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Translational Science

Cancer Research

Phase Ib Results of the Rational Combination of Selumetinib and Cyclosporin A in Advanced Solid Tumors with an Expansion Cohort in Metastatic Colorectal Cancer

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Abstract

MEK inhibition is of interest in cancer drug development, but clinical activity in metastatic colorectal cancer (mCRC) has been limited. Preclinical studies demonstrated Wnt pathway overexpression in *KRAS*-mutant cell lines resistant to the MEK inhibitor, selumetinib. The combination of selumetinib and cyclosporin A, a noncanonical Wnt pathway modulator, demonstrated antitumor activity in mCRC patient-derived xenografts. To translate these results, we conducted a NCI Cancer Therapy Evaluation Program–approved multicenter phase I/IB trial (NCT02188264) of the combination of selumetinib and cyclosporin A. Patients with advanced solid malignancies were treated with the combination of oral selumetinib and cyclosporin A in the dose escalation phase, followed by an expansion cohort of irinotecan and oxaliplatin-refractory mCRC. The expansion cohort utilized a single-agent selumetinib "run-in" to evaluate FZD2 biomarker upregulation and *KRAS*-WT and *KRAS*-MT stratification to identify any potential predictors of efficacy. Twenty and 19 patients were enrolled in dose escalation and expansion phases, respectively. The most common adverse events and grade 3/4 toxicities were rash, hypertension, and edema. Three dose-limiting toxicities (grade 3 hypertension, rash, and increased creatinine) were reported. The MTD was selumetinib 75 mg twice daily and cyclosporin A 2 mg/kg twice daily on a 28-day cycle. *KRAS* stratification did not identify any differences in response between *KRAS*-WT and *KRAS*-MT cancers. Two partial responses, 18 stable disease, and 10 progressive disease responses were observed. Combination selumetinib and cyclosporin A is well tolerated, with evidence of activity in mCRC. Future strategies for concept development include identifying better predictors of efficacy and improved Wnt pathway modulation.

Significance: These findings translate preclinical studies combining selumetinib and cyclosporin into a phase I first-in-human clinical trial of such a combination in patients with advanced solid malignancies. *Cancer Res; 78(18); 5398–407. ©2018 AACR.*

Introduction

Colorectal cancer is the third leading cause of malignancy and the fourth common cause of cancer-related death worldwide (1). In the United States, colorectal cancer is the fourth most common cancer, and this year, an estimated 140,250 new cases of colorectal cancer will be diagnosed (2). Approximately, 20% of patients have metastatic or stage IV disease and only 13.9% of patients are alive at 5 years (2). Current treatment options include initial treatment with a 5-flurouracil (5-FU) and leucovorin backbone accompanied by oxaliplatin or irinotecan. Bevacizumab, a VEGF inhibitor, is administered along with 5-FU–based therapy and is commonly continued beyond progression. Rat sarcoma (*RAS*) gene wild-type patients with metastatic colorectal cancer (mCRC) have been shown to benefit from mAbs directed against EGFR. Other agents used in later lines of therapy include regorafenib, a multi-kinase inhibitor, and TAS-102, a combination of a thymidine-based nucleic acid analogue and a potent thymidine phosphorylase inhibitor.

Despite these therapeutic advances, mCRC is often incurable with a sobering median survival of 28–30 months (3). There is an unmet need for research and development of new and more effective therapies. A better understanding of the resistance mechanisms to targeted therapy has led to rational combination strategies (4). One of the distinctive fundamental capabilities of cancer is the ability to sustain proliferative signaling. The MAPK pathway (RAS/RAF/MEK/ERK) is one such proliferation pathway

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that is frequently dysregulated in cancer through gain-of-function mutations in the RAS and RAF (rapidly accelerated fibrosarcoma) proteins. *RAS* mutations are found in roughly 55% of colorectal cancers and its downstream effector pathways include the MAPK/ERK, the PI3K, and the Ral-GDS pathways. MEK is a critical MAPK enzyme in the downstream pathway from RAS and RAF that phosphorylates and activates ERK/p-ERK, its only known substrate, which in turn translocates to the nucleus where it activates many transcription factors, resulting in growth and proliferation (4–6). Unfortunately, activation of this downstream signaling pathway is associated with lack of beneficial responses to EGFR antibody blockade in patients with mutations in these proteins (7, 8).

Therefore, MEK inhibition has been an attractive therapeutic target for cancer treatment and has been tested in clinical trials since 2000. The safety, tolerability, and efficacy of MEK inhibition has been established from numerous studies investigating selumetinib, as well as other MEK inhibitors such as trametinib and cobimetinib (9–13). Single-agent activity has been somewhat modest except for trametinib, which demonstrated improved median progression-free survival (4.8 vs. 1.5 months, $P < 0.001$) and 6-month survival rates (81% vs. 67%) in patients with advanced *BRAF* V600E- or V600K-mutated melanoma (10, 14). This lack of convincing clinical activity of single-agent MEK inhibition could be due to simultaneous dysregulation of multiple signaling pathways and/or compensatory pathways that overcome the effect of MEK inhibitors (5, 6, 15–17). The combination of MEK inhibitors with other targeted agents or chemotherapy may overcome resistance and thus improve efficacy.

Selumetinib (AZD6244; ARRY-142886) is an orally active small-molecule MEK inhibitor that has been studied in many clinical trial settings. In the initial phase I study, selumetinib was found to be well tolerated with a recommended phase II dose (RP2D) of 100 mg twice daily (16). Bennouna and colleagues conducted a phase II randomized open label study that compared selumetinib at 100 mg twice daily to 1,250 mg/m^2 twice daily of oral capecitabine in patients with refractory mCRC. Disease progression was described in 80% of patients in both treatment groups with a very modest progression-free survival (PFS) in both (17). MEK inhibitors have been combined with other therapies to enhance clinical efficacy. Hochster and colleagues combined selumetinib and irinotecan in patients with *KRAS*-MT colorectal cancer showing improved clinical activity with the combination, but the study was terminated prior to full accrual (18). The combination of selumetinib and cetuximab has also been shown to be safe and well tolerated in another phase I study, but minimal antitumor activity was noted in *KRAS*-MT refractory mCRC (19).

Previous studies have identified the Wingless integrated (Wnt) signaling pathway as a resistance mechanism to MEK inhibition (6). The Wnt pathway is an evolutionarily conserved signal transduction pathway that regulates several cellular processes including stem cell renewal through canonical and noncanonical pathways (Fig. 1; refs. 6, 20, 21). The canonical pathway signals via the frizzled (FZD) family of G-protein coupled receptors. In the absence of Wnt ligand binding to FZD, the β-catenin destruction complex is degraded through proteolytic destruction. Binding of Wnt to the FZD-LRP5/6 coreceptor complex disrupts the APC/Axin/GSK3 complex that is required for the destruction of β-catenin. Stabilized β-catenin translocates to the nucleus where it mediates transcription of target genes (Fig. 1A). Aberrant Wnt pathway activation through the loss of function mutation of adenomatous polyposis coli (*APC*) is an early event in the development of colorectal cancer (20). The two well-known noncanonical or β-catenin–independent pathways are the Wnt/Ca2$^+$ pathway (Fig. 1B) and the planar cell polarity pathway (Fig. 1C). The Wnt/Ca2$^+$ pathway also acts through Wnt-FZD activation of Dsh. Dsh through PLC activates IP3, which leads to release of intracellular Ca2$^+$, and the Ca2$^+$ in turn activates CamK11 and a serine/threonine phosphatase, calcineurin. Calcineurin-induced dephosphorylation of NFAT results in the translocation of NFAT to the nucleus where it regulates transcription of genes (Fig. 1B). Preclinical studies of selumetinib-resistant *KRAS*-MT colorectal cancer cell lines show overexpression of several members of the Wnt pathway including Frizzled (FZD). Both gene set enrichment analysis and a synthetic lethal screen demonstrated that many of the genes involved in the canonical and noncanonical Wnt pathways were upregulated in selumetinib-resistant colorectal cancer cell lines (22).

Recent studies have shown that cyclosporin A, a calcineurin inhibitor, traditionally used for its immunosuppressive effects, inhibits the activity of the noncanonical Wnt/Ca^{++}/NFAT signaling pathway (23–25). DeGregori and colleagues identified the Wnt/Ca2$^+$ pathway genes as being synthetically lethal in combination with imatinib in RNAi-based screens and NFAT inhibition by cyclosporin A resulted in the sensitization of leukemia cells to Bcr-Abl inhibition (23). Synergistic antitumor effects with the combination of cyclosporin A and selumetinib were observed in *KRAS*-MT colorectal cancer xenografts that were known to be resistant to selumetinib monotherapy. These xenografts were noted to have increased expression of FZD2 by qRT-PCR when treated with selumetinib monotherapy. Given the predilection of cyclosporin A to inhibit P-glycoprotein drug efflux pumps, the abovementioned preclinical study also measured selumetinib concentrations in plasma, tumor, and liver when treated with selumetinib alone and in combination with cyclosporin A and reported no significant difference in selumetinib or its metabolite (6).

The theoretical rationale and the data suggest that the primary and secondary resistance to selumetinib-driven MEK inhibition may be overcome through concurrent noncanonical Wnt inhibition with cyclosporin A. On the basis of these intriguing preclinical data, we pursued the next step of translation in a multicenter phase IB study of selumetinib and cyclosporin A with an expansion cohort in patients with mCRC.

Patients and Methods

This study was conducted according to the ethical guidelines laid out by the Belmont Report and was approved by the institutional review board. Written informed consent was obtained from patients during the course of the study.

Patients

Patients with a histologic or cytopathologic diagnosis of an advanced solid cancer that is refractory to standard therapy or for which there is no standard therapy were included in the dose escalation cohort of the study. Once the MTD was identified, patients who had progressed on oxaliplatin- and irinotecan-based therapies with a histologic or cytopathologic diagnosis of advanced/metastatic unresectable colorectal cancer with known *RAS* mutational status, no known *BRAF* mutation and measurable disease were eligible for the expansion cohort. Patients were

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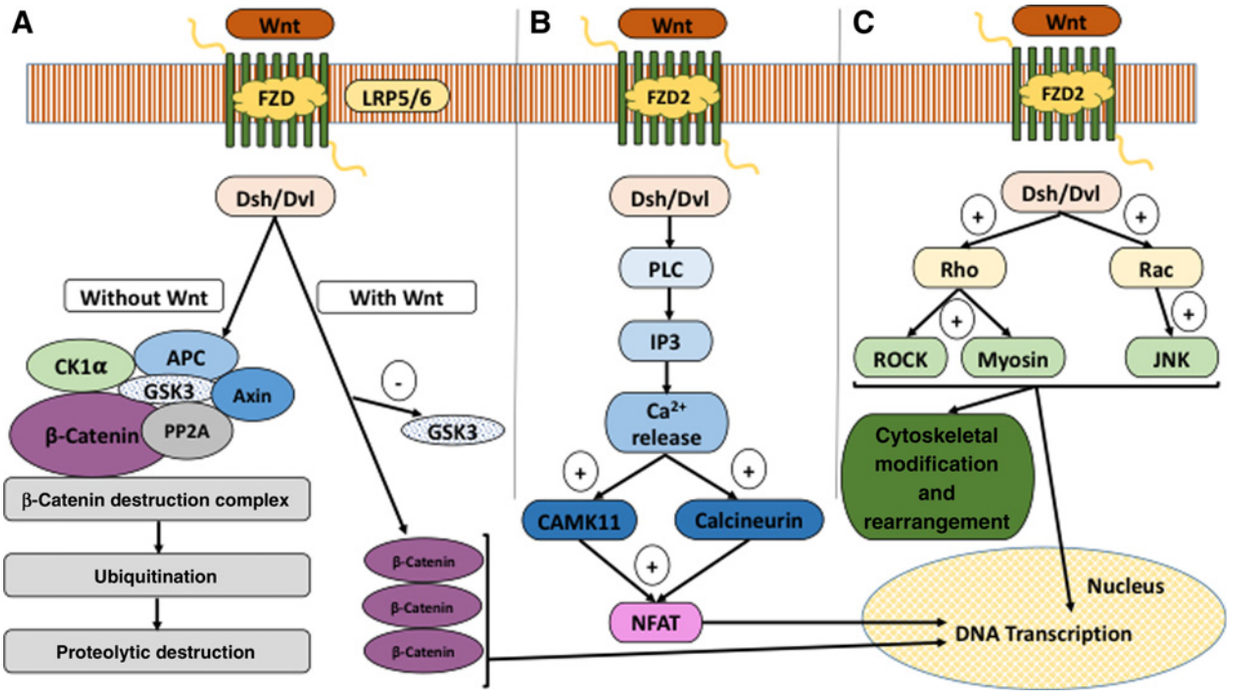

Figure 1.

The Wnt signaling pathway. **A,** The canonical pathway signals via the FZD family of G-protein–coupled receptors. **B,** Wnt/$Ca2^{+}$ pathway (noncanonical). **C,** planar cell polarity (noncanonical).

required to be ≥18 years, Eastern Cooperative Oncology Group (ECOG) Performance Status 0–1, and have an estimated life expectancy >3 months. Adequate marrow function, renal function, hepatic function, and serum albumin ≥ 2.5 g/dL were required. Study-specific exclusion criteria included chemotherapy or radiotherapy within 4 weeks, unstable brain metastases, less than 1 month from definitive therapy of brain metastases, uncontrolled inter-current illness, known ophthalmologic conditions especially current or past history of serious retinopathy or retinal vein occlusion, major surgical procedure within less than 3 weeks or minor surgical procedure within 1 week of first study drug administration, inability to swallow capsules, known history of HIV, hepatitis B, and/or hepatitis C, pregnancy, and electrolyte abnormalities that are refractory to therapy.

Study design, drug, and treatment

This was a multicenter phase I/IB study with escalation and expansion cohorts. The trial incorporated a standard 3+3 design with a cohort expansion to 6 patients if a dose-limiting toxicity (DLT) was reported. All patients were treated with selumetinib and cyclosporin A. In the dose escalation phase, the starting dose level of selumetinib and cyclosporin A was 50 mg twice daily and 2 mg/kg twice daily, respectively. The cyclosporin A trough levels were measured 6–8 days after initiating treatment and the steady-state trough level goals were 125 to 250 ng/mL. The MTD was defined as the highest dose at which no more than 1 of 6 experienced a DLT. Once the RP2D/MTD was identified, the dose expansion cohort of 20 patients with metastatic irinotecan and oxaliplatin-refractory colorectal cancer was initiated. *KRAS* wild-type (WT) and MT colorectal cancer objective responses were assessed to determine whether there were differential responses between the subsets. All patients in the expansion cohort were required to have a baseline tumor biopsy prior to starting treatment. The first 10 patients within the expansion cohort had a 7-day run-in of selumetinib alone at the RP2D followed by a repeat tumor biopsy during the cycle #1 of treatment to assess whether there was a correlation between FZD upregulation and tumor response. Five of these 10 patients were *KRAS* MT and the other 5 were *KRAS* WT to determine whether *RAS* status was predictive of response. The remaining 10 patients in the dose expansion cohort received selumetinib and cyclosporin A concurrently from the start of enrollment and had a tumor biopsy at the time of restaging prior to cycle #3.

Toxicities were defined as per the National Cancer Institute's Common Terminology Criteria for Adverse Events v4.0. Patients were monitored for DLTs through the course of therapy but only DLTs occurring in the first 28 days were used to determine dose escalation and ultimately, MTD. The following adverse events if attributed to either selumetinib or cyclosporin A were considered a DLT: grade 4 neutropenia for ≥7 days, grade 3 or 4 neutropenia with a single temperature reading ≥ 38.3°C or sustained temperature reading > 38°C for >1 hour, grade 3 thrombocytopenia associated with clinically significant bleeding that required transfusion therapy, grade 4 thrombocytopenia, ≥ grade 3 nausea or vomiting that persisted beyond 72 hours despite use of optimal antiemetics, ≥ grade 3 diarrhea that persisted beyond 72 hours despite use of optimal antidiarrheal agents, ≥ grade 3 hyponatremia, hypokalemia, hypomagnesemia, and hypophosphatemia that persisted for or beyond 7 days despite maximal medical

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management, any intolerable adverse event regardless of grade that did not resolve within 7 days despite maximal medical management or other nonhematologic toxicities grade 3 or higher except for adverse events related to underlying disease, alopecia, fatigue, lymphopenia without significant infection, and isolated asymptomatic grade 3 electrolyte abnormalities.

Dose modifications

Selumetinib. Treatment with selumetinib was withheld if patient experienced a DLT or any intolerable adverse event regardless of grade that was considered related to selumetinib despite optimal supportive care. Treatment was not restarted until the toxicity improved to grade 1 or baseline except in the case of rash where treatment was restarted with a grade 2 rash. Treatment was resumed at the original dose or at a permanently reduced dose at the discretion of the investigator. Drug was withheld and then restarted at a permanently reduced dose in patients who experienced recurrence of a specific toxicity. If previous dose reductions had already taken place due to recurring toxicity or patient was already receiving the lowest possible dose of selumetinib (50 mg once daily), then selumetinib was discontinued.

Cyclosporin A. Cyclosporin A dosing was changed according to trough levels although the range was not absolute and trough levels outside this range were also accepted at the discretion of the treating physician. If the patient had a known toxicity to cyclosporin A such as renal toxicity or hypertension, then the dose adjustment was determined by the toxicity and not the cyclosporin A level. Dose adjustment recommendations were: if the cyclosporin A trough level was below 125 ng/mL then the dose of cyclosporin A was increased by 0.5–1 mg/kg not to exceed 50 mg per dose adjustment; if the cyclosporin A trough levels were above 250 ng/mL then dose was decreased by 0.5–1 mg/kg not to exceed 50 mg per dose adjustment; if the cyclosporin A trough level was above 350 mg/mL then cyclosporin A was held and levels were monitored until the level was below 125 ng/mL and then cyclosporin A was restarted at 67% of previous dose with repeated drug levels at 48–72 hours. If patients contracted or were exposed to infectious diseases like herpes viruses or *Pneumocystis jiroveci* pneumonia (PJP) then cyclosporin A was discontinued because of concerns about cyclosporin A related immunosuppression.

Pharmacologic assessments

Pharmacokinetic assessments. Because the pharmacokinetics of selumetinib and cyclosporin A have already been studied in humans, the pharmacokinetic analysis in our study specifically evaluated the pharmacokinetic effects of selumetinib, its active metabolite N-desmethyl AZD6244 and cyclosporin A on each other. All patients in cycle 1 of the dose escalation phase received selumetinib alone on day -7 and cyclosporin A on day -3 with plasma sampling at 0.5, 1, 2, 4, 8, and 24 hours after each of those treatments. Pharmacokinetic sampling was also performed at the same time intervals after both drugs were given to patients on day 1 of the first cycle. Steady-state pharmacokinetic measurements were performed on weeks 2 and 4 of cycle 1. All plasma samples were analyzed with LC/MS-MS assays (26, 27).

Pharmacodynamic analyses. Biomarker analysis for p-ERK and FZD1/2 was performed in the expansion cohort of 20 patients to determine whether there were any associations between p-ERK and FZD1/2 expression and antitumor activity of the selumetinib–cyclosporin A combination. All patients had tumor biopsies at baseline. The first 10 patients had a second biopsy after the selumetinib run-in while the other 10 patients had a second biopsy at the time of restaging. Two core biopsies of tumor were analyzed by IHC for p-ERK and FZD1/2. P-ERK IHC utilized a primary p44/p42 ERK1/2 rabbit mAb while FZD1/2 IHC was achieved with Santa Cruz Biotechnology Goat Polyclonal IgG FZD1/2 primary antibody. A light to dark brown staining of the membrane and/or cytoplasm along with a pale to dark blue coloration of the nuclei with hematoxylin counterstaining was considered a positive IHC reaction. Predominantly stained compartments were identified in pre- and posttreatment tissue specimens and Histology scores (H-scores) were calculated using the proportion and intensity of stained tumor cells. The H-scores pre- and posttreatment were then compared. The H-score range was from 0 to 300 and a value over 50 was considered to be positive while anything below that was considered a negative test.

Statistical analyses

All patients who had received at least one dose of the study medication were included in the safety analyses. The primary objective of the study was to find the MTD and the MTD was defined as the highest dose at which 0 or 1 of 6 patients or 2 of 12 patients had a DLT. Descriptive statistics were used to analyze patient characteristics, safety, pharmacodynamics, and efficacy. Adverse events were tabulated by type and grade. Antitumor activity was assessed on the basis of objective tumor response as per Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 criteria and PFS. Response rates were tabulated with 95% exact binomial confidence intervals. PFS was evaluated using the product limit method of Kaplan and Meier. Pharmacokinetic analyses included examination of the area under the curve (AUC) and C_{max} of the combination therapy of the study drugs. Pharmacodynamic studies included pre- and posttreatment evaluation of MEK activity and noncanonical Wnt signaling by using IHC to measure p-ERK and FZD1/2, respectively. Posttreatment specimens were expected to demonstrate $\geq$ 30% inhibition in p-ERK to be considered significant while a 1.5-fold or greater increase in FZD1/2 was considered a cutoff for the selumetinib only arm.

Results

Patient characteristics

Twenty patients were enrolled in the dose escalation cohort while 19 patients were enrolled in the dose expansion cohort (Table 1). The majority of patients were 18 to 64 years of age with a smaller proportion of patients being over the age of 65. Colorectal cancer was the most common tumor type and comprised 31 (79.5%) patients included in the study. Other tumor types that were included in the dose escalation cohort were renal cell (1), prostate (1), hepatocellular (1), cervical (1), endometrial (1), ovarian (2), and pancreatic cancer (2). The rates of *RAS* mutation on the study were similar to what is found in the general population. Enrollment occurred over a period of 24 months and patients were on the study for a median duration of 3.15 months.

Drug exposure

All patients received at least one dose of study medication. 6 patients received the 50 mg twice-daily dosing while the rest of the patients received the 75 mg twice-daily dosing of selumetinib.

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Table 1. Patient baseline characteristics

Characteristics	Escalation cohort ($n = 20$) No. of patients (%)	Expansion cohort ($n = 19$) No. of patients (%)
Age (years)		
18–64	13 (68.4%)	12 (57.1%)
65+	6 (31.6%)	9 (42.8%)
Sex		
Male	9 (47.4%)	10 (47.6%)
Female	10 (52.6%)	11 (52.4%)
Tumor primary site		
Colorectal	13	
Renal	1	
Prostate	1	
Pancreas	1	
ECOG Performance Status		
0	7 (36.8%)	10 (47.6%)
1	12 (63.2%)	11 (52.4%)

Cyclosporin A was maintained within trough levels of 125–250 ng/mL in all patients during the course of the study. The dosing of cyclosporin A was maintained at 2 mg/kg throughout the study.

Safety and tolerability

DLTs were grade 3 hypertension, rash, and elevated creatinine. The grade 3 hypertension was noted at dose levels of 75 mg orally twice daily of selumetinib and 2 mg/kg orally twice daily of cyclosporin A. The grade 3 rash and the grade 3 elevated creatinine occurred at the same abovementioned dose levels as well. Hence, MTD was determined to be 75 mg twice daily of selumetinib and 2 mg/kg of cyclosporin A, and this is also determined to be the RP2D.

Treatment-related adverse events are described in Table 2. Adverse events such as acneiform rash, maculopapular rash, diarrhea, and edema can be attributed to selumetinib as these adverse events have been reported by other MEK inhibitor trials while hypertension and elevated creatinine are well-known side effects of cyclosporin A. Nine grade 3 and one grade 4 toxicity were reported on the study. The grade 3 adverse events comprised of hypertension, rash, decreased lymphocyte count, anemia, hyponatremia, fatigue, anorexia, anal mucositis, myositis, peripheral motor and sensory neuropathy, peripheral edema, acute kidney injury, lung infection, dyspnea, and acute coronary syndrome. Of those, hypertension, anemia, peripheral edema, and rash were definitely attributed to study drugs. The grade 4 adverse events included hyponatremia and increased alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Hyponatremia was possibly related to the study drug but the elevated AST and ALT were thought to be unrelated. Two patients died while still on study, one due to intracranial hemorrhage and the other due to tumor progression but none of the deaths were study drug-related.

Pharmacokinetics

Because the pharmacokinetics of selumetinib and cyclosporin A have already been studied in humans, the pharmacokinetic analysis in our study specifically evaluated the pharmacokinetic effects of selumetinib and cyclosporin A on each other. Pharmacokinetics for selumetinib was evaluated on cycle 1 day -7 when it was administered alone and on cycle 1 day 1 (C1D1) when it was administered along with cyclosporin A (Fig. 2A and B). When administered alone, the mean $t_{1/2}$ of selumetinib at the MTD was 6.4 hours while mean C_{max} was observed to be 1,550 ng/mL. For cyclosporin A, mean C_{max} on day -3 was 858 ng/mL, whereas it was noted to be 919 ng/mL on C1D1 when it was administered with selumetinib (Fig. 2C and D). Mean $t_{1/2}$, C_{max}, and area under the plasma concentration–time curve increased with increase in dosing of selumetinib (Fig. 3A). C_{max} for the MTD of selumetinib when given with cyclosporin A was 1,250 ng/mL. The mean C_{max} of N-desmethyl-AZD6244, an active metabolite of selumetinib, was 70 ng/mL when selumetinib was given alone and 58 ng/mL on C1D1 when selumetinib was given along with cyclosporin A. Hence, it is appropriate to conclude that there is no substantial difference in the pharmacokinetics of selumetinib when given alone versus in conjunction with cyclosporin A (Fig. 3B and C).

Mean half-life of cyclosporin A was determined to 8.24 hours on day -3 when it was administered alone (Fig. 3D). Selumetinib does not appear to affect the pharmacokinetics of cyclosporin A when they are administered together (Figs. 2 and 3).

Steady-state pharmacokinetic measurements were done on weeks 2 and 4 of cycle 1. All plasma samples were analyzed with LC/MS-MS assays.

Table 2. Treatment-related adverse events occurring in more than 10% of patients

Adverse event	No. of patients (%)
Any grade ≥ 3	10 (26%)
Blood and lymphatic system disorders	
Anemia	11 (28%)
Neutropenia	5 (13%)
Thrombocytopenia	7 (18%)
Gastrointestinal disorders	
Abdominal pain	8 (21%)
Nausea	26 (67%)
Vomiting	15 (38%)
Diarrhea	18 (46%)
Constipation	8 (21%)
Anorexia	9 (23%)
Mucositis	7 (18%)
Dry mouth	5 (13%)
Hepatic disorders	
AST increased	14 (36%)
ALT increased	5 (13%)
ALP increased	8 (21%)
Total Bilirubin increased	5 (13%)
Generalized disorders of well-being	
Fatigue	19 (49%)
Hypoalbuminemia	8 (21%)
Weight gain	5 (13%)
Skin disorders	
Rash acneiform	17 (44%)
Rash maculopapular	16 (41%)
Renal disorders	
Elevated creatinine	13 (33%)
Peripheral edema	17 (44%)
Facial edema	7 (18%)
Hypertension	19 (49%)
Respiratory disorders	
Dyspnea	10 (26%)
Neurologic disorders	
Dizziness	7 (18%)
Headache	6 (15%)
Peripheral sensory neuropathy	5 (13%)
Electrolyte abnormalities	
Hyponatremia	5 (13%)
Hypomagnesemia	11 (28%)

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Pharmacodynamics

p-ERK and FZD1/2, the two biomarkers of interest, were evaluated pre- and posttreatment because p-ERK downregulation and FZD overexpression was expected with MEK inhibition on the basis of prior preclinical studies. FZD overexpression posttreatment with selumetinib alone would confirm the hypothesis that Wnt pathway upregulation is a means of resistance to MEK inhibition. Decreased FZD expression with cyclosporin A would be an indication of cellular antitumor activity.

Twenty-one patients received pretreatment biopsies per protocol. Of these, 4 pretreatment biopsies were not evaluable because partner block was not submitted, or no tumor cells were detected on evaluation. Pretreatment, p-ERK was positive in all 17 patients but FZD1/2 testing was positive in only 11 patients. Fifteen of the 21 pretreatment biopsy patients had second biopsies either in the run-in arm or in the combined therapy arm. When patients from the run-in and the concurrent treatments were combined together for posttreatment biomarker assessment, 14 of 15 patients were positive for p-ERK and 11 of 15 patients were positive for FZD1/2 testing. One patient who was positive for p-ERK at baseline converted to being negative on the biopsy posttreatment with selumetinib alone on the run-in arm. Of all the patients who were positive for p-ERK, 46.6% demonstrated $\geq$30% inhibition of p-ERK posttreatment. None of the patients who were positive for FZD1/2 at baseline converted to being negative for FZD1/2 posttreatment. One patient who was previously negative for FZD1/2 at baseline became positive posttreatment. After treatment with selumetinib alone, 28.6% of patients had a 1.5-fold or greater increase in FZD1/2 expression.

Overall, p-ERK downregulation was noted but statistically significant inhibition was seen in approximately half of the evaluable patients. FZD1/2 overexpression with selumetinib treatment was noted (Fig. 4A and B) but correlation between FZD overexpression and overall response rate was not evaluable from our study because of limited sequentially associated biopsy samples.

Antitumor effect

Objective response rates per RECIST v1.1 were measurable in 30 of 39 patients enrolled in both dose escalation and expansion cohorts. Twenty-six of the 30 patients with measurable disease had metastatic colorectal cancer. Among the 30 patients, there were no complete responses (CR), two patients

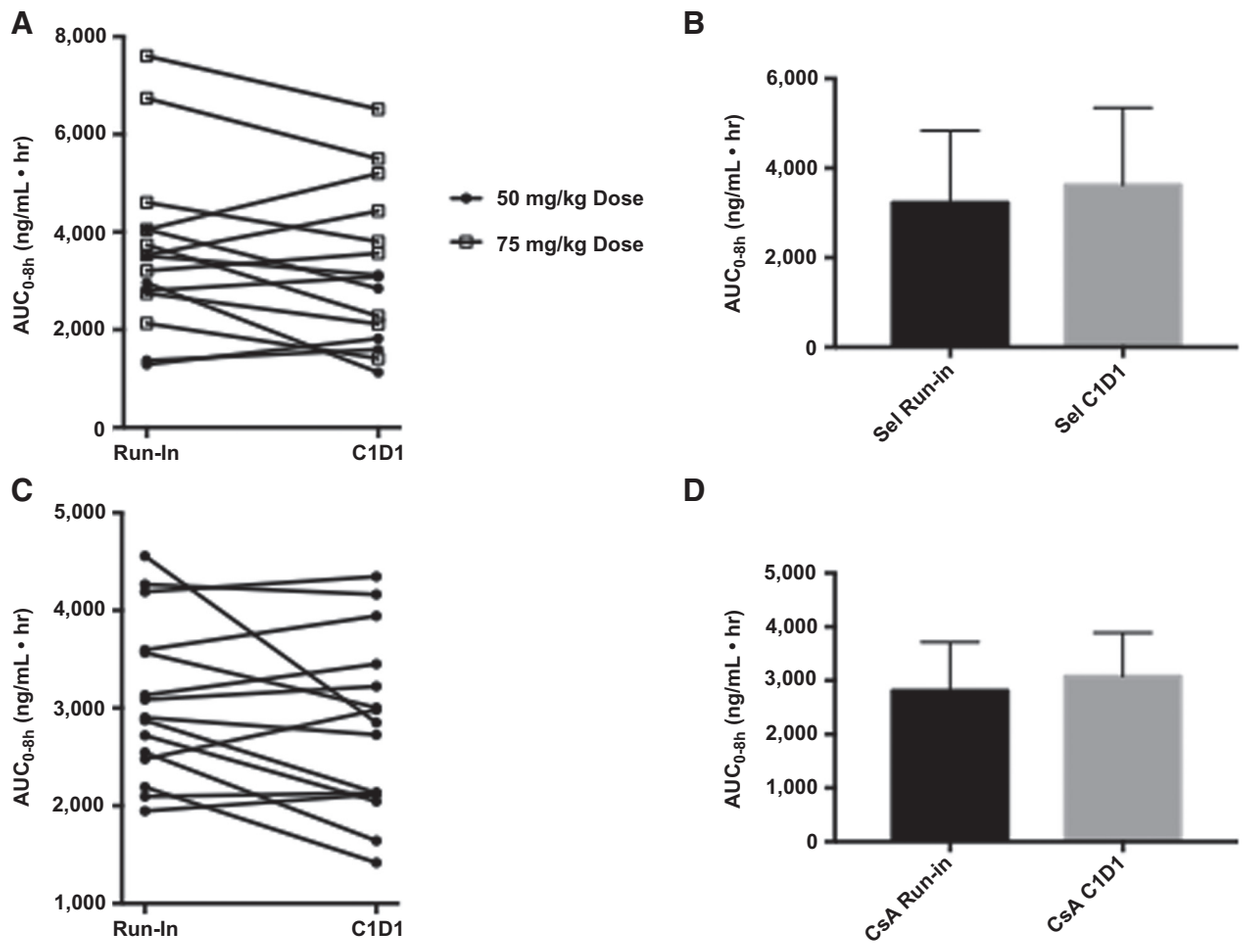

Figure 2.

A and **B,** AUC for selumetinib run-in and C1D1 when administered with cyclosporin A. **C** and **D,** AUC of cyclosporin A alone and C1D1 when administered with selumetinib.

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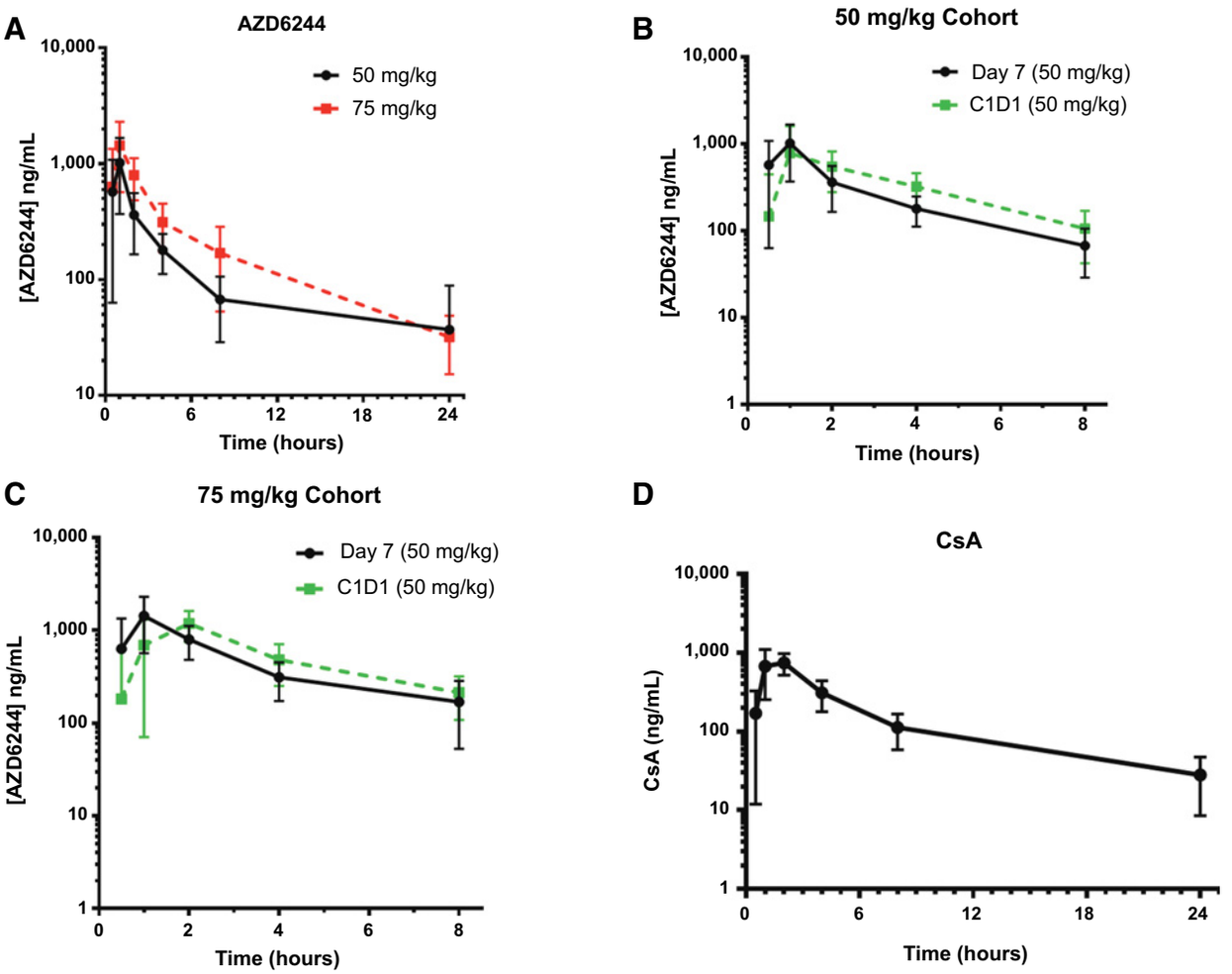

Figure 3.

A, AUC over a 24-hour period of selumetinib alone at the 50 mg and 75 mg dose. **B,** AUC over an 8-hour period of selumetinib 50 mg dosing when administered alone at day -7 and on C1D1 with cyclosporin A. **C,** AUC over an 8-hour period of selumetinib 75 mg dosing when administered alone at day -7 and on C1D1 with cyclosporin A. **D,** AUC over a 24-hour period of cyclosporin A at 2 mg/kg dosing when administered alone.

with colorectal cancer had partial responses (PR), and 18 other patients had stable disease (SD; Fig. 5). The combined clinical benefit rate (CR + PR + SD) for both cohorts was 67%. The dose expansion cohort alone comprised of 16 patients with mCRC with measurable disease. Nine of the 16 patients had SD with no PR or CR. The clinical benefit rate for the dose expansion cohort alone was 56%.

KRAS mutations were observed in 58% of study patients with colorectal cancer, which is in keeping with what is seen in the general population. One patient with colorectal cancer who had a *KRAS*-MT had a PR. SD was noted in 15 patients, of which 10 were *KRAS*-MT, and progressive disease was observed in a total of 8 patients, of which 3 patients were *KRAS*-MT. No statistically significant variations were noted in antitumor effect between *KRAS*-MT and *KRAS*-WT populations.

Median PFS, a secondary endpoint, was calculated to be 3.15 months (95% confidence interval, 2.48–3.82) in all patients combined from the dose escalation and dose expansion cohorts. However, the value of PFS in an early-phase clinic trial is limited.

Discussion

In recent years, new treatment options have been developed for mCRC, but the gains in overall survival have been limited with median survival being 28 to 30 months. At this time, more effective and personalized therapies are needed to improve survival and limit toxicity. In line with these objectives, preclinical studies were done to identify means of resistance to various therapies including MEK inhibition that showed canonical and noncanonical Wnt pathway overexpression in MEK inhibitor–resistant *KRAS*-MT colorectal cancer cell lines and this preclinical work formed the basis of our trial. The primary objective of this study was to identify a MTD and determine DLTs. The study design of the expansion cohort was adapted to not only evaluate safety, pharmacokineticss, and response but also clinically validate Wnt pathway overexpression seen in the preclinical data, enunciate molecular evidence of Wnt suppression with cyclosporin A, and differentiate efficacy on the basis of presence or absence of *RAS* mutations.

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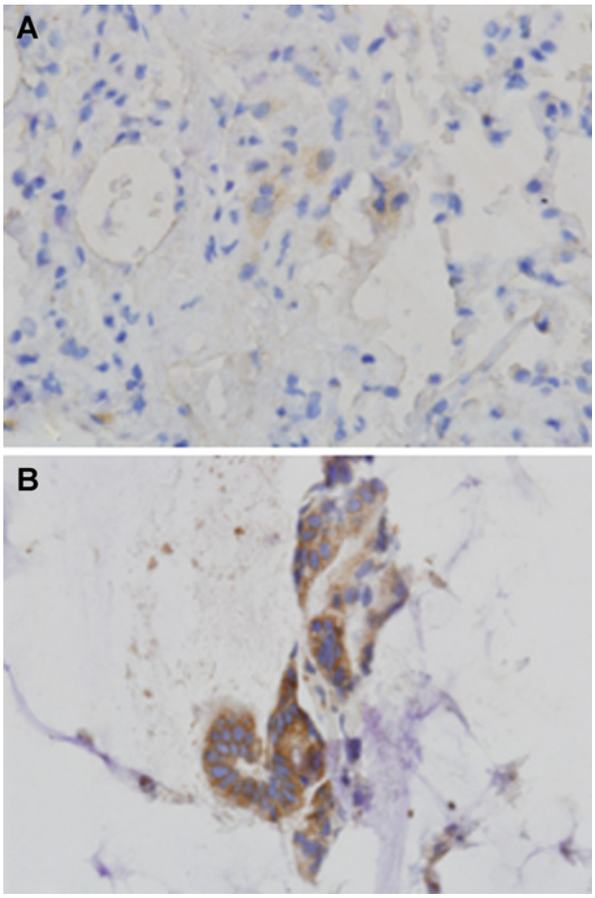

Figure 4.
A, FZD 1/2 expression at baseline prior to selumetinib therapy. **B,** Elevated FZD 1/2 expression posttreatment with selumetinib.

This study recommends a phase II dose of 75 mg twice daily and 2 mg/kg twice daily for selumetinib and cyclosporin A, respectively. The DLTs were hypertension, elevated creatinine, and rash. Hypertension and elevated creatinine are well known toxicities of cyclosporin A while rash is a common toxicity associated with MEK inhibitors including selumetinib. Studies have shown that cyclosporin A causes a decrease in the glomerular filtration rate through vasoconstriction of glomerular arterioles that results in increased creatinine and hypertension. Transplant patients on chronic cyclosporin treatment are typically managed with calcium channel blockers that can prevent the renal vasoconstriction (28, 29). Patients in the study with hypertension were managed with antihypertensives with good effect and there was no known long-term renal dysfunction secondary to cyclosporin on the study.

The DLT of selumetinib in the original single-agent phase I trial was grade 3 acneiform rash and pleural effusion, and the most common adverse events were fatigue, acneiform dermatitis, nausea, diarrhea, and peripheral edema (30). All these adverse events were commonly noted in this study as well. Visual changes, a well-known class effect of MEK inhibitors, were noted in only 6% of patients on our study compared with 12% in the phase I study of selumetinib but this is probably because selumetinib dosing did not exceed 75 mg twice daily (30). The MTD identified in this combination trial for selumetinib was 75 mg twice daily, which is in keeping with findings of the phase I trial of selumetinib monotherapy.

The phase I trial of selumetinib alone has shown previously the mean half-life ($t_{1/2}$) of selumetinib administered in the capsule form to be 5 to 8 hours. Our study showed a similar $t_{1/2}$ life for the single agent as well as the combination (30). Cyclosporin A, which is primarily metabolized through the liver, also had a similar half-life when administered alone and in combination with selumetinib (31). Overall, the pharmacokinetic profile supports the RP2D scheme.

Preclinical studies have demonstrated Wnt pathway overexpression with MEK inhibition. Pretreatment p-ERK positivity is indicative of an active MAPK pathway in the tumors and therefore an active substrate for selumetinib. Phase I studies of selumetinib have previously shown significant p-ERK inhibition, but in our study, significant p-ERK inhibition posttreatment could be demonstrated in only half the patients, suggesting that the MAPK pathway may have still been activated despite treatment with selumetinib. Differences in duration of MEK inhibition, types of samples obtained, variation in methodology of testing for p-ERK, and phosphorylation of ERK by kinases other than MEK1/2 could be some of the reasons why all patients did not show significant inhibition of p-ERK compared with prior studies investigating selumetinib (30). The extent of ERK inhibition in our study is also limited by selumetinib that is a second-generation MEKi. There are other MEK inhibitors in development that have shown greater potency in inhibiting MEK1/2 (9, 32). We observed FZD expression in 65% of patients at baseline that suggests an activated Wnt pathway. About 30% of tissue specimens obtained from patients posttreatment with selumetinib monotherapy showed significant upregulation of FZD2, which is in keeping with the hypothesis of our study that Wnt pathway overexpression is a means of resistance to MEK inhibition therapy in colorectal cancer. All tumor specimens did not display FZD2 overexpression with MEK inhibition, which confirms our current understanding that multiple pathways are involved in treatment resistance. Our pharmacodynamic analyses could not clearly establish suppression of the Wnt pathway with cyclosporin A treatment at the molecular level because of inadequate sample size.

The two PRs and the 18 patients with SD noted on this study are consistent with the promising activity seen in the preclinical studies. The clinical benefit rate for the combination of selumetinib and cyclosporin A in this study is modest but encouraging given that the response rate is higher than would be expected for either single-agent alone (33). However, the results do not mirror the robust responses seen in our preclinical models, thus highlighting the difficulty of translating promising preclinical data into clinical trials. Therefore, it is critically important to investigate resistance mechanisms in patient samples obtained during the course of the study, and these studies are ongoing at this time.

In summary, this phase I study establishes that the combination of selumetinib and cyclosporin A has a manageable safety profile at the RP2D for future studies and provides preliminary evidence of antineoplastic activity. Future directions include not only studying the selumetinib/cyclosporin A combination in a phase II setting but also combining MEK inhibition with canonical Wnt inhibitors. Many inhibitors of the canonical Wnt signaling

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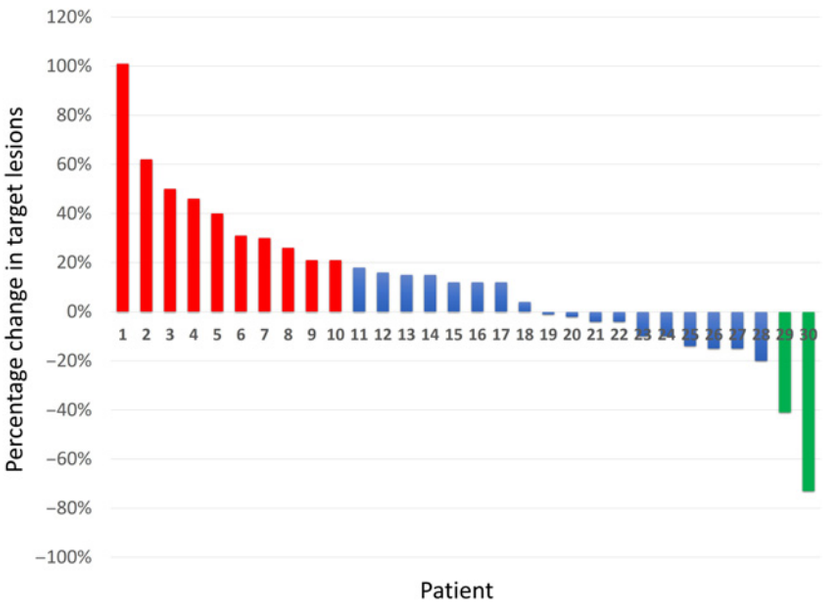

Figure 5.
Objective response rate as per RECIST criteria v1.1 by patient. Green bars, PR; blue bars, SD; red bars, progressive disease.

pathway are currently being investigated in the preclinical and early clinical studies. Antibodies to Wnt ligands, overexpression of naturally occurring Wnt ligand antagonists, FZD antibodies, promotion of β-catenin degradation are just some of the many ways in which the Wnt pathway is being targeted (21).

Disclosure of Potential Conflicts of Interest

M.N Stein is a consultant/advisory board member for Merck Sharp & Dohme. H.K. Sanoff reports receiving a commercial research grant from Bayer and Merck. No potential conflicts of interest were disclosed by the other authors.

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Phase Ib Results of the Rational Combination of Selumetinib and Cyclosporin A in Advanced Solid Tumors with an Expansion Cohort in Metastatic Colorectal Cancer

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