A Prospective Pilot Study of Target-guided Personalized Chemotherapy with Intensity-modulated Radiotherapy in Patients With Early Rectal Cancer

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Purpose: To investigate the feasibility of personalizing chemotherapy in patients with rectal cancer.

Methods: Patients with cT3 or cN1 and cM0 rectal cancer were eligible. A set of 6 molecular markers including *KRAS*, *BRAF*, and *PI3K* mutations and expression of topoisomerase-1 (*Topo-1*), *ERCC-1*, and thymidylate synthase (*TS*) using immunohistochemistry were performed in a tumor biopsy. All patients were treated with capecitabine 625 to 825 mg/m^2/12 h M-F in combination with either irinotecan or oxaliplatin based on *Topo-1* and *ERCC-1* expression plus either bevacizumab or cetuximab based on the mutation status. All patients received intensity-modulated radiation therapy. A surgery was performed 6 to 8 weeks after the treatment.

Results: Fifteen patients (94%) had T3 tumor and 10 (62%) N+ disease of 16 patients enrolled. In all patients, the full set of markers was analyzed within 10 days. Seven patients had *K-ras* mutation, and 4, 5, and 10 expressed *Topo-1*, *ERRC-1* and *TS*, respectively. All patients had wild-type *BRAF* and *PI3K* tumors. The median time from obtaining informed consent to the treatment period was 18 days and all patients completed the chemoradiation treatment. Fifty percent achieved a complete pathologic response to treatment. Four patients (25%) developed grade 3 proctitis or diarrhea. There were no relevant surgical complications. Sixty-nine percent of the patients received adjuvant XELOX.

Conclusions: The individualization of neoadjuvant chemotherapy in patients with rectal cancer is feasible and leads to a high rate of pathologic response.

Key Words: molecular targets, personalized treatment, rectal cancer

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The medical treatment of colorectal cancer has greatly improved over the last few years with the introduction of effective chemotherapy agents such as oxaliplatin, irinotecan, cetuximab, and bevacizumab.[1] Current standard management is based on combinations of these agents with several potential equally effective regimens. Treatment decisions remain largely empirical. The only universally accepted biomarker is *KRAS* genotyping supported by the lack of efficacy of cetuximab in patients with certain activating mutations in this oncogene.[2] Recent data also suggest that mutation in other genes in the Ras pathway, such as *BRAF* and *PI3K* also confer resistant to cetuximab, but this is not yet standard of care.[3–5] For all other agents, including 5-fluoropyrimidines, oxaliplatin, and irinotecan, there is no clinically accepted biomarker. However, for each one of these drugs, there are putative biomarkers, with different levels of validation, in the literature.[6–9]

Rectal cancer, defined as a tumor arising in the distal large bowel <12 cm from the anal verge by rigid sigmoidscopy,[10] affects 40,000 new patients per year in the United States.[11] Current recommended management of patients with localized rectal cancer includes combined chemoradiation with 5-fluorouracil or capecitabine and external-beam radiation therapy, followed by surgical resection. In rectal cancer, there is a very well-established correlation between disease-free survival (DFS) in patients with localized rectal cancer and the pathologic TNM staging (ypTNM) after chemoradiotherapy. Thus, the long-term DFS is 97% for patients with ypT0N0M0 (ypCR), whereas only 42% in patients with ypN+.[12] With standard treatment, the ypCR is 15% with grade 3 diarrhea and proctitis occurring in 10% to 15% of the patients.[13] In newer approaches such as capecitabine and oxaliplatin (CAPOX) or irinotecan or intensity-modulated radiation therapy, the ypCR increases from 25% to 30%, whereas grade 3 toxicities can be as high as 40%.[14–16] The excellent correlation between the pathologic response and DFS makes for a very interesting intermediate endpoint to test new interventions. Thus, a number of recent studies have taken advantage of this clinical management scenario to perform the biological and imaging studies.[17–20]

Our group has been interested in personalizing the treatment of colon cancer by applying a full panel of biomarkers in a treatment algorithm that provides a guide for treatment selection.[21] In this study, we have tested the feasibility of applying in real time a panel of biomarkers in patients with operable rectal cancer. The results show that the approach is feasible, leading to recommendation of individual patient treatment in real time with promising therapeutic results.

PATIENTS AND METHODS

Eligibility Criteria

Patients with rectal adenocarcinoma stage T3-T4 and/or N+ candidates to receive preoperative chemoradiation were

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eligible for the study. Other eligibility criteria included availability of tumor tissue or possibility of a tumor biopsy to determine therapeutic targets and adequate renal ($Cr < 1.5$ mg/d), liver (bilirubin ≤ 1.5 mg/dL, AST and ALT $\leq 3.0\times$ the upper limit of normal), and normal bone marrow function (absolute neutrophil count $\geq 1500/\mu L$, hemoglobin ≥ 9.0 g/dL, and a platelet count of $\geq 100{,}000/\mu L$). The exclusion criteria included contraindication for the administration of any of the drugs used in the study including capecitabine, irinotecan, oxaliplatin, cetuximab, or bevacizumab. The study was approved by the local ethics committee and the Spanish Health authorities in as per European Regulations and conducted in accordance with the Declaration of Helsinki (October 2000). The trial was registered with the Clinical Trials.gov identifier NCT01366118.

Pretreatment Assessments

In addition to evaluating patients on the basis of medical history, physical examination, hematology, biochemistry, and tumor markers, patients were studied using colonoscopy, endoscopic ultrasound (EUS), pelvic magnetic resonance imaging, computed tomography of the chest, abdomen, and pelvis, and whole-body positron emission tomography (PET) scan, with assessment of standardized uptake value (SUV). In case of discordance of clinical locoregional staging between the EUS and the magnetic resonance the worse stage was chosen. Similar studies were conducted after treatment, before surgery, to determine the clinical response.

Biological Studies

A set of 6 molecular therapeutic targets were determined in pretreatment tumor tissues including mutation analysis of *KRAS*, *BRAF*, and *PI3K* and immunohistochemical determination of topoisomerase-1 (*Topo-1*), *ERCC-1*, and thymidylate synthase (*TS*). These markers were determined according to well-established and well-published methods. Immunohistochemistry data were analyzed on the basis of the percentage of tumor cells stained (0 = 0% of tumor cells, 0.1 = 1% to 9% of tumoral cells, 0.5 = 10% to 49%, 1 ≥ 50% of tumor cells) and intensity of staining (0 = no staining, 1 = mild staining, 2 = moderate staining, 3 = strong staining). These 2 parameters were added to generate a final score. The expression of marker was considered positive if the aggregate score was ≥ 1.[22]

Sections were deparaffinized in xylene and rehydrated in graded alcohol. Antigen retrieval was performed using EDTA, pH 9.0, in a pressure chamber (Pascal; Dako Cytomation) except for TP. All tissues were immunostained using the Autostainer (Dako Cytomation). The duration of antibody incubation was 60 minutes for *ERCC-1* (dilution 1:100; Neomarkers; clone: 8F1) and *Topo-1* (dilution 1:50; Novocastra; clone 1D6) and 30 minutes for *TP* (dilution 1:50; Neomarkers; clone PGF44C). Immunodetection was done using the Dako Envision + dual-link polyper-horseradish peroxidase (Dako Cytomation) visualization method with diaminobenzidine chromogen (DAB +) as the substrate. The sections were counterstained with hematoxylin. *KRAS*, *BRAF*, and *PI3K* mutations were identified from the formalin-fixed and paraffin-embedded tumors. Hematoxylin and eosin staining of each tumor was reviewed by a pathologist (E.G.G.) to assess the percentage of tumor cells before DNA extraction. Macrodissection of tumors was performed in samples with small percentage of tumor tissue to enrich the final amount of tumor DNA. Mutation screening was performed using polymerase chain reaction and an automatic direct sequencing as previously described.[23]

Treatment Decision-making Tree

All patients were treated with 625 to 825 mg/m^2 capecitabine for 12 hours daily from Monday to Friday. Patients with high *Topo-1*-positive tumors received 50 mg/m^2 irinotecan every week. If *Topo-1* was negative, *ERCC-1* status was considered. Patients with *ERCC-1*-negative tumors received oxaliplatin. If *ERCC-1* expression was positive, patients received capecitabine alone. In addition, patients with *KRAS*-mutated or *BRAF*-mutated tumor received bevacizumab, 5 mg/kg every 2 weeks. If both *KRAS* and *BRAF* were wild type, patients received either cetuximab 400/250 mg/m^2 weekly or bevacizumab at investigators' discretion (Fig. 1).

Surgery, Radiation Therapy, Adjuvant Treatment, and Follow-up

A Belly Board immobilization device was used either for simulation or for treatment. Contouring was performed on the simulation computed tomography with PET fusion when available. Two treatment volumes were defined: the first one includes mesorectal tissue and standard pelvic nodes (common iliac, internal iliac, presacral, obturator, and perirectal lymph nodes), planning target volume margin was 1 cm in all directions; the second one includes the macroscopic tumor and malignant nodes observed on TC and/or PET-TC with a planning target volume margin of 0.5 to 1 cm. The patients underwent radiotherapy in 23 daily fractions from Monday to Friday; a concomitant boost technique allows treatment of the target volumes with different dose intensities. Treatment dose was 46 Gy for the pelvic volume and 57.5 Gy for the tumor and node volume. Surgery was performed 6 to 8 weeks after completion of treatment using standard surgical approaches. Low anterior resection was the preferred surgical technique. An abdominoperineal resection was performed in distal tumors (1 to 2 cm from anal verge). Adjuvant treatment with CAPOX was recommended at the investigator's discretion. Toxicity was assessed weekly and are reported according to the NCI CTC Ver3.0

Evaluation of Response

EUS and pathologic staging was determined using the TNM system. Clinical downstaging was done by comparing the posttreatment EUS with the pretreatment staging. Primary tumor and lymph node downstaging was defined as reductions in T and N stage by at least one level. PET responses were compared on the basis of variation in the SUV and reported as per the EORTC criteria.[24] Pathologic regression was reported according to the grading system of Rödel et al[25] comprising 4 categories ranging from 0, the absence of pathologic regression, to 4, complete tumor regression.

Microvessel Status

We studied the microvessel status before and after treatment in 5 patients. Paraffin-embedded tumor samples were collected from diagnostic biopsy (pretreatment) and surgical specimen (posttreatment) and processed according to standard procedure.[26] The sections were counterstained with hematoxylin. After searching the areas of highest neovascularization, hot spots, individual microvessels were counted on 3 $\times 200$ fields. Any brown-stained endothelial cell or endothelial cell cluster distinct from adjacent microvessels, tumor cells, and connective tissue elements was considered as a single countable microvessel. The number of *CD31* vessels was counted in each $\times 200$ field. The medium containing the total number of vessels of the 3 fields of view was the final result.

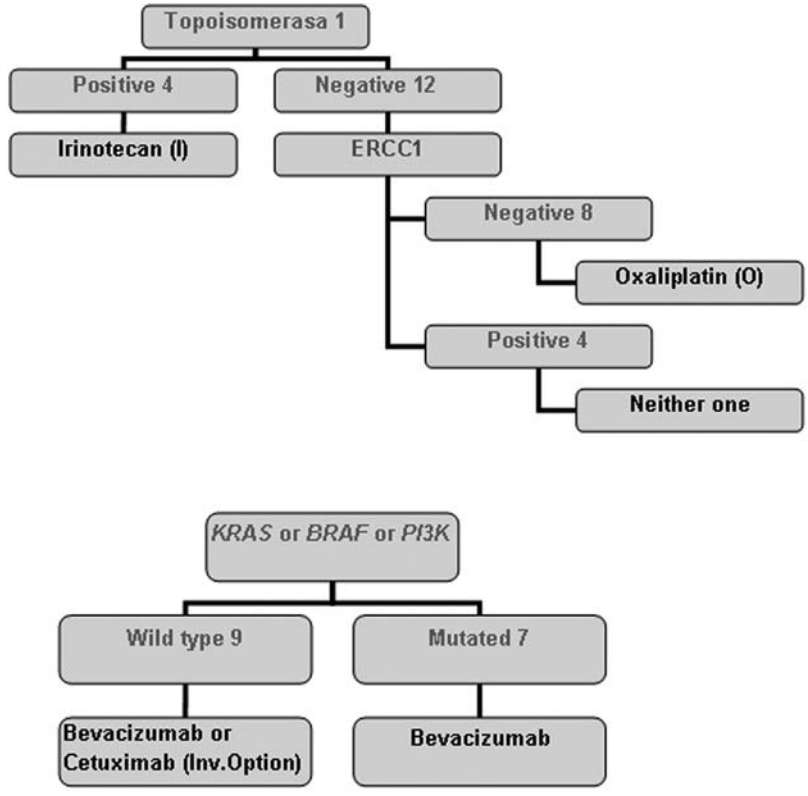

FIGURE 1. Decision-making tree for guided chemotherapy based on molecular marker expression of the 16 patients. Patients with positive Topo-1 expression received capecitabine and irinotecan (CAPIRI). Patients with negative Topo-1 were treated with capecitabine and oxaliplatin (CAPOX) if ERCC-1 expression was negative or with capecitabine alone if ERCC-1 was positive. Patients with mutated KRAS or BRAF received bevacizumab, whereas patients with wild-type genotype for both genes had the option to receive bevacizumab or cetuximab.

RESULTS

Patients

A total of 16 patients, whose principal characteristics are listed in Table 1, were enrolled. All patients except 1 had T3 disease and 10 had positive lymph node disease detected by EUS. The tumors were FDG positive with a median SUVmax of 11 (6.8 to 19.9). Four patients had *Topo-1*-positive cancers and 11 had *ERCC-1*-negative tumors. All subjects had wild-type *BRAF* and *PI3K* tumors and there were 7 patients with mutated *KRAS*.

TABLE 1. Patients Characteristics

No. patients	16
Age (median, range)	(56, 33-74)
Sex (M/F)	(8/8)
Stage	
T2N1	1
T3N0	6
T3N1	7
T3N2	2
Median distant from anal verge	5.5
Median (range) PET SUV uptake	12 (6.8-19)
Immunohistochemistry biomarker analysis (+/−)	
TS	10/4
ERCC-1	5/11
Topo-1	4/12
Mutation status (mut/wt)	
KRAS	7/9
BRAF	0/16
PI3K	0/16

SUV indicates standardized uptake value; Topo-1, topoisomerase-1; TS, thymidylate synthase.

Biomarker Assessment and Treatment Allocation

Tumor material of sufficient quantity and quality was obtained from all patients. The assessment of the full set of markers was completed in all patients within 10 days after receiving a signed informed consent from them, allowing to personalize the patient's treatment. Treatment was initiated 18 days (mean range, 10 to 27 d) after the consent was signed. On the basis of the results of the molecular analysis, 4 patients received capecitabine and irinotecan (CAPIRI), 8 CAPOX, and 4 capecitabine alone. A total of 10 patients received bevacizumab, including 7 patients with *KRAS*-mutated tumors and 3 patients with wild-type cancer. Six patients received cetuximab. Table 2 lists the treatment of each individual patient. Eleven patients received adjuvant CAPOX after surgery.

Outcome

Eleven patients were found to have downstaging of T stage and 8 patients were found to have downstaging of N stage on comparing pretreatment clinical staging with pathologic staging. In 1 patient the T stage advanced from T2 to T3. No patient presented new lymph node involvement. Eight (50%) patients had ypT0N0 and 13 (81%) had ypN0 after treatment. In 8 patients, there was a complete pathologic

TABLE 2. Patients' Treatment and Outcome

Patient Number	cTN	Treatment	SUV PET % Decrement	ypTN	TRG
1	T3N1	Cap + Bev	−100	T0N0	4
2	T3N1	CAPOX + Bev	−100	T3N1	3
3	T3N1	CAPIRI + Cet	−62	T0N0	4
4	T3N0	CAPOX + Bev	−68	T0N0	4
5	T3N0	CAPOX + Cet	−100	T0N0	4
6	T2N1	CAPOX + Bev	−100	T3N0	0
7	T3N0	CAPOX + Cet	−1	T0N0	4
8	T3N0	CAPIRI + Bev	−40	T3N0	3
9	T3N1	CAPOX + Cet	−100	T3N1	3
10	T3N1	CAPIRI + Cet	−66	T0N0	3
11	T3N1	CAPOX + Bev	−56	T0N0	4
12	T3N1	CAPIRI + Cet	−65	T0N0	4
13	T3N2	CAPOX + Bev	−75	T2N0	3
14	T3N0	Cap + Bev	−73	T2N0	3
15	T3N2	Cap + Bev	−100	T3bN1b	3
16	T3N0	Cap + Bev	−78	T0N0	4

Bev indicates bevacizumab; Cap, capecitabine; CAPIRI, capecitabine and irinotecan; CAPOX, capecitabine and oxaliplatin; cTN, clinical TN stage; SUV, standardized uptake value; TRG, tumor regression grade; ypTN, pathologic stage posttreatment.

response (50%) and in 3 additional patients there was grade 3 tumor regression grade (TRG). Of the 3 patients with positive lymph node disease after treatment, 2 had TRG 3 and 1 had TRG 2. Fifteen patients had a PET response including 6 patients with complete responses. PET response, however, was a poor predictor of TRG with only 2 patients with negative PET after treatment having a TRG 4. In addition, the only patient with no treatment response had a negative PET after treatment and the patient with no PET response had a complete pathologic response. Seven patients with a mean DAV pretreatment of 2.5 cm underwent an abdominoperineal resection. All under study had B-raf-native and TP-negative results. Among the 8 patients with ypT0N0, k-ras status was wild type in 5 and *ERCC-1* and *Topo-1* were negative, and thymidylate synthase was positive in 5 patients. Six of these 8 patients received a 3-drug-combination treatment, 3 with bevacizumab and 3 with cetuximab. Two of them received 2-drug-treatment, both with bevacizumab.

Microvessel Density

Table 3 summarizes *CD31* results. Two of 3 patients treated with bevacizumab had an increase in microvessel density after treatment. These patients showed a complete tumor regression in surgical specimen. No increment in MVD was observed in patients who did not receive bevacizumab.

TABLE 3. Microvessel Density

Patient Number	CD31 Count (Diagnostic Biopsy)	CD31 (Surgical Specimen)	TRG	Bevacizumab
2	27	21	3	Yes
4	6	12.3	4	Yes
7	16.6	6.6	4	No
10	10.3	7.6	3	No
11	5.3	15.6	4	Yes

TRG indicates tumor regression grade.

Toxicity

Treatment was in general well tolerated with side effects as expected by this type of treatment. The most common toxicity was diarrhea that was grade 3 in 1 patient treated with CAPIRI + cetuximab and that required a 1-week delay in irinotecan dose. Twelve patients had grade 2 diarrhea. Grade 3 proctitis developed in 3 patients, all of them with cancers in the lower region of the body (6 cm from the anal verge). They recovered completely at the end of the treatment and only one of them had a delay in surgery for this reason.

DISCUSSION

This pilot study aimed to incorporate a set of well-defined biomarkers to guide the selection of chemotherapy regimen in patients with rectal cancer. The results show that this approach was feasible in all patients tested with a rapid turnaround of biomarker assessment results that allowed the identification of individual chemotherapy regimens for each one of the patients. The data show that this approach led to a high pathologic complete response (pCR) rate and that SUV uptake does not seem to be a good predictor of pathologic outcome. These 2 findings, however, need to be taken cautiously given the small sample size and uncontrolled nature of the trial.

The choices of chemotherapy treatment for CRC, like many other solid tumors, are several. The current process to select a treatment modality for a patient is largely empirical. However, for many agents putative response markers have been identified.[6,9,27–29] Although the data are often not definitive and sometimes even controversial, it is possible to select a set of markers to guide treatment selection. In this project, we constructed an algorithm for treatment selection on the basis on published information that could be practically used. It is clear that other algorithms are possible and that, indeed, we do not know which one is better, but our goal was to test that the approach, overall, is feasible.

The clinical results are quite remarkable (ypCR 50%). The results of standard radiation chemotherapy are quite variable in the literature (ypCR ranging from 0% to 46%); however, results of ypCR from a larger series and of meta-analysis are in the range of 16%. Because of the limited number of patients enrolled, it is not possible to draw any conclusion other than to launch an efficacy-oriented randomized trial. The disappointing PET scan results of our study in terms of respond prediction could be because of performing it at the end of the treatment. Other recent studies concluded that it is more convenient to do the PET study earlier, after 1 week of preoperative treatment, to get a better noninvasive prediction of pathologic response.[30] It could help to decide the most appropriate time for surgery in some patients. We observed an increase in microvessel density in 2 patients treated with bevacizumab who achieved pCR. This was not expected as previous studies with bevacizumab as a single agent in rectal cancer have shown the opposite outcome.[31–33] It should be noted, however, that bevacizumab was used here in combination with chemotherapy and radiation therapy, as well as for a prolonged period of time. In addition, other studies show that rather than decreasing angiogenesis, antivascular agents lead to vessel normalization.[33–35] Furthermore, the healing process that occurred in these tumors with pCR may also, per se, increase the number of vessels.

In conclusion, these data show that it is feasible to guide the selection of chemotherapy in patients with rectal cancer applying a simple, affordable, and practical algorithm. The clinical outcome of these patients was remarkable. This

strategy should be tested in a randomized phase II study compared with conventional 5-fluorouracil-based chemoradiation. In this trial, a wait-and-see strategy in ypCR patients could be implemented.

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