected. Nevertheless, the clinical impact of this parallel pathways blockade still remains unclear. We reviewed the records of 1,558 consecutive patients and investigated safety, efficacy, and correlations between tumor genetic alterations and clinical benefit in 236 patients with advanced cancers treated with first-in-human phase I clinical study drugs targeting phosphoinositide 3-kinase (PI3K) and/or mitogen—activated protein kinase (MAPK) pathways in our Phase I Clinical Trials Program. Our data suggest that dual inhibition of both pathways may potentially exhibit favorable efficacy compared with single inhibition of either pathway alone, at the expense of greater toxicity in a clinical setting. Furthermore, this parallel pathway targeting strategy may be especially important in patients with coexisting PI3K pathway genetic alterations and *KRAS* or *BRAF* mutations.

oncogenic *RAS* and appear to provide some compensatory signaling when one or the other is inhibited. Recent findings have shown that when *mTOR*, a downstream target of *AKT*, is inhibited, PI3K can activate MAPK via *RAS* (7). Frequent coactivation of these 2 pathways has also been seen in a number of different tumor types, including melanoma, prostate, and CRC (8, 9). These results show further that these 2 pathways operate as a complex network and also provide the rationale for combining therapeutic agents that could simultaneously block both the pathways.

Preclinical studies provide a clear rationale for the coinhibition of these frequently activated, "semi-parallel" pathways. This includes strong evidence for: (i) cross-talk between RAF/MEK/ERK and PI3K/AKT signaling via an MEK/EGFR/PI3K feedback loop, (ii) oncogenic mutations that activate MEK/ERK signaling being associated with resistance to receptor tyrosine kinases inhibition, and (iii) PI3K pathway activation conferring resistance to MEK inhibition.

Horizontal (parallel) blockade or the combined use of inhibitors of multiple signaling pathways may also be important. Deregulation of the RAS/RAF/MEK pathway may be a key regulator in cancer cell resistance to PI3K inhibitors and suggests combined targeting of PI3K and MEK as an effective anticancer strategy (10); however, the clinical relevance of these findings needs to be evaluated. Therefore, we investigated the clinical effect of this horizontal blockade involving PI3K/AKT/mTOR and RAS/MEK/ERK pathways in patients with advanced solid tumors.

Materials and Methods

We reviewed the electronic medical records of 1,558 consecutive patients treated in the Phase I Clinical Trials Program at the START (South Texas Accelerated Research Therapeutics) Center, in San Antonio, TX, from April 2007 through April 2011, and we assessed the safety, efficacy, and correlations between tumor genetic alterations and clinical benefit in 236 patients with advanced solid tumors treated in first-in-human phase I study drugs targeted to PI3K/AKT/mTOR or/and RAS/MEK/ERK pathways. Data were collected from transcribed notes in the electronic database. Patient records were reviewed from the time of presentation in the phase I clinic.

Patients' eligibility and trial enrollment

Patients had pathologically confirmed solid malignancies refractory to standard therapy or for which no standard therapy existed. They were enrolled on specific clinical trials on the basis of the available results of tumor genetic alterations by comprehensive tumor genomic testing as well as logistical factors, including study availability and eligibility criteria. Patient demographic information, safety/toxicity data, and efficacy assessments were all collected prospectively as per individual clinical trial protocols. The formulation of the patient database and formal collection of data for the purpose of this study were conducted retrospectively. The patients were classified into 2 groups; single pathway inhibition group (S; received either PI3K/AKT/mTOR or RAS/MEK/ERK pathway inhibitor as a single-agent therapy) and dual pathway inhibition group (D; received PI3K/AKT/mTOR pathway inhibitor and RAS/MEK/ERK pathway inhibitor as combination therapy). All studies were conducted in accordance with the guidelines of an Institutional Review Board.

Treatment and evaluation

All studies were conducted through standard procedures of first-in-human phase I clinical study in accordance with the Declaration of Helsinki (1964, amended in 2000). Assessments, including history, physical examination, and laboratory evaluations, were conducted as specified in each protocol, typically before the initiation of therapy, weekly during the first cycle, and then, at a minimum, at the beginning of each new treatment cycle. The electronic medical records were reviewed for patient characteristics, adverse events related to investigational drugs during study periods, and clinical benefit (tumor response and/or stabilization). Patient characteristics included sex, age, Eastern Cooperative Oncology Group performance status at the beginning of treatment, tumor type, the number of previous therapies, and tumor genetic alterations, if available. Treatment continued until disease progression or unacceptable toxicity occurred. Treatment was carried out according to the specific requirements in each study protocol.

All drug-related adverse events and dose-limiting toxicities (DLT) were recorded and graded according to the National Cancer Institute-Common Toxicity Criteria

(NCI-CTCAE; version 3.0/4.0). DLTs were defined toxicities related to study drug occurring during cycle 1 after administration of the first dose that met any of the specific criteria in each study protocol. The definition of DLTs differed slightly on each protocol. The DLTs in this study population were mostly nonhematologic, typically defined in phase I studies as any grade >III toxicity, except for nausea/vomiting/diarrhea that responds to maximal medical therapy. Serious adverse event was defined as any untoward medical occurrence that results in death, life-threatening, requires in-patients hospitalization or prolongation of existing hospitalization, and results in persistent or significant disability/incapacity.

Efficacy was assessed routinely by computed tomographic scanning and/or MRI at baseline before treatment initiation and then every 2 cycles (6–8 weeks) per each phase I study protocol. All radiographic studies were read in the Department of Radiology at South Texas Radiology Imaging Centers (STRIC) and reviewed by the START treating oncologists. In accordance with Response Evaluation Criteria in Solid Tumors (RECIST) version 1.0 or 1.1 (depending on study protocol), tumor responses were classified as complete response, partial response, stable disease, or progressive disease. A designation of response required confirmation after 4 to 8 weeks. To further assess activity of the different phase I treatment regimens, waterfall plot analyses (WPA) were also conducted in our study. The percentage change according to RECIST from baseline was evaluated at the time of best response. Average enlargement in tumor burden (AETB) was calculated as the sum in waterfall area under curve divided by the number of patients. In addition, the median enlargement in tumor burden (METB) was also calculated.

Tumor tissue samples and comprehensive tumor genomic testing

With patient's informed consent, and depending on the available amount of archived formalin-fixed paraffin-embedded (FFPE) tumor tissues or fresh tumor tissue, comprehensive tumor genomic testing was conducted by DNA array–based comparative genomic hybridization (DNA array-CGH) and/or multiplex PCR mutation analysis through mutation screening panels.

DNA array-CGH

DNA array-CGH analysis of the tumor genome was conducted at Combimatrix Molecular Diagnostics (CMDX) using the DNAarray tumor profile for copy number change, a 3,039-probe whole genome bacterial artificial chromosome (BAC) microarray.

Multiplex mutation screening panels

Tumor genetic mutations were analyzed using the OncoCarta Panel v 1.0, a multigene panel with 19 key oncogenes and 238 mutations, via the MassARRAY System (Sequenom, Inc.). The MassARRAY System involves the following steps: multiplex PCR of gene exons of interest, primer extension reactions using IPLEX chemistry, and analysis of primer extension products by matrix-assisted laser desorption/ionization—time-of-flight (MALDI-TOF) mass spectrometry. The MassARRAY system testing panels we used were also previously indeed validated with known positive controls for relevant oncogenic mutations.

Statistical considerations

Two-sided Fisher exact tests were used to compare safety result between the 2 treatment groups. Exact two-sided 95% confidence intervals (CI) were calculated for the disease control rate. Time to progression (TTP) was defined as the time interval from the start of therapy to the first observation of disease progression or death, whichever occurred first. These analyses were conducted without adjustments for multiple comparisons. All statistical analyses were conducted using SAS 9.2 (SAS Institute).

Results

Patient characteristics

Overall, the records of 1,558 consecutive patients were examined. Two hundred and thirty-six (15.1%) of the treated patients received first-in-human phase I study drugs targeted to PI3K/AKT/mTOR and/or RAS/MEK/ERK pathways. One hundred and thirty-eight (58%) patients were female, 98 (42%) were male, and the median age was 58.9 years (range, 18–90 years). One hundred and eighty-two (77%) were Caucasians, 46 (19%) Hispanic, 6 (3%) African-Americans, and 2 (1%) Asians. Fifty-six (24%) had CRC, 29 (12%) breast cancer, 24 (10%) soft tissue sarcomas, 17 (7%) pancreatic cancer, 16 (7%) melanoma, 12 (5%) neuroendocrine tumor, 11 (5%) ovarian cancer, and 8 (3%) prostate cancer. A variety of other tumors made up the rest of the patients (Table 1).

Treatments

Of the 236 patients in 11 first-in-human phase I clinical trials, 160 patients (68%) received either PI3K pathway inhibitors or MAPK pathway inhibitors as a single agent and 76 patients (32%) received a combination of a PI3K pathway inhibitor with a MAPK pathway inhibitor. One hundred and twenty-four patients (52%) received a single-agent PI3K pathway inhibitor (pan-class I PI3K, $n = 9$; dual PI3K and mTORC1, $n = 41$; AKT, $n = 33$; p70S6K, $n = 15$; and mTORC1, $n = 26$), 36 patients (15%) received a single-agent MAPK pathway inhibitor (MEK1/2, $n = 14$; and p38MAPK, $n = 22$) and 76 patients (33%) received the combination therapy (AKT + MEK1/2, $n = 45$ and mTORC1 + MEK1/2, $n = 31$; Table 2).

Tolerability and safety

All 236 patients were available for evaluation of toxicities and had at least one adverse event. Overall, the most frequent drug-related grade >III adverse events were transaminase elevations, mucositis, and skin rash in both treatment groups. The rates of drug-related grade >III adverse events were 18.1% (29 of 160) for S group and 53.9% (41 of 76) for the D group ($P < 0.001$); the rates of

Table 1. Patient characteristics

Characteristics	No. of patients (N = 236)	Single pathway inhibition group (N = 160)	Dual pathway inhibition group (N = 76)
Sex (male/female)	98/138	65/93	33/45
Age, y			
Median	58.9	60.5	58.1
Range	18–90	26–90	18–81
ECOG performance status			
0	67	50	17
1	159	100	59
2	10	10	0
Ethnicity			
Caucasian	182	115	67
Hispanic	46	40	6
African-American	6	4	2
Asian	2	1	1
Tumor types ($n \geq 5$)			
Colorectal	56	40	16
Breast	29	22	7
Soft tissue sarcoma	24	14	10
Pancreatic	17	5	12
Melanoma	16	7	9
Neuroendocrine tumor	12	8	4
Ovarian	11	7	4
Prostate	8	6	2
Head and neck	7	3	4
Uterus endometrial	6	5	1
Renal cell	6	6	0
Genitourinary	6	6	0
Other[a]	38	31	7
No. of prior chemotherapy			
Median	4	4	3
Range	1–10	1–10	1–8

NOTE: Single pathway inhibition group refers to patients who received either PI3K/AKT/mTOR or RAS/MEK/ERK pathway inhibitor as a single-agent therapy and dual pathway inhibition group refers to patients who received PI3K/AKT/mTOR pathway inhibitor and RAS/MEK/ERK pathway inhibitor as combination therapy.

Abbreviation: ECOG, Eastern Cooperative Oncology Group.

[a]Other includes lung, gastric, esophageal, and unknown primary.

DLTs were 9.4% (15 of 160) for S group and 18.4% (14 of 76) for the D group ($P = 0.06$). Of the D group patients, 40 (52.6%) had grade III adverse events [mostly transaminase elevations ($n = 11$) and mucositis ($n = 9$)] and only 1 patient developed a grade IV adverse event (reversible thrombocytopenia), whereas 28 patients (17.5%) had grade III adverse events [mostly skin rash ($n = 7$)] and 1 patient experienced grade IV reversible transaminase elevation in the S group. Seven D group patients had serious adverse events, including grade III mucositis ($n = 2$), vomiting ($n = 2$), diarrhea ($n = 1$), nausea ($n = 1$), cardiac arrhythmia ($n = 1$), bacterial pneumonia ($n = 1$), and grade IV thrombocytopenia ($n = 1$); all events were deemed at least possibly related to treatment. A 61-year-old man with metastatic colon cancer treated with a combination of an AKT inhibitor and a MEK inhibitor had a grade III ventricular arrhythmia requiring hospitalization during the first course, and this event was considered to be a DLT. This patient had a history of both hypertension and cardiac arrhythmia (premature ventricular contractions and supraventricular tachycardia), but these cardiac arrhythmias were stable more than 2 years before enrollment in the study. Although the rates of study discontinuation due to drug-related adverse events were similar for both groups, dose interruptions and dose reductions due to adverse events were required for 19.4% and 16.9%, respectively, of patients in the S group and 48.7% and 39.5%, respectively, of patients in

Table 2. Administration protocols of first-in-human phase I drugs

Study no.	Molecular targets	Single agent/combination	N	Trial registration ID
1	PI3K (pan-class I)	Single agent	9	NCT00962611
2	PI3K/mTORC1	Single agent	41	NCT00485719
3	AKT	Single agent	33	NCT00670488
4	mTORC1	Single agent	15	NCT00694083
5	mTORC1	Single agent	11	NCT01071304
6	p70S6K	Single agent	15	NCT01394003
7	MEK1/2	Single agent	14	NCT00959127
8	p38MAPK	Single agent	22	NCT01393990
9	AKT + MEK1/2	Combination	27	NCT01021748
10	AKT + MEK1/2	Combination	18	NCT01138085
11	mTORC1 + MEK1/2	Combination	31	NCT00955773
Total			236	

Abbreviations: p38MAPK, p38 mitogen-activated protein kinase; p70S6K, ribosomal protein S6 kinase.

the D group. There were no treatment-related deaths in either treatment groups (Table 3).

Comprehensive tumor genomic testing

One hundred and twenty of the 236 (51%) patients were tested for the presence of tumor genetic alterations by DNA array-CGH and/or multiplex PCR mutation screening panels. Of 120 patients, 106 patients (88%) had available results of tumor genetic alterations. PI3K pathway genetic alterations were detected in 19 of the 106 (18%) patients. In 19 patients, 13 homozygous *PTEN* deletions were detected (11 in 10q23 and 2 in 10q24), *AKT* amplifications were detected in 5 individuals (*AKT2*, $n = 4$ and *AKT3*, $n = 1$), and *PIK3CA* mutation (H1047R) was found in only one patient with breast cancer.

PTEN deletions were most frequent in CRC [5 of 13 patients (38%)]. *PTEN* deletions were also present in patients with neuroendocrine tumor ($n = 2$), sarcoma ($n = 2$), breast cancer ($n = 1$), melanoma ($n = 1$), merkel cell carcinoma ($n = 1$), and ovarian cancer ($n = 1$). *AKT* amplifications were present in CRC ($n = 2$), breast cancer ($n = 1$), chorangiocarcinoma ($n = 1$), and thyroid cancer ($n = 1$). Of 106 patients, *RAS* mutations were identified in 19 patients [*KRAS*, $n = 18$ (G12A, $n = 1$; G12C, $n = 1$; G12D, $n = 6$; G12V, $n = 5$; G12S, $n = 2$; and G13D, $n = 3$) and *HRAS* (Q61R), $n = 1$] and 5 *BRAF* exon 15 mutations (V600E) were identified in patients with melanomas ($n = 3$), CRC ($n = 1$), and thyroid cancer ($n = 1$). Nine of the 19 (47%) patients with PI3K pathway genetic alterations harbored simultaneous *KRAS* or *BRAF* mutations (CRC, $n = 7$; melanoma, $n = 2$).

Antitumor activity

Of the 236 patients, 177 [75%; S, 70% (112 of 160); D, 85% (65 of 76)] patients were evaluable for response by RECIST. In the D group, 5 (7.7%) confirmed partial responses were observed, including 2 patients with melanoma, and one patient each with head and neck cancer, non–small cell lung cancer, and breast cancer.

A heavily treated male patient with breast cancer with *AKT3* gene amplification treated with an mTOR inhibitor had a partial response with significant improvement in both bony and visceral diseases. This was paralleled by significant symptomatic benefit with reduction in pain from a right chest wall mass. A patient with an adenoid cystic carcinoma harboring an *HRAS* mutation who was treated with a combination of an MEK inhibitor and an mTOR inhibitor had a partial response with 60% tumor reduction in multiple pulmonary metastatic lesions (Fig. 1A and B).

There were no significant differences in both tumor control rate (partial response + stable disease) by best radiographic response for S versus D (52.7% and 64.6%, respectively; S: 95% CI, 43.5–61.7; D: 95% CI, 52.5–75.2; $P = 0.16$) and rates of stable disease >6 months for S versus D (6.3% and 10.8%, respectively; S: 95% CI, 95% CI, 2.9–12.6; D: 95% CI, 5.0–20.9; $P = 0.39$; Table 4). WPAs displayed AETB/METB: 19.9%/18.0% (S) and 9.5%/8.0% (D; AETB; S: 95% CI, 15.0–24.8; D: 95% CI, 2.5–14.2; $P = 0.02$), and there was no obvious difference in efficacy between the patients with known activating tumor genetic alterations and tumor genetic alteration "negative" or "unknown" patients (Fig. 2).

Of the 10 patients with a single PI3K pathway alteration, 2 patients with breast cancer were enrolled in clinical trials with a single PI3K/AKT/mTOR inhibitor. One patient with a *PIK3CA* mutation received an AKT inhibitor and the other patient with an *AKT3* amplification was treated with an mTOR inhibitor. One patient had prolonged stable disease lasting for more than 66 weeks. Of the 15 patients with a single *RAS*/*RAF* mutation, 5 patients with colon cancer were enrolled in clinical trials with a single MEK1/2 inhibitor or p38MAPK inhibitor and all 5 patients had progressive disease (4 with radiological progressions and 1 with clinical progression). In comparison, all of the 9 patients with coactivation of PI3K/AKT and RAS/RAF/MEK pathways

Table 3. Summary of safety and grade III-IV drug-related adverse events of special interest

	Single agent, N (%)	Combination, N (%)	*P*	
Any grade AEs	157 (98.1)	76 (100)	0.55	
Grade III-IV AEs	29 (18.1)	41 (53.9)	<0.001	
DLTs	15 (9.4)	14 (18.4)	0.06	
SAEs	10 (6.3)	7 (9.2)	0.43	
Study discontinuation due to AEs	15 (9.4)	8 (10.5)	0.82	
Dose interruption due to AEs	31 (19.4)	37 (48.7)	<0.001	
Dose reduction due to AEs	27 (16.9)	30 (39.5)	<0.001	
	S of PI3K (N = 124)	**S of MAPK (N = 36)**	**D of PI3K + MAPK (N = 76)**	**Total patients (N = 236)**
Patients with any grade III-IV drug-related AEs, *n* (%)	25 (20.2)	3 (8.3)	41 (53.9)	69 (29.2)
Transaminase elevation	4 (3.2)[a]	0	11 (14.5)	15 (6.4)
Skin rash	6 (4.8)	1 (2.8)	4 (5.3)	11 (4.7)
Mucositis	1 (0.8)	0	9 (11.8)	10 (4.2)
Hyperglycemia	2 (1.6)	0	2 (2.6)	4 (1.7)
Lipase elevation	2 (1.6)	0	2 (2.6)	4 (1.7)
Vomiting	2 (1.6)	0	1 (1.3)	3 (1.3)
Leukopenia	2 (1.6)	0	1 (1.3)	3 (1.3)
Hemoglobin	1 (0.8)	1 (2.8)	1 (1.3)	3 (1.3)
Fatigue	1 (0.8)	0	1 (1.3)	2 (0.8)
Neutropenia	0	0	2 (2.6)	2 (0.8)
Thrombocytopenia	0	0	2 (2.6)[a]	2 (0.8)
Diarrhea	0	0	1 (1.3)	1 (0.4)
Nausea	1 (0.8)	0	0	1 (0.4)
Anorexia	1 (0.8)	0	0	1 (0.4)
Cardiac event	0	0	1 (1.3)	1 (0.4)
Neurologic event	1 (0.8)	0	0	1 (0.4)
Patients with DLTs, *n* (%)	14 (11.3)	1 (2.8)	14 (18.4)	29 (12.3)

Abbreviations: AE, adverse event; D, dual pathways inhibition; S, single pathway inhibition; SAE, serious adverse event.
[a]Grade IV events: one event of transaminase elevation (single-agent group) and one event of thrombocytopenia (combination group).

were enrolled in clinical trials that included single pathway inhibition or dual pathways inhibition. Of 9 patients with coactivation of PI3K/AKT and RAS/RAF/MEK pathways, all 5 patients treated with dual pathways inhibition had tumor regression/stabilization ranging from 2% to 64%, whereas all 4 patients treated with single pathway inhibition developed progressive diseases. Mean TTP in group S was 51 days and 151 days in group D ($P = 0.09$). However, because of the small number of patients, this did not reach statistical significance. Duration of these responses were 12.7, 16.0, 17.2, 45.8, and 15.8 weeks (Table 5).

Discussion

The dual-targeting strategy involving PI3K/AKT/mTOR and RAF/MEK/ERK pathways is particularly intriguing as there is evidence that both pathways are extensively interconnected. Previous studies suggest that combined targeting of these pathways are necessary for optimal therapeutic activity (8, 10). Although a fair number of combination phase I clinical trials currently have been initiated that use this strategy, the clinical impact of this parallel pathway blockade still remains unclear.

In the safety analysis of our study, along with the higher tendency of both dose interruptions and dose reductions due to adverse events in the D group, the rates of drug-related grade >III adverse events were significantly higher in the D group than in the S group (53.9% vs. 18.1%, $P < 0.001$). Nevertheless, there was no statistical difference between the S group and the D group for the rates of DLTs (9.4% vs. 18.4%, $P = 0.06$).

In the efficacy analysis, although WPAs showed that the percentages of both METB and AETB had smaller tendency in the D group than in the S group, WPAs with results of tumor genetic alterations showed that there was no obvious difference in efficacy between the patients with known activating tumor genetic alterations compared with those without tumor genetic alterations or for whom the result

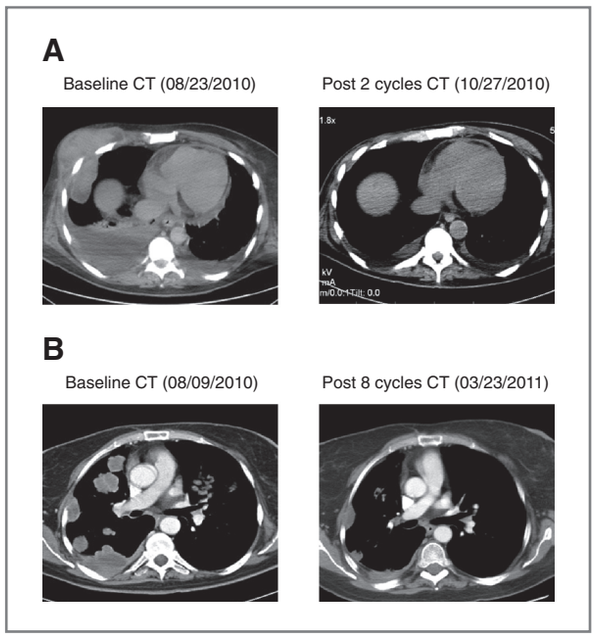

Figure 1. Computed tomographic (CT) scans of responding patients. A, a heavily treated male patient with breast cancer with *AKT3* gene amplification treated with an mTOR inhibitor showing partial response in right chest wall mass. B, a patient with an adenoid cystic carcinoma harboring an *HRAS* mutation treated with a combination of an MEK inhibitor and an mTOR inhibitor showing partial response in multiple pulmonary metastatic lesions.

was unavailable. To assess the activity of different compounds, waterfall analysis may provide a potentially useful new efficacy metric. Given the fact that recent analyses of WPAs have been conducted mostly in phase II or large phase III studies, further validation studies focused on early phase I studies with heterogeneous patient populations are warranted. However, with the response rates being historically low in phase I studies, AETB and/or METB may offer a very preliminary indication as to potential efficacy differences between compounds. This novel type of analysis will require further study and validation.

There are several explanations that might point to the differences in both safety and efficacy in the 2 study arms. First, because this was a retrospective study and because entry criteria for phase I clinical trials are intentionally not overly restrictive, there was considerable heterogeneity in both the overall patient population and between the 2 study arms. For example, breast cancer was somewhat overrepresented in the S group, whereas pancreatic cancer was somewhat overrepresented in the D group. More importantly, there are significant differences in starting doses in single-agent phase I studies compared with combination phase I studies. In combination phase I studies, the maximum tolerated dose (MTD) of each single agent has generally already been defined and thus patients are treated closer to doses in the therapeutic range. On the other hand, doses generally are not escalated as high in combination studies due to greater degrees of toxicity.

For example, in this study, some patients with *KRAS* or *BRAF* mutant cancers received single-agent MAPK pathway inhibitors at doses significantly lower than the MTD or recommended phase II dose (RIIPD) of the respective agents. Indeed, most of the patients who responded to treatment in both groups received higher doses closer to the respective MTD and RPIID. Furthermore, the frequency of severe toxicities tended to occur at higher dose levels, especially in the combination treatment group. Because some of these studies are ongoing, the MTD has not been defined for all treatments. Thus, at the time of publication, these data could not be presented in a more quantitative fashion. One question that also arises is why some patients without any known tumor genetic alterations responded in each treatment group. It is possible that the tumor genomic testing procedures may not be sensitive enough and that some of these patients may have actually harbored pertinent tumor genetic alterations below the limits of detection for the specific test.

Furthermore, some of the study drugs may in fact have multiple mechanisms of action and/or may have inherent antitumor activity without necessarily requiring definable tumor genetic alterations.

Activation of the PI3K signaling pathway is frequently mediated by mutations in the p110α subunit of PI3K (*PIK3CA*), with most mutations (>80%) occurring either in exon 9, which codes for the helical domain, or exon 20, which codes for the kinase domain (11). Preclinical studies suggested that *PIK3CA* mutations could predict response to PI3K inhibitors, although concomitant mutations in *RAS* (*KRAS*, *NRAS*) or *BRAF* might mediate resistance (12, 13). A key aspect is whether or not the detection of additional mutations that might confer resistance would provide more predictive information. While the activation of both pathways is widespread across many solid and hematologic cancer types, the clearest markers of pathway activation are activating genetic lesions within components of the pathways. Frequent coactivation of the PI3K/AKT and RAS/MEK/ERK pathways has also been seen in a number of different tumor types, including melanoma, prostate, and CRC (8, 13–18). Although several preclinical studies suggest that aberrations in the PI3K/AKT/mTOR and the MAP kinase pathway may coexist, only limited studies in patients have been undertaken and have mostly concentrated on CRC (11, 13, 14). In this regard, a recent report also showed that PI3K pathway activation strongly influences the sensitivity of *RAS* mutant cells to MEK inhibitors. Activating mutations in *PIK3CA* reduce the sensitivity to MEK inhibition, whereas *PTEN* mutations seem to cause complete resistance (19). In addition, dual inhibition of the PI3K/AKT and RAF/MEK/ERK pathways also seems to be required for complete inhibition of the downstream mTOR effector pathway (19).

Another recent report also suggested that patients with *PIK3CA* mutant CRC and a simultaneous *KRAS* mutation did not respond to PI3K/AKT/mTOR axis therapy (20), which is in agreement with previous preclinical data suggesting that *KRAS* activation mediates resistance to PI3K

Table 4. Summary of antitumor activity

Treatment group	No. of patients	Patients available by RECIST	% of all patients
Single pathway inhibition	160	112	70
PI3K pathway inhibitors	124	87	70
MAPK pathway inhibitors	36	25	70
Dual pathways inhibition	76	65	85
Best response compared with baseline	**Single agent (N = 112)**	**Combination (N = 65)**	***P***
Tumor growth (% of patients)	85 (76)	48 (74)	0.86
No change (% of patients)	5 (4)	0 (0)	
Tumor shrinkage (% of patients)	22 (20)	17 (26)	
RECIST			
PR	1 (0.9)	5 (7.7)	0.03
SD	58 (51.8)	38 (58.5)	0.44
PD	53 (47.3)	22 (33.8)	0.09
Tumor control rate, % (PR + SD)	52.7	64.6	0.16
95% CI by best radiographic response	43.5–61.7	52.5–75.2	
SD			
≥6 mo, %	6.3	10.8	0.39
95% CI	2.9–12.6	5.0–20.9	
AETB, %	19.9	9.5	0.02
95% CI	15.0–24.8	2.5–14.2	
METB, %	18.0	8.0	

Abbreviations: AETB, PD, progressive disease; PR, partial response; SD, stable disease.

inhibitors (12). These recent findings and studies provide a strong rationale for the combination of PI3K and MEK inhibitors in cancers with coexisting *PIK3CA* and *KRAS* mutations (21). Therefore, we also examined our patients for coexistence of the PI3K pathway genetic alteration with *KRAS* or *BRAF* mutations.

In this study, 9 of the 19 (47%) patients with PI3K pathway genetic alterations had simultaneous *KRAS* or *BRAF* mutations (CRC, $n = 7$; melanoma, $n = 2$), which is consistent with previously published data (22, 23). Of 9 patients with coactivation of both pathways, all 4 patients treated by single pathway inhibition showed tumor progression, whereas all 5 patients treated by dual pathway inhibitions had tumor regression/stabilization ranging from 2% to 64% by RECIST. This result suggests that this horizontal dual pathway targeting strategy may be important in patients with coexisting genetic alterations.

Interestingly, 2 melanoma patients with both $BRAF^{V600E}$ mutation and *PTEN* deletion treated with combination of MEK1/2 inhibitor and mTOR inhibitor remained on study longer than any of the other patients with $BRAF^{V600E}$ mutations who were on MEK1/2 inhibitor combination trials. There is some preclinical rationale which potentially explains this result. Resistance to the antiproliferative effects of RAF and MEK inhibition was observed in approximately 10% of $BRAF^{V600E}$ mutant melanoma cell lines, a fraction consistent with about 20% of patients who fail to derive any clinical benefit from the RAF inhibitor PLX4032 (24). In these resistant cases, *PTEN* loss coexisted with $BRAF^{V600E}$ mutation. Recent preclinical data identified *PTEN* loss as one genetic event that abrogates the effects of ERK pathway inhibition in $BRAF^{V600E}$ tumors. The study of Dankort and colleagues showed the ability of $BRAF^{V600E}$ expression to cooperate with *PTEN* silencing in the genesis of metastatic melanoma (25). Other recent data also showed that tumors with concurrent *BRAF* mutation and *PTEN* loss were typically dependent upon ERK signaling, but the nature of the dependence was altered by concurrent loss of *PTEN*. These data also suggest that tumors with concurrent $BRAF^{V600E}$ and *PTEN*/*RB* loss will be resistant to inhibitors of ERK signaling (26). These data provide a rationale for combined targeting of the RAF/MEK and PI3K/mTOR pathways in the cohorts of patients with concurrent *BRAF* mutation and *PTEN* alteration. It is also anticipated that the ongoing Tumor Cancer Genome Atlas project in melanoma will define the frequency of co-occurrence with *BRAF* mutation and *PTEN* alteration in patients with previously untreated melanoma.

In conclusion, our preliminary results suggest that dual inhibition of PI3K/AKT/mTOR and RAS/RAF/MEK pathways may potentially exhibit favorable efficacy compared with single inhibition of either pathway alone, at the expense of greater toxicity. Furthermore, this parallel pathway targeting strategy may be especially important in patients with coexisting PI3K pathway genetic alterations and *KRAS* or *BRAF* mutations and suggests that molecular

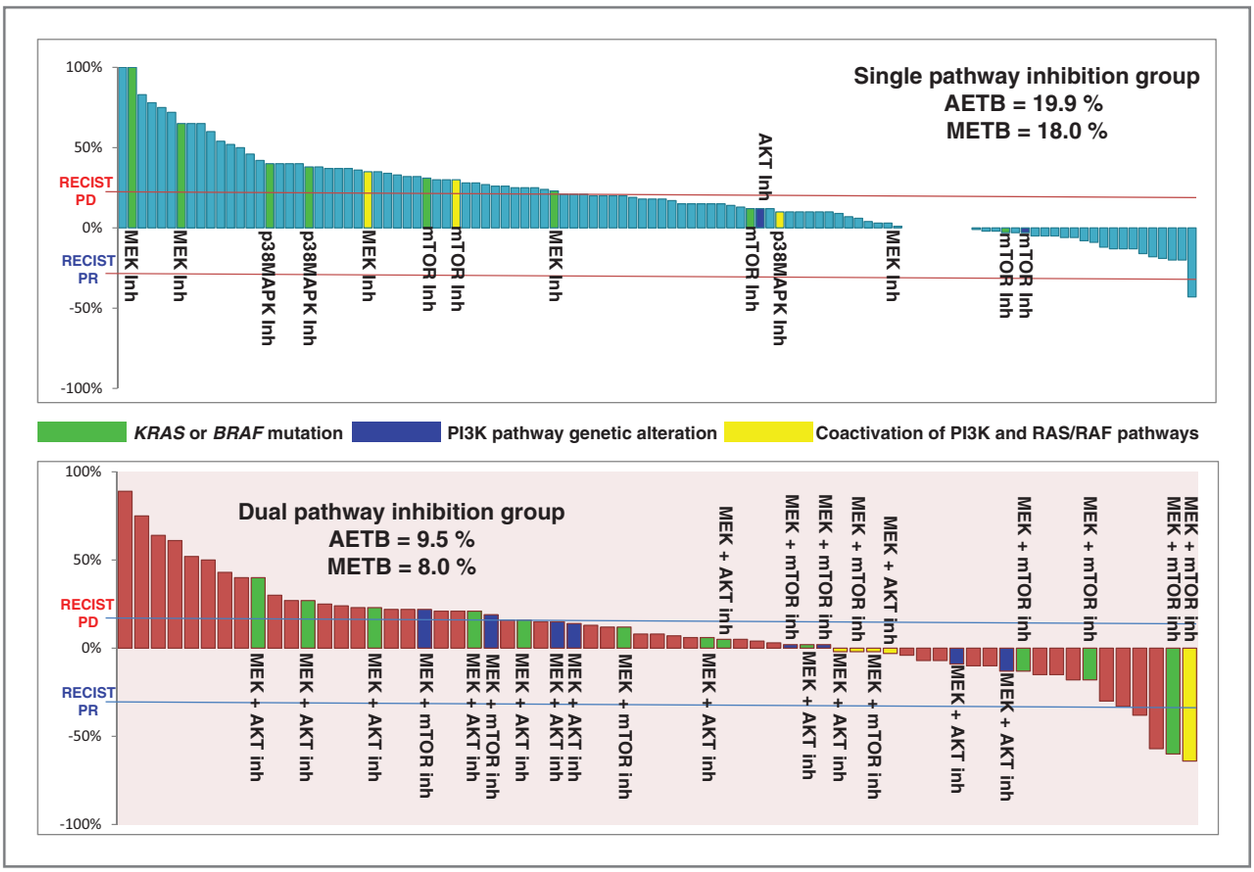

Figure 2. WPAs with available results of tumor genomic alterations. PD, progressive disease.

profiling and matching patients with combinations of these targeted drugs will need to be investigated in depth. These retrospective data, while not definitive, are certainly hypothesis generating and warrant further investigation in larger, appropriately designed prospective clinical trials, in which patients are assigned to treatments on the basis of comprehensive analyses of *KRAS*, *BRAF*, *PIK3CA*, and *PTEN* gene statuses.

Table 5. Clinical outcomes of patients with PI3K/AKT pathway genetic alterations harboring simultaneous RAS/RAF/MEK pathway genetic alterations (N = 9)

Patients no.	Tumor type	PI3K pathway genetic alteration	MAPK pathway genetic alteration	Target of study drug	Response (RECIST %)	TTP, wk
1	Colon	*PTEN* deletion	*KRAS* G12D mutation	MEK1/2	PD (+35)	4.0
2	Colon	*PTEN* deletion	*KRAS* G13D mutation	P38MAPK	SD (+10)	5.2
3	Colon	*PTEN* deletion	*KRAS* G12D mutation	mTORC1	PD (+28)	9.7
4	Colon	*PTEN* deletion	*KRAS* G13D mutation	mTORC1	PD (ascites)	10.4
5	Colon	*PTEN* deletion	*KRAS* G12D mutation	mTORC1 + MEK1/2	SD (−2)	12.7
6	Colon	*AKT2* amplification	*KRAS* G12D mutation	mTORC1 + MEK1/2	SD (−3)	16.0
7	Melanoma	*PTEN* deletion	*BRAF* V600E mutation	mTORC1 + MEK1/2	SD (−2)	17.2
8	Melanoma	*PTEN* deletion	*BRAF* V600E mutation	mTORC1 + MEK1/2	PR (−64)	45.8
9	Colon	*AKT2* amplification	*MAP3K10* amplification	AKT + MEK1/2	SD (−2)	15.8

Abbreviations: PR, partial response; PD, progressive disease; SD, stable disease.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed. No author received any research funding, expert testimony, or any other remuneration.

Acknowledgments

The authors thank Mary Robinson, Cally Claiborne, Cathy Lawton, Dee Cortez, and Rebecca Arcos for their assistance in the preparation of the manuscript.

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 13, 2011; revised December 13, 2011; accepted January 11, 2012; published OnlineFirst January 19, 2012.

References

1. Vivanco I, Sawyers CL. The phosphatidylinositol 3-kinase AKT pathway in human cancer. Nat Rev Cancer 2002;2:489–501.
2. Carpten JD, Faber AL, Horn C, Donoho GP, Briggs SL, Robbins CM, et al. A transforming mutation in the pleckstrin homology domain of AKT1 in cancer. Nature 2007;448:439–44.
3. Davies MA, Stemke-Hale K, Tellez C, Calderone TL, Deng W, Prieto VG, et al. A novel AKT3 mutation in melanoma tumours and cell lines. Br J Cancer 2008;99:1265–8.
4. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, et al. Mutations of the B-RAF gene in human cancer. Nature 2002;417: 949–54.
5. Allen LF, Sebolt-Leopold J, Meyer MB. CI-140 (PD184352), a targeted signal transduction inhibitor of MEK (MAPKK). Semin Oncol 2003; 30:105–66.
6. Wellbrock C, Karasarides M, Marais R. The RAF proteins take center stage. Nat Rev Mol Cell Biol 2004;5:875–85.
7. Carracedo A, Pandolfi PP. The PTEN-PI3K pathway: of feedbacks and cross-talks. Oncogene 2008;27:5527–41.
8. Kinkade CW, Castillo-Martin M, Puzio-Kuter A, Yan J, Foster TH, Gao H, et al. Targeting AKT/mTOR and ERK MAPK signalling inhibits hormone- refractory prostate cancer in a preclinical mouse model. J Clin Invest 2008;118:3051–64.
9. Russo AE, Torrisi E, Bevelacqua Y, Perrotta R, Libra M, McCubrey JA, et al. Melanoma: molecular pathogenesis and emerging target therapies (review). Int J Oncol 2009;34:1481–89.
10. Hoeflich KP, O'Brien C, Boyd Z, Cavet G, Guerrero S, Jung K, et al. In vivo antitumor activity of MEK and phosphatidylinositol 3-kinase inhibitors in basal-like breast cancer models. Clin Cancer Res 2009;15: 4649–64.
11. Engelman JA. Targeting PI3K signalling in cancer: opportunities, challenges and limitations. Nat Rev Cancer 2009;9:550–62.
12. Ligresti G, Militello L, Steelman LS, Cavallaro A, Basile F, Nicoletti F, et al. PIK3CA mutations in human solid tumors: role in sensitivity to various therapeutic approaches. Cell Cycle 2009;8:1352–8.
13. Ihle NT, Lemos R Jr, Wipf P, Yacoub A, Mitchell C, Siwak D, et al. Mutations in the phosphatidylinositol-3-kinase pathway predict for antitumor activity of the inhibitor PX-866 whereas oncogenic Ras is a dominant predictor for resistance. Cancer Res 2009;69: 143–50.
14. De Roock W, Claes B, Bernasconi D, De Schutter J, Biesmans B, Fountzilas G, et al. Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. Lancet Oncol 2010;11:753–62.
15. Velho S, Oliveira C, Ferreira A, Ferreira AC, Suriano G, Schwartz S Jr, et al. The prevalence of PIK3CA mutations in gastric and colon cancer. Eur J Cancer 2005;41:1649–54.
16. Benvenuti S, Frattini M, Arena S, Zanon C, Cappelletti V, Coradini D, et al. PIK3CA cancer mutations display gender and tissue specificity patterns. Hum Mutat 2008;29:284–8.
17. Nosho K, Kawasaki T, Ohnishi M, Suemoto Y, Kirkner GJ, Zepf D, et al. PIK3CA mutation in colorectal cancer: relationship with genetic and epigenetic alterations. Neoplasia 2008;10:534–41.
18. Prenen H, De Schutter J, Jacobs B, De Roock W, Biesmans B, Claes B, et al. PIK3CA mutations are not a major determinant of resistance to the epidermal growth factor receptor inhibitor cetuximab in metastatic colorectal cancer. Clin Cancer Res 2009;15:3184–8.
19. Wee S, Jagani Z, Xiang KX, Loo A, Dorsch M, Yao YM, et al. PI3K pathway activation mediates resistance to MEK inhibitors in KRAS mutant cancers. Cancer Res 2009;69:4286–93.
20. Janku F, Tsimberidou AM, Garrido-Laguna I, Wang X, Luthra R, Hong DS, et al. PIK3CA mutations in patients with advanced cancers treated with PI3K/AKT/mTOR axis inhibitor. Mol Cancer Ther 2011;10:558–65.
21. Engelman JA, Chen L, Tan X, Crosby K, Guimaraes AR, Upadhyay R, et al. Effective use of PI3K and MEK inhibitors to treat mutant Kras G12D and PIK3CA H1047R murine lung cancers. Nat Med 2008;14: 1351–6.
22. Ogino S, Nosho K, Kirkner GJ, Shima K, Irahara N, Kure S, et al. PIK3CA mutation is associated with poor prognosis among patients with curatively resected colon cancer. J Clin Oncol 2009;27:1477–84.
23. Janku F, Lee JJ, Tsimberidou AM, Hong DS, Naing A, Falchook GS, et al. PIK3CA mutations frequently coexist with RAS and BRAF mutations in patients with advanced cancers. PLoS One 2011;6: e22769.
24. Flaherty KT, Puzanov I, Kim KB, Ribas A, McArthur GA, Sosman JA, et al. Inhibition of mutated activated BRAF in metastatic melanoma. N Engl J Med 2010;363:809–19.
25. Dankort D, Curley DP, Cartlidge RA, Nelson B, Karnezis AN, Damsky WE Jr, et al. Braf (V600E) cooperates with Pten loss to induce metastatic melanoma. Nat Genet 2009;41:544–52.
26. Xing F, Persaud Y, Pratilas CA, Taylor BS, Janakiraman M, She QB, et al. Concurrent loss of the PTEN and RB1 tumor suppressors attenuates RAF dependence in melanomas harboring (V600E) BRAF. Oncogene. 2011 Jul 4. [Epub ahead of print].