

## Antitumor Activity in RAS-Driven Tumors by Blocking AKT and MEK

Anthony W. Tolcher<sup>1</sup>, Khurum Khan<sup>2</sup>, Michael Ong<sup>2</sup>, Udai Banerji<sup>2</sup>, Vassiliki Papadimitrakopoulou<sup>3</sup>, David R. Gandara<sup>4</sup>, Amita Patnaik<sup>1</sup>, Richard D. Baird<sup>2</sup>, David Olmos<sup>2</sup>, Christopher R. Garrett<sup>3</sup>, Jeffrey M. Skolnik<sup>5</sup>, Eric H. Rubin<sup>6</sup>, Paul D. Smith<sup>5</sup>, Pearl Huang<sup>6</sup>, Maria Learoyd<sup>5</sup>, Keith A. Shannon<sup>6</sup>, Anne Morosky<sup>6</sup>, Ernestina Tetteh<sup>6</sup>, Ying-Ming Jou<sup>6</sup>, Kyriakos P. Papadopoulos<sup>1</sup>, Victor Moreno<sup>2</sup>, Brianne Kaiser<sup>1</sup>, Timothy A. Yap<sup>2</sup>, Li Yan<sup>6</sup>, and Johann S. de Bono<sup>2</sup>

### Abstract

**Purpose:** *KRAS* is the most commonly mutated oncogene in human tumors. *KRAS*-mutant cells may exhibit resistance to the allosteric MEK1/2 inhibitor selumetinib (AZD6244; ARRY-142886) and allosteric AKT inhibitors (such as MK-2206), the combination of which may overcome resistance to both monotherapies.

**Experimental Design:** We conducted a dose/schedule-finding study evaluating MK-2206 and selumetinib in patients with advanced treatment-refractory solid tumors. Recommended dosing schedules were defined as MK-2206 at 135 mg weekly and selumetinib at 100 mg once daily.

**Results:** Grade 3 rash was the most common dose-limiting toxicity (DLT); other DLTs included grade 4 lipase increase, grade 3 stomatitis, diarrhea, and fatigue, and grade 3 and grade 2 retinal pigment epithelium detachment. There were no meaningful pharmacokinetic drug–drug interactions. Clinical antitumor activity included RECIST 1.0–confirmed partial responses in non–small cell lung cancer and low-grade ovarian carcinoma.

**Conclusion:** Responses in *KRAS*-mutant cancers were generally durable. Clinical cotargeting of MEK and AKT signaling may be an important therapeutic strategy in *KRAS*-driven human malignancies (Trial NCT number NCT01021748). *Clin Cancer Res*; 21(4): 739–48. ©2014 AACR.

### Introduction

Kirsten rat sarcoma viral oncogene homolog (*KRAS*) is frequently mutated. This results in deregulated signaling via the Ras/Raf/MEK/ERK pathway in human cancers, which promotes neoplastic transformation and maintenance of a malignant pheno-

type. Ras signaling may be activated through direct interactions with numerous growth factor receptors, or independently stimulated by somatically acquired mutations in approximately 20% of human cancers, making this protein an important therapeutic target (1, 2). Nevertheless, to date, direct targeting of Ras activation by guanosine triphosphate (GTP) interaction has not been clinically feasible despite substantial research efforts (3). Furthermore, single-agent inhibition of downstream effector pathways through the use of MEK or protein kinase B (AKT) inhibitors has not led to significant clinical antitumor activity in *KRAS*-mutant tumors (4, 5).

Numerous preclinical models have suggested that *KRAS*-mutant tumors require cotargeting of the Ras/Raf/MEK/ERK and PI3K/AKT pathways due to multiple points of cross-talk, negative feedback, and redundancy (6, 7). Inhibition of MEK by selumetinib in *KRAS*-mutant cancers can result in reactive upregulation of AKT phosphorylation (8), while cotargeting of PI3K and MEK ablates this compensatory effect and results in superior antitumor efficacy, in contrast with inhibition of either pathway alone (9). Mutations in the PI3K/AKT and Ras/Raf pathways frequently coexist in advanced cancers (10), whereas coactivating mutations are often found in treatment-resistant *KRAS*-mutant tumor models (11). For example, in *KRAS*-mutant cell lines treated with MEK inhibitors, activating *PIK3CA* mutations or *PTEN* loss lead to MEK inhibition resistance, which can be reversed by coinhibition of the PI3K/AKT pathway (12). *PIK3CA* mutations concurrent with *KRAS* mutations appear to drive AKT signaling, restoring cyclin D1 expression and allowing G<sub>1</sub>–S cell-cycle progression by underlying mechanisms independent of *KRAS*-mediated MEK/ERK

<sup>1</sup>South Texas Accelerated Research Therapeutics, START Center for Cancer Care, San Antonio Texas. <sup>2</sup>Royal Marsden NHS Foundation Trust and The Institute of Cancer Research, London, United Kingdom. <sup>3</sup>MD Anderson Cancer Center, Houston, Texas. <sup>4</sup>UC Davis Cancer Center, Sacramento, California. <sup>5</sup>AstraZeneca, Alderley Park, Macclesfield, Cheshire, United Kingdom. <sup>6</sup>Merck & Co., Inc., Whitehouse Station, New Jersey.

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

Prior presentation: Preliminary data were presented in part as oral and poster presentations at the 47th and 48th American Society of Clinical Oncology Annual Meetings, Chicago, Illinois, USA (2011 and 2012, respectively).

Current address for J.M. Skolnik: Tetralogic Pharmaceuticals, Malvern, Pennsylvania; current address for P. Huang: Roche, Basel, Switzerland; current address for Y.-M. Jou: Bristol-Myers Squibb, Hopewell, New Jersey; and current address for A. Morosky: ExecuPharm on assignment at GlaxoSmithKline, Collegeville, Pennsylvania

**Corresponding Author:** Johann S. de Bono, Royal Marsden NHS Foundation Trust and The Institute of Cancer Research, 15 Cotswold Road, Sutton, Surrey SM2 5PT, United Kingdom. Phone: 44-20-8722-4028; Fax: 44-20-8642-7979; E-mail: johann.de-Bono@icr.ac.uk

doi: 10.1158/1078-0432.CCR-14-1901

©2014 American Association for Cancer Research.

### Translational Relevance

RAS mutations that result in the unregulated activation of RAS signaling are common in cancer and a key unmet medical need. Therapeutic strategies that block RAS signaling could benefit many patients with cancer suffering from KRAS-mutant cancers. Preclinical studies indicate that combined MEK and AKT blockade can abrogate KRAS signaling. We conducted a combination phase I study of an MEK inhibitor (selumetinib) and an AKT inhibitor (MK2206). To minimize the drug toxicities of this combination, while maximizing antitumor activity, we evaluated several drug schedules. This trial demonstrates the complexity of drug combination trials and demonstrates that this combination strategy has antitumor activity against KRAS-mutant cancers at tolerable doses.

signaling (13). Indeed, in a KRAS-mutant lung cancer mouse model insensitive to MEK inhibition alone, combined MEK and PI3K/mTOR inhibition resulted in synergistic induction of tumor regression (14). Combinatorial inhibition of key nodes within signal transduction networks to overcome both *de novo* and acquired resistance has wide clinical applications (15), and preclinical data supporting the present combination are a successful example of this treatment strategy.

We approached targeting KRAS-mutated tumors through the dual inhibition of two major downstream signaling pathways, RAF/MEK/ERK and PI3K/AKT. Two potent, allosteric, selective, and orally administered inhibitors were combined: MK-2206, an allosteric inhibitor of AKT 1, 2, and 3 ( $IC_{50}$  = 8, 12, and 65 nmol/L, respectively), and selumetinib (AZD6244; ARRY-142886), a non-ATP competitive inhibitor of MEK ( $IC_{50}$  = 10–14 nmol/L; refs. 16, 17).

## Materials and Methods

### Preclinical studies

***In vitro cell line models.*** For determining the combination index (CI) by the Chou–Talalay method for cell lines treated with selumetinib and MK-2206, combinations of MK-2206 with AZD6244 were evaluated in 9 cancer cell lines with various mutational activations in the PI3K and/or MAPK pathways: 3 colon cancer cell lines (HCT116, HCT15, and HT29), 3 pancreatic cancer cell lines (AsPC-1, BxPC-3, and MIA-Pa-Ca2), 2 lung cancer cell lines (Calu-6, NCI-H460), and 1 melanoma (A2058) cell line. Human cell lines were purchased from the American Type Culture Collection (ATCC), which employs short tandem repeat (STR) profiling to ensure cell line authenticity. The fixed-ratio experimental design originally described by Chou–Talalay was used (18). The *in vitro* antiproliferative potencies ( $IC_{50}$ ) of selumetinib and MK-2206 as single agents were first determined separately to yield the  $IC_{50\text{-selumetinib}}/IC_{50\text{-MK-2206}}$  ratio. A dilution series of selumetinib/MK-2206 combinations in which the ratio of selumetinib/MK-2206 was fixed and equal to the  $IC_{50\text{-selumetinib}}/IC_{50\text{-MK-2206}}$  ratio was then prepared. Corresponding single-agent dilution series of selumetinib and MK-2206 were also prepared. The 3 dilution series were tested in the proliferation/viability assay (CellTiter-Glo Luminescent Cell Viability Assay; Promega). The data were analyzed using CalcuSyn software that calculates the CI for each combination of selume-

tinib/MK-2206.  $CI < 0.9$  indicates synergism;  $CI = 0.9$  to  $1.1$  indicates additivity; and  $CI > 1.1$  indicates antagonism. Enhanced apoptotic cell death was assessed by luminescence assay for caspase-3/7 activity after 24 hours.

***In vivo studies.*** CD1-nude mice bearing HCT116 tumor xenografts were selected as a model for study (Supplementary Table S1). Selumetinib at 25 mg/kg was orally administered twice daily on days 0 to 4 and days 7 to 11. MK-2206 at 120 mg/kg was orally administered once-every-other-day (QOD) for 2 weeks.

CD1-nude mice bearing A2058 tumor xenografts were selected as a model for study. Selumetinib at 25 mg/kg was orally administered twice daily on days 0 to 4 and days 7 to 11. MK-2206 at 120 mg/kg was orally administered QOD for 2 weeks.

### Clinical study

This was a two-part, phase I study (Merck Sharp & Dohme Corp.; MK-2206 Study Number 010; ClinicalTrials.gov identifier: NCT01021748) organized as below to achieve the following:

- Dose finding: Identify the MTD of combination therapy with oral MK-2206 and oral selumetinib (capsule formulation) in patients with locally advanced or metastatic solid tumors.
- MTD expansion: Confirm the MTD of combination therapy of oral MK-2206 and oral selumetinib (capsule formulation) in a select cohort of patients with KRAS-mutant non-small cell lung cancer (NSCLC).

In the dose-finding portion of the study, sequential cohorts of 3 to 6 patients were enrolled into panels representing different dose levels of combination therapy to determine a preliminary MTD. The dose-escalation schedule initially followed a QOD schedule for MK-2206 in combination with selumetinib. Subsequent escalation included evaluation of the once-weekly dosing schedule of MK-2206 in combination with selumetinib.

Patients evaluated in the determination of dose-escalation decisions must have received  $\geq 80\%$  of planned study combination therapy during the first 28-day cycle [unless they experienced a dose-limiting toxicity (DLT) before completing cycle 1]. Patients who failed to begin trial treatment, or who did not complete at least 80% of study therapy, were replaced for determination of the dose-escalation decision. For any initial cohort of 3 patients, up to 3 additional patients were enrolled (to make the total number of evaluable patients = 6) to further evaluate safety and tolerability. The dose level administered to each subsequent cohort of patients was to be determined based on the total number of DLT observed at the current dose relative to the total number of patients treated and evaluable for DLT at the current dose. Dose escalation continued until the MTD or maximum planned dose was reached according to the modified toxicity probability interval (mTPI) approach (Supplementary Table S2).

The study was conducted in accordance with Good Clinical Practice guidelines and in compliance with local and/or national regulations and in accordance with the ethical principles that have their origin in the Declaration of Helsinki. All patients gave informed consent, and approval was obtained from the ethics committees at each participating institution.

Patients were evaluated for tumor response using CT or MRI at baseline and every 8 weeks during the study. Tumor response was assessed by RECIST 1.0 (19). Where appropriate, different tumor markers were used to assess the effects of the combination treatment on the respective tumor types. Patients received MK-

**Table 1.** CI<sup>a</sup> by the Chou–Talalay method for selumetinib and MK-2206<sup>b</sup>

Cell line	Cancer type	KRAS	PIK3CA	BRAF	PTEN	CI: ED <sub>50</sub>	CI: ED <sub>75</sub>	CI: ED <sub>90</sub>
HCT116	Colon	G13D	H1047R	WT	WT	0.20	0.14	0.11
HCT15	Colon	G13D	E545K	WT	WT	0.12	0.12	0.12
HT29	Colon	WT	P449T	V600E	WT	0.68	0.73	0.82
A2058	Melanoma	WT	WT	V600E	Del	0.35	0.19	0.12
AsPC-1	Pancreatic	G12D	WT	WT	WT	0.18	0.09	0.15
MIA-Pa-Ca2	Pancreatic	G12C	WT	WT	WT	0.42	0.25	0.58
Calu-6	Lung	Q61K	WT	WT	WT	0.25	0.20	0.18
HCI-H460	Lung	Q61H	E545K	WT	WT	0.04	0.03	0.02

<sup>a</sup>Cell Lines were studied across 2-fold serial dilutions spanning the IC<sub>50</sub> for each cell line for each individual drug. Shown is the CI, which calculates the combination effects as a function of the slopes of the inhibition curves independent of any specific drug concentration.

<sup>b</sup>An additional analysis was performed on an expanded set of colorectal cancer cell lines harboring either wild-type or mutant *K-RAS*, *B-RAF*, or *PIK3CA*, which were analyzed for sensitivity to MEKi or AKTi + MEKi. The results suggest that *PIK3CA* mutant status predicts resistance to single-agent MEKi treatment, whereas combination treatment of AKTi + MEKi reversed this effect. MEKi sensitivity was not altered in the presence of either wild-type or mutant *K-RAS* or *B-RAF* status in these lines.

2206 and selumetinib until disease progression, intolerable adverse event, or consent withdrawal.

Sampling for pharmacokinetic determinations of MK-2206 and selumetinib (including the metabolite *N*-desmethyl selumetinib) was conducted during the first cycle of combination therapy. Predose and serial postdose specimens were taken on either days 1 and 22 (once-weekly dosing schedules) or days 1 and 27 (QOD dosing schedules). Additional predose collection time points included days 2, 3, 5, 8, 15, 23, 24, 26, and 29 for patients on the MK-2206 once-weekly dosing schedule, and days 2, 3, 7, 15, 21, 28, and 29 for patients on the QOD dosing schedule.

Plasma (circulating nucleic acid) and archival tumor (formalin-fixed, paraffin-embedded tissue) specimens were collected at baseline. Analysis focused on PI3K or BRAF pathway activation events such as the mutation status of *KRAS*, *BRAF*, and *PIK3CA*.

## Results

Previous preclinical studies of MK-2206 and selumetinib monotherapy each identified dose-dependent growth inhibition in tumor-bearing mouse models and showed evidence of target inhibition (20). We evaluated synergism between MK-2206 and selumetinib by conducting a fixed-ratio experiment as described by the Chou–Talalay method in 8 cancer cell lines derived from colon, pancreatic, melanoma, and lung cancers (18). Table 1 shows combination indices <0.9, the threshold for synergy, in all *KRAS*-mutant cell lines and cell lines driven by Raf/MEK/ERK and PI3K/AKT signaling.

We then examined the effects of combined treatment on cell survival of *KRAS*-mutant HCT116 cells (containing the *KRAS* G13D mutation). Induction of activated caspase-3 and -7 in HCT116 cells was significantly higher after 24 hours of cotreatment with MK-2206 and selumetinib compared with either agent alone, as determined by luminescence assay (Fig. 1A). FACS cell-cycle profiles of HCT116 cells exposed to MK-2206 and/or selumetinib for 72 hours showed that cotreatment resulted in the accumulation of a significantly greater proportion of cells in the sub-G<sub>1</sub> phase than either MEK or AKT blockade alone (Fig. 1B). Inhibition of downstream signaling biomarkers was assessed by immunoblotting following incubation of cells with MK-2206 and/or selumetinib for 24 hours (Fig. 1C). Selumetinib alone blocked pT202/Y204 ERK completely, but incompletely suppressed pS235/236 S6 ribosomal protein (S6RP) and pT70 4E-BP1, and did not affect pS473 AKT. In contrast, monotherapy with MK-2206 completely abrogated pS473 AKT, but incompletely inhibited pT70 4E-BP1, and had no effect on pT202/Y204 ERK or

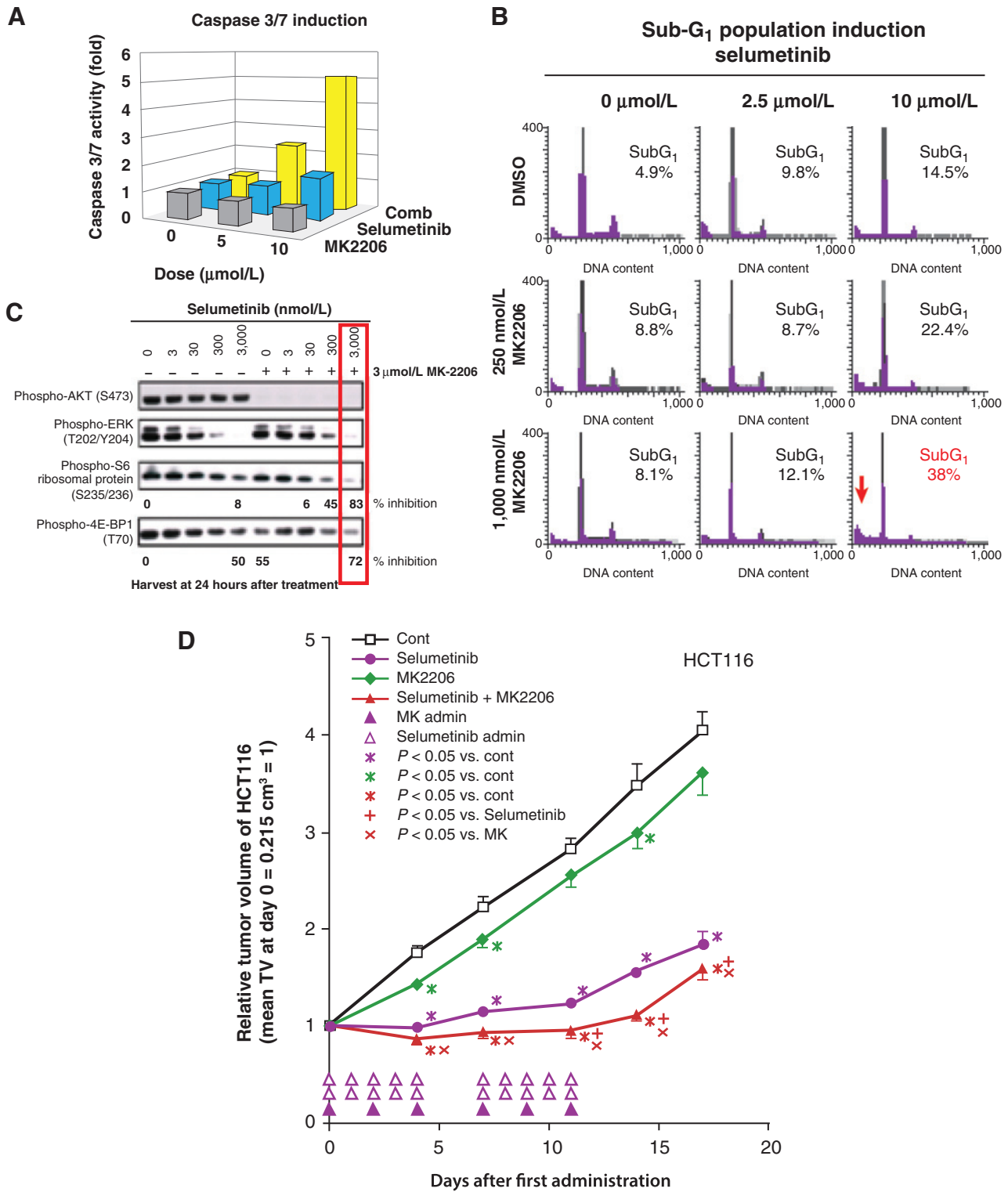
pS235/236 S6RP. Combined treatment resulted in significant inhibition of pS473 AKT, pT202/Y204 ERK, pS235/236 S6RP, and pT70 4E-BP1.

Xenograft studies of *KRAS*-mutant HCT116 demonstrated inhibition of tumor growth by selumetinib, but little antitumor activity with MK-2206 alone (Fig. 1D; Supplementary Table S1). Combined treatment with MK-2206 and selumetinib resulted in moderate but significant increase in tumor regression over time compared with selumetinib alone. All treatments were well tolerated by the animals, with no mortalities or adverse clinical signs. A decrease in body weight was observed in tumor-bearing nude mice. The body weight reduction was very slightly enhanced by the combination (Supplementary Fig. S1).

In A2058 melanoma xenograft mouse models harboring a *BRAF* V600E mutation and *PTEN* loss, but not *KRAS* or *PIK3CA* mutations, only modest antitumor activity was observed with MK-2206 and selumetinib monotherapy, whereas combination treatment again demonstrated enhanced antitumor responses (Supplementary Fig. S2; Supplementary Table S3). Preclinical *in vivo* toxicity studies for each agent alone indicated little evidence of overlapping histomorphologic changes, with the exception of alterations in hemolymphatic and gastrointestinal tract systems at poorly tolerated doses in nonrodents. A decrease in body weight was observed in tumor-bearing nude mice with no mortalities or adverse clinical signs. The body weight reduction was very slightly enhanced by the combination (Supplementary Fig. S1).

## Clinical studies

Based upon the strong scientific rationale for the coinhibition of AKT and Ras/Raf pathways, and preclinical evidence of synergy and tolerability, we initiated a phase I clinical study combining MK-2206 and selumetinib. No formal drug–drug interaction studies were conducted to assess the potential for an interaction between selumetinib and MK-2206, as the risk of a drug–drug interaction was considered low based upon the metabolism of each drug. Specifically, neither of these drugs is a potent inhibitor of CYP1A2, CYP2C19, or CYP3A4, and although both selumetinib and MK-2206 are substrates of P-glycoprotein, selumetinib is not an inhibitor and MK-2206 is only a weak inhibitor of this transporter. We had previously characterized the pharmacokinetic and pharmacodynamic profile of each drug in studies with paired tumor biopsies (16, 17, 21). The MTD for MK-2206 is 60 mg QOD or 200 mg weekly. At 60 mg QOD, the terminal half-life (*t*<sub>1/2</sub>) of MK-2206 was 71.3 hours, and the median suppression of post-dose tumor pS473 AKT was 81%. For selumetinib, the hydrogen sulfate oral capsule formulation was used, with prior phase I trials



**Figure 1.** Activated caspase induction, cell-cycle profiles, and pERK/pAKT profiles of HCT116 cell lines and HCT116 xenografts in rodents treated with MK-2206 and/or selumetinib. A, caspase-3/7 activity after 24 hours was determined by luminescence assay. Data were indicated as fold induction of caspase against the signals of DMSO-treated cells. B, percent of sub-G<sub>1</sub> population in cell cycle was determined with FACS when HCT116 cells were exposed with MK-2206 and/or selumetinib for 72 hours. C, HCT116 colon cancer cells were treated with MK-2206 and/or selumetinib at the indicated concentration for 24 hours. The cell lysates were analyzed by Western blot with the indicated antibodies. D, selumetinib alone showed potent antitumor efficacy in this animal model. However, MK-2206 had only marginal antitumor effect. The combination therapy produced a tumor regression with statistically significant increase in the antitumor response as compared with monotherapy ( $P < 0.05$ ).

Downloaded from <http://aacrjournals.org/clinccancerres/article-pdf/21/4/739/2027896/739.pdf> by guest on 28 May 2024

having established the MTD as 75 mg twice daily and the terminal  $t_{1/2}$  as 5.3 hours (16) with this formulation; previously, a dose of 100 mg twice daily with freebase powder formulation had been established (21). Suppression of pERK at 6 hours was observed in both peripheral blood mononuclear cells and paired tumor biopsies. Clinical toxicities of MK-2206 were mainly erythematous maculopapular rash and stomatitis, whereas those for selumetinib included acneiform dermatitis, diarrhea, fatigue, nausea, and peripheral edema. Rash was a DLT for both drugs, likely reflecting on-target effects of AKT and MEK inhibition for MK-2206 and selumetinib, respectively.

In this phase I study, initial cohorts of 3 to 6 evaluable patients with advanced, treatment-refractory solid tumors were recruited and given combinations of MK-2206 and selumetinib. Additional patients were enrolled to evaluate tolerability according to the mTPI approach as reflected in Supplementary Table S2. Fifty-one patients received treatment during the dose-escalation portion of the study, with 46 evaluable for dose-escalation safety assessment (Table 2). Five patients were considered nonevaluable for the dose-escalation safety assessment because they did not complete  $\geq 80\%$  of the first cycle of treatment or due to either noncompliance with study medication ( $n = 2$ ), or non-DLT ( $n = 3$ ; 1 of the 3 patients experienced rapid disease progression and was discontinued after 1 week of therapy). Dose escalation of either or both drugs aimed to define the MTD as the highest dose at which  $< 20\%$  patients experienced a DLT (22). A dose-expansion cohort at the MTD recruited an additional 11 patients with *KRAS*-mutant NSCLC; this cohort was selected based on preclinical antitumor activity (20)—observed antitumor activity during dose escalation (Fig. 2)—and was further supported by data from a parallel randomized phase II trial showing activity of selumetinib in combination with docetaxel in advanced *KRAS*-mutant NSCLC (23).

The starting dose for combination therapy was MK-2206 45 mg QOD, which represented 75% of the QOD MTD (60 mg), administered with the monotherapy MTD of 75 mg twice daily of selumetinib. However, combined treatment at this dose resulted in an unacceptably high rate of DLT, with 2 of 3 evaluable patients having National Cancer Institute Common Toxicity Criteria (NCI-CTCAE) version 3.0 grade 3 maculopap-

**Table 2.** Patient demographics

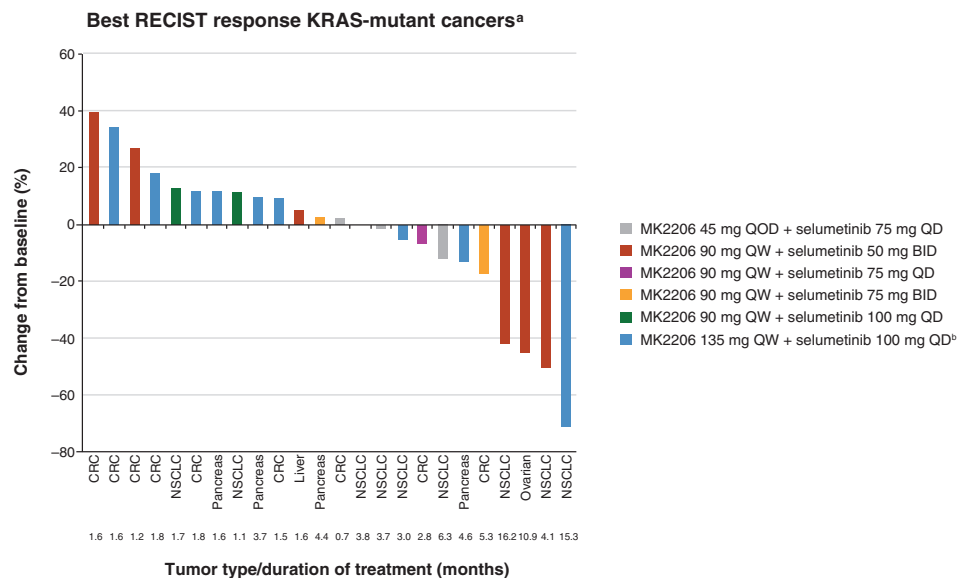
Patient characteristics	Patients (N = 62), n (%)
Age, years	
Median	58
Range	33–81
Performance status	
0	22 (35)
1	40 (65)
Male/females	26/36 (42/58)
Patients with prior chemotherapy	62
Median number of prior chemotherapy regimens	3
Range	1–10
Tumor types	Total
Non–small cell lung	19 (31)
<i>KRAS</i> mutant	13 (21)
Colorectal	14 (23)
<i>KRAS</i> mutant	9 (15)
Ovarian	5 (8)
<i>KRAS</i> mutant	2 (3)
Pancreatic	5 (8)
<i>KRAS</i> mutant	4 (6)
Breast	3 (5)
Leiomyosarcoma	3 (5)
Ewing sarcoma	2 (3)
Prostate	2 (3)
Melanoma	2 (3)
Appendix, cervical (squamous), chondrosarcoma, esophageal, liver, Merkel cell, thyroid	7 total [1 each](11 [2])
<i>KRAS</i> mutant	1 (2)

ular rash (Supplementary Fig. S3). At 45 mg QOD of MK-2206 and selumetinib 75 mg once daily, grade 1 rash and grade 3 diarrhea were reported, but no DLTs were observed. This dose was determined to be the MTD of QOD dosing of MK-2206 with selumetinib.

The MK-2206 schedule was then changed to once-weekly dosing based on monotherapy studies demonstrating its long terminal elimination half-life, reduced drug accumulation, improved tolerability, and pharmacodynamic data suggesting ongoing target inhibition at day 5 after dose (17). The initial combination schedule of MK-2206 90 mg once weekly with selumetinib at

**Figure 2.**

Waterfall plot of RECIST responses in evaluable patients with *KRAS*-mutant cancers. <sup>a</sup>These data only reflect subjects who were re-evaluated after the baseline scan. <sup>b</sup>Recommended phase II dose for the combination. BID, twice daily; CRC, colorectal cancer; QD, once daily; QW, once weekly.



75 mg twice daily was not tolerable, with DLT of grade 2 retinal pigment epithelium detachment and grade 3 stomatitis and dermatitis acneiform observed in 3 of 7 evaluable patients. Subsequent dose levels required dose and schedule modifications from this starting dosing schedule. Doses of MK-2206 at 135 mg once weekly with selumetinib 100 mg once daily resulted in only 1 of 6 DLT, comprising grade 3 fatigue, and this was therefore defined as the recommended phase II dose. Further exploration of this dose level in an additional 11 patients (all evaluable) confirmed this dose to be well tolerated, with only 2 further patients experiencing DLT of grade 3 rash and stomatitis (Table 3).

The most common drug-related adverse events (occurring in >5 patients) are summarized in Supplementary Table S4. Rash was the most frequent adverse event and DLT; two distinct appearances were noted: a reversible maculopapular rash associated with pruritus consistent with our previous experience with MK-2206 (17) and an erythematous acneiform rash associated with selumetinib that improved with topical steroids or oral tetracycline therapy (24). Other DLT included diarrhea and stomatitis, which appeared to be dose related. No drug-related hematologic toxicities were observed in this study. Infrequent asymptomatic grade 3 increases in circulating hepatic transaminases also were reported ( $n = 5$ ), but fully normalized on temporary discontinuation of the drug. Dose-limiting detachment of retinal pigment epithelium, which was observed in 2 patients, was reversible following discontinuation of treatment.

Pharmacokinetics suggested no meaningful drug–drug interaction between MK-2206, selumetinib, and the active metabolite *N*-desmethyl selumetinib. At the combination MTD, the terminal  $t_{1/2}$  of MK-2206 was  $61.7 \pm 15.2$  hours, within range of that seen in monotherapy ( $88.9 \pm 26.9$ ). Published monotherapy pharmacokinetic data for selumetinib 100 mg capsule are not available for direct comparison with the combination MTD. However, exposure (mean  $C_{max}$  and  $t_{max}$ ) following 75 mg selumetinib plus MK-2206 was within the range previously reported for monotherapy at the same dose (16). Exposure to selumetinib increases proportionally with dose (16), and therefore was slightly higher in the combination MTD cohort treated with selumetinib 100 mg once daily [mean  $C_{max}$  1,140 ng/mL (range, 554–2,540) and  $AUC_{0-10h}$  4,500 ng·h/mL (range, 2,297–7,875)] than cohorts given 75 mg selumetinib.

Interpatient variability was moderate for MK-2206 [% coefficient of variation (CV) of  $C_{max}$  and  $AUC_{0-168h}$  ranged from 33% to 47%], and high for selumetinib (%CV of  $C_{max}$  and  $AUC_{0-10h}$  ranged from 19% to 74%). Preclinical models associated antitumor activity with MK-2206 concentrations above 57 nmol/L, a concentration at which >70% inhibition of pS473 AKT was achieved; this target steady-state trough concentration of >57 nmol/L was achieved in 100% of patients in 48 hours at the 135 mg once-weekly MTD level. In previous monotherapy studies, we have shown that pAKT and pERK were robustly suppressed at the exposures achieved at the combination MTD of MK-2206 (135 mg once weekly) and selumetinib (100 mg once daily), respectively (16, 17, 25).

In this study, 29 patients with *KRAS*-mutant cancers were treated, for whom confirmed RECIST 1.0 partial responses were observed in 3 of 13 (23%) patients with NSCLC, and 1 of 2 (50%) patients with ovarian cancer. The best antitumor responses were observed in a 59-year-old Caucasian female with chemotherapy-refractory *KRAS*-mutant lung adenocarcinoma. Overall, she had a 71% RECIST response and remained

on treatment for 15 months. A 63-year-old patient of Asian ethnicity with chemotherapy-refractory *KRAS*-mutant lung adenocarcinoma also had a 45% RECIST response and remained on study for 20 weeks (Fig. 3). One additional patient with pancreatic cancer achieved a RECIST partial response, and although *KRAS* mutations are known to occur in approximately 85% in pancreatic ductal carcinoma (26), mutation status was not available for this patient. RECIST stable disease >6 months was observed in 1 patient with NSCLC and another with low-grade ovarian cancer. In contrast, none of the 33 patients with confirmed *KRAS* wild-type tumors achieved a confirmed RECIST partial response or stable disease >6 months. Interestingly, no confirmed objective responses were observed in the colorectal cancers with *KRAS* mutations ( $n = 11$ ; Fig. 2).

The majority of *KRAS* mutations were amino acid substitutions to cysteine (21%), valine (34%), or aspartate (14%) in codon 12. The type of *KRAS* mutation, or presence of concurrent *PIK3CA* or *BRAF* mutations, did not appear to influence whether an objective response was achieved, though notably, the limited number of responders in this study prevented us from drawing a definitive conclusion. Among patients with NSCLC, 2 of 13 patients (15.4%) had concurrent *PIK3CA* mutations detected, whereas 0 of 9 patients with colorectal cancer had concurrent *PIK3CA* mutations detected.

## Discussion

Although single-agent activity for MEK inhibitors in clinical *KRAS*-mutant disease has been modest, Raf/MEK/ERK signaling is considered to be a major Ras effector pathway (4, 5). The activity we observed in *KRAS*-mutant models appeared largely driven by selumetinib, which is consistent with findings from other preclinical models combining MEK and mTOR inhibitors in this setting (8). Therefore, based on these data, we attempted to prioritize maintenance of MEK blockade, while also attempting to combine AKT inhibition. This trial highlights the multiple challenges of combining targeted agents (27). The numerous permutations of dose, schedule, and sequence result in significant complexities, and there are currently no standardized trial designs to assess optimal combination strategies. The particular challenge of combining MEK and AKT inhibition included known overlapping monotherapy toxicities, especially rash and diarrhea that were observed at the MTD of both selumetinib and MK-2206 (17, 21). Rash and diarrhea expectedly limited dosing and ultimately required de-escalation of both MK-2206 and selumetinib to improve tolerability of the combination. Although de-escalation of both selumetinib and MK-2206 from recommended monotherapy doses was required to mitigate tolerability issues, both drug doses at the combination MTD were previously shown to be biologically active in their respective single-agent studies. In this study, we successfully changed schedules of administration of both drugs (compared with single-agent phase I schedules and doses) to improve tolerability and is an example of how flexible design of early clinical trials can help circumvent toxicity (27). The pharmacokinetic data in this study suggested no drug–drug interaction between selumetinib and MK-2206, which supported the preclinical assessment of the combination having a low potential for interaction. However, as no formal drug–drug interaction assessment studies were conducted, this result must be interpreted with caution. Clinical antitumor activity was observed with durable RECIST

Table 3. Related grade 3/4 adverse events (AEs)

Dose level	MK-2206	Selumetinib	n (evaluable for safety)	DLT n (%)	Patients with C1-related grade (Gr) 3/4 AEs		Patients with C≥2-related Gr 3/4 AEs	
					Event	n	Event	n
QOD-1	45 mg QOD	75 mg BID	4 (3)	2 (66.7)	Gr 3 rash	2 <sup>a</sup>	Gr 3 ALT/AST elevation	1
QOD-1	45 mg QOD	75 mg QD	6 (6)	0 (0)	Gr 3 diarrhea	1	None	0
QW-1	90 mg QW	75 mg BID	9 (7)	3 (42.8) <sup>b</sup>	Gr 3 stomatitis	1 <sup>a</sup>	Gr 3 anemia	1
					Gr 3 rash	1 <sup>a</sup>	Gr 3 fatigue	1
							Gr 3 retinal pigment epithelium detachment <sup>b</sup>	1
QW-1a	90 mg QW	75 mg QD	7 (6)	1 (16.7)	Gr 3 diarrhea	1 <sup>a</sup>	Gr 3 ALT elevation	1
QW-1b	90 mg QW	50 mg BID	7 (6)	2 (33.3)	Gr 3 rash	2 <sup>a</sup>	Gr 3 CPK elevation	1
QW-1c	90 mg QW	100 mg QD	3 (3)	0 (0)	None	0	Gr 3 diarrhea	1
QW-1d	90 mg QW	150 mg QD	3 (3)	2 (66.7)	Gr 4 lipase elevation	1 <sup>a</sup>	None	0
					Gr 3 retinal pigment epithelium detachment	1 <sup>a</sup>	None	0
QW-1e <sup>c</sup>	135 mg QW	100 mg QD	17 (17)	3 (17.6)	Gr 3 fatigue	1 <sup>a</sup>	Gr 3 ALT/AST elevation	1
							Gr 3 dry skin	1
							Gr 3 pruritus	1
							Gr 3 rash	3
QW-1f	100 mg QW	100 mg QD	6 (6)	0 (0)	G3 lymphocyte count decreased	1	None	0
					Gr 3 pruritus	1		
					Gr 3 rash	3 <sup>a</sup> (1 DLT)		
					Gr 3 stomatitis	1 <sup>a</sup>		

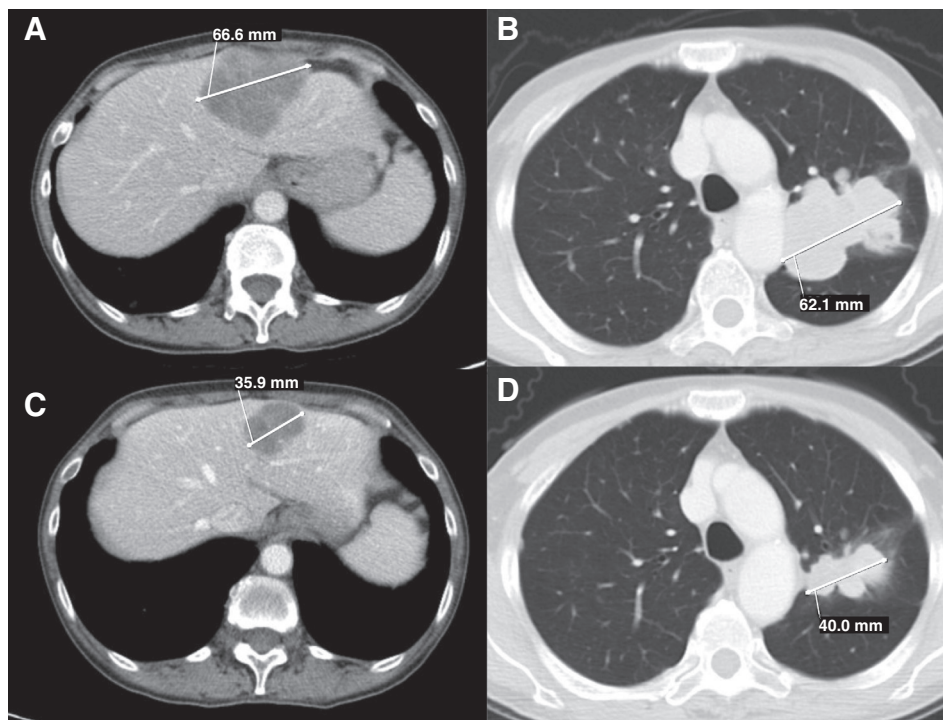
NOTE: No grade 5 events considered by the investigator to be related to either drug were reported.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BID, twice daily; CPK, creatine phosphokinase; DLT, dose-limiting toxicity; QD, once daily; QW, once weekly.

<sup>a</sup>Event counted as DLT.

<sup>b</sup>One grade 2 DLT of retinal pigment epithelium detachment was observed during cycle 1 in the same patient who experienced a grade 3 event after beginning cycle 2.

<sup>c</sup>Dose considered the MTD for the combination; 6 enrolled in dose-escalation period, 11 KRAS-mutant NSCLCs enrolled in confirmation period.



**Figure 3.**

Example responder: *KRAS*-mutant adenocarcinoma of lung. A 63-year-old female never smoker of Asian ethnicity with metastatic adenocarcinoma of the lung, which was *KRAS* G12D mutant, but wild-type for *EGFR*, anaplastic lymphoma kinase (*ALK*), *PIK3CA*, and *BRAF* gene aberrations. A and B represent pretreatment scans, whereas C and D represent the same slices of scans with best response to the treatment. This patient's prior treatments included (i) cisplatin and pemetrexed (2 cycles with disease progression); (ii) carboplatin and paclitaxel (8 cycles with partial response); (iii) docetaxel (2 cycles with disease progression); (iv) carboplatin and paclitaxel rechallenge (2 cycles with disease progression); (v) radiotherapy to spine metastases. She was treated with MK-2206 135 mg PO once weekly and selumetinib 100 mg PO once daily without dose reduction, although she had a grade 2 skin rash that was tolerable. She had a RECIST partial response with 45% reduction in the sum of diameters of the target lesions. The patient eventually developed progressive disease after 6 cycles due to the development of symptomatic brain metastases.

tumor shrinkage in *KRAS*-mutant NSCLC and low-grade ovarian carcinoma. However, no responses were observed in *KRAS*-mutant colorectal or small-bowel carcinoma, possibly suggesting distinct biologic context differences in these diseases. As this trial did not include prospective screening of concurrent mutations for study entry, the number of subjects where both *KRAS* and *PIK3CA* mutations were detected was very limited and precluded our ability to draw firm conclusions around whether these concurrent mutations could be associated with response in the clinic. Colorectal cancer has previously shown limited success to treatment with MEK inhibitor monotherapy (28). The observed heterogeneity of response among patients with *KRAS*-mutant cancers likely reflects the complexities of tumor biology and possibly the presence of other aberrant driver mutations or disruption of signaling feedback loops (29), although we have not excluded poor drug penetration to tumor in these studies. Furthermore, there may be mechanisms independent of *PI3K* and *MEK* that lead to maintenance of cellular proliferation, such as upregulation of p21-activated kinase (30) or *LKB1* mutations (31). Ultimately, the selection of molecularly targeted agents to combine on the basis of molecular profiling remains a challenging and imperfect strategy, requiring the everevolving application of an array of modern technologies, including DNA sequencing, genomics, bioinformatics, and computational approaches (15).

A combination of selumetinib and docetaxel was recently evaluated in a phase II study with patients with *KRAS*-mutant NSCLC, demonstrating an improvement in response rate, progression-free survival (5.3 months vs. 2.1 months), and median overall survival in the selumetinib combination arm compared with docetaxel alone (9.4 vs. 5.3 months), although the combination resulted in more toxicities (23).

In conclusion, these are the first clinical data to demonstrate that different *KRAS*-mutant cancers may show differential sensitivity to the cotargeting of *MEK* and *AKT* and to present a novel and rational antitumor strategy against cancers driven by a common driver mutation. Multiple other drug combinations targeting different components of the *Ras/Raf* and *PI3K/AKT* signaling pathways have also entered clinical development based on robust preclinical biology. We envision that the complex and multifaceted clinical evaluation of these drug combinations will lead to a new therapeutic avenue for many *RAS*-mutant cancers, including *KRAS* mutation-driven NSCLC and low-grade ovarian cancer. Moreover, as with other rationally designed molecularly targeted strategies (e.g., *PARP* inhibitors in *BRCA1/2*-mutant cancers), we have observed antitumor activity in patients whose tumors have the same molecular defect, but which arise from diverse geographical origins (lung, ovary, and pancreas; ref. 32). Due consideration must continue to be given in oncological drug development and registration studies to select patients based, not



simply on disease origin, but also on the underlying cancer biology.

### Disclosure of Potential Conflicts of Interest

A.W. Tolcher is a consultant/advisory board member for AbbVie, Adnexus, Ambit, AP Pharma, Aragon, Ariad, ArQule, Astellas, Astellas-Japan, Astex, Bayer, Bind Therapeutics, BioMed Valley Discoveries, Blend Therapeutics, Bristol-Myers Squibb Japan, Celator, Clovis, Curis, Daiichi Sankyo, Dicerna, Eisai, Eli Lilly, Emergent Product Development, Endo, Five Prime, Galapagos NV, Janssen, MedImmune, Merck Sharp & Dohme, Merus, Micromet, Nanobiotix, Nektar, Neumedical, Novartis, OncoGenex, Onyx, Otsuka, Pfizer, Pharmacyclics, Pierre Fabre, ProNAi, Proximagen, Sanofi-Aventis, Santaris, Symphogen, Vaccinex, and Zyngenia. U. Banerji is a consultant/advisory board member for Merck. V. Papadimitrakopoulou reports receiving commercial research grants from Astellas, AstraZeneca, Bayer, Bristol Myers Squibb, Celgene, Clovis Oncology, ITO - EN, Janssen, Merck, Novartis, and Pfizer and is a consultant/advisory board member for Amgen, Biothera, Clovis, Eli Lilly, Genentech, GlaxoSmithKline, Janssen, and Merck. D.R. Gandara reports receiving other research grants from GlaxoSmithKline and Merck. J.M. Skolnik has ownership interest (including patents) in GlaxoSmithKline. P.D. Smith has ownership interest (including patents) in AstraZeneca. P. Huang and E. Tetteh have ownership interest (including patents) in Merck. M. Learoyd is an employee of AstraZeneca. J.S. de Bono reports receiving commercial research grants from and is a consultant/advisory board member for AstraZeneca and Merck. No potential conflicts of interest were disclosed by the other authors.

### Authors' Contributions

**Conception and design:** A.W. Tolcher, K. Khan, U. Banerji, J.M. Skolnik, P. Huang, K.A. Shannon, L. Yan, J.S. de Bono

**Development of methodology:** A.W. Tolcher, J.M. Skolnik, P. Huang, K.A. Shannon, L. Yan, J.S. de Bono

**Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.):** A.W. Tolcher, K. Khan, M. Ong, U. Banerji, V. Papadimitrakopoulou, A. Patnaik, R.D. Baird, D. Olmos, C.R. Garrett, A. Morosky, K.P. Papadopoulos, V. Moreno, B. Kaiser, T.A. Yap, L. Yan, J.S. de Bono  
**Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis):** A.W. Tolcher, K. Khan, M. Ong, U. Banerji,

V. Papadimitrakopoulou, A. Patnaik, R.D. Baird, J.M. Skolnik, P. Huang, M. Learoyd, E. Tetteh, Y.-M. Jou, T. A. Yap, L. Yan, J.S. de Bono

**Writing, review, and/or revision of the manuscript:** A.W. Tolcher, K. Khan, M. Ong, U. Banerji, V. Papadimitrakopoulou, D.R. Gandara, A. Patnaik, R.D. Baird, D. Olmos, C.R. Garrett, J.M. Skolnik, E.H. Rubin, P.D. Smith, P. Huang, M. Learoyd, K.A. Shannon, A. Morosky, E. Tetteh, Y.-M. Jou, K.P. Papadopoulos, V. Moreno, T. A. Yap, L. Yan, J.S. de Bono

**Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases):** K. Khan, M. Ong, C.R. Garrett, J.M. Skolnik, M. Learoyd, K.A. Shannon, L. Yan, J.S. de Bono

**Study supervision:** A.W. Tolcher, K. Khan, M. Ong, U. Banerji, R.D. Baird, C.R. Garrett, J.M. Skolnik, K.A. Shannon, A. Morosky, L. Yan, J.S. de Bono

**Other (acted as the primary physician on the study):** K. Khan

### Acknowledgments

The authors thank Michael Lam and Emmet Schmidt, both of Merck & Co., Inc., for their contributions in interpreting the preclinical data presented in this article. Editorial assistance was provided by Amy O. Johnson-Levonas, PhD, and Kristen Lewis, both of Merck & Co., Inc., as well as Jennifer Granit, PhD, of Integrus Scientific, a division of Medicus International New York.

### Grant Support

This study was sponsored by Merck & Co., Inc., in collaboration with AstraZeneca.

The Drug Development Unit of the Royal Marsden NHS Foundation Trust and The Institute of Cancer Research is supported in part by a program grant from Cancer Research UK (grant number: C347/A18077). Support was also provided by an Experimental Cancer Medicine Centre grant (no grant number) and the National Institute for Health Research Biomedical Research Centre (jointly to the Royal Marsden NHS Foundation Trust and The Institute of Cancer Research; grant numbers: A46/CCR-CCR4057 and CCR4058).

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 July 24, 2014; revised October 30, 2014; accepted November 24, 2014; published OnlineFirst December 16, 2014.

### References

- Downward J. Targeting RAS signalling pathways in cancer therapy. *Nat Rev Cancer* 2003;3:11–22.
- Pylayeva-Gupta Y, Grabocka E, Bar-Sagi D. RAS oncogenes: weaving a tumorigenic web. *Nat Rev Cancer* 2011;11:761–74.
- Steckel M, Molina-Arcas M, Weigelt B, Marani M, Warne PH, Kuznetsov H, et al. Determination of synthetic lethal interactions in KRAS oncogene-dependent cancer cells reveals novel therapeutic targeting strategies. *Cell Res* 2012;22:1227–45.
- Carter CA, Rajan A, Szabo E, Khozin S, Thomas A, Brzezniak CE, et al. Two parallel randomized phase II studies of selumetinib (S) and erlotinib (E) in advanced non-small cell lung cancer selected by KRAS mutations. *J Clin Oncol* 2013;31. Abstract 8026.
- Hochster HS, Messersmith WA, O'Neill BH, Groshen SG, Lenz H-J, Cohen DJ, et al. The MEK inhibitor selumetinib ([SEL], AZD6244, ARRY-142886) plus irinotecan (IRI) as second-line therapy for KRAS-mutated (KRASm) metastatic colorectal cancer (CRC). *J Clin Oncol* 2013;31. Abstract 3587.
- Carracedo A, Pandolfi PP. The PTEN-PI3K pathway: of feedbacks and cross-talks. *Oncogene* 2008;27:5527–41.
- She QB, Halilovic E, Ye Q, Zhen W, Shirasawa S, Sasazuki T, et al. 4E-BP1 is a key effector of the oncogenic activation of the AKT and ERK signaling pathways that integrates their function in tumors. *Cancer Cell* 2010;18:39–51.
- Holt SV, Logie A, Davies BR, Alferrez D, Runswick S, Fenton S, et al. Enhanced apoptosis and tumor growth suppression elicited by combination of MEK (selumetinib) and mTOR kinase inhibitors (AZD8055). *Cancer Res* 2012;72:1804–13.
- Hofmann I, Weiss A, Elain G, Schwaederle M, Sterker D, Romanet V, et al. KRAS mutant pancreatic tumors show higher sensitivity to MEK than to PI3K inhibition in vivo. *PLoS One* 2012;7:e44146.
- 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.
- Jaiswal BS, Janakiraman V, Kljavin NM, Eastham-Anderson J, Cupp JE, Liang Y, et al. Combined targeting of BRAF and CRAF or BRAF and PI3K effector pathways is required for efficacy in NRAS mutant tumors. *PLoS One* 2009;4:e5717.
- 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.
- Halilovic E, She QB, Ye Q, Pagliarini R, Sellers WR, Solit DB, Rosen N. PIK3CA mutation uncouples tumor growth and cyclin D1 regulation from MEK/ERK and mutant KRAS signaling. *Cancer Res* 2010;70:6804–14.
- 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.
- Al-Lazikani B, Banerji U, Workman P. Combinatorial drug therapy for cancer in the post-genomic era. *Nat Biotechnol* 2012;30:679–92.
- Banerji U, Camidge DR, Verheul HM, Agarwal R, Sarker D, Kaye SB, et al. The first-in-human study of the hydrogen sulfate (Hyd-sulfate) capsule of the MEK1/2 inhibitor AZD6244 (ARRY-142886): a phase I open-label

- multicenter trial in patients with advanced cancer. *Clin Cancer Res* 2010;16:1613–23.
17. Yap TA, Yan L, Patnaik A, Fearen I, Olmos D, Papadopoulos K, et al. First-in-man clinical trial of the oral pan-AKT inhibitor MK-2206 in patients with advanced solid tumors. *J Clin Oncol* 2011;29:4688–95.
  18. Chou TC, Talalay P. Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. *Adv Enzyme Regul* 1984;22:27–55.
  19. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* 2000;92:205–16.
  20. Meng J, Dai B, Fang B, Bekele BN, Borrmann WG, Sun D, et al. Combination treatment with MEK and AKT inhibitors is more effective than each drug alone in human non-small cell lung cancer in vitro and in vivo. *PLoS One* 2010;5:e14124.
  21. Adjei AA, Cohen RB, Franklin W, Morris C, Wilson D, Molina JR, et al. Phase I pharmacokinetic and pharmacodynamic study of the oral, small-molecule mitogen-activated protein kinase kinase 1/2 inhibitor AZD6244 (ARRY-142886) in patients with advanced cancers. *J Clin Oncol* 2008;26:2139–46.
  22. Ji Y, Li Y, Nebiyu Bekele B. Dose-finding in phase I clinical trials based on toxicity probability intervals. *Clin Trials* 2007;4:235–44.
  23. Jänne PA, Shaw AT, Pereira JR, Jeannin G, Vansteenkiste J, Barrios C, et al. Selumetinib plus docetaxel for KRAS-mutant advanced non-small-cell lung cancer: a randomised, multicentre, placebo-controlled, phase 2 study. *Lancet Oncol* 2013;14:38–47.
  24. Banerji U, van Doorn L, Papadatos-Pastos D, Kristeleit R, Debnam P, Tall M, et al. A phase I pharmacokinetic and pharmacodynamic study of CHR-3996, an oral class I selective histone deacetylase inhibitor in refractory solid tumors. *Clin Cancer Res* 2012;18:2687–94.
  25. Yap TA, Patnaik A, Fearen D, Olmos D, Papadopoulos N, Tunariu D, et al. First-in-class phase I trial of a selective Akt inhibitor, MK2206 (MK), evaluating alternate day (QOD) and once weekly (QW) doses in advanced cancer patients (pts) with evidence of target modulation and antitumor activity. *J Clin Oncol* 2010;28:15s. Abstract 3009.
  26. Bos JL. RAS oncogenes in human cancer: a review. *Cancer Res* 1989;49:4682–9.
  27. Yap TA, Omlin A, de Bono JS. Development of therapeutic combinations targeting major cancer signaling pathways. *J Clin Oncol* 2013;31:1592–1605.
  28. Bennouna J, Lang I, Valladares-Ayerbes M, Boer K, Adenis A, Escudero P, et al. A Phase II, open-label, randomised study to assess the efficacy and safety of the MEK1/2 inhibitor AZD6244 (ARRY-142886) versus capecitabine monotherapy in patients with colorectal cancer who have failed one or two prior chemotherapeutic regimens. *Invest New Drugs* 2011;29:1021–28.
  29. Prahallad A, Sun C, Huang S, Di Nicolantonio F, Salazar R, Zecchin D, et al. Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR. *Nature* 2012;483:100–3.
  30. Tabusa H, Brooks T, Massey AJ. Knockdown of PAK4 or PAK1 inhibits the proliferation of mutant KRAS colon cancer cells independently of RAF/MEK/ERK and PI3K/AKT signaling. *Mol Cancer Res* 2013;11:109–21.
  31. Ji H, Ramey MR, Hayes DN, Fan C, McNamara K, Kozlowski P, Torrice C, et al. LKB1 modulates lung cancer differentiation and metastasis. *Nature* 2007;448:807–10.
  32. Fong PC, Boss DS, Yap TA, Tutt A, Wu P, Mergui-Roelvink M, et al. Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. *N Engl J Med* 2009;361:123–34.