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Phase I/II study of neoadjuvant bevacizumab, erlotinib and 5-fluorouracil with concurrent external beam radiation therapy in locally advanced rectal cancer

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Background: To determine the maximal tolerated dose of erlotinib when added to 5-fluorouracil (5-FU) chemoradiation and bevacizumab and safety and efficacy of this combination in patients with locally advanced rectal cancer.

Patients and methods: Patients with Magnetic resonance imaging (MRI) or ultrasound defined T3 or T4 adenocarcinoma of the rectum and without evidence of metastatic disease were enrolled. Patients received infusional 5-FU 225 mg/M2/day continuously, along with bevacizumab 5 mg/kg days 14, 1, 15 and 29. Standard radiotherapy was administered to 50.4 Gy in 28 fractions. Erlotinib started at a dose of 50 mg orally daily and advanced by 50 mg increments in the subsequent cohort. Open total mesorectal excision was carried out 6–9 weeks following the completion of chemoradiation.

Results: Thirty-two patients received one of three dose levels of erlotinib. Erlotinib dose level of 100 mg was determined to be the maximally tolerated dose. Thirty-one patients underwent resection of the primary tumor, one refused resection. Twenty-seven patients completed study therapy, all of whom underwent resection. At least one grade 3–4 toxicity occurred in 46.9% of patients. Grade 3–4 diarrhea occurred in 18.8%. The pathologic complete response (pCR) for all patients completing study therapy was 33%. With a median follow-up of 2.9 years, there are no documented local recurrences. Disease-free survival at 3 years is 75.5% (confidence interval: 55.1–87.6%).

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Conclusions: Erlotinib added to infusional 5-FU, bevacizumab and radiation in patients with locally advanced rectal cancer is relatively well tolerated and associated with an encouraging pCR.
Clinicaltrials.gov: NCT00307736.
Key words: rectal cancer, radiation, bevacizumab, erlotinib

introduction

Neoadjuvant chemoradiation is a standard of care for locally advanced rectal cancer [1]. Because of the observation of association with pathologic complete response (pCR) with improved disease outcomes, there has been considerable interest in evaluating new agents with chemoradiation to improve response rates [2]. Agents targeting vascular endothelial growth factor (VEGF) and the epidermal growth factor receptor (EGFR) have shown efficacy in metastatic colorectal cancer and have led to the approval of bevacizumab [3], aflibercept [4], cetuximab [5] and panitumumab [6].

Targeting VEGF and EGFR with chemoradiation for rectal cancer has been extensively studied in phase II trials. Bevacizumab, while improving survival in metastatic colorectal cancer, has failed to convincingly improve pCR rates in multiple phase II studies with fluoropyrimidine-based chemoradiation [7–9]. Likewise, cetuximab, a chimeric monoclonal antibody to EGFR, has also been evaluated in multiple neoadjuvant rectal studies, but also has disappointing response rates [10–15]. However, preclinical data suggest that dual inhibition of EGFR and VEGF receptor pathways in combination with radiation may be super-additive in head and neck cancers [16]. Erlotinib is a small molecule tyrosine kinase inhibitor of EGFR and has been studied in combination with bevacizumab, chemotherapy and radiation in head and neck cancer, non-small cell lung cancer and esophageal cancer [17–19]. The purpose of this study was to evaluate the safety and efficacy of adding erlotinib and bevacizumab to 5-fluorouracil (5-FU)-based neoadjuvant chemoradiation for locally advanced rectal cancer.

patients and methods

eligibility criteria

Patients enrolled on to this study had histologically confirmed adenocarcinoma of the rectum that begins within 15 cm of the anal verge as determined by sigmoidoscopy and colonoscopy. Tumors must be T3 or T4 (see Evaluation). Patients could not have evidence of metastatic disease as determined by computerized tomography (CT) scan of the chest, abdomen and pelvis and must have had adequate hematologic, renal and hepatic function (absolute neutrophil count $\geq$1500, hemoglobin $\geq$9 g/dl, platelet count $\geq$100 000, creatinine $\leq$2, total bilirubin <1.5 and SGOT $\leq$2.5 $\times$ the upper limit of normal). Patients may not have had a history of poorly controlled hypertension (blood pressure >150/100), unstable angina, myocardial infarction or stroke within 6 months, clinically significant peripheral vascular disease, urinary protein–creatinine ratio $\geq$1, evidence of a bleeding diathesis or coagulopathy). The protocol was approved by the Dana–Farber Harvard Cancer Center Investigational Review Board and all patients signed informed consent.

evaluation

In addition to the above eligibility evaluation, rectal tumor staging was carried out with either an MRI of the rectum (with endorectal coil for low tumors and surface phase array for mid- to upper tumors) or endorectal ultrasound within 28 days of treatment initiation. Colonoscopy was carried out in all patients. All patients underwent weekly assessments by medical oncology and radiation oncology clinicians with weekly CBC and chemistries. On days when bevacizumab was administered, a protein–creatinine ratio was also obtained. All patients underwent clinical evaluation in addition to CBC and chemistries, as well as repeat CT 4–6 weeks following the completion of chemoradiation. CEA was carried out every 3 months for the first 3 years and every 6 months for the next 2 years following resection and CT surveillance every 6 months for the first 3 years following resection and then once yearly for the next 2 years.

radiotherapy

Radiation was delivered in daily treatments Monday through Friday. Three-dimensional CT planning was carried out for all patients. Initial large fields included the entire mesorectum, internal iliac nodes and presacral space. After 25 treatments, a boost to the mesorectum and presacral space was carried out for three more treatments. Patients received 45 Gy in 1.8 Gy/fraction to the initial field and 5.4 Gy in 1.8 Gy/fraction to the boost field to a total dose of 50.4 Gy. Photon energy was 6 MV or greater for all treatments.

chemotherapy

All patients underwent placement of a central venous access device. Patients received three cycles of chemotherapy which consisted of 5-FU 225 mg/M2/day by continuous infusion days 1–14, bevacizumab 5 mg/kg on day 1 and erlotinib days 1–14. 5-FU and erlotinib were discontinued on the last day of radiotherapy. Patients were not given prophylactic anti-emetics and were instructed on the use of loperamide for diarrhea.

surgery

It was recommended that surgical resection be carried out 6–9 weeks following the completion of chemoradiation. A total mesorectal excision was mandated.

pathology

Resected specimens were evaluated for the degree of residual tumor and classified according to the American Joint Committee on Cancer/International Union Against Cancer (AJCC/IUAC). A pCR was defined by the absence of invasive viable tumor cells in the rectum or lymph nodes.

study design, definition and end points

phase I. This study had a phase I/II study design. Dose limiting toxic effects (DLTs) in the phase I portion of the study were defined as any grade 4 neutropenia, grade 3 thrombocytopenia or any grade $\geq$3 toxicity resulting in greater than a 7-day interruption in therapy. Cohorts of 3–6 patients were treated. If no DLT occurred, then subsequent patients were treated at the next highest dose level. Only the erlotinib was to be advanced in this study, with cohort 1 receiving 50 mg, cohort 2 100 mg and cohort 3 150 mg.

phase II. An additional 19 patients were enrolled at the maximum tolerated dose (MTD) for a total of up to 32 patients, in order to analyze at

least 25 assessable patients. With 25 patients treated at the MTD, the toxicity rate could be estimated to within at most ±0.16 with 90% confidence.

Disease-free survival (DFS) was measured from protocol entry until documented recurrence or death from any cause. DFS was estimated using the Kaplan–Meier method and 95% confidence intervals (CIs) were generated using Greenwood's formula. Because the pCR rate in Sauer *et al.* was 8%, the accepted pathologic CR rate at the time of study conception with standard 5-FU and chemotherapy was estimated to be in the range 10–15%. Therefore, the regimen was to be deemed worthy of further investigation if 5 or greater pCR's occurred among the 25 patients at the MTD. Assuming the true pCR rate for this regimen was 30%, the probability of observing five or greater pCR's in 25 patients studied was 0.91. If the true pCR rate for the regimen was only 10%, the probability of observing five or more pCRs in 25 patients was only 0.10.

Mutational analysis of oncogenes and tumor suppressor genes was carried out using SNaPshot (Applied Biosystems) for resected patients, using a previously described multiplexed DNA sequencing platform [20].

results

patient characteristics

Thirty-two patients were enrolled on to the study between October 2006 and December 2009. Patient characteristics are listed in Table 1.

toxicity

phase I. The phase I portion of the study enrolled nine patients. Two patients on dose level 1 experienced grade 2 mucositis for which the clinician reduced the 5-FU dose by 25%. On dose level 3 (erlotinib 150 mg), two patients withdrew consent. One patient had grade 3 diarrhea and refused to wait 1 week before reassessing toxicity. The second had a grade 3 rash. The third patient on the cohort also experienced a grade 3 rash which required a 14-day hold of erlotinib followed by a reduction in the erlotinib to 100 mg. We decided not to expand that cohort and declared 100 mg as the MTD.

phase II. Twenty-three patients were enrolled on the phase II portion of the trial. One patient was removed from the trial for cardiac ischemia presumably due to fluoropyrimidine related coronary vasospasm. Two additional patients did not complete study therapy due to toxicity; one for grade 3 dehydration, diarrhea and grade 2 mucositis and one for grade 3 diarrhea.

Overall, 46.9% of patients experienced grade 3 or 4 toxicity (Table 2). The most common grade 3–4 toxicity excluding lymphopenia was diarrhea, in 18.8% of patients, followed by rash in 6.3%. Ten of 27 (33.3%) patients completing protocol therapy required a dose reduction. Five (18.5%) patients only had the 5-FU dose reduced, three (11.1%) only had the erlotinib dose reduced and two (7.4%) had both the 5-FU and erlotinib dose reduced. No patient had a dose reduction in the bevacizumab.

All 31 patients who underwent surgery had an R0 resection with the following surgical procedures: abdominal perineal resection 38.7%, low anterior resection in 35.5% and low anterior

Table 1. Patient characteristics

Variable	Value	*n* (%)
Gender (*n* = 32)	Female	8 (25)
	Male	24 (75)
Race (*n* = 32)	White	29 (90.3)
	Other	3 (9.3)
Ethnicity (*n* = 32)	Non-Hispanic	28 (87.5)
	Hispanic	2 (6.3)
	Ethnicity Not Known	2 (6.3)
Stage grouping (*n* = 32)	II	11 (34.3)
	III	21 (65.6)
Clinical staging (*n* = 32)	T3N0	6 (18.8)
	T3N1	15 (46.8)
	T3N2M0	4 (12.5)
	T3Nx	4 (12.5)
	T4N0	2 (6.3)
	T4N1	1 (3.1)
Surgical pathologic (N staging) (*n* = 28)	N0	21 (75)
	N1	7 (25.0)
Surgical pathologic T staging (*n* = 28)	T0	7 (25.0)
	T1	2 (7.1)
	T2	4 (14.3)
	T3	13 (46.4)
	T4	1 (3.6)
	Tis	1 (3.6)
Surgical post-treatment histologic grade[a] (*n* = 19)	Moderately differentiated	17 (89.5)
	Poorly differentiated	2 (10.5)

[a]Not assessable in nine patients due to pCR [7], Tis [1] or insufficient viable tumor to grade [1].

Table 2. Grade 3 or greater toxicity

Toxicity description	Grade		
Frequency (*n*)	3	4	Total
Lymphopenia	16	5	21
Diarrhea w/o prior colostomy	6	0	6
Hypophosphatemia	3	0	3
Rash: acne/acneiform	2	0	2
ALT-SGPT	1	0	1
AST-SGOT	1	0	1
Cardiac-ischemia	1	0	1
Colitis	1	0	1
Dehydration	1	0	1
Fatigue	1	0	1
Febrile neutropenia	0	1	1
Hypertension	1	0	1
Hyperuricemia	0	1	1
Hypokalemia	1	0	1
Hyponatremia	1	0	1
Muco/stomatitis (symptom) oral cavity	1	0	1
Muco/stomatitis by exam-oral cavity	1	0	1
Proteinuria	1	0	1
Radiation dermatitis	1	0	1
Rash/desquamation	1	0	1
Rectum-pain	1	0	1
Total (including lymphopenia)	42	7	49
Total (excluding lymphopenia)	26	2	28

resection with coloanal anastomosis in 25.8%. Post-operative complications included: 4 (14.3%) anastomotic leaks, 2 (7.1%) intra-abdominal infection, 2 (7.1) wound infections, 1 (3.6%) pulmonary embolus, 1 (3.6%) small bowel obstruction, 5 (17.9%) urinary obstruction/retention and 1 fever (3.6%).

radiation interruptions. Of the 32 patients who initiated study therapy, 7 of 32 patients who started treatment experienced a treatment break. In three patients, the cause was diarrhea, in one patient, acneiform skin rash, in two patients oral mucositis and in one patient hand-foot syndrome. The mean length of break for these seven patients was 11.8 days (range 7–15 days).

efficacy. A pCR was achieved in 9 of 27 (33.3%) of patients who completed study therapy and 10 of 31 (32.2%) of patients undergoing resection. In the pre-defined efficacy cohort of patients treated at the MTD, 7 of 26 (26.9%) patients had a pCR. A total of seven patients have experienced distant progression. With a median follow-up of 2.9 years, there are no confirmed local (pelvic) recurrences. The DFS at 1, 2 and 3 years is 93.4% (95% CI: 76.2%, 98.3%), 83.4% (95% CI: 64.7%, 92.7%) and 75.5% (95% CI: 55.1%, 87.6%), respectively (Figure 1).

discussion

Preoperative chemoradiation became the standard of care for locally advanced rectal cancer based on superior local control, treatment tolerability and sphincter preservation when compared with post-operative chemoradiation [1]. Numerous phase III trials have shown improved outcomes in patients achieving a

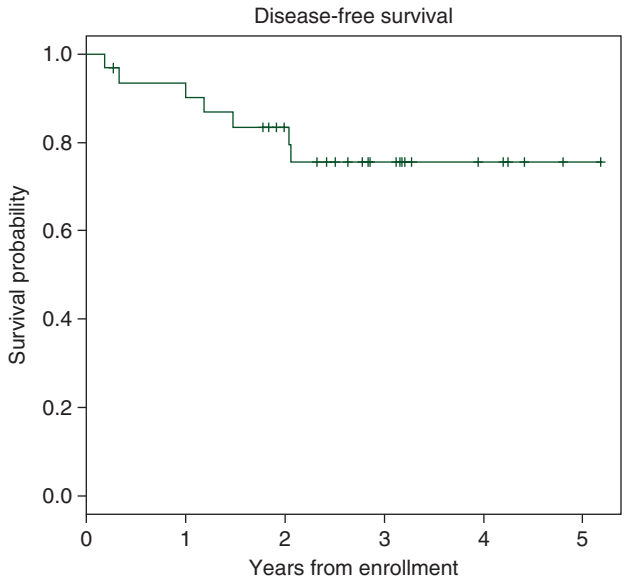

Figure 1. Disease-free survival.

pCR [2, 21]. Consequently, there have been many efforts to improve the response to radiation by adding various agents to radiation and a fluoropyrimidine in the preoperative setting; however, no drug has convincingly improved the pCR rate. Oxaliplatin has been the most widely studied agent in this setting but has failed to improve the pCR rate in multiple phase III studies [22, 23].

This current study sought to capitalize on potential synergy in combined anti-EGFR and anti-VEGF therapy in radiosensitization seen in preclinical studies [16]. Anti-EGFR and anti-VEGF therapies have been studied separately with chemoradiation as they were each attractive strategies to improve pCR rates in rectal cancer because of the clinical benefit seen in the metastatic setting. The clinical results with bevacizumab (Table 3) and cetuximab (Table 4), however, have been modest.

The role of erlotinib in colorectal cancer remains undefined. In a randomized phase III study in 700 patients with metastatic colorectal cancer who received first-line chemotherapy with FOLFOX, capecitabine/oxaliplatin or FOLFIRI with bevacizumab, patients without progression were randomized to maintenance therapy with bevacizumab alone or bevacizumab with erlotinib, with a primary end point of progression-free survival (PFS). In the 446 patients who did not have progression and were randomized, PFS was improved with the addition of erlotinib as maintenance therapy, suggesting that erlotinib may be an active drug in colorectal cancer though further maturation of the data is clearly needed.

This current study achieved its pre-defined pCR goal of ≥5 of 25 patients achieving a pCR. The clinical significance was benchmarked against of the number of phase III trials that have reported pCR rates of 8–15% [1, 21–23]. However, recent data from a large phase III trial suggest that the true pCR rate achievable with 5-FU or capecitabine with radiation may be closer to 20% (290). If a baseline assumption of 20% is used, the one-sided *P*-value of 0.253 and is no longer significant. Thus, while the 33.3% pCR rate in patients who completed therapy and 26.9% pCR in the predefined cohort treated at the MTD are

Table 3. Select studies of bevacizumab-containing chemoradiation regimens for rectal cancer

Author	Number of patients	Regimen	pCR
Willett *et al.* [7]	32	5-FU/bevacizumab	5/32
Czito *et al.* [8]	11	Capecitabine/oxaliplatin/ bevacizumab	2/11
Crane *et al.* [9]	25	Capecitabine/bevacizumab	8/25
Kennecke [24]	42	Capecitabine/oxaliplatin/ bevacizumab	7/42

Table 4. Select studies of cetuximab-containing chemoradiation regimens for rectal cancer

Author	Number of patients	Regimen	pCR
Debucquoy *et al.* [10]	40	Capecitabine/cetuximab	2/40
Bengala *et al.* [11]	40	Capecitabine/cetuximab	3/40
Sartore-Bianchi *et al.* [12]	40	Capecitabine/cetuximab	3/38
Colakoglu *et al.* [13]	37	Capecitabine/cetuximab	3/37
Hofheinz *et al.* [14]	20	Capecitabine/irinotecan/ cetuximab	5/20
Rodel *et al.* [15]	58	Capecitabine/ oxaliplatin/cetuximab	4/45

encouraging, enthusiasm must be tempered with the observation that other single arm phase II studies have achieved pCR rates of ~25% but have failed to improve pCR rates when tested in randomized trials (30). However, our long-term local control of 100% with a median follow-up of 2.9 years is the most encouraging outcome of the trial.

Because of the potential for added toxicity, the best niche for agents with convincing radiosensitization properties, but unproven systemic benefit, may be in high-risk patients with low tumors or a threatened/involved mesorectal fascia with a high risk for a positive margin. However, this additional therapy must be balanced against added toxicity, which was substantial in this study. The NSABP has accordingly proposed a trial evaluating the use of targeted therapies with chemoradiation in selected high-risk rectal cancer patients, who would be most likely to benefit from treatment intensification. As the grade 3 or greater toxicity rate of this regimen (46.9%) is similar to the preoperative arm of NSABP R-03 (41.9%) (30), this current regimen is planned as the first experimental arm in this new clinical trial platform.

funding

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disclosure

LSB received clinical trial support from Genentech. DRB is a paid consultant for Bio-Reference Laboratories, the licensee of SNaPshot, the technology used in this study. All remaining authors have no conflict of interest.

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Rare *EGFR* exon 18 and exon 20 mutations in non-small-cell lung cancer on 10 117 patients: a multicentre observational study by the French ERMETIC-IFCT network

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Background: There is scarce data available about epidermal growth factor receptor (*EGFR*) mutations other than common exon 19 deletions and exon 21 (L858R) mutations.

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