Molecular biomarkers in colorectal cancer in pursuit of personalizing treatment

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Abstract

Introduction

Recent and rapid advancements made in our understanding of the biology of colorectal cancer (CRC) has pointed towards a more personalized way of treating cancer, utilizing targeted treatment based on the tumour molecular profile. Epidermal Growth Factor Receptor (EGFR) has been found to play an important role in CRC tumorigenesis with its activation stimulating key signal transduction cascades involved in tumour growth and progression. Cetuximab and panitumumab are monoclonal antibodies that inhibit EGFR activation, leading to inhibition of downstream signalling. Both agents have been approved in the setting of metastatic CRC. KRAS mutations have been established to be negative predictors of response to these EGFR-targeted therapies. However, 40–60% of wild-type KRAS cases do not respond to anti-EGFR therapy, suggesting the involvement of other genes that act downstream of EGFR such as BRAF, NRAS or PI3K, that may be causative for the lack of response to anti-EGFR antibodies. In this article, we review the current literature investigating the potential predictive or prognostic values of these molecular biomarkers that are downstream of EGFR.

Conclusion

Tumour molecular profiling is integral to treatment personalization in CRC. Identifying predictive and prognostic biomarkers help to tailor therapy to better patient selection, in order to maximize clinical outcomes and minimize exposure to unnecessary toxicity. Most of the data presented show the need for selection strategies beyond the current reliance on KRAS to include biomarkers downstream of EGFR. However, as the data have been mostly exploratory and retrospective in nature, the clinical significance of these molecular markers will need to be validated in prospective, randomized clinical trials.

Introduction

Advancements in gene sequencing have fuelled expectations regarding the promise of personalized cancer treatment. The ability to molecularly profile a patient’s tumour to better tailor targeted therapies have improved response rates, in addition to avoiding unnecessary toxicity and cost. Significant progress has been made in unravelling complex signalling pathways that govern resistance to treatment. In particular, the identification of molecular biomarkers is proving integral to help with patient selection for and in predicting clinical benefit to targeted therapies in metastatic colorectal cancer (mCRC). Here we review the current literature investigating the potential predictive or prognostic values of these molecular biomarkers that are downstream of EGFR.

Discussion

The Epidermal Growth Factor Receptor (EGFR) was proposed as a potential therapeutically target: it is over-expressed in a variety of malignancies including colorectal cancer (CRC) and its activation triggers a cascade of downstream signalling pathways that result in tumour proliferation, metastasis, angiogenesis, and resistance to chemotherapy. This led to the development of monoclonal antibodies that bind to the extracellular portion EGFR, thus inhibiting EGFR activation. Cetuximab, a human-murine chimeric monoclonal antibody (mAb), and panitumumab, a fully human mAb, have both been extensively studied in multiple trials involving patients with advanced CRC. These studies evaluated the efficacy of each agent as monotherapy as well as in combination with chemotherapy, both as first line therapy and as therapy in previously treated patients who have progressive disease. The BOND-1 trial of cetuximab alone, or in combination with irinotecan, in patients with irinotecan refractory disease and with EGFR positive tumours, demonstrated response rates (RR) of 10.8% and 22.9% respectively (P = 0.007). Based on the BOND-1 study, cetuximab received FDA approval for use in patients with mCRC refractory or intolerant to Irinotecan. In a similar study comparing panitumumab with best supportive care (BSC) in patients with progressive CRC receiving conventional chemotherapy, panitumumab was found to significantly prolong progression-free survival (PFS; hazard ratio [HR] 0.54, P < 0.0001), and this led to FDA approval of panitumumab. Surprisingly, there was no relationship found between the level of EGFR expression by immunohistochemical techniques and response to the monoclonal antibody, a finding confirmed in other trials.

EGFR blockade and KRAS

During these initial studies, the relatively modest RR derived from these anti-EGFR mAbs, combined with the lack of correlation of response with EGFR expression, led to the investigation of signalling pathways downstream of EGFR. This was to assess for molecular biomarkers that mediated resistance or that could predict response to therapy with anti-

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EGFR mAbs. KRAS was found to be an important downstream effector of EGFR, which when mutated in its constitutionally active form, would lead to downstream signalling independent of EGFR inhibition, leading to cell proliferation. The KRAS status is thus critical in predicting response to EGFR blockade. In retrospective studies, wild-type KRAS gene was predictive of tumour response to both cetuximab and panitumumab. Karapetis and colleagues found that patients with KRAS wild-type (WT) tumours treated with cetuximab had significantly improved overall survival OS (9.5 versus [vs.] 4.8 months; hazard ratio [HR] 0.55; P = 0.001) and progression-free survival (PFS) (3.7 vs. 1.9 months [mo]; HR 0.40; P < 0.001) compared with supportive care alone.5

Conversely, in patients with mutated KRAS tumours, there were no significant differences between these two study arms, with respect to OS (HR 0.98; P = 0.89) or PFS (HR 0.99; P = 0.96). Similarly, the effect of panitumumab on PFS in KRAS WT tumours (HR 0.45) was significantly greater (P < 0.0001) than in the mutant group (HR 0.99).6 The influence of KRAS status on response to these agents has been confirmed in additional retrospective as well as prospective randomized studies. The OPUS study compared 5-fluorouracil, leucovorin and oxaliplatin (FOLFOX-4) to FOLFOX-4 plus cetuximab as first line treatment in patients with mCRC.7 Patients with KRAS WT tumours showed significantly improved PFS (HR 0.57; P = 0.0064) and RR (odds ratio 2.551; P = 0.0027) with the addition of cetuximab to FOLFOX-4.8 A subgroup analysis of the CRYSTAL study, which evaluated the efficacy of 5-fluorouracil, leucovorin and irinotecan (FOLFIRI) vs. FOLFIRI plus cetuximab led to similar findings: the addition of cetuximab resulted in an improvement in PFS for cetuximab resulted in an improvement in PFS for patients with KRAS mutant tumours, PFS was significantly reduced in the panitumumab + FOLFOX4 arm compared to the FOLFOX4-only arm (HR, 1.29; P < 0.02), and median OS was 15.5 months vs. 19.3 months, respectively (HR, 1.24; P < 0.068).9 A similar negative effect of cetuximab was seen when used with chemotherapy in patients with KRAS-mutant in the OPUS study.8 The repeated demonstration of the influence of KRAS status, as a negative predictor of response to anti-EGFR mAbs has led to the recommendation of restricting anti-EGFR mAbs to patients with KRAS WT tumours.

De Roock et al. suggested that the KRAS Gly13Asp (G13D) mutation may predict tumour response to EGFR inhibitors, demonstrating an improvement in OS (7.6mo vs. 5.7mo; HR 0.5; P = 0.005) and PFS (4 mo vs. 1.9mo; HR 0.51; P = 0.004) for patients with G13D mutations compared with other KRAS mutations treated with cetuximab.10 However, a number of studies have demonstrated no effect on response to either cetuximab or panitumumab due to specific KRAS mutations.12,13 Peeters et al. performed a retrospective analysis from four pooled data sets that showed no consistent associations between tumours with specific KRAS mutations and patient outcome (PFS or OS).12 These findings support the increasing body of evidence that not all KRAS mutations have the same effects on tumour biology and clinical outcome in CRC. It is clear from emerging data that KRAS mutation status alone is not sufficient to fully describe the heterogeneity underlying CRC biology.

Anti-EGFR mAbs in combination with chemotherapy regimens

We would like to highlight the importance of the choice of agent to use in combination with EGFR-targeted therapies. For example, it appears that meaningful benefit to patients is achieved only when anti-EGFR therapy is combined with infusional 5-fluorouracil (5-FU). The COIN study highlighted a potential negative interaction between cetuximab and capcitabine (an orally administered prodrug of 5-FU).14 In this study, most patients received capcitabine and oxaliplatin (XELOX) regimen. No survival benefit was found with the addition of cetuximab, irrespective of tumour mutational status. In patients with KRAS WT tumours treated with infusional 5-FU, benefit in PFS was evident with the addition of Cetuximab with HR of 0.77; p=0.06, compared with HR for capcitabine-based therapy of 1.06; p=0.56 (p for interaction 0.07). It may be hypothesized that different fluoropyrimidine regimens can influence the clinical effect of EGFR-targeted therapy differently. The NORDIC study also showed no survival benefit with the addition of cetuximab to FLOX (5-FU bolus and oxaliplatin) in first line mCRC.13 FLOX, involving 5-FU bolus administration, is in contrast with the more commonly used FOLFOX regimens, which utilises infusional 5-FU. It is hard explain why cetuximab would have an effect when combined with the FOLFOX regimen and not with FLOX or XELOX. These results suggest that the benefit of cetuximab may be restricted to combination with infusional 5-FU, but not IV bolus 5-FU or the orally administered 5-FU prodrug (capcitabine).

VEGF and EGFR inhibitor combination therapy

Bevacizumab is a monoclonal antibody that targets and inhibits VEGF (vascular endothelial growth factor). VEGF plays a role in angiogenesis and has been shown to improve PFS and OS in combination with chemotherapy (fluoropyrimidine-based chemotherapy with either oxaliplatin or irinotecan).

Preclinical data indicate that EGFR stimulation increases VEGF expression, thus providing a potential mechanistic hypothesis for synergy between EGFR- and VEGF- targeted therapies. Subsequently, studies have been
undertaken to evaluate the safety and efficacy of adding a dual antibody combination (anti-EGFR mAb + anti-VEGF mAb) to chemotherapy: The first trial of such was the randomized BOND-2 trial, which showed promising clinical efficacy (37% RR and an OS of 14.5 months) after administration of cetuximab + bevacizumab + irinotecan (arm 1) compared with cetuximab + bevacizumab only (arm 2) (20% RR and 11.4 month OS).\(^{15}\)

However, both the PACCE and CAIRO-2 trials revealed unexpected negative results.\(^{16,17}\) The CAIRO-2 study showed that the addition of cetuximab to capcitabine, oxaplatin and bevacizumab resulted in a significant decrease in PFS and a poorer quality of life.\(^{17}\) The PFS was 10.7 months in the chemotherapy and bevacizumab (CB) group versus 9.4 months in the CB with cetuximab (CBC) group, corresponding to a HR of 1.22 (p=0.01). This was surprising considering earlier pre-clinical and clinical study results.\(^{18,19}\) A similar result with anti-EGFR therapy was observed in the PACCE trial where previously untreated patients with mCRC were randomly assigned to receive chemotherapy (either FOLFOX or FOLFIRI) with bevacizumab, and then randomly assigned further to receive concomitant panitumumab or no additional treatment (1:1).\(^{16}\) The PACCE trial was prematurely discontinued because of worse efficacy and decreased PFS in the panitumumab arms.

The results from both the CAIRO-2 study and the PACCE study argue against the use dual antibody therapy (anti-VEGF plus anti-EGFR agents) with chemotherapy in mCRC, given the negative interactions that may exist between bevacizumab and cetuximab or panitumumab when combined with chemotherapy.

EGFR-targeted therapies are currently approved only for mCRC, where their role is clear. Its role in the adjuvant setting in patients with resected stage III CRC has unfortunately, proven to be without any clinical benefit, based on two trials: The United States National Cancer Institute Intergroup Study N1047 trial\(^{20}\) and the European Intergroup PETA2CB trial\(^{21}\).

Henceforth, anti-EGFR mAbs are utilized only in the metastatic setting for CRC.

### Beyond KRAS: NRAS, BRAF and PIK3CA mutations

KRAS mutations have been established as a highly specific negative biomarker for benefit to anti-EGFR mAb treatment. However, 40-60% of KRAS-wt cases do not respond to anti EGFR therapy and not all KRAS mutations are equal in their biological characteristics, suggesting the involvement of other genes that act downstream of EGFR mediating resistance to anti-EGFR mAbs. EGFR activation stimulates key signal transduction cascades, which include the RAS-RAF-MEK-ERK and PI3K/ AKT/mTOR pathways, all involved with tumour growth and progression. Particularly, it has been suggested that downstream mutations of BRAF, NRAS or PIK3CA can lead to lack of response to anti-EGFR mAbs.

NRAS mutations
NRAS is a member of the KRAS oncogene family and bears similar homology to KRAS. Its mutation rate in CRC is 3-5%. Mutations in KRAS, NRAS and BRAF are mutually exclusive. NRAS mutations are associated with a lack of response to anti-EGFR therapy. In the PICCOLO trial, there appeared to be a panitumumab-related trend toward potential harm to patients with tumours harbouring NRAS mutations, although this was not statistically significant (HR for death, 1.97 (p = 0.23).\(^{22}\) In the European Consortium study—where effects of mutations downstream of EGFR on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory mCRC was studied—2.6% of patients were shown to harbour an NRAS mutation. KRAS WT tumours with NRAS mutations had a significantly lower RR (7.7% vs. 38.1%, p = 0.013) than did those with NRAS-WT, and a trend toward shorter PFS (median PFS 14 vs. 26 weeks; HR 1.82, p = 0.055) and OS (median OS 38 vs. 50 weeks; HR 1.89, p = 0.051).\(^{23}\)

A recently published retrospective analysis on the PRIME study evaluated the role of RAS mutations as a negative predictive biomarker.\(^{24}\) The study revealed that RAS mutations not only predicted lack of response with the addition of panitumumab to FOLFOX4, but also indicated a potentially detrimental effect, associated with inferior PFS and OS. The retrospective, exploratory nature of these data as well as the small numbers of patients with these mutations provides insufficient power to confidently claim that NRAS mutations have a true predictive effect. However it is evident that better tumour profiling for patient selection is important to improve the benefit-risk profile of anti-EGFR therapy.

### BRAF mutations

BRAF is a primary effector of KRAS signalling downstream. Activating BRAF mutations are present in 4-15% of sporadic CRC. This frequency is heavily dependent on the patient population studied because BRAF mutations confer poor prognosis and the percentage of patients with these mutations decline with later lines of therapy.

Initial studies suggested that BRAF mutations were associated with resistance to anti-EGFR mAb therapy.\(^{23,25}\)

For example, in a retrospective analysis performed by De Roock and colleagues, 2 of 24 (8.3%) patients with BRAF mutation responded to cetuximab compared with 124 of 326 (38%) of patients who were BRAF WT.\(^{23}\) This led to the suggestion that BRAF status should be used in combination with KRAS to select patients suitable for anti-EGFR mAb, although the pooled analysis of OPUS - CRYSTAL trials found no predictive value in BRAF status.\(^{26}\)

In this pooled analysis, patients with KRAS WT tumours harbouring BRAF mutations were found to have poor prognosis across all outcome measures in both treatment arms (with/without cetuximab), compared with those with KRAS WT/BRAF WT tumours.\(^{26}\) Similarly, a retrospective biomarker...
analysis of the patients in the CAIRO-2 trial by Tol et al., revealed significantly shorter PFS and OS in patients with BRAF mutated tumours compared to BRAF WT tumours, irrespective of treatment arm (CB or CBC), concluding its role as a poor prognostic marker.27

As a consequence of the prognostic significance of BRAF mutations as well as the low prevalence of this mutation, it has been difficult to clarify whether BRAF has value as a predictive biomarker.

**PIK3CA mutation**

Activating mutations are found in approximately 10-20% of unselected CRC patients, and can occur with KRAS and BRAF mutations.28 There is data to suggest a role for exon 20 mutated PIK3CA in the prediction of resistance to anti-EGFR therapy. The largest data set held by the European Consortium indicates that 14.5% (108/743) of patients harbour PIK3CA mutations.23 60-65% of PIK3CA mutations are in exon 9 and 20-25% in exon 20. This study revealed that exon 20 mutations were associated with a poorer outcome compared with PIK3CA WT, with RR of 0.0% vs. 36.8% (p = 0.029), PFS of 11.5 vs. 24 weeks (HR 2.52; p = 0.013), and median OS of 34 vs. 51 weeks (HR 3.29; p = 0.0057).23 Exon 9 mutations had no effect on the measured RR, PFS or OS.

Mao et al.29 carried out a systematic review and meta-analysis of thirteen studies evaluating the association between PIK3CA mutations and resistance to anti-EGFR mAbs in KRAS WT mCRC. The analysis similarly showed a lower overall response rate (ORR) in patients with exon 20 mutations compared with KRAS WT (ORR 0% vs. 37%; p = 0.082) when treated with anti-EGFR therapy. The small sample size partly contributed to the statistical insignificance. These data highlight the potential role of PIK3CA exon 20 mutations in predicting resistance to anti-EGFR therapy.

**Conclusion**

The introduction of KRAS testing as a diagnostic tool in the selection of patients for EGFR-targeted treatment has been validated and is considered one of the biggest advancements made toward personalizing CRC treatment based on tumour biology. However, KRAS WT status does not guarantee benefit from these agents. The investigation of additional biomarkers downstream of EGFR signalling thus provides a platform to 1) identify causes of primary resistance to anti-EGFR therapy; 2) better select patients who are more likely to benefit from EGFR-targeted therapy; 3) improve overall outcomes as well as minimize toxicity; and 4) potentially identify new therapeutic targets.

The data available to date are mostly retrospective in nature, with small sample sizes that provide insufficient power to confidently confirm or refute the roles of these individual mutations as positive or negative, predictive or prognostic biomarkers.

It is imperative for these mutations (NRAS, BRAF and PIK3CA) and others to be further investigated in large, randomized controlled trials conducted in KRAS-WT mCRC patients before the clinical significance of these biomarkers can be fully validated. Only then can an algorithm be created incorporating the optimal combination of these biomarkers needed for effective use of EGFR- and possibly other targeted therapies.

**Acknowledgement**

I would like to thank Marion L. Hartley of the Ruesch Center for her editing expertise.

**Conflict of interests**

Dr JL Marshall speaks and consults for Genentech, Amgen and Bayer.

**Competing interests**

Dr JL Marshall speaks and consults for Genentech, Amgen and Bayer.

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