Molecular Profiling of Patients with Colorectal Cancer and Matched Targeted Therapy in Phase I Clinical Trials

Rodrigo Dienstmann1, Danila Serpico1, Jordi Rodon1, Cristina Saura1, Teresa Macarulla1, Elena Elez1, Maria Alsina1, Jaume Capdevila1, Jose Perez-Garcia1, Gessami Sánchez-Ollé2, Claudia Auras3, Ludmila Prudkin3, Stefania Landolf4/C211

Abstract

Clinical experience increasingly suggests that molecular prescreening and biomarker enrichment strategies in phase I trials with targeted therapies will improve the outcomes of patients with cancer. In keeping with the exigencies of a personalized oncology program, tumors from patients with advanced chemorrefractory colorectal cancer were analyzed for specific aberrations (KRAS/BRAF/PIK3CA mutations, PTEN and pMET expression). Patients were subsequently offered phase I trials with matched targeted agents (MTA) directed at the identified anomalies. During 2010 and 2011, tumor molecular analysis was conducted in 254 patients: KRAS mutations (80 of 254, 31.5%), BRAF mutations (24 of 196, 12.2%), PIK3CA mutations (15 of 114, 13.2%), KRAS and PIK3CA mutations (9 of 114, 7.9%), low PTEN expression (97 of 183, 53.0%), and high pMET expression (38 of 64, 59.4%). In total, 68 patients received 82 different MTAs: phosphoinositide 3-kinase (PI3K) pathway inhibitor (if PIK3CA mutation, n = 10; or low PTEN, n = 32), PI3K pathway inhibitor plus MEK inhibitor (if KRAS mutation, n = 10; or BRAF mutation, n = 1), second-generation anti-EGF receptor monoclonal antibodies (if wild-type KRAS, n = 11), anti-hepatectye growth factor monoclonal antibody (if high pMET, n = 10), mTOR inhibitor plus anti-insulin-like growth factor-1 receptor monoclonal antibody (if low PTEN, n = 3), and BRAF inhibitor (if BRAF mutation, n = 3). Median time-to-treatment failure on MTA was 7.9 versus 16.3 weeks for their prior systemic antitumor therapy (P < 0.001). Partial response was seen in 1 patient (1.2%, PI3K inhibitor with PIK3CA mutation) and stable disease >16 weeks in 10 cases (12.2%). These results suggest that matching chemorrefractory patients with colorectal cancer with targeted agents in phase I trials based on the current molecular profile does not confer a significant clinical benefit. Mol Cancer Ther; 11(9); 2062–71. ©2012 AACR.
cancers. As many as 70% involve the exon 9 helical domain (such as E542K or E545K) or the exon 20 kinase domain (such as H1047R; ref. 3). Loss-of-function mutations, deletions, or epigenetic silencing by promoter methylation of PTEN, a negative regulator of phosphoinositide 3-kinase (PI3K), have also been linked to resistance to anti-EGFR mAbs in colorectal cancers (4–6). Loss of PTEN expression, assessed by immunohistochemistry, is found in as many as 40% of colorectal cancer tumors (4–6). Importantly, the RAS/RAF/MEK and PI3K/akt/mTOR pathways also interact extensively: (i) each shows activation of upstream receptor tyrosine kinases; (ii) mutations in KRAS or BRAF and PI3K coexist in a significant percentage of colorectal tumors; and (iii) complex networks allow compensatory parallel signaling when one or the other pathway is inhibited (7).

Complex cross-talk involving EGFR and other pathways has also been identified in the past few years. Mesenchymal–epithelial transition (MET) activation, for example, occurs in 50% to 80% of colorectal tumors with KRAS activation of upstream receptor tyrosine kinases; (ii) mutations in KRAS or BRAF and PI3K coexist in a significant percentage of colorectal tumors; and (iii) complex networks allow compensatory parallel signaling when one or the other pathway is inhibited (7). The same is true for the insulin-like growth factor-1 receptor (IGF1R) as well as HER2 and HER3 upregulation (12–14).

Multiple novel targeted agents have entered clinical development. These include innovative mAbs targeting EGFR, EGFR/HER3, MET (or HGF), and IGF1R, as well as kinase inhibitors downstream of these pathways, mainly BRAF, MEK, PI3K, mTORC1/2, and AKT. These agents are being investigated in phase I trials using biomarker enrichment strategies. Their study designs are based on preclinical and early clinical response data showing the potential predictive value of specific molecular aberrations. Examples include PIK3CA-activating mutations and loss of PTEN expression and benefit with PI3K pathway inhibitors (15, 16), synergistic antitumor activity with combination blockade of mTOR and IGF1R signaling (17, 18), RAS/RAF mutations and response to PI3K pathway inhibitors plus MEK inhibitors (19–21), BRAF mutations linked to antitumor activity of selective BRAF inhibitors (22, 23), and MET hyperactivation and benefit with MET/HGF targeting agents (24, 25).

Molecular biomarker-based patient selection in early clinical trials has many putative advantages over an unselected population-based approach. These include (i) early clinical qualification of potential predictive biomarkers for molecularly targeted agents; (ii) further delineating underlying cancer biology; (iii) providing clinically relevant therapeutic opportunities for patients with advanced-stage cancer; and (iv) impacting novel drug development by assisting in go versus no go decisions and changing drug approval registration strategies for promising agents. A caveat is, however, that finding a potential target in a patient’s tumor does necessarily correlate with response to a therapeutic agent directed toward the target. Early clinical validation is, therefore, a key component of the drug development process (26).

Recent studies have examined the feasibility of using a real-time molecular profile of patient tumors and matching the identified aberration(s) with treatments targeted to the specific aberration(s). Von Hoff and colleagues and Tsimberidou and colleagues showed that patients who received MTAs had better response rates and improved time to treatment failure (TTF; ref. 27, 28). As part of our personalized oncology program, tumors from patients with advanced chemorefractory metastatic colorectal cancer were analyzed for specific molecular aberrations [KRAS/BRAF/PIK3CA mutations, PTEN, and phosphorylated MET (pMET) expression] in the Vall d’Hebron Molecular Pathology and Cancer Genomics Labs (Barcelona, Spain). Patients with advanced cancer were then offered enrollment on a molecularly appropriate phase I trial with an MTA based on the hypothesis that biomarker enrichment strategies might improve their outcomes. We retrospectively evaluated the benefit of MTA only in patients with metastatic colorectal cancers, unlike studies enrolling patients with any advanced tumor type (27, 28). Our primary objective was to compare time TTF using a therapy selected by the molecular profile of a patient’s tumor with the TTF for the most recent unmatched therapy on which the patient had experienced progression. We also describe the clinical benefit rate with an MTA in patients with metastatic colorectal cancers treated in phase I clinical trials.

**Patients and Methods**

**Patients and matched targeted therapy**

All patients with pathologically confirmed metastatic colorectal cancers refractory to standard therapy referred to phase I clinical trials at the Molecular Therapeutic Research Unit of Vall d’Hebron Institute of Oncology during 2010 and 2011 had archived formalin-fixed, paraffin-embedded (FFPE) tumor samples analyzed for specific molecular aberrations. Informed consent was obtained at baseline. In total, 254 patients/tumor samples were analyzed, and 68 patients were enrolled on specific first-in-human phase I trials based on the results of tumor genetic alterations/protein expression levels as well as logistic factors, including study availability and eligibility criteria. If more than one molecular deregulation was identified or 2 different matched targeted therapies were available for one specific aberration, patients could receive more than one MTA. These included second-generation anti-EGFR mAbs [if (wt) KRAS, PI3K pathway inhibitors (if PIK3CA mutation or low PTEN expression), mTORC1 inhibitor plus anti-IGF1R mAb (if low PTEN expression), PI3K pathway inhibitors plus MEK inhibitors (if KRAS or BRAF mutation), BRAF inhibitor (if BRAF mutation) and anti-HGF mAb (if high pMET expression). All phase I studies were conducted in accordance with the guidelines of the Vall d’Hebron Institute of Oncology Institutional Review Board.
Tissue samples and molecular analyses

PTEN and pMET expression levels were assessed by immunohistochemistry using a histopathologic scoring (H-score) system, which is based on a sum of the proportion of stained cells multiplied by the staining reactivity (H-score = (0 × % staining cells negative) + (1 × % staining cells weakly positive) + (2 × % staining cells moderately positive) + (3 × % staining cells strongly positive); ref. 29). An anti-PTEN antibody (Cell Signaling Technologies, clone 138G6, cat# 9559) was used in a 1:100 dilution and incubated at room temperature for 60 minutes. We considered low PTEN expression when there was an absence of immunostaining (PTEN-null) or PTEN H-score ≤50 (range, 0–300) compared with the internal control. The subgroup with PTEN-null expression (H-score = 0) is described separately. Paraffin-embedded cell pellets were used as controls in each run (MDA231 and PC3 as positive and negative controls, respectively) and also considered as internal positive controls were nerve sheaths and vessels. An antibody against pMET (pMET Tyr1349; Epitomics, EP2367Y, cat# 2319-1) was used in a 1:10 dilution and incubated at room temperature for 60 minutes. Positive expression was defined as H-score >30 (range, 0–300; ref. 30).

For mutation detection, KRAS and PIK3CA were initially assessed by real-time PCR using a DxS Scorpions technology (TheraScreen KRAS Mutation Kit and DxS PI3K Mutation Test Kit; DxS). In this technique, allele- or mutation-specific amplification is achieved by ARMS (amplification refractory mutation system) technology and its detection is conducted using Scorpions (bifunctional molecules containing a PCR primer covalently linked to a probe; ref. 31). The following mutations were evaluated: KRAS G12A, G12D, G12R, G12C, G12V, G13D and PIK3CA H1047R, E542K, E545D, E545K. Standard Sanger sequencing of exons 9 or 20 of the gene confirmed each PIK3CA mutation. Sanger sequencing was also used to assess BRAF mutations in exon 15. Later on, tumor genetic analyses by PCR-based and deep sequencing techniques were substituted by the OncoCarta Panel v 1.0, a multigene panel with 19 key oncogenes and 238 mutations, via the MassARRAY System (Sequenom, Inc.). The MassARRAY System involves the following steps: multiplex PCR of gene exons of interest, primer extension reactions using IPLEX chemistry, and analysis of primer extension products by matrix-assisted laser desorption/ionization—time-of-flight (MALDI-TOF) mass spectrometry. A Vall d’Hebron pathologist evaluated total tumor area in the tissue block, and a minimum 30% tumor content was required for further processing. DNA was extracted from five 10-μm slices of FFPE tumor samples using the RecoverAll Total Nucleic Acid Isolation Kit (Ambion). DNA was quantified with nanodrop (ND-1000), 260:280 and 260:230 ratios were used for quality control (QC) of sample purity. Additional QC was obtained in low concentration samples by quantitative PCR of 100-bp long amplicons in AKT1 (exon 1), KRAS (exon 1), and PIK3CA (exon 20), dsDNA quantification with Quant-iT DNA BR (Invitrogen) and lab-on-a-chip using High Sensitivity DNA Chips (Agilent).

The full mutation panel is not available for all patients included in this study. This is because the prescreening program was initially constructed on the basis of the clinical trials available at the time of screening and their inclusion criteria (i.e., there were no trials focused on patients with PIK3CA- or BRAF-mutated tumors actively recruiting in early 2010). In addition, we gradually added new technologies (OncoCarta Panel v 1.0 via the MassARRAY System) to simultaneously assess multiple mutations.

Treatment evaluation

We reviewed the electronic medical records of patients enrolled in clinical trials according to molecular prescreening results, sex, age, Eastern Cooperative Oncology Group performance status at the beginning of treatment and detailed information on prior treatments. The formulation of the patient database and collection of data for the purpose of this study were conducted retrospectively. Previous therapies were classified into different groups according to National Comprehensive Cancer Network (NCCN) guidelines: (i) standard regimens, including capecitabine/5-fluorouracil, oxaliplatin, irinotecan, bevacizumab, and cetuximab (monotherapies or combinations) or (ii) nonstandard, if not an approved chemotherapy regimen (such as single-agent gemcitabine or pemetrexed used off-label, or mitomycin or capecitabine after progression to regimens containing infusional 5-fluorouracil) or an unmatched agent in another early-phase clinical trial. Treatment on phase I studies continued until disease progression or death or unacceptable toxicity occurred and was carried out according to the specific requirements of each protocol. Efficacy was assessed routinely by computed tomographic scans and/or MRI at baseline before treatment initiation and then every 2 cycles (6–8 weeks) as per each phase I study protocol. In accordance with Response Evaluation Criteria in Solid Tumors (RECIST) v. 1.0 or 1.1 (depending on study protocol), tumor responses were classified as complete response, partial response, stable disease, or progressive disease. TTF was defined as the time interval from the start of a therapy to its discontinuation for any reason, including disease progression, treatment toxicity, or death, whichever occurred first. Patients with TTF on MTA longer than 16 weeks (>2 radiologic RECIST assessments) were described separately. In addition, if the TTF on MTA/TTF on prior therapy ratio was ≥1.3, then the matched targeted therapy selected was defined as having benefit for the patient (27).

Statistical analysis

Survival analyses were conducted using the Kaplan–Meier method and compared with a log-rank test (TTF with MTA vs. previous unmatched therapy). Subgroup analysis was conducted considering standard versus nonstandard previous therapy. Exact 2-sided 95% confidence
intervals (CI) were calculated. All tests were 2-sided, and \( P < 0.05 \) was considered statistically significant. All statistical analyses were conducted using SPSS v.15.0 software (SPSS).

Results

Molecular prescreening

During 2010 and 2011, we analyzed tumor samples from 254 patients with metastatic colorectal cancers referred to phase I trials at our institution. Archived biopsy specimens were the only source of tissue used for analysis. The median age of patients was 61.9 years (range, 32.8–85.1) and 65% were men. Detailed molecular profiles of patients’ tumors are presented in Table 1. KRAS, BRAF, and PIK3CA mutation rates are similar to previous reports (3). PTEN expression was evaluated as being low in 53% (97 of 183) of tumor samples. Of these, 46.4% (45 of 97) had PTEN-null expression. The coexistence of KRAS mutations and PIK3CA mutations (7.9%) or KRAS mutations and PTEN-low/null expression (20.8%/7.6%) confirms...
the frequent parallel activation of RAS/RAF/MEK and PI3K/AKT/mTOR pathways in colorectal cancers. Compared with (wt) KRAS patients, those harboring a KRAS mutation did not have a significantly higher prevalence of PIK3CA mutations (7.9% vs. 5.3%, \( P = 0.5 \)). In addition, approximately 60% of the tumors analyzed had high pMET expression according to the cutoff value for inclusion on the anti-HGF trial. This aberration was not mutually exclusive with KRAS, BRAF, or PIK3CA mutations.

**Matched targeted therapy**

Sixty-eight patients were enrolled in 15 different phase I clinical trials. There were a total of 82 matched targeted therapies, with 8 patients participating in 2 clinical trials and 3 who were enrolled in 3 clinical trials, as shown in Fig. 1. Table 2 presents patients’ demographics and clinical characteristics. Phase I trials included PI3K pathway inhibitors (National Clinical Trial numbers: NCT01115751, NCT01219699, NCT01090960, NCT00940498, NCT01058707, NCT00620594, NCT01068483, NCT01449370), PI3K pathway inhibitors plus MEK inhibitors (NCT01363232, NCT01155453), second-generation anti-EGFR mAbs (NCT01207323, NCT0117428), anti-HGF mAb (NCT00969410), mTORC1 inhibitor plus anti-IGF1R mAb (NCT00730379), and BRAF inhibitor (NCT01143753). Detailed descriptions of matched targeted therapies, dose levels, and patient outcomes are presented in Table 3.

**Time to treatment failure on MTA**

Median TTF on MTA was 7.9 weeks (95% CI, 7.6–8.1) compared with 16.3 weeks (95% CI, 13.9–18.7) with the prior systemic antitumor therapy (\( P < 0.001 \)). As shown in Fig. 2, analysis according to type of previous therapy showed that if it was considered nonstandard (\( n = 39 \)), TTF with MTA was 7.0 weeks (95% CI, 5.7–8.3) and TTF with prior therapy was 8.7 weeks (95% CI, 7.3–10.1). Nonstandard therapy was another phase I trial with unmatched agent in 21 patients and off-label chemotherapy regimens.
Table 3. Matched targeted therapies in phase I clinical trials based on molecular profile, dose level, and patient outcome

<table>
<thead>
<tr>
<th>Targeted therapy</th>
<th>Rationale</th>
<th>Molecular profile</th>
<th>No.</th>
<th>Dose level</th>
<th>Response rate</th>
<th>Reasons for lack of response</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI3K pathway inhibitor (n = 42)</td>
<td>PTEN low or PIK3CA mutation</td>
<td>PTEN-low and KRAS wild-type</td>
<td>22</td>
<td>&lt;1/3 MTD: 5</td>
<td>PR: 1 (2.4%)</td>
<td>Wrong biomarker?</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PTEN-low and KRAS mutation</td>
<td>10</td>
<td>1/3 to &lt;MTD: 1; MTD: 36</td>
<td>SD &gt;16 wks: 5 (11.9%)</td>
<td>Need for combination therapy. Resistance to monotherapy in the presence of KRAS mutations.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PIK3CA mutation and KRAS wild-type</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PIK3CA mutation and KRAS mutation</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PI3K pathway inhibitor plus MEK inhibitor (n = 11)</td>
<td>KRAS or BRAF mutation</td>
<td>KRAS mutation</td>
<td>6</td>
<td>1/3 to &lt;MTD: 6</td>
<td>PR: 0 SD &gt;16 wks: 0</td>
<td>Treatment at biologically inactive doses.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KRAS mutation and PTEN-null</td>
<td>3</td>
<td>MTD: 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>KRAS mutation and PIK3CA mutation</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>BRAF mutation and PTEN-null</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Second-generation anti-EGFR mAb (n = 11)</td>
<td>KRAS wild-type</td>
<td>KRAS wild-type</td>
<td>5</td>
<td>MTD: 11</td>
<td>PR: 0 SD &gt;16 wks: 3 (27.3%)</td>
<td>Presence of downstream pathway aberrations (PTEN-low and BRAF mutation). Acquired resistance to anti-EGFR mAbs</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KRAS wild-type and PTEN-low</td>
<td>5</td>
<td>MTD: 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>KRAS wild-type - BRAF mutation</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-HGF mAb (n = 10)</td>
<td>pMET high</td>
<td>pMET high and KRAS mutation</td>
<td>5</td>
<td>&lt;1/3 MTD: 1</td>
<td>PR: 0 SD &gt;16 wks: 1 (10%)</td>
<td>Wrong biomarker? Need for combination therapy. Treatment at biologically inactive doses.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>pMET high and KRAS wild-type</td>
<td>4</td>
<td>1/3 to &lt;MTD: 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>pMET high and BRAF mutation</td>
<td>1</td>
<td>MTD: 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mTORC1 inhibitor plus anti-IGF1R mAb (n = 5)</td>
<td>PTEN low</td>
<td>PTEN-low and KRAS mutation</td>
<td>3</td>
<td>MTD: 5</td>
<td>PR: 0 SD &gt;16 wks: 1 (20%)</td>
<td>Wrong biomarker? Target or pathway redundancy?</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PTEN-low and KRAS wild-type</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRAF inhibitor (n = 3)</td>
<td>BRAF mutation</td>
<td>BRAF mutation</td>
<td>3</td>
<td>&lt;1/3 MTD: 1</td>
<td>PR: 0 SD &gt;16 wks: 0</td>
<td>Need for combination therapy.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>MTD: 2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: PR, partial response; SD, stable disease.
in 18 patients. On the other hand, if previous therapy was
an approved standard regimen \((n = 43)\), TTF with MTA
was 8.1 weeks \((95\% \text{ CI}, 7.2–9.1)\) and TTF with prior therapy
was 21.9 weeks \((95\% \text{ CI}, 15.0–28.7)\). Overall, 64 patients
\((78\%)\) received maximum tolerated doses \((\text{MTD})\) or recom-
manded phase 2 doses \((\text{RP2D})\), 9 patients \((11\%)\) had doses
lower than one third of the MTD and 9 patients \((11\%)\) received intermediate doses (that ranged from one third to
less than the MTD). In total, 10 matched therapies had to be
discontinued because of adverse events \((12.2\%)\).

**Tumor response and clinical benefit with MTA**

One patient whose tumor harbored PIK3CA R88Q and
KRAS G12C mutations (detected using the MassARRAY
system) had a confirmed partial response to a specific
PI3K-α inhibitor as monotherapy. This is the only patient
who has continued on treatment for longer than 24 weeks.
Stable disease longer than 16 weeks was seen in 10 cases
\((12.2\%)\): 2 patients on specific PI3K-α inhibitor (both with
PIK3CA mutations), 2 patients on PI3K/mTOR inhibitors,
and one patient on mTORC1/2 inhibitor [all with low
PTEN and a (wt) KRAS/BRAF/PIK3CA profile], one patient
on mTORC1 inhibitor plus anti-IGFIR mAb (PTEN-null and
KRAS mutant), 3 patients on second-generation anti-EGFR mAb [all with (wt) KRAS/BRAF profiles and refractory to cetuximab or panitumumab], and one patient on anti-HGF mAb [the one with the highest pMET H-score \((200)\) plus (wt) KRAS status; only
2 patients had an H-score < 100]. None of the 11 patients
who received a PI3K pathway inhibitor plus an MEK
inhibitor had disease stabilization for longer than 16
weeks \((3 \text{ had a simultaneous KRAS mutation and
PTEN-null expression, one had a KRAS mutation plus a
PIK3CA mutation, and one had a BRAF mutation and
PTEN-null expression})\). A TTF ratio \(\geq 1.3\) was achieved in
13 of the 82 matched therapies \((15.9\%)\). There was no
difference in terms of TTF on MTA according to site of
mutation analysis [primary \((n = 53)\) vs. metastasis \((n = 29)\); 7.9 weeks in both subgroups], number of previous
lines of chemotherapy \([1–3 \quad (n = 52) \text{ vs. } \geq 4 \quad (n = 30)]; 7.9 \text{ and } 8.0 \text{ weeks, respectively})], number of molecular aberrations
\([0 \text{ or } 1 \quad (n = 44) \text{ vs. } \geq 2 \quad (n = 38)]; 8.4 \text{ and } 6.1 \text{ weeks, respectively})], and dose level [MTD \((n = 64)\) vs. lower
than MTD \((n = 18)]; 7.9 \text{ weeks in both subgroups}].

**Discussion**

Our results suggest that matching patients with chemor-
efractory metastatic colorectal cancers with targeted agents
in early clinical trials based on their current molecular
profiles does not result in longer TTF compared with their
prior systemic therapy. In addition, only a small percent-
age of study patients derived benefit in tumor response or
disease stabilization, comparable with statistics reported
in the literature of patients treated on phase I trials without
molecular selection \((32)\). These findings are disparate from
those in a recently published series of patients with PIK3CA
mutant breast and gynecologic malignancies enrolled in
phase I trials with PI3K/mTOR inhibitors \((33)\). However,
all responses in this study were observed in patients
treated with combination therapies, mainly mTORC1 inhi-
bitors plus cytotoxic agents or bevacizumab.

Some caveats deserve consideration when looking at
the results of this study. First, it is a retrospective study in
a single institution. Despite the homogeneous patient pop-
ulation, the mechanisms of action of the targeted agents
are heterogeneous. Notably, the biomarkers selected for
enrichment of the phase I trials were primarily explor-
dy. Different techniques (DxS Scorpions, sequencing,
MassARRAY system) with different sensitivities were
used to identify the most frequent mutations in KRAS,
BRAF, and PIK3CA \((\text{of note, all PIK3CA mutations}
detected by DxS Scorpions or the MassARRAY system
were confirmed by Sanger sequencing})\). In addition, ana-
lyzing archival tumor samples from the time of diagnosis
may not reflect the molecular aberrations that drive met-
astatic disease as a result of clonal evolution and selection
pressure from previous treatments \((34)\). This is particu-
larly true in regard to protein expression levels, such as
PTEN \((5)\). Furthermore, a limitation inherent to all phase I
clinical trials is that patients may have been treated at
nonbiologically active doses, even though 78% received
MTD/RP2D doses and were treated in the expansion

![Figure 2. Comparisons of TTF on MTA (light gray bars) and prior unmatched therapy (dark gray bars). Subgroup analysis according to type of prior therapy. NCCN, National Comprehensive Cancer Network; MTA, matched targeted agent; TTF, time to treatment failure.](image-url)
cohorts of each protocol. Finally, the timing of tumor assessment during a phase I trial (every 6–8 weeks) is usually shorter than restaging intervals for patients receiving standard therapies (every 8–12 weeks), which is another possible bias.

Many patients in our series received PI3K pathway inhibitors based on PTEN expression levels, irrespective of coexisting oncogenic aberrations (e.g., KRAS mutations). The predictive value of PTEN expression for determining response to PI3K pathways inhibitors in colorectal cancer is, however, still pending. Moreover, the optimal method for assessing PTEN loss of function has not yet been established, that is, a cutoff point to define positive and negative expression levels according to immunohistochemical assay results. Preclinical experiments showed that PIK3CA mutations and PTEN aberrations render tumors sensitive to PI3K pathway inhibitors, whereas simultaneous mutations in the mitogen-activated protein kinase (MAPK) pathway (KRAS, BRAF) can mediate resistance to therapy (35, 36). Preliminary clinical data suggested a negative predictive role for tumors harboring KRAS mutations in response to mTORC1 inhibitors (37). Nevertheless, efficacy of mTORC1/2 inhibitors was observed in KRAS/BRAF-mutant colorectal cancer xenograft models, and PI3K/mTOR inhibitors induced tumor regressions in genetically engineered (wt) PIK3CA mouse models (38, 39). Interestingly, the only patient with colorectal cancer achieving a partial response in our series had a molecular profile with a simultaneous PIK3CA and KRAS mutation. PI3K pathway inhibitors have also shown activity in KRAS-mutant breast and ovarian cancer (33, 40). Despite this positive result, most of our patients with PI3K pathway activation treated with single-agent kinase inhibitors did not derive benefit from therapy, especially those with low PTEN expression and coexisting KRAS mutations.

It is possible that a treatment approach with a combination of agents could overcome resistance to PI3K pathway inhibitors in colorectal cancers. In preclinical models, inhibiting the PI3K pathway showed synergy with cytotoxic agents, enhancing its efficacy by potentiating apoptosis (41). In addition, dual inhibition of the RAF/MEK and PI3K/AKT/mTOR pathways seems to be required for complete abrogation of downstream effectors in RAS-mutant tumors (19–21). PIK3CA and PTEN mutations strongly reduce the sensitivity of RAS-mutant cells to MEK inhibitors (42). Preclinical studies provide a clear rationale for the co-inhibition of these frequently co-activated pathways, and many early clinical trials testing this hypothesis are underway. The clinical impact of such a parallel pathway blockade remains unclear, but none of the 11 patients treated with PI3K pathway inhibitors plus MEK inhibitors in our series had benefit in terms of response or disease stabilization. A recently published large data set reported similarly disappointing results, with no partial responses or disease stabilizations for more than 16 weeks in 16 patients with metastatic colorectal cancers treated using the same approach (43).

Remarkably, as in our series, some patients who received PI3K pathway inhibitors plus MEK inhibitors had simultaneous RAS/RAF and PI3K/AKT/mTOR activation. It is also important to highlight the fact that the use of full doses of both targeted agents was rarely achieved because of overlapping toxicities. Interestingly, in patient-derived metastatic colorectal cancer xenografts exhibiting KRAS and BRAF mutations, concomitant inhibition of PI3K/mTOR and MEK pathway activity proved to be more effective than single pathway inhibition, although the best response was limited to tumor growth arrest rather than overt tumor regression (21). This observation is potentially important for further drug development in RAS/RAF-mutant colorectal cancers.

Regarding BRAF inhibitors in BRAF-mutant colorectal cancers, none of the 4 patients treated in our series with selective BRAF inhibitors or PI3K pathway inhibitors plus MEK inhibitors had signs of antitumor activity. In an expansion cohort of the phase I study vemurafenib at the RP2D, only 1 of 19 patients had a confirmed partial response (44). These findings support preclinical data suggesting that BRAF-mutant colorectal cancers might respond to combined targeted therapy with BRAF and EGFR inhibitors (45). A combination strategy also appears hopeful when agents targeting the MET/HGF pathway are used. Preliminary clinical data suggest that adding an MET tyrosine kinase inhibitor or an anti-HGF mAb to an approved anti-EGFR mAb might increase the response rate in (wt) KRAS metastatic colorectal cancer (46, 47). Interestingly, the patients who had the greatest benefit with a MTA in our data set had a molecular profile with (wt) KRAS/BRAF and received second-generation anti-EGFR mAbs (3 of 11 patients refractory to cetuximab or panitumumab had stable disease for more than 16 weeks).

These retrospective data, while not definitive, are certainly hypothesis-generating. With the switch from histology- to molecular-driven therapy, phase I trials in clinical oncology are providing an arena for early hypothesis testing. The one-size-fits-all approach for drug development of molecular targeted agents in solid tumors has yielded disappointing results, and patients with metastatic colorectal cancers enrolled in biomarker-driven early clinical trials do not appear to derive significant benefit. A strong biologic hypothesis, solid preclinical data on the mechanisms of primary/acquired resistance and tumor models that better mimic the clinical disease are needed to further drug development. Our data confirm that prescreening strategies for early clinical trials and molecular profiling in patients with metastatic colorectal cancers are feasible. For personalized therapy to become a reality for these patients, a more comprehensive analysis of molecular aberrations appears to be critically important.

Disclosure of Potential Conflicts of Interest

J. Tabernero has had a role as consultant/advisor for Amgen, Genentech, Merck-Serono, Novartis, Roche, and Sanofi-Aventis. No potential conflicts of interest were disclosed by the other authors.
Authors' Contributions

Conception and design: R. Dienstmann, D. Serpico, J. Rodon, C. Saura, E. Elez, J. Tabernero
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): R. Dienstmann, J. Rodon, C. Saura, T. Macarrulla, E. Elez, M. Alsina, J. Capdevila, G. Sánchez-Ollé, C. Aura, L. Prudkin, S. Landolfi, J. Herrán-Zosa, A. Vivancos, J. Tabernero
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): R. Dienstmann, J. Rodon, M. Alsina, G. Sánchez-Ollé, A. Vivancos, J. Tabernero
Writing, review, and/or revision of the manuscript: R. Dienstmann, J. Rodon, C. Saura, E. Elez, J. Capdevila, J. Perez-Garcia, J. Herrán-Zosa, A. Vivancos, J. Tabernero

References


Acknowledgments

The authors thank Debora Moreno and Nuria Murtura from Database Management Office, Jose Jimenez from Molecular Pathology Lab of Vall d’Hebron Institute of Oncology, and Joann Aaron, MA, for editorial support.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received March 21, 2012; revised May 14, 2012; accepted May 23, 2012; published OnlineFirst June 21, 2012.
Colorectal Cancer Patients in Phase I Trials of Targeted Agents


Molecular Profiling of Patients with Colorectal Cancer and Matched Targeted Therapy in Phase I Clinical Trials

Rodrigo Dienstmann, Danila Serpico, Jordi Rodon, et al.


Updated version
Access the most recent version of this article at:
doi:10.1158/1535-7163.MCT-12-0290

Cited articles
This article cites 42 articles, 19 of which you can access for free at:
http://mct.aacrjournals.org/content/11/9/2062.full#ref-list-1

Citing articles
This article has been cited by 7 HighWire-hosted articles. Access the articles at:
http://mct.aacrjournals.org/content/11/9/2062.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.