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Permalink

<https://escholarship.org/uc/item/6c2779pg>

Journal

Cancer Chemotherapy and Pharmacology, 85(4)

ISSN

0344-5704

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Publication Date

2020-04-01

DOI

10.1007/s00280-020-04038-8

Supplemental Material

<https://escholarship.org/uc/item/6c2779pg#supplemental>

Peer reviewed

Article Type: Original Article

Title: Phase I Dose-Escalation Trial of the Oral AKT Inhibitor Uprosertib in Combination With the Oral MEK1/MEK2 Inhibitor Trametinib in Patients With Solid Tumors

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Running Head: Phase I Trial of Uprosertib and Trametinib in Patients With Solid Tumors

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Abstract

Purpose: This study aimed to determine the safety, tolerability, and recommended phase II doses of trametinib plus uprosertib (GSK2141795) in patients with solid tumors likely to be sensitive to MEK and/or AKT inhibition.

Methods: This was a phase I, open-label, dose-escalation, and dose-expansion study in patients with triple-negative breast cancer or *BRAF*-wild type advanced melanoma. The primary outcome of the expansion study was investigator-assessed response. Among 126 enrolled patients, 63 received continuous oral daily dosing of trametinib and uprosertib, 29 received various alternative dosing schedules, and 34 were enrolled into expansion cohorts. Doses tested in the expansion cohort were trametinib 1.5 mg once daily (QD) + uprosertib 50 mg QD.

Results: Adverse events (AEs) were consistent with those reported in monotherapy studies but occurred at lower doses and with greater severity. Diarrhea was the most common dose-limiting toxicity; diarrhea and rash were particularly difficult to tolerate. Overall, 59% and 6% of patients reported AEs with a maximum severity of grade 3 and 4, respectively. Poor tolerability prevented adequate delivery of uprosertib with trametinib at a concentration predicted to have clinical activity. The study was terminated early based on futility in the continuous-dosing expansion cohorts and a lack of pharmacological or therapeutic advantage with intermittent dosing. The objective response rate was <5% (1 complete response, 5 partial responses).

Conclusions: Continuous and intermittent dosing of trametinib in combination with uprosertib was not tolerated, and minimal clinical activity was observed in all schedules tested.

Keywords: AKT inhibitor, *BRAF*-wild type melanoma, MEK inhibitor, trametinib, triple-negative breast cancer, uprosertib

Introduction

The mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K)/AKT pathways interact extensively and are involved in the regulation of cell proliferation, apoptosis, metabolism, and immunity [1]. These pathways share common signaling inputs, are activated by oncogenic RAS, and appear to provide reciprocal compensatory signaling when one pathway is inhibited [1-3]. Co-activation of both pathways occurs in melanoma, prostate cancer, and colorectal cancer [4-6], and dual inhibition may be required for complete inhibition of the downstream effector pathway [2, 7]. Preclinical studies suggest that co-targeting both pathways can result in additive or synergistic inhibition and can induce apoptosis [8, 9]. Recent clinical studies combining MAPK kinase (MEK) and AKT inhibitors provided evidence of antitumor activity, although with significant toxicity [10, 11]. This study was designed to determine whether alternative dosing schedules or careful patient selection would demonstrate a more favorable risk to benefit ratio.

Trametinib is an oral, reversible, noncompetitive MEK1/2 kinase inhibitor that has been approved as a single agent for metastatic melanoma and in combination with dabrafenib (BRAF inhibitor) for *BRAF* V600-mutant unresectable or metastatic melanoma, adjuvant melanoma, non-small cell lung cancer (NSCLC), and anaplastic thyroid cancer [12, 13]. Uprosertib is an adenosine triphosphate-competitive, reversible pan-AKT inhibitor that binds

to the adenosine triphosphate-binding pocket of AKT1, 2, and 3 [14, 15]. Uprosertib, as monotherapy, showed modest clinical activity in an exploratory study in patients with platinum-resistant ovarian cancer, although RAS/RAF pathway mutations segregated with resistance to AKT inhibition [16], suggesting activation of the MAPK pathway. Preliminary pharmacokinetic data suggests that uprosertib (10 mg to 150 mg once daily dose levels) is rapidly absorbed with a median maximum observed concentration (C_{max}) occurring 3 hours following multiple doses and a mean effective half-life of approximately 3 days. The likelihood of drug-drug interactions between trametinib and uprosertib is low because the in vitro IC_{50} values for inhibition and EC_{50} values for induction of CYP450 enzymes are 3- to 100-fold higher than the anticipated clinical C_{max} values for either drug. In nonclinical studies, combining trametinib and uprosertib induced cytotoxic responses, with additive or synergistic effects observed in colon, pancreatic, and lung cell lines, independent of *RAS/RAF* and *PI3K/PTEN* genotypes [15]. Additionally, the combination treatment enhanced the inhibition of tumor growth in pancreatic tumor xenografts compared with either single agent [17].

This open-label, phase I, dose-expansion clinical trial sought to determine the maximum tolerated dose (MTD) and evaluate the safety and clinical efficacy of uprosertib in combination with trametinib in patients with solid tumors likely to be sensitive to MEK and/or AKT inhibition. The two pathways targeted—MEK and PI3K/AKT are commonly co-activated in cancer and

believed to be drivers. We explored different schedules and specific expansion cohorts to try and find a tolerable dose and/or a tumor type that would be responsive. As overlapping toxicities were expected and clinical experience with combinations of MEK and PI3K pathway inhibitors have highlighted tolerability concerns, starting doses were reduced to $\geq 50\%$ of the MTD identified in previous phase I monotherapy dose-escalation trials [10, 11, 18-20]. Continuous trametinib dosing with intermittent uprosertib was also evaluated in this study, to potentially permit greater exposure and for more complete PI3K/AKT inhibition while maintaining tolerability.

Materials and Methods

This phase I, open-label study comprised a dose-escalation phase in patients with solid tumors, followed by an expansion phase in patients with unresectable or metastatic melanoma or triple-negative breast cancer (TNBC; estrogen receptor negative/progesterone receptor negative/human epidermal growth factor receptor-2 negative). Patients were enrolled from May 2010 to November 2014 at 8 study centers in the United States (GlaxoSmithKline study TAC113886; NCT01138085). This study was conducted in accordance with the provisions of the Declaration of Helsinki and Good Clinical Practice guidelines. The protocol was approved by the institutional review board or human research ethics committee at each study

center. Written informed consent was obtained from each patient before any study-specific procedures were performed.

Study design and patients

Dose escalation for continuous daily dosing (part 1A) followed a zone-based approach, incorporating a modified 3+3 cohort design until an MTD or a recommended phase II dose (RP2D) of the combination regimen was identified (Figure 1a) [21]. Multiple MTDs could be identified from part 1A given the nature of the zone-based dose-escalation process. Six patients were initially enrolled in Cohort 1, Zone 1, and the starting dose was trametinib 0.5 mg once daily [QD] + uprosertib 25 mg QD, both on a continuous basis. MTD was defined as the highest dose at which 1 or fewer of up to 6 enrolled patients experienced a dose-limiting toxicity (DLT) at the specified dose and schedule of the combination therapy. A DLT was defined as any of the following occurring within the first 28 days of combination therapy and having a possible causal relationship to the study drug: (1) grade 4 neutropenia lasting ≥ 5 days, (2) febrile neutropenia of any grade or duration, (3) grade 4 thrombocytopenia or anemia, (4) grade 4 alanine aminotransferase $> 3 \times$ upper limit of normal with bilirubin $> 2 \times$ upper limit of normal, (5) grade 3 clinically significant nonhematologic toxicity (except grade 3 electrolyte disturbances that responded to correction within 24 hours; grade 3 diarrhea, nausea, vomiting, or mucositis/esophagitis that responded to maximal supportive care within 48 hours; grade 3 hypertension

that was adequately controlled within 2 weeks with up to 2 additional antihypertensive medications; or grade 3 rash that improved to grade 2 within 7 days without a dose reduction within 2 weeks of restarting the study treatment), (6) failure to receive $\geq 75\%$ of the scheduled doses in the treatment period due to toxicity, or (7) ventricular ejection fraction below the lower limit of normal, with an absolute decrease of $>20\%$ from baseline. The MTD was exceeded if ≥ 2 patients in a cohort of up to 6 patients experienced DLTs.

Once the continuous daily MTD or RP2D was identified in part 1A, dose escalation with intermittent dosing schedules (part 1B) was initiated, in which the dosing schedule was adjusted for uprosertib while trametinib 2 mg QD was administered on a continuous basis (Figure 1b). Dose escalation of uprosertib proceeded using a traditional 3+3 cohort design until an MTD or RP2D of the combination regimen was identified. For part 1B, 2 intermittent schedules of uprosertib were assessed: 4-days-on/10-days-off (with and without loading dose) and weekly dosing.

Enrollment into planned expansion cohorts, including the continuous QD dosing (part 2A) and intermittent dosing (part 2B) schedules, began once the recommended dosing regimens were identified in parts 1A and 1B. Part 2A included 2 separate tumor-specific cohorts of patients with TNBC or melanoma. A 2-stage design was used to allow for the enrollment of additional patients to better characterize the combination profile or to allow

for early termination if sufficient clinical activity was not demonstrated. An interim analysis was planned for part 2 on each schedule after 15 patients had either confirmed response data (available from 2 postbaseline scans) or withdrawn from the study before response was assessed (nonresponder), with early stopping rules for futility. At least 3 responses in 15 patients in the TNBC cohort needed to be observed or the arm was terminated; at least 2 responses in 15 patients in the melanoma cohort needed to be observed or the arm was terminated.

Patient eligibility criteria

Key inclusion criteria for part 1A were patients aged ≥ 18 years with histologically or cytologically confirmed diagnosis of a solid tumor malignancy not responsive to standard therapies, for which there was no approved or curative therapy, or for which patients refused standard therapy; Eastern Cooperative Oncology Group performance status of 0 or 1; and adequate organ system function. In part 1B, patients were required to have a confirmed diagnosis of 1 of the following cancers: colorectal, NSCLC, pancreatic, endometrial, ovarian, squamous cell carcinoma of the head and neck, *BRAF*-wild type melanoma (as determined locally), melanoma resistant to *BRAF* inhibition, or TNBC. Patients with colorectal cancer or NSCLC had to have *KRAS* mutation status confirmed by a local laboratory. *PTEN* genetic status was also assessed using Ventana Medical Systems, Tucson, Arizona, US at enrollment to facilitate further patient selection. In part 2A, patients

were required to have a histologically or cytologically confirmed diagnosis of either TNBC or melanoma and known tumor PTEN status determined by a central laboratory. Patients were tested for the expression of PTEN in paired tumor biopsies (baseline and on treatment). An additional tumor biopsy at the time of disease progression was also required, if feasible. Any patient with type 2 diabetes mellitus must have been diagnosed ≥ 6 months before enrollment and had a glycated hemoglobin of $\leq 8\%$ at screening.

Key exclusion criteria included treatment with anticancer therapy, including investigational drugs, within 28 days or 5 half-lives before the first dose of study drugs; prior exposure to an MEK inhibitor or an AKT inhibitor; presence of any condition that could affect gastrointestinal absorption or predispose to gastrointestinal ulceration; type 1 or type 2 diabetes mellitus (part 1A or 1B only); human immunodeficiency virus infection positive status; history of positivity for hepatitis B virus surface antigen or positivity for hepatitis C virus antibody; leptomenigeal disease; brain metastases if symptomatic or treated (eg, surgery, radiation therapy) but not clinically and radiographically stable 1 month after therapy or if asymptomatic and untreated but >1 cm in the longest dimension; cardiac QTcF interval of ≥ 480 ms; class II-IV heart failure as defined by the New York Heart Association functional classification system; or history or current evidence/risk of retinal vein occlusion or central serous retinopathy.

Study endpoints and assessments

The primary endpoints for part 1 were to determine the safety, tolerability, and RP2D using changes in the safety parameters, including laboratory values and vital signs. Adverse events (AEs), serious AEs (SAEs), and clinical laboratory data were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) v4.0. The primary endpoint for part 2 was the investigator-assessed tumor response, using Response Evaluation Criteria in Solid Tumors (RECIST) v1.1 [22], including objective response rate (ORR). Tumor assessments for parts 1A and 1B were conducted at screening, at the start of week 9 (part 1B only) and every 8 weeks thereafter, and at the final study visit.

Secondary a priori endpoints included pharmacokinetic (PK) parameters following repeat-dose administration of trametinib and uprosertib alone and in combination, including area under the curve to the end of the dosing interval, predose (trough) concentration at the end of the dosing interval, maximum concentration, and time to maximum concentration; duration of response and progression-free survival (PFS); change from baseline levels of pharmacodynamic (PD) parameters, biomarkers downstream of AKT and MEK pathways (eg, pERK, pAKT, pPRAS40, and pS6RP) in tumor biopsies; and change from baseline levels in glucose insulin or 1,5-anhydroglucitol values. RNA-Seq was used to identify somatic mutations in the pretreatment tumor biopsy samples from the TNBC cohort. Each sample was paired-end sequenced using Illumina HiSeq 2500 (Illumina Inc, San Diego, CA).

Statistical methods

Part 1 sample size was determined by the number of patients required to adequately characterize the safety and tolerability of the trametinib and uprosertib combination and was not driven by statistical considerations. Part 2 sample size was determined using the Green-Dahlberg design [23]. A maximum of 140 patients were planned to be enrolled in parts 2A and 2B, estimating up to 70 patients per tumor type in the 2 dosing schedules ($n = 35$ patients per schedule).

The all-treated population included all patients who received ≥ 1 dose of trametinib or uprosertib, and safety analyses were performed on this population. The PK population comprised all patients in the all-treated population from whom a PK sample was obtained and analyzed. PK parameters were calculated by standard noncompartmental analysis using WinNonlin Pro v5.2 or higher (Pharsight Corp, Mountain View, CA). The PD population comprised all patients in the all-treated population from whom evaluable paired biopsies were available for ≥ 1 candidate biomarker.

Patients with unknown or missing tumor assessments were treated as nonresponders, and exact 95% confidence intervals (CIs; Clopper-Pearson) were computed. For part 2A, PFS was defined as the time between the first dose and disease progression or death due to any cause.

To allow for early termination of the part 2 cohorts, interim data were evaluated based on the Green-Dahlberg design [23] after 15 patients per schedule had either confirmed response data or withdrawn from the study before assessment of response (nonresponder). At least 3 responses (TNBC cohort) or ≥ 2 responses (*BRAF*-wild type cohort) from the 15 patients were needed at the interim analysis to continue enrolling patients in part 2.

Results

Patient characteristics

Overall, 126 patients were enrolled. Of these, 63 patients received continuous trametinib and uprosertib in part 1A, 29 received an alternative dosing schedule with continuous trametinib and intermittent uprosertib in part 1B, and 34 were enrolled into the part 2A expansion cohort. Across all cohorts, the median duration of therapy was 56.5 days (range, 3–557 days) with trametinib and 52 days (range, 2–370 days) with uprosertib. Enrollment for the *BRAF*-wild type cohort was halted early for futility, and study part 2B was never opened for enrollment.

Table 1 presents patient demographics, baseline characteristics, and disposition by treatment cohorts. The most common primary tumor types at screening were breast (25%), melanoma (23%), and colon/rectum (11%). Most patients (90%) had stage IV, IVb, or IVc disease, and almost all patients

(96%) had received prior anticancer therapy, including 11% of patients who received prior targeted therapy. Overall, the most common reasons for treatment discontinuation were disease progression (61%), AEs (19%), and investigator discretion (12%).

DLTs and MTD

A total of 28 DLTs were reported in 14 patients; the most commonly reported were diarrhea ($n = 5$), fatigue ($n = 3$), vomiting ($n = 2$), mucosal inflammation ($n = 2$), and stomatitis ($n = 2$) (Supplementary Table S1). Most DLTs ($n = 23$ [82%]) resolved with dose interruption, dose reduction, or treatment withdrawal; 5 DLTs (18%) remained unresolved despite these interventions.

Dose escalation—part 1A

In part 1A with continuous QD dose escalation, 8 patients experienced 17 DLTs. All but 1 DLT (anemia in cohort 10) were considered treatment related by the investigator. Most DLTs ($n = 11$) resulted in dose interruption, and 2 DLTs led to dose reduction. Two DLTs in 1 patient in cohort 7 (chest pain and ventricular tachycardia) resulted in treatment discontinuation. No DLTs were of grade 4 or 5. Using the zone-based approach, which allowed concurrent enrollment of cohorts (Figure 1), 3 MTDs were identified with QD dosing for both drugs: trametinib 0.5 mg + uprosertib 75 mg, trametinib 1.5 mg + uprosertib 50 mg, and trametinib 2 mg + uprosertib 25 mg. Based on the minimal dose needed for trametinib activity and an intermediate active and

tolerable dose of uprosertib, the regimen selected for evaluation in the expansion cohorts was trametinib 1.5 mg QD + uprosertib 50 mg QD.

Dose escalation—part 1B

In part 1B, alternative intermittent-dosing schedules of uprosertib were evaluated with continuous dosing of trametinib (2 mg; Figure 1). Six patients experienced 11 DLTs (Supplementary Table S1). All DLTs in cohorts 12 through 16 were considered treatment related and resulted in 5 dose interruptions, 5 dose reductions, and 1 treatment discontinuation (grade 2 fatigue). No DLTs were of grade 4 or 5. Two MTDs were identified: (a) uprosertib 100 mg loading dose on days 1 and 15, and uprosertib 50 mg on days 2 to 4 and days 16 to 18 of a 28-day cycle + trametinib 2 mg QD, and (b) uprosertib 225 mg on days 1, 8, 15, and 22 of a 28-day cycle + trametinib 2 mg QD. None of the schedules were evaluated further due to study closure.

Safety and tolerability

Across all cohorts, the most common AEs regardless of causality included diarrhea (67%), fatigue (46%), nausea (45%), vomiting (35%), and dermatitis acneiform (33%) (Table 2). Rash AEs were unresolved (at study closure) in 40% of patients, and the median duration of the first occurrence of rash was 88 days (range, 68–113 days). Grade 3–5 AEs (all causality) occurred in 87 patients (69%; grade 3, 59%; grade 4, 6%; grade 5, 4%). Causes of death

included failure to thrive, hypoxia, sepsis, fall, craniocerebral injury, and cardiac arrest; none were treatment related.

Overall, 70 patients (56%) experienced ≥ 1 AE that required dose interruption. The most commonly reported AEs leading to dose interruptions were diarrhea (14%), nausea (7%), vomiting (6%), rash maculopapular (6%), and increased aspartate aminotransferase levels (5%).

The proportion of patients reporting a treatment-related AE with a maximum severity grade of 3 was 30% in part 1A, 55% in part 1B, and 44% in part 2A (Supplementary Tables S2-S4). The most commonly reported ($\geq 10\%$ of all patients) treatment-related grade 3 AE was diarrhea (10%, 14%, and 12%, in parts 1A, 1B, and 2A, respectively). Ten of the 14 grade 3 diarrhea events were considered SAEs. Dermatitis acneiform was the most common grade 3 treatment-related AE in the melanoma cohort. Of the 96 nonfatal SAEs reported in 43 patients (34%), 29 were considered treatment related, most commonly diarrhea (7%), nausea (3%), vomiting (3%), and dyspnea (3%). Fatal SAEs were reported in 5 patients: 4 in part 1A and 1 in part 2A (all unrelated to treatment).

Pharmacokinetics

Limited observations from continuous dosing in part 1A suggested that there was increased exposure to both trametinib and uprosertib with increasing dose (Supplementary Table S5). Due to study termination, a formal analysis

of the drug interaction between trametinib and uprosertib was not performed. In part 1B, both 7-day and 10-day washout periods were sufficient to return uprosertib concentrations to negligible levels (Supplementary Table S6). Limited sampling of the continuous dosing expansion cohort in part 2A suggested steady-state predose (C_{trough}) concentrations were similar in the TNBC and melanoma cohorts following administration of trametinib 1.5 mg QD + uprosertib 50 mg QD and ranged between 0 and 277 ng/mL and 0 and 24 ng/mL, respectively. No PK analysis was performed in part 2A due to limited sample availability.

Pharmacodynamics

Twelve patients (4 melanoma and 8 TNBC) enrolled in part 2A had evaluable pretreatment and on-treatment tumor tissue biopsies available for biomarker analysis. Two additional patients with TNBC had pretreatment or posttreatment evaluable samples, which were also included for biomarker analysis (Figure 2). There was a ≥ 5 -fold decrease from baseline in the phosphorylated/total protein ratio for ERK (3/14 patients [21%]; TNBC, $n = 2$; melanoma, $n = 1$), PRAS40 (21%; TNBC, $n = 2$; melanoma, $n = 1$), and S6RP (21%; TNBC, $n = 1$; melanoma, $n = 2$). All patients with decreased protein phosphorylation were treated at the RP2D of trametinib 1.5 mg QD + uprosertib 50 mg QD. A ≥ 2 -fold increase from baseline in the phosphorylated AKT/total AKT ratio was observed in 5 of 14 patients (36%), indicating AKT inhibition by uprosertib (Supplementary Table S7).

Clinical activity

The ORR was 4.8% (6/126) across all cohorts, with 1 complete response (CR) and 5 partial responses (PRs). In part 2A, CR was observed in 1 patient, a 76-year-old woman with TNBC, who remained on treatment (trametinib 1.5 mg QD + uprosertib 50 mg QD) for >11.3 months (Supplementary Table S8). PR was observed in 2 patients in part 1A (with endometrial cancer and adenocarcinoma of unknown primary), 2 patients in part 1B (with melanoma and thyroid cancer), and 1 patient in part 2A (with melanoma). In patients with PR, duration of response ranged from 3.7 months (at the last follow-up due to study closure) to 12.6 months.

Median PFS (95% CI) was 69 days (56-105 days) in part 1A ($n = 63$), 114 days (57-253 days) in part 1B ($n = 29$), 54 days (33-116 days) in patients with TNBC in part 2A ($n = 20$), and 106 days (54-164 days) in patients with melanoma in part 2A ($n = 14$).

PTEN tumor status

The cohort expansion portion of the study (part 2A) preferentially enrolled patients with treatment-refractory TNBC or melanoma. Of the 59 evaluable archived specimens from unique patients with detectable cytoplasmic staining (TNBC, $n = 24$; melanoma, $n = 35$), the average H-score was 169 (range, 5-300) and the majority (67%) had midrange scores (101-200). Of the 53 specimens with detectable nuclear H staining, the average score was 61 (range, 1-280) and the majority (79%) had low H-scores (range, 1-100).

The correlation coefficient between the cytoplasmic and nuclear H-scores for the evaluable specimens was 0.5908. Only 2 TNBC specimens were PTEN-null, as defined by <30% of tumor cells staining at 1+ intensity and <10% tumor cells staining at 2+ or 3+ intensity. All melanoma samples tested were positive for PTEN expression; in general, PTEN levels were higher in the melanoma specimens than in the TNBC specimens.

Somatic mutation analysis

Results of an analysis of the RNA-Seq tumor transcriptome sequencing data from the TNBC cohort identified 96 mutations (nonsynonymous, stop gain/loss, indel [DNA base insertion or deletion], and nonsynonymous + indel) in a total of 84 genes (Supplementary Table S9). The majority of the identified genes appeared in only 1 patient. The highest incidence of mutations was noted for *TP53* (5/12 patients) and *RBL2* (5/12 patients). Numerous mutations were identified in the patient with TNBC who had a CR (patient 17), including nonsynonymous mutations in *ERBB2* (including L755S) and indel mutations in *PIK3R1*.

Discussion

This study aimed to determine an acceptable dose and schedule for the combination of the MEK1/2 inhibitor trametinib and pan-AKT inhibitor uprosertib, and to evaluate the preliminary activity of this combination in

patients selected for predicted likelihood of responding to this dual pathway inhibition. Because some toxicities were expected to overlap, numerous combinations of doses and schedules were explored; however, continuous dosing of trametinib and uprosertib was not well tolerated, and minimal clinical activity was observed at the achievable doses. The planned cohort expansion of an alternative dosing schedule testing intermittent uprosertib with continuous trametinib was not completed. Co-targeting MAPK-dependent and MAPK-independent BRAF inhibitor resistance may be effective in some settings, as demonstrated by a phase I trial evaluating dabrafenib (150 mg twice daily) with dose escalation of uprosertib (50 mg QD starting) alone or with trametinib (1.5 mg QD starting) in patients with *BRAF* V600E/K-mutant advanced solid tumors [24]. With a DLT observed only at the highest dose of the triple combination, dual pathway inhibition was well tolerated and objective responses were observed.

The most commonly reported AEs in this study were consistent with those reported in monotherapy studies, albeit at much lower doses than those with either single agent [18, 19]. Gastrointestinal side effects, particularly diarrhea, and skin-related AEs were frequently observed in uprosertib plus trametinib and monotherapy trials in patients with solid tumors and *BRAF*-mutant melanoma, respectively. In the current trial, diarrhea was the most common DLT, reported as a grade 3 AE in 11% of patients. Diarrhea, nausea and vomiting, and maculopapular rash were the most common AEs causing dose interruptions. These AEs were managed adequately with prophylactic

treatment, dose reductions and interruptions. The patient population had advanced disease; 90% of patients had stage IV disease and 53% had received ≥ 3 prior regimens. A better tolerability profile could have been achieved in patients with less advanced disease, potentially allowing for higher dosing or a longer treatment duration.

Our results are largely consistent with those of other trials that evaluated combinations of drugs targeting both the MAPK and PI3K/AKT pathways [10, 11, 20, 24-27]. Evaluation of trametinib plus the pan-AKT inhibitor afuresertib in 20 patients was terminated owing to poor tolerability at doses well below the MTD of each single-agent [11]. In another trial evaluating dual MAPK and PI3K/AKT inhibitors, toxicities limited escalation to the doses needed to achieve clinical activity [25]. Similar to our study, gastrointestinal, hepatic, dermatologic, and hematologic AEs most commonly resulted in dose interruptions, dose reductions, or treatment discontinuations [11, 20, 25]. Another study evaluating a combination of vemurafenib and everolimus also demonstrated clinical activity across histologies, with partial responses noted in advanced NSCLC, melanoma, optic nerve glioma, and xanthoastrocytoma, including patients who previously experienced progression on BRAF and/or MEK inhibitor therapy. The vemurafenib-everolimus combination was well tolerated in this patient population [28].

Median trough concentrations of trametinib and uprosertib were mostly in the range of steady-state trough concentrations reported in monotherapy

studies [18, 19]; however, with uprosertib continual dosing, its MTD in combination with trametinib was at most 67% of the target dose identified in the monotherapy trial [19]. To potentially achieve higher drug exposure with better tolerability, alternate schedules with intermittent uprosertib dosing were evaluated. Intermittent dosing regimens of MEK and PI3K/AKT inhibitors in preclinical mouse cancer models have resulted in similar in vitro/in vivo activity to continuous dosing schedules and may be better tolerated [29]. Alternate dosing schedules could potentially allow more complete PI3K/AKT pathway inhibition in combination with MEK inhibition, which may result in tumor cell death, while maintaining tolerability of the combination. However, in this study, intermittent dosing did not achieve higher uprosertib exposure than the steady-state concentrations achieved with continuous dosing. Uprosertib concentrations returned to negligible levels after 7-day washouts. Limited sampling with the continuous dosing indicated that steady-state predose (C_{trough}) concentrations were similar in the TNBC and melanoma cohorts. Furthermore, >50% of patients required dose modifications and/or interruptions of trametinib and/or uprosertib.

PD results using paired tumor biopsies from patients with TNBC or melanoma showed a >2-fold change in the phosphorylation rate of several markers in the MEK and AKT pathways in only a small subset of patients, including increases in pAKT (Supplementary Table S7). These results suggest that the drug concentrations achieved in part 2A may not have been sufficient for a robust pathway inhibition, and because the intermittent schedule in part 1B

did not show any increase in drug concentration relative to QD dosing at the respective MTDs, the planned expansion cohorts were not opened.

Identification of somatic mutations in tumor samples from the TNBC cohort showed that the majority of genes with mutations appeared only once in individual patients. *TP53* represented 1 of 2 genes occurring at a higher frequency (5/13) in the tumors assessed. This was expected considering the high *TP53* mutation rate (78%) in TNBC [30]. Mutations in *ERBB2* and *PI3KR1* were identified in the patient with a CR and may warrant further study. The *ERBB2* L755S mutation is known to be recurrent in breast cancer [31].

Of note, the clinical activity of uprosertib plus trametinib in this study (ORR, 4.8%) was not better than that previously reported with monotherapy [18, 19], despite patient selection based on predicted likelihood of responding to dual pathway inhibition. PTEN loss is associated with increased AKT activation [32]; however, none of the patients in part 2A had tumors that met the study definition of PTEN low/null. One limitation of the study was that tumor samples were not uniformly available.

In conclusion, trametinib plus uprosertib was associated with a high incidence of AEs at the doses and schedules tested. Diarrhea was the most common DLT, and diarrhea, nausea, vomiting, and maculopapular rash were the most common AEs leading to dose interruptions. Minimal antitumor activity was observed despite the enriched patient population. Since co-

activation of MAPK and PI3K/AKT pathway signals are common in cancer, it will be important for future studies to determine if these pathways can be simultaneously suppressed by alternative drug combinations.

Acknowledgments

The authors thank all participating patients and their families. The authors also thank William Fazzino, PhD, of ArticulateScience, LLC, for editorial assistance, which was funded by Novartis Pharmaceuticals Corporation.

Conflict of Interest

A.W.T. has received fees for consulting and board memberships, as well as research funding (paid to NEXT Oncology) from AbbVie, ADC Therapeutics, Adagene, Agenus, Aminex, AroBioTX, Arrys, Asana, Ascentage, Aximmune, Bayer, BioInvent, Birdie, Boston Bio, CStone, Deciphera, EMD Serono, Forbius, GSK, HBM Partners, Ignyta, Inhibrx, Immunome, Immunomet, Innate, Jazz Pharmaceuticals, Kiromet, Mekanistic, Mersana, Nanobiotix, NatureWise, NBE Therapeutics, NextCure, Nitto Biopharma, Nuvalent, Pelican, Pfizer, Pierre Fabre, Pieris, Ridgeway, Scitemex, Sesen Bio, Seattle Genetics, Sunshine Guojian, Symphogen, Syndax, Syneos, Tizone, and Zymeworks. R.K. has received research funding from Boehringer Ingelheim, DeBiopharm, Foundation Medicine, Genentech, Grifols, Guardant Health, Incyte, Konica Minolta, Merck Serono, OmniSeq, Pfizer, and Sequenom; has consulting or

advisory role in Actuate Therapeutics, Gaido, LOXO, NeoMed, Pfizer, Roche, Soluventis, and X-Biotech; has received speaker fees from Roche; is a board member of CureMatch, Inc.; and has stock and other equity interests in CureMatch, Inc., IDbyDNA, and Soluventis. V.V. received grant and honorarium from Novartis. R.G. received research funding from Novartis, GSK and grant and personal fees from Array, Bristol-Myers Squibb, Merck, and Roche/Genentech. R.S.H. received consulting honoraria from Boehringer Ingelheim, Novartis, Tarveda, Apollomics and received research funding (to his institution) from Daiichi Sankyo, Agios, Novartis, Corvus, Mirati, Millennium, Genentech Roche, AbbVie, Exelixis, Celgene, and Incyte. A.R.T. received research funding from GSK. C.W. and S.K-S. are employees of GSK. C.L. and L.Y. were former employees of GSK. J.F.K. was an employee of Novartis. J.G. and A.M.D. Jr are employees of Novartis Pharmaceuticals and have stock ownership in Novartis and GSK. C.E. is an employee and stock owner of GSK. N.I. was an employee of GSK and currently is an employee of Merck. All remaining authors have declared no conflicts of interest. All authors received assistance with manuscript preparation from ArticulateScience, LLC.

References

1. Burotto M, Chiou VL, Lee JM, Kohn EC (2014) The MAPK pathway across different malignancies: a new perspective. *Cancer* 120:3446–3456.
<https://doi.org/10.1002/cncr.28864>
2. Wee S, Jagani Z, Xiang KX, Loo A, Dorsch M, Yao YM et al (2009) PI3K pathway activation mediates resistance to MEK inhibitors in KRAS mutant cancers. *Cancer Res* 69:4286–4293. <https://doi.org/10.1158/0008-5472.CAN-08-4765>
3. Lim SY, Menzies AM, Rizos H (2017) Mechanisms and strategies to overcome resistance to molecularly targeted therapy for melanoma. *Cancer* 123:2118–2129. <https://doi.org/10.1002/cncr.30435>
4. Hu Y, Gu Y, Wang H, Huang Y, Zou YM (2015) Integrated network model provides new insights into castration-resistant prostate cancer. *Sci Rep* 5:17280. <https://doi.org/10.1038/srep17280>
5. Liu Y, Sheikh MS (2014) Melanoma: molecular pathogenesis and therapeutic management. *Mol Cell Pharmacol* 6:228.
6. Lan YT, Jen-Kou L, Lin CH, Yang SH, Lin CC, Wang HS et al (2015) Mutations in the RAS and PI3K pathways are associated with metastatic location in colorectal cancers. *J Surg Oncol* 111:905–910.
<https://doi.org/10.1002/jso.23895>

7. Greger JG, Eastman SD, Zhang V, Bleam MR, Hughes AM, Smitheman KN et al (2012) Combinations of BRAF, MEK, and PI3K/mTOR inhibitors overcome acquired resistance to the BRAF inhibitor GSK2118436 dabrafenib, mediated by NRAS or MEK mutations. *Mol Cancer Ther* 11:909–920. <https://doi.org/10.1158/1535-7163.MCT-11-0989>
8. McCubrey JA, Steelman LS, Chappell WH, Abrams SL, Franklin RA, Montalto G et al (2012) Ras/Raf/MEK/ERK and PI3K/PTEN/Akt/mTOR cascade inhibitors: how mutations can result in therapy resistance and how to overcome resistance. *Oncotarget* 3:1068–1111. <https://doi.org/10.18632/oncotarget.659>
9. Sanchez-Hernandez I, Baquero P, Calleros L, Chiloeches A (2012) Dual inhibition of (V600E)BRAF and the PI3K/AKT/mTOR pathway cooperates to induce apoptosis in melanoma cells through a MEK-independent mechanism. *Cancer Lett* 314:244–255. <https://doi.org/10.1016/j.canlet.2011.09.037>
10. Tolcher AW, Khan K, Ong M, Banerji U, Papadimitrakopoulou V, Gandara DR et al (2015) Antitumor activity in RAS-driven tumors by blocking AKT and MEK. *Clin Cancer Res* 21:739–748. <https://doi.org/10.1158/1078-0432.CCR-14-1901>
11. Tolcher AW, Patnaik A, Papadopoulos KP, Rasco DW, Becerra CR, Allred AJ et al (2015) Phase I study of the MEK inhibitor trametinib in combination

with the AKT inhibitor afuresertib in patients with solid tumors and multiple myeloma. *Cancer Chemother Pharmacol* 75:183–189.

<https://doi.org/10.1007/s00280-014-2615-5>

12. Flaherty KT, Robert C, Hersey P, Nathan P, Garbe C, Milhem M et al (2012) Improved survival with MEK inhibition in BRAF-mutated melanoma. *N Engl J Med* 367:107–114.

<https://doi.org/10.1056/NEJMoa1203421>

13. Mekinist (trametinib) [prescribing information] Novartis Pharmaceuticals Corporation East Hanover, NJ, USA. 2018. Accessed 30 July 2019.

14. Pachi F, Plattner P, Ruprecht B, Médard G, Sewald N, Kuster B (2013) Characterization of a chemical affinity probe targeting Akt kinases. *J Proteome Res* 12:3792–3800. <https://doi.org/10.1021/pr400455j>

15. Dumble M, Crouthamel MC, Zhang SY, Schaber M, Levy D, Robell K et al (2014) Discovery of novel AKT inhibitors with enhanced anti-tumor effects in combination with the MEK inhibitor. *PLoS One* 9:e100880.

<https://doi.org/10.1371/journal.pone.0100880>

16. Gungor H, Saleem A, Babar S, Dina R, El-Bahrawy MA, Curry E et al (2015) Dose-finding quantitative ¹⁸F-FDG PET imaging study with the oral pan-AKT inhibitor GSK2141795 in patients with gynecological

malignancies. J Nucl Med 56:1828–1835.

<https://doi.org/10.2967/jnumed.115.156505>

17. Data on file. Study TAC113886 (2015) <http://www.gsk-clinicalstudyregister.com>. Accessed 16 February 2016.
18. Infante JR, Fecher LA, Falchook GS, Nallapareddy S, Gordon MS, Becerra C et al (2012) Safety, pharmacokinetic, pharmacodynamic, and efficacy data for the oral MEK inhibitor trametinib: a phase 1 dose-escalation trial. Lancet Oncol 13:773–781. [https://doi.org/10.1016/S1470-2045\(12\)70270-X](https://doi.org/10.1016/S1470-2045(12)70270-X)
19. Burris HA, Siu LL, Infante JR, Wheler JJ, Kurkjian C, Opalinska J et al (2011) Safety, pharmacokinetics (PK), pharmacodynamics (PD), and clinical activity of the oral AKT inhibitor GSK2141795 (GSK795) in a phase I first-in-human study. J Clin Oncol 29:3003. https://doi.org/10.1200/jco.2011.29.15_suppl.3003
20. Bedard PL, Taberero J, Janku F, Wainberg ZA, Paz-Ares L, Vansteenkiste J et al (2015) A phase Ib dose-escalation study of the oral pan-PI3K inhibitor buparlisib (BKM120) in combination with the oral MEK1/2 inhibitor trametinib (GSK1120212) in patients with selected advanced solid tumors. Clin Cancer Res 21:730–738. <https://doi.org/10.1158/1078-0432.CCR-14-1814>

21. Huang X, Biswas S, Oki Y, Issa JP, Berry DA (2007) A parallel phase I/II clinical trial design for combination therapies. *Biometrics* 63:429–436.
<https://doi.org/10.1111/j.1541-0420.2006.00685.x>
22. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R et al (2009) New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer* 45:228–247.
<https://doi.org/10.1016/j.ejca.2008.10.026>
23. Green SJ, Dahlberg S (1992) Planned versus attained design in phase II clinical trials. *Stat Med* 11:853–862.
<https://doi.org/10.1002/sim.4780110703>
24. Algazi AP, Moon J, Chmielowski B, Lo R, Kendra KL, Lao CD et al (2017) SWOG S1221: a phase 1 dose escalation study co-targeting MAPK-dependent and MAPK-independent BRAF inhibitor resistance in BRAF mutant advanced solid tumors with dabrafenib, trametinib, and GSK2141795 (ClinicalTrials.gov NCT01902173). *J Clin Oncol* 35:2578.
https://doi.org/10.1200/JCO.2017.35.15_suppl.2578
25. Do K, Speranza G, Bishop R, Khin S, Rubinstein L, Kinders RJ et al (2015) Biomarker-driven phase 2 study of MK-2206 and selumetinib (AZD6244, ARRY-142886) in patients with colorectal cancer. *Invest New Drugs* 33:720–728. <https://doi.org/10.1007/s10637-015-0212-z>

26. Tolcher AW, Baird RD, Patnaik A, Garcia VM, Papadopoulos KP, Garrett CR et al (2011) A phase I dose-escalation study of oral MK-2206 (allosteric AKT inhibitor) with oral selumetinib (AZD6244; MEK inhibitor) in patients with advanced or metastatic solid tumors. *J Clin Oncol* 29:3004. https://doi.org/10.1200/jco.2011.29.15_suppl.3004
27. Yap TA, Omlin A, de Bono JS (2013) Development of therapeutic combinations targeting major cancer signaling pathways. *J Clin Oncol* 31:1592–1605. <https://doi.org/10.1200/JCO.2011.37.6418>
28. Subbiah V, Sen S, Hess KR, Janku F, Hong DS, Khatua S et al (2018) Phase I study of the BRAF inhibitor vemurafenib in combination with the mammalian target of rapamycin inhibitor everolimus in patients with BRAF-mutated malignancies. *JCO Precision Oncology* 2, 1-12. <https://doi.org/10.1200/PO.18.00189>
29. Hoeflich KP, Merchant M, Orr C, Chan J, Den Otter D, Berry L et al (2012) Intermittent administration of MEK inhibitor GDC-0973 plus PI3K inhibitor GDC-0941 triggers robust apoptosis and tumor growth inhibition. *Cancer Res* 72:210–219. <https://doi.org/10.1158/0008-5472.CAN-11-1515>
30. Wang X, Guda C (2016) Integrative exploration of genomic profiles for triple negative breast cancer identifies potential drug targets. *Medicine (Baltimore)* 95:e4321. <https://doi.org/10.1097/MD.0000000000004321>

31. Bose R, Kavuri SM, Searleman AC, Shen W, Shen D, Koboldt DC et al (2013) Activating HER2 mutations in HER2 gene amplification negative breast cancer. *Cancer Discov* 3:224–237. <https://doi.org/10.1158/2159-8290.CD-12-0349>

32. Song MS, Salmena L, Pandolfi PP (2012) The functions and regulation of the PTEN tumour suppressor. *Nat Rev Mol Cell Biol* 13:283–296. <https://doi.org/10.1038/nrm3330>

Tables and Figures

Table 1 Patient demographics, baseline characteristics, and disposition (all-treated population, $N = 126$)

Parameters	Continuous dose- escalation cohorts		Expansion cohort ^a		
	Part 1A ($n = 63$)	Part 1B ($n = 29$)	Part 2A		
			Part 2A TNBC ($n = 20$)	Part 2A melanom a ($n = 14$)	Total ($N = 126$)
Age, years, median (min-max)	60 (24-82)	65 (38-81)	49 (33-76)	54 (35-73)	58 (24-82)
Gender, n (%)					
Male	22 (35)	10 (34)	0	9 (64)	41 (33)
Female	41 (65)	19 (66)	20 (100)	5 (36)	85 (67)
Tumor stage, n (%)					
IV	58 (92)	27 (93)	18 (90)	11 (79)	114 (90)
III	5 (8)	1 (3)	1 (5)	3 (21)	10 (8)
Ic or II	0	1 (3)	1 (5)	0	2 (2)
Ethnicity, n (%)					
Hispanic or Latino	2 (3)	3 (10)	2 (10)	1 (7)	8 (6)
Not Hispanic or Latino	61 (97)	26 (90)	18 (90)	13 (93)	118 (94)
Race, n (%) ^b					
White/Caucasian/ European	57 (92)	26 (90)	17 (89)	13 (100)	113 (92)
African American	5 (8)	0	2 (11)	0	7 (6)
Asian	0	3 (10)	0	0	3 (2)
Primary tumor type, n					
Melanoma	11	4	-	14	29
Colon/rectum	11	3	-	-	14
Other ^c	8	3	-	-	11
Ovary	7	3	-	-	10
Pancreas	6	4	-	-	10
Breast	4	7	20	-	31
Endometrium/uterus	3	2	-	-	5
Thyroid	3	2	-	-	5
Non-small cell lung	2	1	-	-	3
Prostate	2	-	-	-	2
Fallopian tube	1	-	-	-	1
Head and neck	1	-	-	-	1
Kidney	1	-	-	-	1
Liver	1	-	-	-	1
Neuroendocrine	1	-	-	-	1
Small intestine	1	-	-	-	1

Prior anticancer therapy, <i>n</i> (%)					
Any	60 (95)	28 (97)	20 (100)	13 (93)	121 (96)
Chemotherapy	52 (83)	24 (83)	20 (100)	8 (57)	106 (84)
Biologic therapy	30 (48)	16 (55)	4 (20)	5 (36)	47 (37)
Hormonal therapy	9 (14)	8 (28)	6 (30)	0	16 (13)
Immunotherapy	3 (5)	1 (3)	0	3 (21)	8 (6)
Radiotherapy	24 (38)	9 (31)	13 (65)	6 (43)	55 (44)
Small-molecule- targeted therapy	7 (11)	3 (10)	4 (20)	1 (7)	14 (11)
Prior anticancer regimens, <i>n</i> (%)					
None	5 (8)	1 (3)	0	4 (29)	10 (8)
1-2	17 (27)	12 (41)	3 (15)	4 (29)	36 (28)
3-4	20 (32)	6 (21)	10 (50)	3 (21)	42 (33)
>4	18 (29)	10 (34)	7 (35)	3 (21)	38 (30)
Patients discontinued treatment for any reason, <i>n</i> (%)					
Adverse event	10 (16)	6 (21)	5 (25)	3 (21)	24 (19)
Disease progression	46 (73)	14 (48)	11 (55)	6 (43)	77 (61)
Study closed/terminated	0	1 (3)	1 (5)	0	2 (2)
Investigator discretion	4 (6)	4 (1)	2 (10)	5 (36)	15 (12)
Withdrew consent	3 (5)	4 (14)	1 (5)	0	8 (6)

^aThe study design included an expansion cohort part 2B. No patients were enrolled in part

2B

^b*n* = 123; race was not known for 3 patients (1 patient in part 1A and 2 patients in part 2A)

^cPart 1A “other” included peripheral nerve sheath tumor, uterine cancer, uveal and ocular melanoma, radiation-associated sarcoma of the left posterior, nasopharyngeal cancer, adenoma of unknown primary; part 1B “other” included mucinous adenocarcinoma, appendix cancer, and non-small cell lung cancer

TNBC triple-negative breast cancer

Table 2 Most common adverse events reported in $\geq 10\%$ of patients across all cohorts (all-treated population, $N = 126$)

AE, n (%)	All grades	Grade 3 or 4^a
Patients with any event	126 (100)	82 (65)
Diarrhea	84 (67)	14 (11)
Fatigue	58 (46)	8 (6)
Nausea	57 (45)	5 (4)
Vomiting	44 (35)	5 (4)
Dermatitis acneiform	42 (33)	4 (3)
Decreased appetite	35 (28)	1 (<1)
Maculopapular rash	32 (25)	4 (3)
Mucosal inflammation	31 (25)	4 (3)
Peripheral edema	29 (23)	1 (<1)
Stomatitis	26 (21)	5 (4)
Increased AST	24 (19)	10 (8)
Hypokalemia	22 (17)	7 (6)
Rash ^b	21 (17)	1 (<1)
Anemia	21 (17)	9 (7)
Constipation	20 (16)	0
Dry skin	20 (16)	1 (<1)
Dizziness	19 (15)	0
Dyspnea	18 (14)	5 (4)
Dehydration	17 (13)	3 (2)
Hypomagnesemia	17 (13)	0
Abdominal pain	16 (13)	1 (<1)
Dry mouth	16 (13)	0
Pyrexia	16 (13)	0
Pruritus	15 (12)	2 (2)
Hyponatremia	14 (11)	9 (7)
Increased blood alkaline phosphatase	13 (10)	8 (6)
Cough	13 (10)	0

^aGrade 4 AEs included hypoglycemia ($n = 1$), increased AST level ($n = 2$), increased blood alkaline phosphatase level ($n = 1$), increased alanine aminotransferase level ($n = 1$), increased blood creatinine level ($n = 1$), decreased neutrophil count ($n = 1$), anemia ($n = 1$), and retinal tear ($n = 1$)

^bRash includes all AEs of rash, with the exception of dermatitis acneiform and rash maculopapular.

AE adverse event, AST aspartate aminotransferase

Figure Legend

Figure 1 Dose-escalation schedules of trametinib (T) and uprosertib (U).

(a) In part 1A, a zone-based dose-escalation model with a modified 3+3 cohort design with a continuous QD dosing schedule of trametinib and uprosertib was used. Dose escalation was based on tolerability, and dose escalation proceeded until the maximum tolerated dose or the recommended phase II dose of the combination regimen was identified. Cohorts along each axis were enrolled simultaneously.

(b) In part 1B, an alternate escalation dosing schedule was used to evaluate a continuous daily dosing regimen of trametinib 2 mg QD with a 4-days-on/10-days-off schedule of uprosertib with or without a loading dose or a once weekly dose of uprosertib. ^aCohort 10 (not shown in diagram):
trametinib 2 mg + uprosertib 50 mg
QD once-daily

Figure 2 Change from baseline in AKT and MEK pathway biomarkers. Total phosphorylated/total H-score ratios of pERK, pAKT, pPRAS40, and pS6RP were determined from available tumor tissue biopsies obtained before and after treatment. A ratio of >1 indicated an increase in phosphorylation, a ratio of 1 indicated no change, and a ratio of <1 indicated a decrease in phosphorylation.

ERK extracellular signal-regulated kinases, *MEK* mitogen-activated protein kinase kinase, *S6RP* S6 ribosomal protein

