Relevance of BRAF and extended RAS mutational analyses for metastatic colorectal cancer patients

G Aprile1*, M Macerelli1, G De Maglio2, S Pizzolitto2, G Fasola1

Abstract

Introduction

In the past 15 years, the treatment of advanced colorectal cancer has markedly advanced, leading to a median survival improvement from 1 year with 5-Fluorouracil alone to over 24 months with modern therapies. Recently, we have learned that specific genomic alterations have a clear prognostic role and are closely linked to response to specific anticancer agents. Specifically, antibodies targeting the epidermal growth factor receptor pathway have produced stunning survival improvements for advanced colorectal cancer patients. To optimise their use, however, a more profound knowledge of tumour molecular oncology is crucial. In this short viewpoint the authors depict the biological, clinical and economic relevance of v-Raf murine sarcoma viral oncogene homologue B1 and extended rat sarcoma mutational analyses when focusing on epidermal growth factor receptor inhibitor-based therapy.

Conclusion

Colorectal cancer patients who are most likely to benefit from epidermal growth factor receptor inhibitors may be selected with a deeper molecular biology, encompassing not only exon 2 Kirsten rat sarcoma viral oncogene homologue, but also neuroblastoma rat sarcoma viral oncogene homologue and BRAF mutational status. This fine-tuning in patients’ selection has produced striking implications.

Introduction

Although, colorectal cancer (CRC) still remains a significant healthcare problem, major survival improvements have been noted in the past two decades. More specifically, the life expectancy of patients diagnosed with advanced, unresectable disease has progressively increased, along with the possibility of receiving all available treatments, including antiangiogenics and epidermal growth factor receptor inhibitors (EGFR-I). Between 2008 and 2010, a growing amount of data demonstrated that patients harbouring mutations in codon 12 or 13 on the Kirsten rat sarcoma viral oncogene homologue (KRAS) have a negligible chance to benefit from EGFR-I, regardless these target drugs were administered alone or in combination with chemotherapy. This body of information has promptly induced the international community and the Regulatory Agencies to limit the use of EGFR-I in KRAS wild-type tumours only. Soon after, this evolving landscape has broadened frenetically beyond KRAS exon 2 mutational status. It was demonstrated that less frequent mutations occurring in other KRAS codons, such as exon 61 on exon 3 or exon 146 on exon 4, may also result in impaired EGFR-I efficacy. Also, v-Raf murine sarcoma viral oncogene homologue B1 (BRAF) mutations seem to limit the benefit from EGFR-I, as may the loss of phosphatase and tensin homolog (PTEN) expression. At the same time, many issues regarding technical and practical aspects have swiftly raised and have created new challenges. Among these, we list the debates on the optimal methodology for molecular status assessment, the most appropriate site to obtain tissue samples, the testing turnaround time and the need for reliability of molecular testing, ensured with external and internal quality assessment programmes. Recently, the huge clinical value of a deeper focusing on tumour molecular biology when deciding the first-line treatment strategy has emerged, rendering the scenario even more complex than before. How a more profound molecular knowledge, including the analysis of human rat sarcoma (RAS) viral oncogene family, may dramatically impact on the molecularly selected patients’ outcome when exposed to EGFR-I has now reached very compelling evidence, with median improvement in overall survival (OS) surpassing 6 months.

All these practice-changing shifts have immediately produced significant consequences and have reshaped our treatment paradigms. Within the blurred borders of this evolving landscape, the aim of this concise viewpoint is to report and discuss the biological, clinical and economic relevance of the extended molecular testing in CRC patients.

Discussion

The authors have referenced some of their own studies in this review. These referenced studies have been conducted in accordance with the Declaration of Helsinki (1964) and the protocols of these studies have been conducted in accordance with the Declaration of Helsinki (1964) and the protocols of these studies have
been approved by the relevant ethics committees related to the institution in which they were performed. All human subjects, in these referenced studies, gave informed consent to participate in these studies.

Which molecular biomarkers should be analysed?
Since CRCs have complex and highly heterogeneous genomic profiles that may change over time or evolve under treatment pressure\(^2\), the Cancer Genome Atlas Network has promoted a more profound molecular knowledge of this cancer and has recently published a comprehensive molecular disease characterisation\(^1\). To expand the understanding of the disease, pathobiology and its molecular underpinnings allow medical oncologists to use robust and useful biomarker information\(^3\). EGFR is a member of erythroblastic leukaemia viral oncogene homologue (ErbB1) family with a pronounced tyrosine kinase activity. Its downstream intracellular signalling triggers two different pathways (Figure 1): the first is linked to the RAS-RAF proto-oncogene serine/threonine-protein kinase) mitogen-activated protein kinase (MAPK) axis, the other involves the lipid kinase phosphatidylinositol 3-kinase (PI3K) and the phosphoinositide-dependent kinase-AKT signalling pathway. Although EGFR over-expression is detected in up to 70% of CRC\(^4\), EGFR gene amplification lies in approximately 50% of cases\(^5\). When the EGFR pathway is activated, the tumour phenotype becomes more aggressive and may acquire resistance to treatments\(^6\). RAS oncogenes have a well-established role in cell growth and proliferation, with its three isoforms, mainly neuroblastoma rat sarcoma viral oncogene homologue (NRAS) KRAS and Harvey rat sarcoma viral oncogene homologue (HRAS). In CRC, RAS mutations occur in 30%-50% of cases. KRAS is by large the most mutated isoform (35%-45%) followed by NRAS (3%-5%), whereas somatic mutations on HRAS are anecdotic events. Also NRAS mutations lead to constitutive activation of the MAPK pathway, and several studies have shown that these mutations predict resistance to EGFR\(^7\). However, over 40% among CRC patients harbouring wild-type KRAS gene fail to respond to EGFR\(^1\)\(^2\)\(^3\)\(^4\)\.

BRAF mutation is an early event in CRC and there is a high concordance between primary and metastatic tissue. The predominant mutation (90%) is a single-base substitution of valine by glutamic acid at position 600 (V600E) within the activation segment. Since BRAF and KRAS mutations are mutually exclusive and BRAF mutations occur in approximately 8% of these patients, the two markers together may identify up to 55% of non-responders\(^7\). Products of BRAF gene act as downstream agents of KRAS in the MAPK pathway.

There is growing interest on the analysis of PI3K pathway and PTEN, since activating mutations are frequently detected and may confer a more aggressive clinical behaviour\(^8\)\(^9\). The usefulness of these biomarkers in the clinical practice, however, is still not validated.

Which method should be preferred to perform the molecular characterisation?
Point mutations are the most frequent genotypic alterations in CRC. Molecular analyses of predictive or prognostic biomarkers require standardisation,
accuracy, reproducibility, with adequate sensitivity and specificity. Since the introduction of commercial tests for KRAS, Sanger sequencing has been considered the gold standard approach, despite this the technique suffers from a low analytic sensitivity. Preanalytic keypoints that may influence on laboratory results are correct DNA preservation, accurate sample selection and precise macrodissection to obtain adequate tumour cells enrichment. Analytical sensitivity and specificity are also important, and depend upon the system used. Many studies compared different approaches and defined their reliability in routine clinical practice. Several systems differ for being screening techniques or targeted on known mutations only, for their sensitivity (minimum amount of mutant allele detectable), for the amount of DNA required for the analysis, for the number of mutations they can identify and for being either laboratory-based techniques or commercially available diagnostic systems.

New commercially available high-throughput techniques have recently been presented. They are multiplexed tests that simultaneously analyse multiple gene panels by using very low amounts of DNA with high sensitivity and specificity. The major advantage of these next-generation techniques is that KRAS/BRAF/NRAS/PIK3CA mutations may be analysed in a single run, instead of using a time-consuming conventional gene-by-gene approach. Available methodologies for KRAS testing are summarised in Table 1.

Evidence coming from recent randomised clinical trials: Will molecular biomarkers help in selecting patients? While prognostic biomarkers help clinicians to identify patients with a specific disease outcome regardless of received treatments, predictive biomarkers may suggest the most appropriate therapeutic option. Beyond classical chemotherapeutic agents, patients with metastatic, unresectable CRC may benefit from an increased list of targeted biologic drugs, including two antiangiogenics (bevacizumab, aflibercept), two EGFR-I (cetuximab, panitumumab) and a new orally available multitarget molecule (regorafenib). All those agents may significantly impact on progression-free survival (PFS) and/or OS in multiple treatment lines, enhancing the possibility for a personalised strategy of treatment. If specific molecular targets for biologic agents are still under discussion, BRAF and extended RAS mutational status analysis have become increasingly important to choose the optimal upfront therapy.

Both cetuximab and panitumumab bind to the extracellular domain of EGFR, inhibiting its downstream signal, and have proven efficacy when used upfront in combination with chemotherapy or alone in pretreated patients. Clinical trials are ongoing to verify if cetuximab could be useful beyond disease progression or patients may be rechallenged with the same EGFR-I after a period of time.

Initial studies have consistently confirmed that CRC patients harbouring KRAS mutations do not benefit from EGFR-I. In the following years, a number of randomised trials have established that significant response advantages and survival gain for the upfront use of EGFR-I in combination with standard chemotherapy are confined to KRAS

Table 1 Overview of main techniques for rat sarcoma and v-Raf murine sarcoma viral oncogene homologue B1 testing used in colorectal cancer

<table>
<thead>
<tr>
<th>Technique</th>
<th>Sensitivity</th>
<th>Detected mutations</th>
<th>CE-IVD commercially available kits</th>
<th>Multiples genes analysed in a single run</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sanger sequencing</td>
<td>Low</td>
<td>Known and new</td>
<td></td>
<td>V</td>
</tr>
<tr>
<td>Pyrosequencing</td>
<td>Medium</td>
<td>Known and new</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HRMA</td>
<td>Medium</td>
<td>Known and new</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TaqMelt Real Time PCR</td>
<td>High</td>
<td>Known and new</td>
<td></td>
<td>V</td>
</tr>
<tr>
<td>PNA/LNA Clamp</td>
<td>High</td>
<td>Known only</td>
<td></td>
<td>V</td>
</tr>
<tr>
<td>ASLNaqPCR</td>
<td>High</td>
<td>Known only</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scorpions ARMS</td>
<td>High</td>
<td>Known only</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ion Torrent, Ion AmpliSeq™</td>
<td>High</td>
<td>Known only</td>
<td></td>
<td>V</td>
</tr>
<tr>
<td>Maldi-TOF, Sequenom *</td>
<td>High</td>
<td>Known only</td>
<td></td>
<td>V</td>
</tr>
</tbody>
</table>

ARMS, amplified refractory mutation system; ASLNaqPCR, allele specific locked nucleic acid quantitative polymerase chain reaction; CE-IVD, European economic area in vitro diagnostic; HRMA, high-resolution melting analysis; LNA, locked nucleic acid; Maldi-TOF, matrix-assisted laser desorption / ionisation time-of-flight; PNA, peptide nucleic acid.

Licensee OA Publishing London 2013. Creative Commons Attribution License (CC-BY)

Figure 2: EGFR-inhibitors (cetuximab and panitumumab) that may block the EGFR pathway. BRAF, v-Raf murine sarcoma viral oncogene homologue B1; EGFR, epidermal growth factor receptor; Grb2, growth factor receptor-bound protein 2; MAPK, mitogen-activated protein kinases; MEK, methyl ethyl ketone; PDK, phosphoinositide-dependent kinase; PI3K, phosphatidylinositol 3-kinase; PTEN, phophatase and tensin homologue; RAS, rat sarcoma; SOS, salt overly sensitive.

wild-type tumours. Among these, the CRystal (Cetuximab Combined with Irinotecan in First-Line Therapy for metastatic colorectal cancer) study\textsuperscript{13} randomised 1198 previously untreated advanced CRC patients to folinic acid, fluorouracil and irinotecan (FOLFIRI) plus cetuximab or FOLFI-RI alone. Trial results demonstrated that patients with KRAS wild-type tumours had a significant improvement in OS (hazard ratio HR 0.79; \( P = 0.009 \)); PFS (HR 0.69; \( P = 0.001 \)) and a higher response rate (adjusted odds ratio 1.4; \( P < 0.001 \)) compared with those with mutated KRAS. More recently, the prospective-retrospective analyses of the phase III PRIME trial\textsuperscript{34} that enrolled 1183 patients to upfront folinic acid, fluorouracil and oxaliplatin regimen (FOLFIRI) with either panitumumab or placebo show that patients harbouring KRAS mutations in exon 3 (codons 59/61) and 4 (codons 117/146), or NRAS mutations in exon 2 (codons 12/13), 3 (codons 59/61) and 4 (codons 117/146) may not benefit from the EGFR-I. The same conclusion came from the preplanned analysis of the phase II PEAK study that randomised in first-line 285 patients to FOLFOX plus either bevacizumab or panitumumab\textsuperscript{35}. PRIME Investigators reported a 2.2 months median advantage in median PFS (10.1 months vs. 7.9 months, HR 0.73, 95% confidence interval (CI) 0.59–0.9, \( P=0.004 \)) and a 5.8 median advantage in OS (26 months vs. 20.2 months, HR 0.78, 95% CI 0.62–0.99, \( P=0.04 \)) for patients without RAS mutations. In the same study, patients with no RAS or BRAF mutations (\( n=446 \)) exposed to FOLFOX and panitumumab derived a 7.6-month median survival benefit (28.3 months vs 20.9 months, HR 0.74, 95% CI 0.57–0.96, \( P=0.02 \)). An exploratory biomarker tumour analysis\textsuperscript{10} of patients enrolled in the panitumumab versus best supportive care (BSC) randomised phase III study\textsuperscript{46} reported similar results. Of note, the addition of panitumumab to first-line FOLFOX might be even detrimental in patients with less common RAS mutations and should be cautiously avoided. A similar extended mutational analysis (including KRAS mutations on exons 2, 3 and 4, NRAS mutations on codons 2, 3 and 4 and BRAF mutations on codons 11 and 15) has been conducted in FIRE-3, a first-line randomised trial that tested the combination of FOLFIRI and cetuximab versus FOLFIRI and bevacizumab in 592 CRC patients with KRAS wild-type tumours\textsuperscript{41}. The analysis was conducted on 407 patients included in the trial. Once again, median OS was significantly longer in the RAS wild-type population exposed to cetuximab, with a notable 7.5-month improvement (33.1 months vs. 25.6 months; HR 0.7; 95% CI 0.53–0.92; \( P=0.01 \)). Moreover, a disappointing median OS of only 16.5 months was reported for the 65 patients harbouring RAS mutated tumours exposed to FOLFIRI and cetuximab. Although, the survival figure was not significantly different from that of patients treated with FOLFIRI and bevacizumab (16.4 months vs. 20.6 months; HR 1.2; 95% CI 0.64–2.28; \( P=0.57 \)) it should alert us that treating RAS mutated patients with EGFR-I may be dangerous and that RAS mutations may retain an overall negative predictive value.

On the basis of these data, marketing authorisation for EGFR-I use has been amended, and now includes a complete RAS status analysis before prescription, restraining the availability of cetuximab and panitumumab to RAS wild-type CRC patients alone. Such significant data have
immediately shrunk the population, but this enriched cohort certainly includes those who may benefit the most and, importantly, exclude those who may have detrimental effects.

Although there is common agreement on the negative prognostic value of BRAF mutational status\textsuperscript{52,53}, its negative predictive role with regard to EGFR-I therapy is not universally accepted.\textsuperscript{6,23,29} However, prospective–retrospective analysis of several randomised trials confirmed that this signature identifies a different CRC subtype that not only has a poor prognosis but also a poor response to both targeted and chemotherapeutic regimens. The rate of response for CRC patients exposed to specific BRAF-I (such as PLX-4720\textsuperscript{44}) was overall poor, mainly because of the feedback regulation of EGFR\textsuperscript{43} (Figure 3). Many specific oncogene inhibitors that may impact on tumour growth are being tested (Figure 4). While phase II trials are ongoing to verify if a combination of BRAF-I and MEK-I (methyl ethyl ketone Inhibitor) or EGFR-I may overcome this hurdle and proteasome inhibitors (such as bortezomib) are being tested at a preclinical level\textsuperscript{45}, an upfront intense treatment encompassing 5-Fluorouracil, oxaliplatin, irinotecan and bevacizumab may be useful for CRC patients harbouring BRAF mutations\textsuperscript{46}.

Cost-effectiveness of rat sarcoma and v-Raf murine sarcoma viral oncogene homologue B1 testing: Are we moving towards a more rational use of the epidermal growth factor receptor-I?

The increasing burden of cancer is producing growing costs that significantly impact on healthcare expenditures, and value-based approaches are among the possible solutions to bend the cost curve. Recently, a 20% increase in cancer drug expenditures was reported in the United States, mainly caused by the introduction of novel targeted therapies\textsuperscript{47}. Moreover, 90% of the new cancer drugs approved by the Food and Drug Administration in the last 5 years exceeded 20,000 USD for 12 weeks of treatment\textsuperscript{48}, rising controversies on their cost-effectiveness. Since 2009, both the American Society of Clinical Oncology and the European Society of Medical Oncology have recommended that advanced CRC patients who are candidates for EGFR-I therapy have their tumours tested for KRAS mutations. Accordingly, Regulatory Agencies asked for the test before EGFR-I prescription. Nevertheless, health economic evaluations of the procedure conducted with cost-utility studies and cost-effective analyses seemed initially unconvincing. In the molecular unselected population, a National Cancer Institute of Canada prospective cost-effective analysis of cetuximab compared with best supportive care alone for pretreated CRC patients enrolled in the CO.17 trial\textsuperscript{49} showed an unacceptable incremental cost-effectiveness ratio close to 200,00 USD per with the incremental cost-utility ratio of 300,00 USD per quality-adjusted life-year (QALY) gained\textsuperscript{50}. When repeating such economic analyses in the KRAS wild-type population, the incremental cost-effectiveness ratio life-year gained and the incremental cost-utility ratio per QALY gained figures, although reduced by approximately 40%, still remained above the generally accepted threshold of 100,000 USD per QALY\textsuperscript{50}. A Japanese cost-effective analysis brought to similar conclusions\textsuperscript{51}. With the improvements in technology and the addition of BRAF mutational analysis testing, the results might be more favourable, as more recent cost-effective analyses\textsuperscript{52–55} and a large literature review of cost-effective and cost-utility

**Figure 3:** Feedback regulation of EGFR that may limit the clinical effect of BRAF-inhibitors or MEK-inhibitors when used as single-agent in patients with advanced colorectal cancer. BRAF, v-Raf murine sarcoma viral oncogene homologue B1; EGFR, epidermal growth factor receptor; ERK, extracellular-signal-regulated kinase; MEK, methyl ethyl ketone; RAS, rat sarcoma.

Licensee OA Publishing London 2013. Creative Commons Attribution License (CC-BY)

studies published between 2000 and 2013 have both suggested. The evidence, however, remains controversial and extensively debated. Although reasonable, if the addition of NRAS testing may increment the cost-effectiveness of the use of EGFR-I is currently unproven.

Conclusion
The landscape of CRC treatment is changing very fast, and the availability of new therapeutic options has created new challenges and generated more complicated treatment algorithms. How to integrate the prognostic and predictive biologic information in the everyday clinical practice has been long debated. In the past 20 years, patient-related factors such as age, performance status and the presence of comorbidities have played a crucial role in the treatment-decision making. Also, cancer-related features such as disease extension and aggressiveness usually drive clinical judgement towards available options. Arguably, the current overwhelming new data on BRAF and RAS testing make now the overall picture more complex than ever. Medical oncologists should be adequately trained to combine molecular biology results with more classical decision factors in order to use an inclusive information and optimise their treatment decision. Indeed, there are many reasons that reinforce the need for a deeper molecular understanding of CRC. First of all, the analysis of RAS and BRAF allow oncologists to identify patients with the highest chance to benefit from EGFR-I. Second, a more appropriate treatment selection helps avoiding useless toxicities and may prevent impaired outcomes. In the majority of cases with RAS and BRAF wild-type status, a first-line combination with an EGFR-inhibitor seems to be the preferred treatment option, whereas the antiangiogenic strategy should be pursued in those with RAS mutated tumours or when a less aggressive treatment is favoured. Third, the extended molecular analysis appears to be cost-saving, although to definitely establish its economic value, a more comprehensive cost-effective analysis including NRAS testing is eagerly awaited.

Abbreviations list
BRAF, v-Raf murine sarcoma viral oncogene homologue B1; CRC, colorectal cancer; EGFR, epidermal growth factor receptor; ERK, extracellular-signal-regulated kinase; GSK, glaxo-smithkline. IGF, insulin-like growth factor; MEK, methyl ethyl ketone; PI3K, phosphatidylinositol 3-kinase; PTEN, phasmatase and tensin homologue; RAS, rat sarcoma; RTK, receptor tyrosine kinase.

References


36. Giardiello F. Molecular profiling of the CAPRI GOIM trial in KRAS wild type (wt) metastatic colorectal cancer (mCRC) patients (pts): cetuximab + FOLFIRI followed by FOLFOX4 + cetuximab. Late breaking abstract LBA 31. In: European Cancer Congress; Amsterdam, Netherlands; 29 September 2013.


Critical review