Onvansertib in Combination with FOLFIRI and Bevacizumab in Second-Line Treatment of *KRAS*-Mutant Metastatic Colorectal Cancer: A Phase Ib Clinical Study

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ABSTRACT

Purpose: Onvansertib is a highly specific inhibitor of polo-like kinase 1 (PLK1), with demonstrated safety in solid tumors. We evaluated, preclinically and clinically, the potential of onvansertib in combination with chemotherapy as a therapeutic option for *KRAS*-mutant colorectal cancer.

Patients and Methods: Preclinical activity of onvansertib was assessed (i) *in vitro* in *KRAS* wild-type and -mutant isogenic colorectal cancer cells and (ii) *in vivo*, in combination with irinotecan, in a *KRAS*-mutant xenograft model. Clinically, a phase Ib trial was conducted to investigate onvansertib at doses 12, 15, and 18 mg/m^2 (days 1–5 and 14–19 of a 28-day cycle) in combination with FOLFIRI/bevacizumab (days 1 and 15) in patients with *KRAS*-mutant metastatic colorectal cancer who had prior oxaliplatin exposure. Safety, efficacy, and changes in circulating tumor DNA (ctDNA) were assessed.

Results: In preclinical models, onvansertib displayed superior activity in *KRAS*-mutant than wild-type isogenic colorectal cancer cells and demonstrated potent antitumor activity in combination with irinotecan *in vivo*. Eighteen patients enrolled in the phase Ib study. Onvansertib recommended phase II dose was established at 15 mg/m^2. Grade 3 and 4 adverse events (AE) represented 15% of all treatment-related AEs, with neutropenia being the most common. Partial responses were observed in 44% of patients, with a median duration of response of 9.5 months. Early ctDNA dynamics were predictive of treatment efficacy.

Conclusions: Onvansertib combined with FOLIFRI/bevacizumab exhibited manageable safety and promising efficacy in second-line treatment of patients with *KRAS*-mutant metastatic colorectal cancer. Further exploration of this combination therapy is ongoing.

See related commentary by Stebbing and Bullock, p. 2005

Introduction

The pace of progress in advancing care for patients with metastatic colorectal cancers (mCRC) has been slow. Chemotherapy is still the backbone of treatment for the majority of patients in the first- and second-line settings (1). In the past two decades, development and approval of mAbs that target angiogenesis (e.g., bevacizumab) and EGFR (cetuximab and panitumumab) have been shown to enhance the efficacy of chemotherapy (2, 3). However, the benefit of EGFR inhibitors is limited to patients with *RAS* wild-type tumors (3, 4). *KRAS* mutation is a frequent and early event in colorectal cancer, occurring in approximately 45% of patients (5, 6). Contemporary data suggest that patients with *KRAS*-mutated mCRC have poorer overall survival and progression-free survival (PFS) compared with those with *KRAS* wild-type (7, 8). Efforts for targeting KRAS in colorectal cancer and other cancers has had very limited progress so far (9), with the exception of the recent success in targeting KRAS-G12C, a relatively rare mutation in colorectal cancer (6, 10). Therefore, advancing treatment options for *KRAS*-mutated mCRC is a high priority area.

The most commonly used first-line treatment in patients with mCRC is FOLFOX (folinic acid, fluorouracil, and oxaliplatin) in combination with bevacizumab, and is associated with a median PFS (mPFS) of 8–10 months (11). Following progression, patients generally transition to FOLFIRI (folinic acid, fluorouracil, and irinotecan) + bevacizumab; however, the efficacy of this regimen is limited, with an objective response rate (ORR) of 5%–10% and an mPFS of approximately 6 months (12, 13).

Polo-like kinase 1 (PLK1) is a serine/threonine kinase, and a key regulator of the cell cycle, particularly in mitosis where it controls its entry and progression (14). PLK1 also plays a pivotal role in the DNA damage response. It facilitates repair through the phosphorylation of key DNA repair factors and promotes recovery from the G_2–M checkpoint arrest following DNA damage (15, 16). PLK1 is overexpressed in several tumor types, including colorectal cancer, and its expression is associated with poor survival (17–19). In addition, increased PLK1 protein and phospho-PLK1 levels were detected in relapsed/metastatic colorectal cancer tissues compared with matched primary tissues (20), suggesting that PLK1 confers resistance to chemotherapy and represents a promising target for patients with chemoresistant colorectal cancer.

Onvansertib is a highly potent, reversible, and selective PLK1 kinase inhibitor, inducing mitotic arrest and ultimately apoptosis in cancer cells (21). Onvansertib effectively and broadly inhibited colorectal cancer cell proliferation *in vitro*, and showed potent antitumor activity *in vivo* in colorectal cancer models (21). Moreover, PLK1 inhibitors, including onvansertib, enhanced the efficacy of DNA damage agents such as oxaliplatin and irinotecan in colorectal cancer preclinical models (20–23), supporting the use of PLK1 inhibitors in combination with chemotherapy.

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Translational Relevance

The polo-like kinase 1 (PLK1) has emerged as a promising therapeutic target for *KRAS*-mutant colorectal cancer. In this work, preclinical data were generated that established a rationale to clinically assess the PLK1 inhibitor onvansertib, in combination with FOLFIRI/bevacizumab, in patients with *KRAS*-mutant metastatic colorectal cancer. In the phase Ib study, the combination proved to be tolerable and was associated with a remarkable and durable response. Importantly, the observed benefits extended across a variety of *KRAS* mutations. These results show promise for the treatment of *KRAS*-mutated metastatic colorectal cancer, a disease in dire need of new therapeutic options.

Onvansertib is orally available with a human half-life of approximately 24 hours (24, 25), presenting the opportunity for convenient, well-regulated, and flexible dosing schedules. In a phase I study of single agent, onvansertib dose levels of 6, 12, and 24 mg/m^2/day for 5 out of 21 days were associated with no dose-limiting toxicities (DLT), and 24 mg/m^2 was established as the recommended phase II dose (RP2D) for solid tumors (24).

On the basis of promising preclinical data indicating the efficacy of PLK1 inhibitors in combination with irinotecan in colorectal cancer models, a phase Ib trial was initiated to define the RP2D of onvansertib in combination with FOLFIRI/bevacizumab (NCT03829410). Here we report our findings on the safety, pharmacokinetics, preliminary efficacy, and biomarkers of response to onvansertib in combination with FOLFIRI/bevacizumab, in second-line treatment of patients with *KRAS*-mutated mCRC.

Patients and Methods

Preclinical studies

In vitro studies

The DLD1 isogenic cells *KRAS*$^{WT/G13D}$ and *KRAS*$^{WT/-}$ were obtained from Horizon Discovery (catalog no. HD-105-002, RRID: CVCL_HD62) and cultured in RPMI1640 (ATCC) supplemented with 10% FBS (Gibco). The cell lines were not tested for *Mycoplasma*. For proliferation assays, cells (passages 3–5) were seeded in 96-well plates and cell viability was measured using the CellTiterGlo assay (Promega) 72 hours after treatment, per the manufacturer's instructions. For the analysis of mitotic cells, cells were fixed (BD Cytofix, 15 minutes, BD Biosciences), permeabilized (BD Phosflow Perm Buffer III, 30 minutes), and stained with Histone H3 phospho-Ser28 antibody (dilution 1:200, BioLegend catalog no. 641003, RRID:AB_1279417) and 1 μg/mL DAPI (BD Biosciences). FACSCelesta (BD Biosciences, RRID:SCR_019597) was used for flow cytometry analysis and the data were processed using FlowJo (RRID:SCR_008520).

In vivo studies

All animal studies were reviewed and approved by the Institutional Animal Care and Use Committee of CrownBio prior to being carried out. The care and use of animals were conducted in accordance with the regulations of the Association for Assessment and Accreditation of Laboratory Animal Care. Female 6–9 weeks old Balb/c nude mice (GemPharmatech Co. Ltd, RRID:IMSR_GPT:D000521) were subcutaneously inoculated with 5 million HCT116 cells (RRID: CVCL_0291). Once tumors reached 100–150 mm^3, mice were randomized and treated with vehicle, onvansertib (oral, 45 mg/kg, daily), irinotecan (intraperitoneally, 5 mg/kg, twice a week) or the combination. Onvansertib was provided by Cardiff Oncology and prepared as a suspension in 0.5% methylcellulose (Sigma-Aldrich, M0262) + 0.1%Tween80. Irinotecan was obtained from Shanghai Xudong Haipu Pharmaceutical Co. Ltd and diluted in saline. Tumor volumes and body weights were measured twice a week.

Patient selection

Patients 18 years or older with histologically confirmed metastatic and unresectable colorectal cancer and a *KRAS* mutation in exon 2, 3, or 4 in either the primary tumor or the metastasis (as assessed by a Clinical Laboratory Improvement Amendments–certified lab), were eligible. Patients were required to have received a minimum of 6 weeks of a first-line regimen that included oxaliplatin and a fluoropyrimidine, with or without bevacizumab, and to have failed treatment or have been intolerant to oxaliplatin. Treatment failure was defined as radiographic progression within 6 months after the last dose of first-line therapy. Patients who received adjuvant or neoadjuvant therapy, and progressed within 6 months of treatment were also eligible. Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 or 1, and adequate organ function were required, as demonstrated by an absolute neutrophil count ≥ 1,500 cells/μL, hemoglobin ≥9 g/dL, platelets ≥100,000/μL, creatinine ≤1.5 times the upper limit of normal (ULN) or creatinine clearance > 50 mL/minute as calculated by the Cockcroft-Gault equation, bilirubin ≤1.5 mg/dL or ≤ 2.0 ULN in presence of liver metastases, and alanine transaminases ≤ 3 times the ULN (or ≤5 times the ULN in patients with liver metastases). Key exclusion criteria were patients with microsatellite instability high/deficient mismatch repair, BRAF V600 mutations, more than one prior chemotherapy regimen administered in the metastatic setting, or untreated brain metastasis. The protocol was approved by the Institutional Review Board or independent ethics committees at each participating center and was in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. Written informed consent was obtained from all patients before screening.

Treatment plan and study design

Onvansertib was administrated orally, in a dose-escalation design, on days 1–5 and 15–19 of a 28-day cycle, in combination with FOLFIRI [irinotecan 180 mg/m^2 i.v.; leucovorin 400 mg/m^2 i.v.; 5-fluorouracil (5-FU) 400 mg/m^2 i.v. bolus; 5-FU 2400 mg/m^2 continuous intravenous infusion for 46 hours] and bevacizumab (5 mg/kg i.v.) administrated on days 1 and 15. The primary objectives were to evaluate the DLTs and the MTD or RP2D of onvansertib. The first dose of onvansertib was 12 mg/m^2, which was half of the RP2D established in the phase I single-agent study for solid tumors (24). The study followed a standard 3+3 dose-escalation design in which onvansertib dose was evaluated at 12, 15, and 18 mg/m^2. Three patients were treated, and if there were no DLTs in the first cycle, escalation to the next higher dose occurred. If a DLT was reported, an additional 3 patients were treated at that dose. If ≥ 2 patients experienced a DLT, the dose was considered as non-tolerated, and lower doses were explored in subsequent cohorts. The MTD was defined as the highest dose achieved at which no more than 1 out of 6 patients experienced a DLT. The RP2D was determined on the basis of the assessment of safety, pharmacokinetics, and preliminary efficacy, in patients treated at a dose cleared for safety.

Safety

Patients were evaluable for safety if they received at least one dose of onvansertib. Safety evaluations included physical examinations,

laboratory test results, electrocardiograms, and monitoring of adverse events (AE) graded by the NCI Common Terminology Criteria for Adverse Events (version 4.03). Investigators assessed causality as either unrelated, unlikely, possibly, probably, or definitely related to the study drug. DLTs were defined as grade 4 (G4) hematologic AEs, grade ≥ 3 non-hematologic AEs that are considered related to the study drug and that do not resolve within 14 days following presentation with standard management and care, grade ≥ 3 thrombocytopenia with bleeding, neutropenic fever, any death not clearly due to the underlying disease or extraneous causes, or any change in liver function that meets Hy's law criteria of a DLT. DLTs were evaluated during the first 28 days of treatment.

Efficacy

Radiographic imaging was obtained at screening and during treatment at every other cycle, until end-of-treatment (EOT). Objective response was assessed using RECIST version 1.1 (RECIST v1.1) in patients who received at least one cycle of treatment. For patients who had stable disease (SD) or partial response (PR) at EOT assessment, follow-up information was collected until the patient started new therapy, underwent a procedure, or experienced progressive disease (PD), for up to 1 year.

Pharmacokinetics

Blood samples were collected from patients for pharmacokinetic analysis on day 5 of cycle 1 at the following timepoints: predose; and 1, 2, 3, 4, 8, 24, 48, and 72 hours postdose. Onvansertib plasma concentrations were determined by LC/MS-MS at Icon Bioanalytical Laboratory. The pharmacokinetic parameters were calculated using Phoenix WinNonlin software (version 8.3, RRID:SCR_024504).

Correlative studies

For patients with available archival tumor tissue, tumor DNA was sequenced using the Tempus xT assay (Tempus Labs Inc.), an next generation sequencing–based targeted panel that analyzed 648 cancer-related genes including *KRAS*.

Blood samples were collected from patients on day 1 (predose) of cycles 1 and 2 into Cell-Free DNA BCT blood tubes (Streck, catalog no. 230470) and processed 24–48 hours after collection at Cardiff Oncology's laboratories. Plasma was separated from whole blood by centrifugation. Circulating tumor DNA (ctDNA) was isolated from plasma and the plasma *KRAS* mutant allelic frequency (MAF) was assessed by digital droplet PCR (ddPCR), as described previously (25). *KRAS* MAF changes were calculated as (*KRAS* MAF C2D1)/(*KRAS* MAF C1D1) − 1.

Statistical analysis

Patient characteristics and AEs were summarized using descriptive statistics. Safety analyses included all enrolled patients who received at least one dose of the study treatment. Efficacy and biomarker analyses included all enrolled patients who received at least one cycle of treatment. PFS and duration of response (DOR) were estimated using the Kaplan–Meier method with 95% confidence intervals (95% CI). The AUC of the ROC curve was used to measure the performance of changes in *KRAS* MAF as a predictor of clinical response. The ROC curve was used to select the best threshold for prediction based on the Youden method (26) and the sensitivity and specificity at this threshold were estimated. All analyses were performed using R version 4.2.3 and GraphPad Prism version 10.0.02 (RRID:SCR_002798). *P* values <0.05 were considered significant.

Data availability

The data generated in this study are not publicly available due to patient privacy, but patient deidentified data are available upon reasonable request from the corresponding author.

Results

Preclinical studies

A genome-wide short hairpin RNA screen identified *PLK1* inhibition to be synthetic lethal with mutant *KRAS* in colorectal cancer cells, suggesting that *KRAS*-mutant colorectal cancer cells are particularly sensitive to PLK1 inhibition (27). To confirm this finding, we assessed the effect of onvansertib in DLD1 isogenic $KRAS^{WT/G13D}$ (KRAS MUT) and $KRAS^{WT/-}$ (KRAS WT) colorectal cancer cells. Onvansertib treatment resulted in increased mitotic arrest and cell death in DLD1 KRAS MUT cells compared with KRAS WT cells (**Fig. 1A** and **B**). These data support that *KRAS*-mutant colorectal cancer cells are more dependent on PLK1 activity for proper mitotic progression and survival, and have increased sensitivity to onvansertib.

Next, we assessed the antitumor activity of onvansertib in combination with irinotecan in HCT116 *KRAS*-mutant colorectal cancer xenograft model (**Fig. 1C**). While both onvansertib and irinotecan single agents significantly reduced tumor growth compared with the vehicle-treated group, the combination resulted in a significantly more profound tumor growth inhibition. On day 42, 4 (50%) of the 8 mice treated with the combination exhibited tumor regression. The combination was well tolerated, with no significant body weight loss observed (**Fig. 1D**).

Collectively, these data confirm that onvansertib in combination with irinotecan represents a promising therapeutic strategy for patients with *KRAS*-mutant colorectal cancer.

Patient characteristics

A phase Ib study was designed to explore the safety, pharmacokinetics, and preliminary efficacy of onvansertib in combination with the standard-of-care (SOC) FOLFIRI/bevacizumab in second-line treatment of patients with *KRAS*-mutated mCRC.

A total of 18 patients were enrolled in two centers in the United States between July 18, 2019 and December 9, 2020. As of May 27, 2022, all patients had completed their on-treatment visits and follow-up period. The median follow-up time was 10.0 months (range, 1.0–22.0) and the median duration of treatment for was 5.5 months (range, 0.1–20.1). Baseline characteristics are summarized in **Table 1** and representativeness of study participants are presented in Supplementary Table S1. The median age was 60 years (range, 37–83), and 44% of patients were male. At study entry, 12 (67%) patients had an ECOG PS of 1. Fourteen (78%) patients had received bevacizumab as part of their first-line treatment and 15 (83%) patients had metastasis at time of diagnosis. Eleven (61%) patients had liver metastasis and 16 patients (89%) had more than one metastatic site at the time of study entry.

Dose escalation and toxicity

At each onvansertib dose level (12, 15, and 18 mg/m^2), 6 patients were enrolled following a 3 + 3 dose-escalation trial design (see Patients and Methods). Subsequently, it was noted that 1 patient had received a dose of 12 mg/m^2 instead of the intended 15 mg/m^2. As a result, the patient was reclassified to the 12 mg/m^2 cohort for the purpose of data analysis.

Five DLTs were observed in total: 1 at 12 mg/m^2 (G4 febrile neutropenia), 1 at 15 mg/m^2 (G4 neutropenia), and 3 at 18 mg/m^2

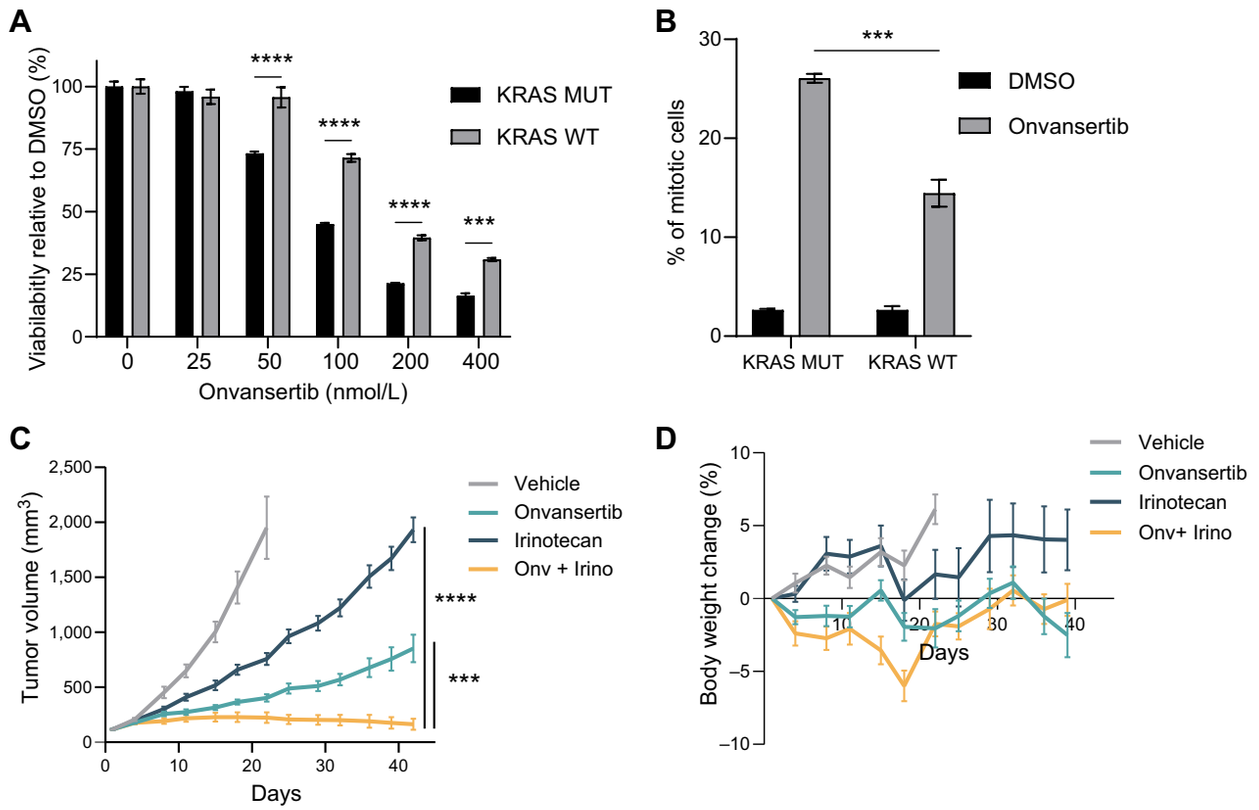


Figure 1.

Effect of onvansertib monotherapy and in combination with irinotecan in colorectal cancer preclinical models. **A,** Isogenic KRAS MUT and KRAS WT DLD1 cells were treated at the indicated concentrations of onvansertib for 72 hours. Bar graph (mean ± SEM, $n = 3$) represents relative viability to DMSO for each cell line. **B,** KRAS MUT and KRAS WT DLD1 cells were treated for 24 hours with DMSO or 100 nmol/L onvansertib. Bar graph (mean ±SEM, $n = 3$) represents the percentage of mitotic cells measured by flow cytometry (positive staining for phospho-H3 Ser28). Data analyzed by two-way ANOVA using Šídák multiple comparisons test. **C** and **D,** BALB/c nude mice inoculated with HCT116 cells were treated with vehicle, onvansertib, irinotecan, or the combination for 42 days. **C,** Mean tumor volumes ± SEM, $n = 7$–8/group. Tumor volumes were compared at day 42 using a one-way ANOVA with Tukey multiple comparison tests. **D,** Mean ± SEM body weight changes from baseline. *** and **** indicate $P \leq 0.001$ and 0.0001, respectively.

Table 1. Patient demographics and baseline characteristics.

Characteristics	*n* = 18 (%)
Median age, years (range)	60 (37–83)
Gender (male)	8 (44)
Race	
White	12 (67)
Asian	4 (22)
Other	2 (11)
ECOG PS	
0	6 (33)
1	12 (67)
Primary tumor site	
Left colon or rectum	11 (61)
Right colon	7 (39)
Metastatic disease at diagnosis	15 (83)
Liver metastasis present	11 (61)
Number of metastatic organs	
Single	2 (11)
Multiple	16 (89)
Received prior bevacizumab	14 (78)

(G4 neutropenia). All patients recovered from their DLTs (range, 1–17 days). On the basis of the dose escalation, onvansertib at 15 mg/m^2 was selected as the RP2D.

Eleven serious adverse events (SAE) were reported in 6 patients (33%) with 5 of them occurring in a single patient (Supplementary Table S2). Three of the 11 SAEs were reported as unlikely related to onvansertib, and the rest were reported as unrelated. Two patients discontinued the study before completion of cycle 1 due to a SAE of grade 3 (G3) intestinal obstruction and G4 febrile neutropenia, respectively.

Most treatment-related AEs (onvansertib and SOC) were grade 1 or 2 (Supplementary Table S3). G3 and G4 AEs represented 15% of total AEs, and the most common across all dose levels was neutropenia (56% of patients; **Table 2**). G4 hyperphosphatemia ($n = 1$) and G4 leukopenia ($n = 1$) were reported in the same patient treated at 18 mg/m^2 and G4 febrile neutropenia was seen in one patient treated at 12 mg/m^2. The other G3 AEs reported in more than one patient included leukopenia (17%), hypertension (17%), nausea (11%), fatigue (11%), and abdominal pain (11%). The most common non-hematologic treatment-related AEs included fatigue (83%), nausea (72%), diarrhea (67%), alopecia (56%), and abdominal pain (50%; Supplementary Table S3).

Table 2. G3 and G4 treatment-related AEs reported in ≥1 patient.

	All doses $N = 18$		12 mg/m^2 $N = 7$		15 mg/m^2 $N = 5$		18 mg/m^2 $N = 6$	
Adverse event	**G3 (%)**	**G4 (%)**	**G3 (%)**	**G4 (%)**	**G3 (%)**	**G4 (%)**	**G3 (%)**	**G4 (%)**
Neutropenia	6 (33)	4 (22)	4 (57)	0 (0)	2 (40)	1 (20)	0 (0)	3 (50)
Leukopenia	3 (17)	1 (6)	0 (0)	0 (0)	2 (40)	0 (0)	1 (17)	1 (17)
Hypertension	3 (17)	0 (0)	1 (14)	0 (0)	1 (20)	0 (0)	1 (17)	0 (0)
Fatigue	2 (11)	0 (0)	2 (29)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Nausea	2 (11)	0 (0)	1 (14)	0 (0)	1 (20)	0 (0)	0 (0)	0 (0)
Abdominal pain	2 (11)	0 (0)	1 (14)	0 (0)	1 (20)	0 (0)	0 (0)	0 (0)
Diarrhea	1 (6)	0 (0)	0 (0)	0 (0)	1 (20)	0 (0)	0 (0)	0 (0)
Thrombocytopenia	1 (6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (17)	0 (0)
Stomatitis	1 (6)	0 (0)	0 (0)	0 (0)	1 (20)	0 (0)	0 (0)	0 (0)
Vomiting	1 (6)	0 (0)	1 (14)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Alanine aminotransferase increased	1 (6)	0 (0)	1 (14)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Aspartate aminotransferase increased	1 (6)	0 (0)	1 (14)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Blood alkaline phosphatase increased	1 (6)	0 (0)	0 (0)	0 (0)	1 (20)	0 (0)	0 (0)	0 (0)
Abdominal abscess	1 (6)	0 (0)	1 (14)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Hepatitis B	1 (6)	0 (0)	1 (14)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Sepsis	1 (6)	0 (0)	0 (0)	0 (0)	1 (20)	0 (0)	0 (0)	0 (0)
Urinary tract infection	1 (6)	0 (0)	0 (0)	0 (0)	1 (20)	0 (0)	0 (0)	0 (0)
Liver abscess	1 (6)	0 (0)	0 (0)	0 (0)	1 (20)	0 (0)	0 (0)	0 (0)
Intestinal obstruction	1 (6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (17)	0 (0)
Hyperphosphatemia	0 (0)	1 (6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (17)
Febrile neutropenia	0 (0)	1 (6)	0 (0)	1 (14)	0 (0)	0 (0)	0 (0)	0 (0)

Across all dose levels, 15 onvansertib-related G3 or G4 AEs were reported. Neutropenia was the only G3/G4 AE reported in more than 1 patient (28% of patients; Supplementary Table S4). The most common non-hematologic onvansertib-related AEs included diarrhea (50%), nausea (50%), fatigue (44%), and vomiting (22%).

Thirteen patients had an AE of neutropenia. The 5-FU bolus was either temporarily interrupted or completely discontinued in 11 patients. Once the 5-FU bolus was stopped, the AE resolved in 10 of the 11 patients in a median of 14 days (range, 1–98). One patient had not recovered from the AE (in 32 days) when they discontinued the study for a non–study-related reason. In the 11 patients where 5-FU bolus was stopped, no changes were made to onvansertib in 6 patients, while onvansertib was temporarily interrupted or dose reduced in 5 patients. In 2 patients, 5-FU bolus was maintained, and the AE resolved on its own in 14 and 82 days, respectively.

Pharmacokinetics

Table 3 shows the mean pharmacokinetic parameters for onvansertib on day 5 of cycle 1. The mean T_{max} values ranged from 1.8 to 5.3 hours, and the terminal half-life of onvansertib in plasma was around 14 hours. Onvansertib C_{max} and AUC values were similar to those reported in the phase Ib dose-escalation study of onvansertib in combination therapy for acute myeloid leukemia (25). One patient with colorectal cancer, treated at 12 mg/m^2, had plasma concentration values more than 10-fold higher than other patients treated at the same dose level (Supplementary Table S5). This patient also experienced a G4 neutropenic fever 10 days after the start of treatment. The reason for the increase in onvansertib plasma concentration is unknown, the patient was considered an outlier and excluded from the summary pharmacokinetic analysis.

Clinical activity

Clinical efficacy was assessed in the 16 patients who completed the first cycle of treatment and is shown in **Fig. 2**. A total of 7 patients achieved PR, including 6 patients with confirmed responses; hence the confirmed and unconfirmed ORR were 37.5% and 43.7%, respectively. The patient with unconfirmed PR was diagnosed with acute hepatitis B shortly after their first PR, and treatment was interrupted; during this time, the patient progressed. Seven patients had SD, and the clinical benefit rate, defined as PR and SD was 87.5%. Three patients discontinued the study to pursue curative surgery ($n = 2$) or targeted liver microwave ablation ($n = 1$). PRs were observed at all dose levels and in patients with diverse *KRAS* mutations (**Fig. 2C**). The median PFS was 12.6 months (95% CI, 9.34–not reached; **Fig. 2D**) and median DOR was 9.5 months (95% CI, 8.9–not reached).

Table 3. Onvansertib pharmacokinetic parameters (mean ± SD) per dose level.

Dose (mg/m^2)	Number of patients	T_{max} (hours)	C_{max} (ng/mL)	AUC_{0-24} (ng/mL.hours)	$t_{1/2}$ (hours)
12[a]	6	5.29 ± 8.32	163 ± 82	2,020 ± 880	13.8 ± 2.1
15	5	1.80 ± 0.84	169 ± 64	1,520 ± 650	13.9 ± 4.3
18[b]	5	2.40 ± 1.20	191 ± 25	2,290 ± 730	13.8 ± 2.3

[a]One patient from the 12 mg/m^2 cohort was excluded from summary statistics as a pharmacokinetic outlier.
[b]One patient from the 18 mg/m^2 cohort was excluded from summary statistics due to incomplete pharmacokinetic samples.

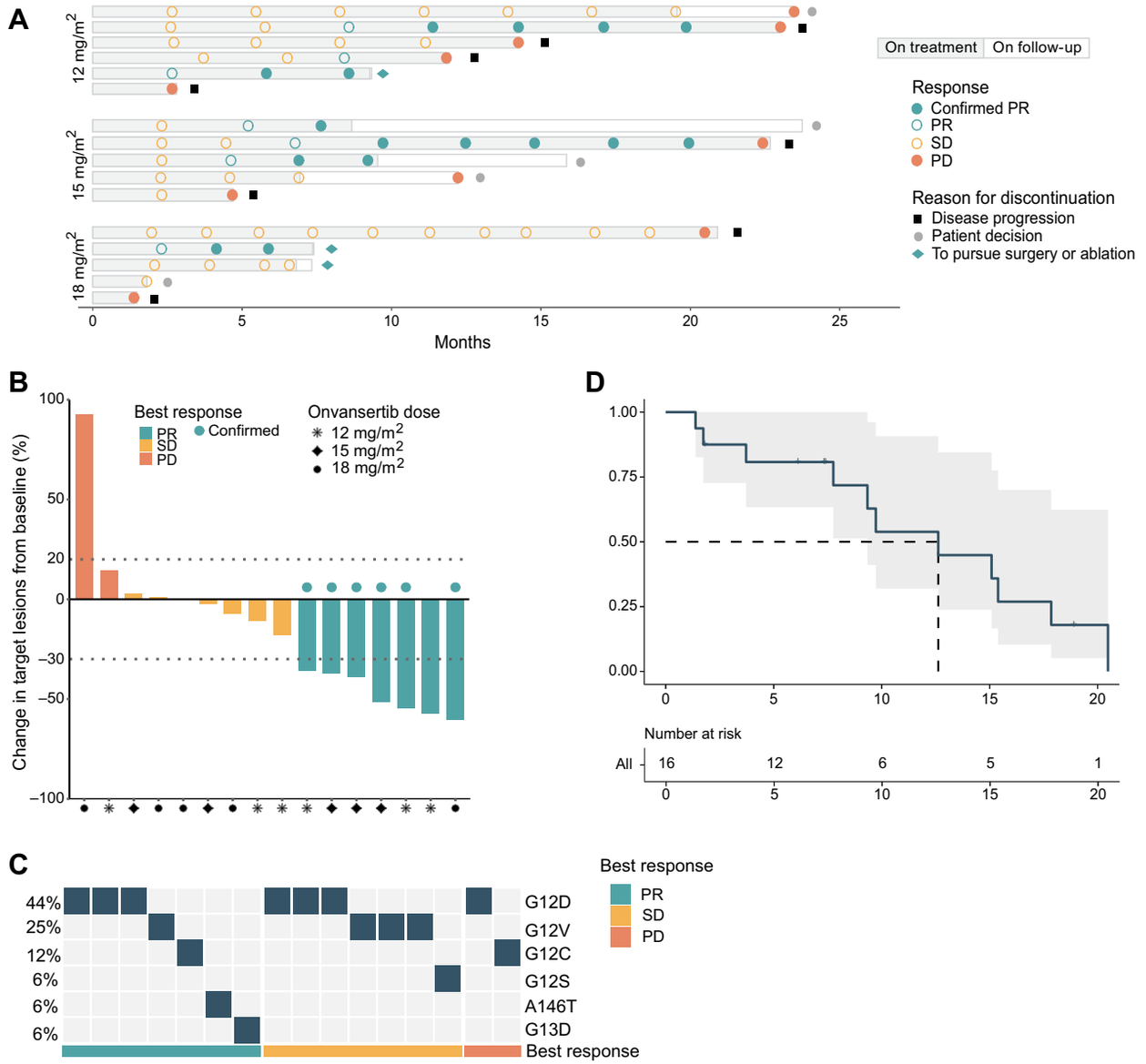


Figure 2.

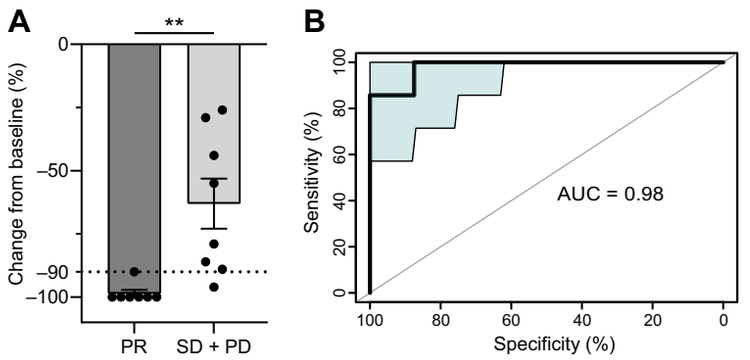
Clinical efficacy of onvansertib in combination with FOLFIRI/bevacizumab. **A,** Swimmer plot according to dose level. Each line represents a patient. **B,** Waterfall plot of maximum percent change in target lesions from baseline. **C,** *KRAS* mutation at baseline according to best response. **D,** Kaplan–Meier curve of PFS. The gray shadow indicates 95% CI. The data are from $n = 16$ patients evaluable for efficacy. PR = partial response, SD = stable disease, PD = progressive disease.

Response biomarker

To confirm the presence of *KRAS* mutations, molecular testing was performed in liquid biopsies for all patients, and on archival tumor tissue for patients with available samples. *KRAS* mutations were detected at baseline in plasma ctDNA using ddPCR in 17 (94%) patients (Supplementary Table S6). Molecular testing on tumor tissue was successfully completed on 8 patients using the Tempus xT assay, where a *KRAS* mutation was detected in all patients and was concordant with the ddPCR results (Supplementary Table S6). One patient had no detectable *KRAS* mutation in plasma and no tissue available for molecular testing.

We assessed the association between baseline *KRAS*-mutant ctDNA and clinical response. *KRAS*-mutant ctDNA was detected at baseline in 15 (94%) of the 16 patients evaluable for efficacy. The levels of baseline *KRAS*-mutant ctDNA assessed as copies/mL of plasma or MAF were not significantly different in patients who achieved clinical response (PR) compared with those who did not (Supplementary Fig. S1).

We next explored whether early changes in ctDNA levels were predictive of clinical response to onvansertib + FOLFIRI/bevacizumab. Changes in *KRAS*-mutant ctDNA levels were assessed by ddPCR in plasma samples collected after one cycle of treatment (~4–6 weeks).



C

	Clinical response		
	PR	SD or PD	
≥90% *KRAS* MAF	7	1	PPV: 87.5%
<90% *KRAS* MAF	0	7	NPV: 100%
	Sensitivity 100%	Specificity 87.5%	Accuracy 93.3%

Figure 3.

Early changes in *KRAS* MAF are associated with clinical benefit. **A,** Percentage change in *KRAS* MAF after one cycle of treatment from baseline according to best response. Fifteen patients were included in the analyses, 7 with PR, and 8 with either SD or PD as best response. Data analyzed by unpaired *t* test with Welch correction, **, $P < 0.01$. **B,** ROC curve for clinical response prediction, showing the sensitivity and specificity of *KRAS*-mutant ctDNA decrease after one cycle to predict clinical response. 95% CIs are shown in blue. **C,** Patient with ≥90% decrease in *KRAS* MAF from baseline were considered molecular responders. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the *KRAS*-mutant ctDNA plasma test to predict clinical response are reported.

Patients achieving PR ($n = 7$) had a significantly greater decrease in *KRAS* MAF compared with those with SD or PD ($n = 8$; **Fig. 3A**). In addition, using a ROC curve analysis, we showed that changes in *KRAS* MAF after one cycle of treatment were predictive of clinical response (AUC = 0.98, $P = 0.0.0009$; **Fig. 3B**). The optimal threshold was calculated to be −89.7%, at which the change in *KRAS* MAF predicted patient clinical response with a specificity of 87.5% (95% CI, 62.5–100) and a sensitivity of 100% (95% CI, 100–100; **Fig. 3C**). The 7 patients achieving PR showed a decrease in *KRAS* MAF of at least 90% after one cycle of treatment, while only 1 of the 8 non-responders did. In addition, 6 of the 7 patients achieving PR had a complete clearance of *KRAS*-mutant ctDNA after one cycle of treatment. Collectively, these data indicate that early changes in ctDNA MAF are associated with clinical response to onvansertib + FOLFIRI/bevacizumab.

Discussion

This is the first study evaluating the safety and preliminary efficacy of onvansertib, an orally available PLK1 inhibitor, in combination with chemotherapy in solid tumors. The rationale of this trial was based on the preclinical data supporting potency of onvansertib in *KRAS*-mutated colorectal cancer cells as well as the antitumor activity of onvansertib and irinotecan in a *KRAS*-mutant colorectal cancer xenograft model. The study established that onvansertib at a dose of 15 mg/m^2 on days 1–5 and 15–19 can be safely administered with FOLFIRI/bevacizumab.

Onvansertib single-agent safety was previously evaluated and a dose of 24 mg/m^2 for 5 days out of 21 was deemed to be safe (24). For this study, onvansertib was initiated at 12 mg/m^2 on days 1–5 and 15–19 with planned dose escalation to 18 mg/m^2. After enrollment of 18 patients, 15 mg/m^2 was shown to be associated with 1 DLT in 5 patients and thus was selected as the RP2D.

Neutropenia was the main toxicity during the DLT period. While neutropenia is a potential toxicity of onvansertib, FOLFIRI/bevacizumab is associated with 30%–40% risk of G3 and G4 neutropenia (28, 29). The main culprit of neutropenia is believed to be the 5-FU bolus, while its contribution to FOLFIRI efficacy has been questioned (30). Exploration of whether the elimination of 5-FU bolus from the onvansertib/FOLFIRI combination reduces the risk of neutropenia and improves the safety of the treatment, will be explored in future studies.

Another significant non-DLT toxicity attributed to onvansertib was fatigue. FOLFIRI/bevacizumab is associated with 40% fatigue (29). Like other agents in the oncology space, fatigue was a cumulative toxicity. The long duration of clinical benefit on onvansertib in combination with FOLFIRI/bevacizumab is a potential contributor to this cumulative toxicity. Strategies to reduce the impact of fatigue on the likelihood of treatment discontinuation should be considered, for example dose reduction and/or patient education for life style changes to reduce the experience of fatigue (31).

At the lowest dose level of onvansertib (12 mg/m^2), one patient experienced G4 neutropenic fever. Pharmacokinetic analysis revealed that the patient was an outlier and had a 10-fold increase in onvansertib exposure. That same individual also experienced G3 intra-abdominal abscess. No other patient in this study experienced febrile neutropenia, including patients receiving higher dose of onvansertib, further suggesting that this individual was an outlier.

The preliminary efficacy of the onvansertib + FOLFIRI/bevacizumab combination is notable. The ORR in patients evaluable for efficacy was 43.7% (including confirmed and unconfirmed responses). This response rate is significantly greater than the historical response rate to FOLFIRI/bevacizumab (12, 13). Furthermore, the median DOR was 9.5 months. The magnitude of response was great enough that 2 patients went on to have curative surgeries and one patient elected to receive targeted liver microwave ablation. While these efficacy data are promising, it should be noted that the study included a small number of patients across limited centers, highlighting the need for larger, multicenter studies to further assess the combination of onvansertib and FOLFIRI/bevacizumab.

Mutated *KRAS* is present in approximately 45% of patients with colorectal cancer with G12D (29%) and G12V (24%) variants being the most common (6). Ongoing drug development efforts for *KRAS*-mutated cancers primarily fall in two categories: direct KRAS inhibitors and inhibitors targeting KRAS upstream or downstream pathways. While targeting *KRAS* G12C variant has made significant progress, it is worth noting that G12C mutations are relatively rare in colorectal cancer (∼3%), and the effectiveness of KRAS G12C inhibitors is limited and accompanied by notable

toxicities (32, 33). In contrast, the combination of onvansertib with FOLFIRI/bevacizumab showed encouraging efficacy across different *KRAS* variants, supporting its potential for the treatment of all patients with *KRAS*-mutant mCRC. Other efforts to develop pan-*KRAS* mutant therapeutics involve the exploration of inhibitors targeting RAS upstream and downstream pathways, including MEK, SHP2, and SOS1 inhibitors, either as single agents or in combination therapies (34). While the study was restricted to *KRAS*-mutated patients, subsequent clinical trials of onvansertib + FOLFIRI/bevacizumab allow enrollment of patients with *NRAS* mutations.

Growing evidence supports that early ctDNA dynamics are associated with treatment benefit to systemic treatment and targeted therapy in colorectal cancer (35, 36). Likewise, we observed a profound decrease in *KRAS*-mutant ctDNA after one cycle of treatment in patients achieving PR compared with patients with SD or PD. In the phase II portion of the study, the utility of liquid biopsies as an early predictor of clinical benefit to onvansertib + FOLFIRI/bevacizumab will be further assessed.

In conclusion, the combination of onvansertib with FOLFIRI/bevacizumab was well-tolerated and showed promising preliminary efficacy in the treatment of patients with *KRAS*-mutant mCRC. Further investigation of the combination's safety and efficacy is ongoing in the phase II portion of the study. In addition, a randomized study is being conducted to assess the efficacy of FOLFIRI/bevacizumab in combination with onvansertib as compared with FOLFIRI/bevacizumab alone.

Authors' Disclosures

D.H. Ahn reports personal fees from Exelixis, Genentech, Eisai, and Advanced Accelerator Applications and other support from Bayer and AstraZeneca outside the submitted work. A. Barzi reports consulting fees from Cardiff Oncology during the conduct of the study. M. Ridinger reports personal fees from Cardiff Oncology during the conduct of the study; in addition, M. Ridinger has a patent for PCT/US2021/031192 pending. E. Samuëlsz reports personal fees from Cardiff Oncology during the conduct of the study; in addition, E. Samuëlsz has a patent for PCT/US2021/031192 pending. R.A. Subramanian reports other support from Cardiff Oncology during the conduct of the study. P.J.P. Croucher reports personal fees from Cardiff Oncology outside the submitted work. T. Smeal reports personal fees from Cardiff Oncology during the conduct of the study. F.F. Kabbinavar reports other support from Cardiff Oncology during the conduct of the study. H.-J. Lenz reports personal fees from Merck, Fulgent, 3T Bioscience, Bayer, BMS, and Repimmune and other support from Breakbio during the conduct of the study. No other disclosures were reported.

Authors' Contributions

D.H. Ahn: Conceptualization, resources, data curation, formal analysis, supervision, investigation, methodology, writing–original draft, writing–review and editing. **A. Barzi:** Conceptualization, data curation, formal analysis, investigation, methodology, writing–original draft, writing–review and editing. **M. Ridinger:** Conceptualization, data curation, formal analysis, supervision, investigation, visualization, methodology, writing–original draft, writing–review and editing. **E. Samuëlsz:** Formal analysis, methodology, writing–review and editing. **R.A. Subramanian:** Data curation, formal analysis, writing–original draft, writing–review and editing. **P.J.P. Croucher:** Methodology, writing–review and editing. **T. Smeal:** Supervision, writing–review and editing. **F.F. Kabbinavar:** Data curation, supervision, writing–review and editing. **H.-J. Lenz:** Conceptualization, resources, data curation, formal analysis, supervision, investigation, methodology, writing–original draft, writing–review and editing.

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Note

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

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