Phase II Trial of Target-guided Personalized Chemotherapy in First-line Metastatic Colorectal Cancer

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Patients and Methods: Patients with untreated metastatic CRC, performance status 0-1, and candidates for systemic chemotherapy were eligible. Tumor tissues were analyzed for KRAS mutations and expression of topoisomerase-1 (Topo-1), excision repair cross-complementing gene 1 (ERCC1), thymidylate synthase (TS), and thymidine phosphorylase (TP). Patients with Topo-1 expression received irinotecan, whereas patients with negative Topo-1 and ERCC1 expression received oxaliplatin. Otherwise, patients received physician’s choice of treatment. If TS was positive, no fluoropyrimidine was administered and if negative, 5-fluorouracil if TP was negative, or capecitabine if TP was positive. KRAS-mutated patients were treated with bevacizumab, whereas KRAS-native received cetuximab. The primary endpoint of the study was progression-free survival (PFS).

Results: A total of 74 patients were enrolled and 67 received personalized treatment including irinotecan (n=27), oxaliplatin (n=16), FOLFIRI (n=12), and FOLFOX (n=12). Thirty-eight patients received cetuximab and 29 bevacizumab. With a median follow-up time of 18.3 months (95% confidence interval [CI], 4-36), the overall median PFS was 8.3 months (95% CI, 6.9-9.7), representing a 12-month PFS rate of 36.5% (95% CI, 25-48). Overall clinical benefit, including response rate and disease stabilization, was 86% (95% CI, 73%-97%). The overall median survival was 21 months (95% CI, 11-40).

Conclusions: Real-time target-guided personalized first-line treatment of patients with advanced CRC is feasible but, with the approached used, did not result in a clear improvement in PFS to warrant phase III testing.

Key Words: molecular targets, personalization, treatment, colon cancer

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The authors declare no conflicts of interest.

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according to RECIST 1.1 criteria, life expectancy of ≥ 6 months, availability of tumor tissue, or possibility to perform a tumor biopsy to determine therapeutic targets and adequate renal (Cr < 1.5 mg/dL), liver (bilirubin level ≤ 1.5 mg/dL, aspartate aminotransferase and alanine aminotransferase levels ≤ 3.0 x the upper limit of normal), and bone marrow function (absolute neutrophil count ≥ 1500/µL, hemoglobin level ≥ 9.0 g/dL, and a platelet count of ≥ 100,000/µL). Exclusion criteria included contraindication for the administration of any of the drugs used in the study including 5-FU, capecitabine, irinotecan, oxaliplatin, cetuximab, or bevacizumab. Previous adjuvant treatment was allowed. The study was approved by the local ethics committee and the Spanish Health Authorities as per European Regulations and conducted in accordance with the Declaration of Helsinki (October 2000). The trial was registered with ClinicalTrials.gov identifier NCT01453257.

**Pretreatment and Follow-up Assessments**

In addition to a medical history, physical examination, hematology, biochemistry, and tumor markers, patients were studied with computed tomography (CT) scan of the chest, abdomen, and pelvis and colonoscopy if primary tumor had not been resected. Other imaging technique (PET-CT, magnetic resonance, or abdominal US) was performed, if needed, for a better assessment of tumor burden and response to treatment. Responses were evaluated according to the RECIST 1.1 criteria every 8 weeks by CT scan and tumor markers if available. Patients were followed up until disease progression. Toxicity was assessed biweekly and reported according to the NCI CTC ver. 4.0 throughout the study.

**Biological Studies**

A set of 7 molecular therapeutic targets were determined in pretreatment tumor tissues including mutation analysis of KRAS, BRF, and PI3K genes and immunohistochemical (IHC) determination of Topo-1, ERCC1, TS, and TP. These markers were determined according to well-established and published methods. IHC data was analyzed according to the percentage of tumor cells staining (0 = 0% of tumor cells; 0.1 = 1% to 9% of tumor 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 multiplied to generate a combined final score. The expression of marker was considered positive if the aggregate score was ≥ 1.15.

Sections were deparaffinised in xylene and rehydrated in graded alcohol. Antigen retrieval was carried out using EDTA, pH 9.0, in a pressure chamber (Pascal; Dako Cytomation), except for TP. All tissues were immunostained using the Dako Autostainer (Dako Cytomation). The antibody incubation was 60 minutes for ERCC1 (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 carried out with the Dako Envision+ dual-link polymer-horseradish peroxidase (Dako Cytomation) visualization method with diaminobenzidine chromogen (DAB+) as the substrate. Sections were counterstained with hematoxylin. KRAS, BRF, and PIK3Ca mutations were analyzed from formalin-fixed and paraffin-embedded tumors (FFPE). A hematoxylin and cosin (H&E) slide of each tumor were reviewed by a pathologist 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 reactions and automatic direct sequencing as previously described.16

**Treatment Decision-making Tree**

Patients with Topo-1-positive tumor received irinotecan. If Topo-1 was negative, ERCC1 status was considered. Patients with ERCC1-negative tumor received oxaliplatin. If ERCC1 expression was positive, patients received irinotecan or oxaliplatin at investigators discretion. Patients with TS-negative tumor received 5-FU if TP was negative or capecitabine if TP was positive. Patients with TS-positive tumor did...
not receive fluoropyrimidines in their treatment schema. In addition, patients with \textit{KRAS}-mutated tumors received bevacizumab, and patients with \textit{KRAS} wild-type received cetuximab (Fig. 1).

**Treatment Schemas**

The chemotherapy regimens were administered according to their different package inserts, using conventional clinical protocols for dose, schedules, concomitant medication, and dose adjustment. Patients with responding tumors and limited anatomic disease spread were considered for locoregional treatments as per standard practice. Patients who underwent any locoregional treatment completed 6 months of chemotherapy after the procedure was completed. Maintenance treatment with bevacizumab or cetuximab plus a fluoropyrimidine, according to the chosen regimen, was recommended, but not mandatory, after completing the 6 months of treatment.

**Statistical Analysis**

The primary endpoint of the study was progression-free survival (PFS). The strategy was considered to be effective if the proportion of patient’s PFS rate at 12 months was $\geq 50\%$ and “non-effective” when the PFS rate at 1 year was $\leq 35\%$. With these parameters, \( H_0 \) was established as PFS at 1 year and “non-effective” when the PFS rate at 1 year was $\leq 35\%$ and \( H_1 \) as PFS at 1 year ($\geq 50\%$). Applying the Fleming method for phase II clinical trials, with a statistical power of 80\% and an $\alpha$ of 0.05, 65 patients need to be included. Assuming a 10\% loss rate, a total of 74 patients was to be included.

**RESULTS**

**Patients**

A total of 74 patients, whose principal characteristics are listed in Table 1, were enrolled in the study between January 2010 and October 2011. The median age was 67 (range, 39 to 84) years and 97\% of the patients had a 0-1 ECOG performance status. Seventeen patients had rectal cancer. Most patients had only 1 or 2 metastatic lesions that were located in the liver in 70\% of them.

**Patient Disposition**

Sixty-seven patients were evaluated. Seven patients (9.5\%) were not evaluable because they did not complete the first 8 weeks of treatment. Reasons for treatment discontinuation included disease progression in 45 patients, drug-related toxicity in 5 (1 infusion reaction, 1 mucositis, and 3 peripheral neuropathy), and serious unrelated events in 2 patients (1 bowel obstruction and 1 pulmonary embolism). Two patients died during the study, one because a surgical complication and another one for an unknown reason. Thirteen patients (19\%) are still on treatment. Twenty-six patients (39\%) received locoregional treatment (surgery, radiosurgery, or radiofrequency ablation) for their metastatic disease during the study.

**Biomarker Assessment and Treatment Allocation**

Tumor material of sufficient quantity and quality was obtained in all patients to perform the full set of pre-specified markers. The results of these studies are summarized in Table 2, and a representative IHC staining example is showed in Figure 2. Median time from informed consent to treatment was 17 (95\% confidence interval [CI], 4-35) days. On the basis of the results of the molecular analysis a total of 39 patients with \textit{KRAS}-native tumors received cetuximab as part of their treatment. The other 28 patients with \textit{KRAS}-mutated tumors received bevacizumab. \textit{BRAF} and \textit{PI3K} mutations were performed in all the samples. Three patients with \textit{BRAF}-mutated \textit{KRAS}-native tumors received cetuximab. The PFS of these patients was 16.5 months (95\% CI, 9.9-23.2), but 2 of these patients received locoregional treatment. Seven patients, 3 with \textit{KRAS}-mutated tumors, had a \textit{PI3K} mutation. As expected, the PFS of patients with \textit{PI3K}-mutated tumor who received cetuximab was very short, that is, 1.9 months (95\% CI, 0.5-4.2).

There were 40 patients with TS-positive tumor who did not receive a fluoropyrimidine in their treatment schema and 27 patients with a TS-negative tumor who received 5-FU. Topo-1 expression was positive in 33 tumors, and these patients received irinotecan-containing regimens. In addition, 6 more patients with Topo-1-negative and ERCC1-positive tumors were treated with irinotecan-containing regimens as per physician discretion. Twenty-eight patients received oxaliplatin as part of their tailored treatment, 24 because of ERCC1-negative, Topo-1-negative tumor expression and 4 with ERCC1-positive, Topo-1-negative tumor because of investigator option.

**Outcome**

With a median follow-up time of 18.3 (95\% CI, 4-36) months, the median PFS was 8.3 (95\% CI, 6.9-9.7) months,

<table>
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<tr>
<th>Table 2. Results of Biomarker Analysis</th>
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<tr>
<td>Topo-1</td>
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<td>Positive</td>
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<td>Negative</td>
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ERCC1 indicates excision repair cross-complementing gene 1; Topo-1, topoisomerase-1; TP, thymidylate phosphorylase; TS, thymidylate synthase.
which is equivalent to a PFS rate of 36.5% (95% CI, 25%-48%) at 12 months (Fig. 3). The RECIST response rate was 34% (95% CI, 22%-46%) and 86% of patients had clinical benefit (95% CI, 74-97). The median overall survival was 21.15 months (95% CI, 11.4-30.9) months. Table 3 shows the resulting tailored schemes in study patients and the resultant PFS of the different treatment groups. The median PFS of patients who had received 5-FU as part of their treatment regimen (8.7 [95% CI, 3.8-13.6] months) was not statistically different to those who did not (8.1 [95% CI, 6.9-9.2 mo; \( P = 0.535 \))).

**Toxicity**

Treatment was, in general, well tolerated with side effects expected by this type of treatments. The most common toxicities were asthenia (39%), rash (31%), diarrhea (31%), and nausea (30%). Toxicities grade \( \geq 3 \) included 18% neutropenia, 4.5% asthenia, and 3% anemia. There were no drug-related fatalities. Five patients (7.5%) discontinued the study because of toxicity, including one patient with an infusion reaction to oxaliplatin, one patient with mucositis, and 3 patients with peripheral neuropathy secondary to oxaliplatin.

**DISCUSSION**

We conducted a phase II trial in patients with chemotheraphy-naive CRC to test the feasibility and the effectiveness of applying in real time a panel of biomarkers (TS, Topo-1, ERCC1, and TP) to guide the selection of first-line treatment. The overall goal was to develop a strategy that will provide for each individual patient a personalized treatment recommendation. The results show that this approach is feasible and
can be performed in a real-time manner. However, the observed efficacy was below the expected therapeutic results of improving PFS compared with modern clinical trials in first-line CRC in which biomarkers were not used.17

We conclude that the approach, in our hands, is feasible. First, tumor was obtained from all patients in enough quantity and quality to assess all the proposed markers. One factor that probably contributed to this high success rate is that most patients came from internal referrals and had been diagnosed and treated at our hospital’s multidisciplinary CRC, greatly facilitating the access or acquisition of tumor materials. In environments in which the patients are mostly referred from outside clinics, real-time tumor acquisition is not that trivial. Second, the median turnaround time to perform all the analysis was 17 days, which, although improvable, is reasonable and greatly facilitated by multidisciplinary clinical groups. Indeed, implementing this patient-centered personalized treatment approach is not possible unless patients are cared by well-orchestrated and truly multidisciplinary groups.

There are several reasons why this strategy may have not resulted in the expected outcome. One is the decision tree selected that led to some patients treated with single-agent oxaliplatin or irinotecan. Although there were no statistically significant differences in PFS between patients who had or who had not received 5-FU as part of their treatments, the numbers are small and this cannot be ruled out. Likewise, as showed in Table 3, the number of patients in each one of the treatment groups is too small to draw conclusion regarding any specific groups. Notwithstanding these limitations, we observed that the group of patients with Topo-1-positive, ERCC-1-positive, and TS-positive tumors (27 patients) who were treated with irinotecan alone had approximately 9 months PFS very similar to patients who received more aggressive, multiagent chemotherapy. This may be of interest to study in patients who are unfit for aggressive chemotherapy and who are mostly managed with fluoropyrimidine alone, if the triple-positive group could be treated with single-agent irinotecan.

Key elements in the design of studies like this are the selection of the biomarkers, tissues, where to measure them, and the laboratory and quantification methods to use. Herein, we selected markers with support in the literature including Topo-1, ERCC1, TS, and TP, in addition to KRAS, BRAF, and PI3K.6,10–12,18 It should be emphasized, however, that the evidence linking expression, or lack off, of these markers and treatment response is for the most part retrospective and obtained in noncontrolled studies.18–26 This has been highlighted by several authors who emphasize that no individual marker reflects accurately all the differences in tumor biology that correspond to disease course or response to treatment. It suggests that other noncontrolled parameters are influencing the biomarker-drug efficacy interactions, as has been described recently for ERCC1.13 We did not consider the status of BRAF and PI3K to treat a patient with cetuximab because their suggested predictive value has not been confirmed in large prospective clinical trials.

Another critical issue is the analytical technique chosen and the methods for quantitation. We focused on IHC assessment of selected targets in the work and applied standardized procedures to assess the markers. It is noteworthy, however, the significant disparity in methodologies and results published in the literature in this field.18–20 Other studies have focused on analysis of gene expression of targets of interest by semi-quantitative reverse transcription polymerase chain reaction. Although this can be perhaps better standardized, no definitive results regarding their predictive power and clinical application are available.8,22 Finally, some studies have explored measuring biomarkers in blood, urine, and other tissues easily accessible and more likely to reflect tumor heterogeneity and dynamics.27,28

Very few studies in the literature have attempted to implement patient-centered personalized medicine programs with the goals to determine the best treatment for each one of the patients enrolled. One of them is our group’s study performed in patients with early rectal cancer with a similar design in the chemotherapy selection plus IMRT radiotherapy that showed a high rate (50%) of pathologic complete responses. The different results could be because of the IMRT radiotherapy used, the molecular disparity between rectal and analgesic, and the combination of single-agent irinotecan.
Colon cancer, or the increase of tumor heterogeneity in metastatic tumors vs. localized ones. Another interesting trial suggests that matching chemorefractory patients with CRC with targeted agents in phase I trials based on the current molecular profile does not confer a significant clinical benefit. A recent study used a comprehensive molecular profile based on IHC and RNA expression analysis of multiple targets to personalize patients’ treatment. In 86 patients enrolled, a target was discovered in 84 and 66 of them received a personalizedized treatment. This approach resulted in improved PFS of these patients as compared with the PFS with their immediate prior treatment, which was the primary goal of the study. Our study had similar goals but with a different patient population, marker selection, and treatment decision tree. As the interest to implement personalized medicine increases, creative studies to achieve these goals will be needed.

In conclusion, this work shows that it is feasible to implement a prospective biomarker guide-decision treatment in the management of patients with advanced CRC. For reasons that are not known and that could include wrong selection of targets, assessment methods, and decision tree algorithm, the study did not meet its primary objective and this strategy is not promising enough to advance to phase III testing. Subgroup analysis such as the Topo-I, ERCC1, and TS-positive group that represented more than one third of the patients reveals an interesting PFS for patients who received single-agent irinotecan combined with biological agents and that could be explored in settings in which single agents management is common and 5-FU is, in general, used such as in poor-risk patients or in maintenance.

REFERENCES