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Phase I Trial of Trametinib with Neoadjuvant Chemoradiation in Patients with Locally Advanced Rectal Cancer

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Abstract

Purpose: The RAS/RAF/MEK/ERK signaling pathway is critical to the development of colorectal cancers, and KRAS, NRAS, and BRAF mutations foster resistance to radiation. We performed a phase I trial to determine the safety of trametinib, a potent MEK1/2 inhibitor, with 5-fluorouracil (5FU) chemoradiation (CRT) in patients with locally advanced rectal cancer (LARC).

Patient and Methods: Stage II/III rectal cancer patients were enrolled on a phase I study with 3+3 study design, with an expansion cohort of 9 patients at the maximum tolerated dose (MTD). Following a 5-day trametinib lead-in, with pre- and post-treatment tumor biopsies, patients received trametinib and CRT, surgery, and adjuvant chemotherapy. Trametinib was given orally daily at 3 dose levels: 0.5 mg, 1 mg, and 2 mg. CRT consisted of infusional 5FU 225 mg/m²/day

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and 28 daily fractions of 1.8 Gy (total 50.4Gy). The primary endpoint was to identify the MTD and recommended phase 2 dose. Immunohistochemistry (IHC) staining for phosphorylated-ERK (pERK) and genomic profiling was performed on the tumor samples.

Results: Patients were enrolled to all dose levels, and 18 patients were evaluable for toxicities and responses. Treatment was well-tolerated, and there was one dose-limiting toxicity of diarrhea, which was attributed to CRT rather than trametinib. At the 2 mg dose level, 25% had pathological complete response. IHC staining confirmed dose-dependent decrease in pERK with increasing trametinib doses.

Conclusions: The combination of trametinib with 5FU-CRT is safe and well-tolerated, and may warrant additional study in a phase II trial, perhaps in a RAS/RAF-mutant selected population.

Keywords

MEK inhibitor; trametinib; radiation; chemotherapy; fluorouracil; 5-FU; rectal cancer

INTRODUCTION

Colorectal cancer is one of the most common malignancies in the Western world, and the second leading cause of cancer-related deaths in the United States. In 2018, there were an estimated 43,030 new cases of rectal cancer in the United States, and the majority have locally advanced disease.¹ Although surgical total mesorectal excision (TME) is the basis of curative-intent treatment for locally advanced rectal cancer, patients with clinically-staged T3/T4 tumors or positive nodal stage also undergo preoperative chemoradiation therapy (CRT) to decrease pelvic recurrence rates and improve tumor downstaging prior to surgery. Phase III trials of neoadjuvant 5-Fluoruracil (5FU) CRT have demonstrated better local-regional control with 5-year local recurrence rates of 6–9% and 5-year overall survival rates of 65–75%.^{2–4} Despite numerous phase II/III trials that have added potentially synergistic agents (e.g. cetuximab and oxaliplatin) to the 5FU CRT backbone, none have shown additional survival benefit so far.^{5–7}

The RAS/RAF/MEK/ERK signaling pathway is a critical signal transduction pathway that contributes to tumor cell proliferation, invasion, migration, and inhibition of apoptosis.^{8,9} Activation of the MAPK pathway is initiated by ligand binding to a receptor tyrosine kinase such as epidermal growth factor receptor (EGFR), leading to KRAS activation, and subsequent activation of RAF isoforms. RAF isoforms then activate MEK-1/2, ultimately leading to ERK-1/2 activation and nuclear translocation. KRAS, NRAS, and BRAF activating mutations have been well-described in colorectal cancer, and occur at a frequency of about 35–45%, 2–5%, and 8–10% respectively.^{10–15} These mutations are mutually exclusive, which may indicate that they play an important role in the MAPK pathway. Thus, inhibition of proteins that are downstream of KRAS, BRAF, and NRAS, such as MEK 1/2, are attractive targets in the treatment of RAS/RAF-mutant colorectal cancer.

Interestingly, ionizing radiation causes activation of the RAS-MAPK pathway in a dose-dependent fashion, and this activation is blunted by MEK inhibition.^{16,17} RAS activating mutations promote resistance to radiation in tumor cells in pre-clinical and clinical studies,

including colorectal cancer and non-small cell lung cancer.^{18–26} Pre-clinical studies demonstrate that genetic downregulation of KRAS or chemical inhibition of KRAS with farnesyltransferase inhibitors re-sensitizes cells to radiation.^{27–29} More recently, MEK inhibitors have been shown to effectively radiosensitize RAS mutant tumor cells *in vitro* and *in vivo*, in many preclinical studies, including colorectal cancer.^{30–34} These studies have elicited potential mechanisms of radiosensitization including altered DNA repair, in part through DNA double-strand break (DSB) repair pathways, and prevention of a HIF1-mediated hypoxic response.^{30,32,34} Preclinical studies have also established the efficacy of combining MEK inhibition with 5FU, with or without radiation both in cell lines and tumor xenografts.^{31,35}

Trametinib (GSK1120212, MEKinist®) is a potent and selective inhibitor of mitogen-activated extracellular signal regulated kinase 1 (MEK1) and MEK2, and is approved for the treatment of BRAF mutant melanoma.³⁶ Trametinib has been shown to effectively radiosensitize RAS mutant tumor cells, and has also been shown to have anti-proliferative properties *in vitro* and *in vivo* when added to human colon cancer cell lines that harbor KRAS or BRAF mutations.^{34,35} Given the substantial preclinical data supporting MEK inhibition as a radiosensitizing strategy for the treatment of colorectal cancer, a cancer enriched with RAS/RAF activating mutations, we conducted a phase I trial to test the safety and tolerability of trametinib in combination with 5FU-based chemoradiation (CRT) prior to surgery for locally-advanced rectal cancer (LARC).

PATIENTS AND METHODS

Participants and eligibility:

Patients with histologically confirmed, locally advanced, not otherwise pretreated stage II or III rectal adenocarcinoma, cT3/4 and/or cN+, M0 (AJCC 7th edition) were recruited to 2 centers (Ohio State University and Vanderbilt University). Investigators obtained informed written consent from each participant or participant's guardian prior to enrollment. The research was conducted in accordance with the recognized ethical guidelines of the Declaration of Helsinki, CIOMS, Belmont report, and U.S. Common Rule. Clinical study was performed after the approval by an institutional review board. Patients were aged 18 years and had adequate hepatic, bone marrow, and renal organ function and an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1. Initially, the trial was open only to patients with a KRAS gene mutation (codon 12, 13, 61), NRAS mutation (codon 12, 13, 61), or BRAF gene mutation, but due to slow accrual and the nature of the phase I trial to determine the recommended phase II dose of trametinib, it was opened to all KRAS, NRAS, and BRAF wild-type patients as well (beginning with patient #6).

Procedures and screening:

Before the amendment to allow for KRAS/NRAS/BRAF wildtype patients, DNA was isolated from primary tumor samples before treatment and tested for KRAS, NRAS, and BRAF mutational status in the local institutional CLIA-certified pathology laboratory by Sanger sequencing. Baseline computed tomography of the thorax/abdomen and pelvis was performed, and all patients underwent either endoscopic rectal ultrasound and/or magnetic

resonance imaging of the pelvis for adequate staging. Adequate archival pre-treatment (diagnostic) tumor tissue was required for correlative studies; if not available, a repeat tumor biopsy was performed prior to study therapy.

Study design:

The primary objective of the trial was to determine the maximally tolerated dose of trametinib in combination with 5FU and radiation in patients with locally advanced rectal cancer. Patients were recruited in a cohort-of-3 trial design (see below for statistical design). Secondary objectives included evaluation of tolerability and safety of the combination, evaluation of pathologic response and clinical outcomes (local control, disease-free survival, and overall survival), evaluation of pharmacodynamics effects of trametinib on RAS/MAPK signaling pathways in tumor biopsies, and performance of tumor mutational profiling to correlate with clinical response.

Trametinib was given orally for a 5-day lead-in period daily Monday-Friday starting at Day -7 and for the duration of radiation therapy (days 1–38). Supplementary Figures S1 and S2 show the flowchart and schema, respectively. Three dose levels of trametinib were tested in escalated fashion: 0.5 mg (dose level 1), 1.0 mg (dose level 2), and 2 mg (dose level 3) [Table 1]. During trametinib monotherapy, research biopsies of tumor and normal tissue for correlative studies were obtained on Day -4 or Day -3. All patients received a total radiation dose of 45 Gy applied in 25 fractions of 1.8 Gy over 5 weeks using conventional 3-dimensional conformal radiation technique or intensity modulated radiation therapy, followed by a cone-down boost of 5.4 Gy in 1.8 Gy fractions to the primary tumor and sacral hollow. Infusional 5FU was delivered as 225 mg/m²/day over 120 hours (Monday-Friday) during days of radiation therapy (days 1–38). An expansion cohort for an additional 6 patients treated at the MTD was completed to provide a total of 12 patients treated at the MTD. Surgery, specifically total mesorectal excision, was recommended to occur after a 6–10 week rest period after completion of chemoradiotherapy. Surgical samples were assessed by a board certified gastrointestinal pathologist and examined for response, including tumor regression grade (score 0–3) based on the modified Ryan scheme.^{37,38} Postoperative chemotherapy was given at the discretion of the treating physician but was encouraged in patients with pN+ disease and to begin between 4–12 weeks following surgical resection.

Toxicities and statistical design:

This is a phase I study designed to determine the maximum tolerated dose (MTD) level of trametinib when given in combination with 5FU and radiotherapy in locally advanced rectal cancer patients, using the standard cohort-of-3 phase I clinical trial design. Dose limiting toxicities (DLTs) were defined according to CTCAE v.4.0 as any grade 4 neutropenia, any grade 3–5 thrombocytopenia, or any non-hematologic toxicity of grade 3–5 attributable to the three therapies (trametinib, 5FU, and RT) that resulted in 7 days or greater interruption in 5FU and RT therapy. The DLT observation period (i.e. the period during which DLTs observed was used to inform dose escalation decisions) was 8.5 weeks in this trial (during trametinib monotherapy, chemoradiation and up to 3 weeks after). The MTD was defined as the maximum dose level of trametinib where at most 1 of 6 patients experience a DLT according to the following rules. If no DLTs were observed at dose level 3 and the MTD

level may indeed be at higher doses, dose level 3 would be considered the MTD. If dose level 2 was deemed safe and dose level 3 was found to exceed the MTD (i.e. 2+ patients with an observed DLT), then the intermediate dose level 2a would be evaluated (see Table 1). Once the MTD was determined, an additional 6 patients (for a total of 12 patients at the MTD) were accrued.

Immunohistochemistry

Sections (4–6 μm thick) of formalin-fixed paraffin-embedded tissue were sectioned, placed on plus slides and baked at 65°C for one hour. Immunostaining was performed on the fully automated Bond RX autostaining system (Leica Biosystems). Briefly, heat-induced antigen retrieval was done using Epitope Retrieval Solution 2 (Leica Biosystems) for 20 minutes, and slides were stained with a rabbit monoclonal antibody to phosphorylated-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (clone D13.14.4E Catalog #4370, Cell Signaling Technology) at a 1:150 dilution for 30 minutes. The Bond Polymer Refine (DAB) detection system (Leica Biosystems) was used. Slides were evaluated for degree of p-ERK staining in cells by acquiring multispectral brightfield images from each tissue section using the automated Vectra® 3.0 automated quantitative pathology imaging system. A board-certified gastrointestinal pathologist (W. Chen) then identified and labeled both the tumor and stromal tissue contained in each image. Using inForm® Cell Analysis™ software, manual tissue segmentation was performed for each image accordingly in order to separately analyze tumor and stromal tissue. Once tissue segmentation was complete, tumor and stromal nuclear segmentations were performed separately by using the unmixed hematoxylin image to find each nucleus. Cytoplasmic segmentation was then performed. In order to separate cytoplasm from the nuclei, only pixels which contained a significant optical density (OD) for DAB, but not hematoxylin, were counted in the cytoplasmic segmentation. For each image, the average DAB per cell of tumor tissue was calculated by extracting the total optical density (OD) within each tissue segment divided by the tissue cell-count. Cells at the edge of either the image or tissue segment were excluded in order to obtain more accurate whole-cell readings. A minimum of 1,000 tumor cells were quantified per patient.

Targeted next-generation sequencing

DNA was extracted from formalin-fixed paraffin-embedded (FFPE) tissue scrolls using the Maxwell 16 FFPE DNA purification kit (Promega, Inc) and quantitated using pico green on a Qubit (Life Technologies). Libraries were generated using the ThruPLEX DNA-seq kit (Rubicon Genomics) and custom-targeted DNA probes from Integrated DNA Technologies (IDT). Briefly, 200ng of FFPE-derived gDNA was sheared using a Covaris instrument, and samples were end-repaired, adaptor and index-ligated and PCR-amplified. Following the PCR, 500 ng of the prepared DNA libraries was hybridized to the probes overnight. Hybridized probes were captured on magnetic beads, washed and PCR-amplified. The target-enriched indexed libraries were pooled and underwent paired-end 150 bp sequencing on an Illumina HiSeq4000 to an average depth of 281.2.

Alignment was performed with bowtie2 v2.2.6 using local option to human reference genome hg19.³⁹ Alignment was converted to bam with Samtools v1.3.1.⁴⁰ After adding read groups, marking duplicates, and sorting with picard 2.4.1 (<http://broadinstitute.github.io/>

picard), GATK v3.6⁴¹ was used to realign around indels. Mpileup format was generated with Samtools v1.3.1 requiring quality > 1 and variants were called with Varscan v2.4.1.⁴² Variants were annotated with Variant Effect Predictor from Ensembl⁴³ and filtered for location (excluding non-coding variants), coding impact (excluding LOW impact variants--those unlikely to change protein behavior), allele frequency in public databases (excluding common variants found in 1000g⁴⁴ and ExAC⁴⁵), and known variants (excluding variants listed in NCBI dbSNP⁴⁶ but not in COSMIC databases). Variants were visually inspected in IGV.⁴⁴

RESULTS

Patients and Toxicities:

Nineteen patients were enrolled in dose levels 1 (cohort 1, 0.5 mg), 2 (cohort 2, 1.0 mg), and 3 (cohort 3, 2.0 mg) between August 2013 and May 2017. One patient was deemed unevaluable due to suspected dihydropyrimidine dehydrogenase (DPD) deficiency. Baseline patient characteristics of the 18 evaluable patients are shown in Table 2. Median follow-up for all patients was 37.1 months. Three patients each were enrolled on dose levels 1 and 2, and no DLTs were encountered. Only 1 DLT was observed in patient #12 in dose level 3, diarrhea (grade 3), which was attributed to 5-FU chemoradiation. The maximum tolerated dose of trametinib was determined to be 2.0 mg daily (dose level 3), and a total of 12 patients were enrolled at this dose, including the expansion cohort. For all dose levels, no grade 4 or 5 toxicities were observed. The most common adverse event observed that was attributed to trametinib was a maculopapular rash (eleven grade 1, two grade 2, and two grade 3) [Table 3]. This maculopapular rash led to trametinib discontinuation after 2 weeks of therapy in 1 patient, and trametinib being held for the last 3 days of chemoradiation for 2 other patients. The other common side effects observed were diarrhea, rectal pain, nausea, oral mucositis, and fatigue, which are commonly encountered with 5-FU chemoradiation.

Surgical toxicities and post-operative chemotherapy:

Six surgeons operated on this clinical trial, and the majority of patients had a temporary ostomy at the time of the initial TME (13 of 18 patients). All patients successfully completed TME (5 abdominoperineal resections and 13 low anterior resections) within the timeframe recommended per the clinical trial, and all patients had R0 resections. Overall, patients healed well after surgery. Patient 1 had T4 involvement requiring cystoprostatectomy during APR, with flap reconstruction. He had an area of poor healing in the flap requiring wound debridement within 4 weeks of initial surgery, which ultimately healed, but he was recommended not to have post-operative chemotherapy. Patient 2 developed a surgical site infection and distal colostomy ischemia within 2 weeks of surgery that was managed expectantly, and this patient went on to receive post-operative chemotherapy. Patient 4 had delayed healing of a perineal wound which eventually healed, but he opted against receiving post-operative chemotherapy. Patients 10, 13, and 14 were re-admitted within 30 days of surgery for dehydration, nausea, and vomiting which resolved with medical management. Patient 11 returned on post-op day 12 for wound infection which ultimately healed, and the patient did receive post-operative chemotherapy. Patient 18 developed an anastomotic leak (coloanal) and abscess within 2 weeks of LAR requiring

takedown of coloanal anastomosis and end colostomy. This patient had a prolonged hospitalization and ultimately did not receive post-operative chemotherapy. Overall, 5 patients did not start post-operative chemotherapy either due to surgical complications or patient refusal. Of note, only one out of the 5 patients who did not receive adjuvant therapy had node-positive disease identified on pathologic surgical specimen evaluation.

Efficacy and outcomes:

Analysis of the whole patient cohort revealed tumor down-staging in 77.8% (14/18) of patients, with pathological complete response seen in 16.7% (3/18) patients. Four patients did not have tumor-downstaging from therapy, one of whom experienced a DLT (diarrhea, patient 12), leading to an interrupted and suboptimal chemoradiation course. In this case, tumor-upstaging was actually observed (from cN1 to pN2b). At the MTD and recommended phase II dose (2 mg dose level), 75% (9/12) of patients had tumor downstaging, 25% (3/12) had pathological complete response (pCR), and an additional 25% (3/12) patients had near pCR (only microscopic foci of residual tumor cells). This led to a 50% (6/12) pCR or near pCR rate at the recommended phase II dose. In terms of disease control outcomes, only 1 patient developed a local (pelvic) failure which occurred in combination with pulmonary metastasis. Another patient developed isolated pulmonary metastasis. One patient died at 62 months after enrollment, but had no signs of recurrence, and death was attributed to non-cancer related causes. Median local recurrence-free survival, median disease-free survival, and overall survival were not reached (Figures 1a–c). Actuarial local-recurrence free survival, disease-free survival, and cancer-specific survival are high at 94.4%, 88.9%, and 100%.

Correlation of phospho-ERK with dose level and response

Quantitative immunohistochemistry (qIHC) was performed on matched tumor specimens from pre-treatment and Day –4/–3 tumor tissue obtained during trametinib monotherapy. For patients with high quality and sufficient tissue available (2 each from dose levels 1 and 2; and 11 from dose level 3), qIHC revealed a dose-dependent decrease in phosphorylated-ERK (p-ERK) compared to pre-treatment p-ERK levels with increasing dose levels of trametinib (Figure 2). Maximal decrease in p-ERK was observed in patients receiving 2 mg daily in cohort 3 which was significantly different compared to pre-treatment levels ($p=0.006$), confirming the pharmacodynamic inhibitory effects of trametinib on activated ERK signaling within tumor cells. We also assessed the levels of pre-treatment p-ERK in tumor and interestingly found a strong correlation with pathologic response. Higher pERK levels at baseline (pre-therapy) were associated with higher tumor regression grade score (TRG, $p=0.0007$), indicating that higher p-ERK levels appear to be correlated with poor response to neoadjuvant therapy (Supplementary Figure S3). However, there was only a trend towards higher pERK levels at baseline being associated with RAS/RAF mutations ($p=0.203$, Supplementary Figure S4). In addition, a higher degree of reduction in pERK levels between pre-treatment and after trametinib monotherapy did not correlate significantly with RAS/RAF mutation status or TRG score, but did show trends for an association with pCR in the whole cohort ($p=0.204$) and 2 mg cohort ($p=0.078$) (Supplementary Figure S5).

Targeted next-generation sequencing

We performed targeted next generation sequencing on DNA isolated from tumor research biopsies using an in-house custom panel of 279 cancer-associated genes.⁴⁶ Mutations were found in 94.4% (17/18) of patients (Supplementary Table 1). We found 38.9% (7/18), 5.6% (1/18), and 0% of patients' tumors had KRAS, BRAF, and NRAS mutations, respectively. TP53 mutations were the most common, occurring in 77.7% (14/18). The next most common mutation was APC (61.1%), followed by lower frequency mutations in SMAD4 (16.7%), mTOR (11.1%), PIK3CA (11.1%), ATM (11.1%), and FBXW7 (11.1%). One patient (#11) was found to have microsatellite instability, indicative of mismatch repair defect and Lynch syndrome, which was confirmed by immunohistochemical studies of MLH1, PMS2, MSH2, and MSH6 as well as germline genetic testing. Of the seven patients (6, 7, 10, 11, 14, 15, and 16) who had an excellent response to neoadjuvant therapy with pCR or near pCR, one patient had a KRAS mutation (14.2%), 86% had TP53 mutations, and 57.1% had APC mutations, but there did not appear to be other frequently occurring mutations in this group of patients that could account for their excellent response. Of note, KRAS and BRAF mutations appeared to be enriched (63.7%) in the 11 patients who did not have a significant response to neoadjuvant therapy. No statistically significant relationship between the presence of KRAS, BRAF, APC, or TP53 mutations and tumor regression grade were identified, although individuals with a KRAS or BRAF mutation in their tumor had a trend towards a worse tumor regression grade ($p=0.076$).

DISCUSSION

In this phase I trial of MEK-1/2 inhibitor trametinib in combination with standard neoadjuvant chemoradiation for locally advanced rectal cancer, we found that the MTD of trametinib was 2.0 mg daily. This dose was chosen, due to the FDA-approved dose of trametinib 2mg daily in melanoma and lung cancer, and due to concern of toxicities at higher doses when given in combination with 5-FU chemoradiation. Overall, treatment was relatively well tolerated, with no grade 4–5 toxicities observed and manageable and reversible grade 1–3 toxicities seen. Only one DLT was observed which was attributed to the standard therapy of chemotherapy and radiation. Furthermore, it did not appear that the addition of trametinib increased post-operative complications. With regards to efficacy, 12 patients were treated at the MTD and recommended phase II dose of 2.0 mg. In this group (cohort 3), we observed a relatively high rate of pCR of 25%, along with an additional 25% of patients with near pCR. Given the historical pathological complete response rate seen for patients with neoadjuvant 5-FU chemoradiation alone is expected to be in the range of 10–15%, these results may be worthy of further exploration, perhaps in a RAS/RAF mutated patient population.

The landmark AIO-94 German rectal trial established the role of neoadjuvant 5FU-based chemoradiation in stage II/III rectal cancers.⁴ The pCR was 8% in this study. NSABP R-03 showed that neoadjuvant therapy attained pCR in 15% of the patients.⁴⁷ Recent strategies to improve neoadjuvant therapy response have included the concept of total neoadjuvant therapy (TNT), in which systemic chemotherapy is delivered in the neoadjuvant setting rather than the adjuvant setting.⁴⁸ The rationale for TNT is that approximately 30–40%

patients do not receive their adjuvant therapy due to post-operative healing problems or complications. Thus, the benefits from the TNT approach are to ensure that patients do receive their intended systemic chemotherapy. In addition, TNT allows for maximal downstaging with both chemotherapy and chemoradiation modalities prior to surgery.

Another strategy is to personalize treatment based on molecular knowledge of the pathways driving therapeutic resistance, such as targeting specific tumor genotypes. Although this strategy has proven to be successful in targeting stage IV disease in many cancers (e.g. EGFR-mutant or ALK fusion positive non-small cell lung cancer), tumor genotype has not yet driven specific radiotherapy treatments in solid tumors. Despite this, there is growing data on how genotype may inform radiotherapy response, particularly KRAS genotype. In early-stage non-small cell lung cancer, localized KRAS mutant lung tumors that were treated with stereotactic body radiotherapy (SBRT) had lower local control, when compared to KRAS wild-type tumors.²¹ A prospective study with SBRT for metastases to the liver from colorectal, gastroesophageal, and pancreatic cancers reported that the presence of a KRAS mutation was predictive of poor local control and radioresistance. One year local control for KRAS mutant tumors was 20% vs 69.2% for KRAS wild-type tumors ($p=0.001$).²⁴ In a study specifically examining neoadjuvant therapy in rectal cancer patients, there was a markedly lower pCR noted in the KRAS mutant patient population (13 vs 33% $p=0.006$).²⁶ Taken together, there is growing clinical evidence that the presence of a KRAS mutation is associated with radioresistance and that these patients have the greatest need for an agent targeting the RAS/RAF pathway.

In our phase I study, most of the patients in the final dose cohort of 2 mg did not harbor KRAS/NRAS/BRAF mutations. In order to expedite patient accrual and establish the R2PD, we amended the protocol to omit the inclusion criteria that patients needed tumors that harbored KRAS, NRAS, or BRAF mutations. As a result, only 2 patients had tumors with KRAS codon 12 mutation in the R2PD dose: one had a pCR and the other had a poor response resulting in tumor up-staging. One patient had a BRAF D594N mutation, and there was evidence of tumor downstaging. As the primary goal of our study was to establish the safe and tolerable dose of trametinib to use in combination with chemoradiation, further study may be needed to truly establish if safety, response and tumor down-staging for patients with locally advanced rectal cancer is improved with MEK inhibition in combination with chemoradiation. Regarding post-operative toxicity, we did note post-operative complications that may or may not be due to the study treatment combination. While we do not have historical institutional data available on surgical complications for patients treated with neoadjuvant chemoradiation, our anecdotal experience is that it likely higher than 5%. We believe a larger study will help us determine if postoperative complications are out of the ordinary for this treatment combination. Due to the limited scope of our study related to small patient numbers, it is difficult to attribute the few wound-healing complications we had to the novel treatment combination. Other studies have shown higher wound complication rates than this study, with complications ranging from 14–80% and need for reoperation in 6–8% of patients, particularly in patients with APR.^{49–50} Thus, we feel that none of the individual surgical complications were outside the range and frequency of colorectal surgical complications we typically observe in historically-treated patients with 5-FU based chemoradiation.

Of particular interest for a future trial with regards to efficacy, is the evaluation of the treatment combination both in RAS/RAF wild-type and RAS/RAF mutant populations to determine if there is an improvement in response in one particular genomic subgroup more than the other, in comparison to historical results. Most of the preclinical data showing efficacy of MEK inhibition in combination with radiation was demonstrated in RAS/RAF mutant cells, except the study by Chung et al. which demonstrated radiosensitization in DU145 prostate cancer cells.^{30–34} Thus, we hypothesize that we would observe a greater degree of benefit with the treatment combination in the RAS/RAF mutant population compared to the RAS/RAF wild-type population since MEK inhibition would abrogate downstream RAS-RAF-MEK-ERK aberrant signaling that promotes radioresistance. Finally, given the increasing interest and role of non-operative management (organ preservation) for rectal cancer, novel strategies to improve clinical complete response and long-term durable pelvic control with definitive chemoradiation are also warranted, and may be achieved with strategies that enhance chemo-radiosensitization.

In conclusion, our phase I evaluation of this treatment combination of trametinib, 5-FU, and radiation appears to be safe, well-tolerated, and demonstrates early signs of clinical efficacy at the 2 mg cohort, with confirmation of on-target biologic effects. Future prospective studies of this treatment combination may be warranted in rectal cancer, perhaps focusing on RAS/RAF mutant rectal cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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TRANSLATIONAL RELEVANCE

Colorectal cancers harboring KRAS, NRAS, or BRAF activating mutations are known to have worse prognoses, and preclinical studies have shown these mutations to promote resistance to radiation. For stage II/III rectal cancers, standard of care includes trimodality treatment with 5FU-based chemoradiation, 5FU and oxaliplatin chemotherapy, and surgery. In this phase I study, we report the safety of combining trametinib, a potent and selective MEK1/2 inhibitor, at the dose of 2mg daily with 5FU chemoradiation in patients with LARC. We also demonstrate pharmacodynamic studies showing a dose-dependent decrease in phosphorylated ERK in the tumor tissue, and preliminary anti-tumor activity. A phase II study may be warranted to determine the efficacy of trametinib and CRT in patients with rectal tumors that harbor KRAS, NRAS, and BRAF mutations, which are indicative of a more aggressive pathology.

Figure 1. Kaplan-Meier curves for (A) local (pelvic) recurrence free survival, (B) disease free survival, and (C) overall survival.

Of the 2 patients with longest follow-up, one patient died at 62 months from non-cancer related causes and had no signs of recurrence prior to death.

Figure 2. Quantitative IHC on pre-treatment and post-treatment tumor biopsies.

(Top left panel) representative photomicrographs of p-ERK-1/2 IHC from pre-treatment biopsy and research tumor biopsies done after 4 or 5 days of trametinib monotherapy. Both patient 7 and 8 were in cohort 3 (2.0 mg trametinib daily dose cohort). Note the considerable decrease in DAB (brown) staining. Nuclei counterstained with hematoxylin. (Top right panel) Quantitation of average optical density of DAB staining per cell comparing pre-treatment to post-treatment (Day -4/-3). Note dose dependent increase in p-ERK staining reduction with increasing dose of trametinib in cohorts 1-3. (Bottom panel) Average optical density of DAB staining/cell in each cohort from pre-treatment to 4 or 5 days of trametinib monotherapy. Note the significant decrease in cohort 3 ($p=0.006$).

Table 1.

Dose levels/cohorts

Dose Level	Trametinib (mg) Monday-Friday	5FU (mg/m ² /day over 120 hr, Monday-Friday)	Total Radiation dose in 28 fractions (Gy)
-1	0.5	200	50.4
1	0.5	225	50.4
2	1	225	50.4
2a	1.5	225	50.4
3	2	225	50.4

Table 2.

Patient characteristics, staging, and surgical outcomes

Patient	Age (yrs at enrollment)	Gender	ECOG at enrollment	Trametinib dose (mg)	Clinical stage	Pathologic stage	Down-staging?	TRG	Mutation status for KRAS, NRAS, BRAF	Type of Surgery
1.	58	M	1	0.5	T4N1	T4N1c	No	3	KRAS G13D	Abdominoperineal resection
2.	48	M	1	0.5	T3N1	T2N0	Yes	3	KRAS G12V	Abdominoperineal resection
3.	59	M	0	0.5	T3N1	T1N0	Yes	2	KRAS G12V	Low Anterior Resection
4.	54	M	1	1	T3N1	T2N0	Yes	2	KRAS G12V	Abdominoperineal resection
5.	52	M	0	1	T3N0	T3N0	No	2	KRAS G12V	Low anterior resection
6.	58	M	1	1	T3N2	T3N0	Yes	1	WT	Low anterior resection
7.	38	M	0	2	T3N0	CR	Yes	0	KRAS G12A	Abdominoperineal resection
8.	55	F	0	2	T3N1	T2N0	Yes	2	BRAF D594N	Low anterior resection
9.	64	M	0	2	T3N0	T3N1	No	2	KRAS G12V	Low anterior resection
10.	52	F	0	2	T3N0	CR	Yes	0	WT	Low anterior resection
11.	34	M	1	2	T3N0	CR	Yes	0	WT	Low anterior resection
12.	65	M	1	2	T3N1	T3N2b	No	2	WT	Low anterior resection
13.	49	F	0	2	T4N2	T1N0	Yes	2	WT	Low anterior resection
14.	74	F	0	2	T3N2b	T3N0	Yes	1	WT	Low anterior resection
15.	48	F	0	2	T3N0	T2N1	Yes	1	WT	Low anterior resection
16.	50	F	0	2	T3N1	T1N0	Yes	1	WT	Low anterior resection
17.	47	M	0	2	T3N1	T1N0	Yes	1	WT	Abdominoperineal resection
18.	66	M	0	2	T4N1	T3N0	Yes	3	WT	Low anterior resection

ECOG= Eastern Cooperative Oncology Group performance score; LAR= low anterior resection; APR= abdominoperineal resection; clinical/pathologic staging is AJCC 7th edition; pCR= pathologic complete response; npCR= near pCR; TRG= tumor regression grade; WT=wild-type

Table 3.

Toxicities for all patients

Toxicity	Grade 1	Grade 2	Grade 3
Maculopapular rash	11	2	2
Diarrhea	10	2	1
Rectal pain	7	3	0
Nausea	7	1	0
Oral mucositis	6	2	0
Fatigue	4	2	0
Pruritus	4	1	0
Abdominal cramps	3	0	0
Anorexia	3	0	0
Proctitis	3	0	0
Hand-foot syndrome	3	0	0
Lymphopenia	0	4	12
Anemia	6	3	0
Leucopenia	5	3	0
Thrombocytopenia	5	0	0