Biomarkers predicting resistance to epidermal growth factor receptor-targeted therapy in metastatic colorectal cancer with wild-type KRAS

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Abstract: EGFR pathway is an important therapeutic target in human tumors, including metastatic colorectal cancer (mCRC). The advent of EGFR-targeted monoclonal antibodies panitumumab and cetuximab has generated promise for the treatment of mCRC and has largely improved patients’ progression-free survival (PFS) and overall survival (OS). However, treatment with anti-EGFR monoclonal antibodies is only effective in a subset of mCRC patients with wild-type KRAS. This indicates that there are other factors affecting the efficacy of anti-EGFR monoclonal antibodies. Existing studies have demonstrated that among colorectal cancer patients with wild-type KRAS, harboring mutations of BRAF, PIK3CA, NRAS, or PTEN-null may demonstrate resistance to anti-EGFR-targeted therapy, and biomarkers detection can provide better-personalized treatment for mCRC patients. How to identify and reverse the secondary resistance to anti-EGFR monoclonal antibody therapy is also another great challenge to improve the anti-EGFR efficacy in wild-type KRAS mCRC patients. Finally, both of the molecular mechanisms of response and acquired resistance would be important for the directions of future research. This review focuses on how to further improve the predictive value of anti-EGFR therapies and how to also try and avoid futile treatment for wild-type KRAS colorectal cancer patients.

Keywords: colorectal cancer, EGFR, BRAF, RAS, cetuximab, panitumumab

Introduction
EGFR is a transmembrane tyrosine kinase receptor belonging to the human epidermal growth factor receptor (HEGFR) family to which ten different ligands can selectively bind.1,2 When ligands bind to the EGFR molecules, the receptor structure is changed, causing receptor autophosphorylation through receptor tyrosine kinase activity.2 The latter triggers a battery of intracellular signaling pathways, including RAS/RAF/MEK/MAPK and the PI3K/AKT pathways, which leads to tumor cell proliferation, inhibition of apoptosis, activation of invasion and metastasis, and stimulation of tumor-induced neovascularization.1,2 Therefore, EGFR is an important therapeutic target in human cancers including metastatic colorectal cancer (mCRC).

Colorectal cancer is the second most common epithelial tumor and in 2012 was also the second leading cause of death, due to cancer, in Europe.3 Over the past decade, systemic chemotherapy has made tremendous progress in the treatment of mCRC patients, and the median overall survival (OS) has increased from less than 9 months without treatment, to more than 20 months with treatment.4 The emergence of the EGFR-targeted monoclonal antibodies panitumumab and cetuximab, is a milestone in the history of the treatment of mCRC and indicates future directions for personalized treatment. Panitumumab and cetuximab have brought promise for the treatment of mCRC and have largely improved progression-free survival (PFS) or OS, as well as quality of life, but
the treatment with anti-EGFR monoclonal antibodies is effective only in a subset of mCRC patients. Up-to-date, KRAS mutational status has been extensively studied to predict the clinical outcome of anti-EGFR-targeted therapy in mCRC patients. Cetuximab or panitumumab monotherapy as well as combination therapy with chemotherapy, have been evaluated in several studies. Cetuximab in combination with standard chemotherapy in mCRC patients carrying wild-type KRAS has proven to improve patients’ OS, PFS, and objective response rate significantly. Similarly, the PFS and objective response rate for mCRC patients with wild-type KRAS have been remarkably improved, when panitumumab is applied in combination with chemotherapy.

However, not all mCRC patients carrying wild-type KRAS respond to anti-EGFR therapy. Thus, batteries of other potential predictive markers have also been investigated to guide this therapy. Two retrospective studies have shown that among wild-type KRAS patients receiving cetuximab or panitumumab, BRAF mutations were significantly and independently associated with patient survival. PIK3CA mutations and the loss of PTEN expression have been reported as predictive markers underlying the response to cetuximab or panitumumab in wild-type KRAS mCRC patients in a number of other studies. In recent years, the relationship between NRAS mutations and the efficacy of anti-EGFR antibodies therapy has also been evaluated. Moreover, the acquired resistance to anti-EGFR antibodies therapy is another urgent problem to improve the efficacy and life quality in mCRC patients. Its underlying mechanism may also relate to BRAF, PIK3CA, NRAS, and PTEN status. In this paper, we reviewed these studies and tried to figure out the most useful biomarkers to help the use of anti-EGFR monoclonal antibodies.

**BRAF mutations**

BRAF is a downstream effector of the RAS signaling pathway. It has been reported that BRAF mutations are related to the resistance of cetuximab or panitumumab in approximately 10% of the cases of colorectal cancer. The most common BRAF mutation in tumors was the BRAF V600E mutation that was mutually exclusive with KRAS mutations. Therefore, the combination of KRAS with BRAF status, can identify further optimized populations that may benefit from anti-EGFR antibodies therapy. Two retrospective studies have reported, whereby BRAF mutations impaired the response to cetuximab or panitumumab in mCRC patients. Di Nicolantonio et al discovered that HT-29 and COLO-205 (both BRAF V600E mutation and wild-type for KRAS) were highly resistant to cetuximab or panitumumab therapy, and the BRAF inhibitor sorafenib could restore sensitivity to anti-EGFR therapy. This implies that combining EGFR and BRAF inhibitors may be more effective for wild-type KRAS/BRAF-mutation populations. Furthermore, Di Nicolantonio et al have also shown that in eleven patients with wild-type KRAS/BRAF-mutation receiving cetuximab or panitumumab treatment, PFS and OS were significantly shorter than both wild-type populations, which was inconsistent with two other retrospective studies. However, Karapetis et al retrospectively analyzed the role of activating mutations of the EGFR signaling pathway in predicting the efficacy of cetuximab-based treatment using the sample obtained from the NCIC CTG/AGITG CO.17 study, and did not discover the predictive significance of BRAF V600E mutation in the year of 2013. Furthermore, the 2013 ASCO (American Society of Clinical Oncology) Annual Meeting reported that in a retrospective analysis of the role of RAS and RAF mutations in the Phase III PRIME study, BRAF V600E mutation found no predictive value. It was also surprising that OS was prolonged in a small number of wild-type KRAS/BRAF-mutant (n=11) patients who were receiving cetuximab and FOLFOX-4, compared with patients treated with only FOLFOX-4 in the OPUS study. This phenomenon is likely related to the heterogeneity of cancer and the small-sample-size. Owing to the small number of BRAF mutation cases and lack of perspective studies, it is difficult to conclude the predictive value of anti-EGFR therapies in colorectal cancer at the current station.

In recent years, three large randomized clinical studies, including the CRYSTAL (cetuximab combined with FOLFOX as first-line therapy for mCRC) study, the COIN (cetuximab combined with oxaliplatin-based first-line chemotherapy for treatment of advanced colorectal cancer) trial, and the NORDIC-VII (cetuximab with Nordic FLOX versus FLOX alone in the first-line therapy for mCRC) study, have consistently demonstrated that the BRAF V600E mutation predicts poor prognosis, which is supported by the data from the pooled analysis of the CRYSTAL and OPUS randomized clinical trials. In the CRYSTAL study, although the addition of cetuximab to FOLFIRI did not show any significant difference in wild-type KRAS/BRAF-mutant patterns, it improved PFS and OS slightly. Thus, cetuximab treatment may not be completely forbidden in terms of BRAF mutations, this is because there is still a survival benefit for such populations. In a word, BRAF mutations are robustly and negatively prognostic factors in mCRC populations according to the studies mentioned earlier. However, it is
controversial with respect to its predictive value in anti-EGFR treatment.

**NRAS mutations**

NRAS, a member of RAS family, is often mutated in human tumors. An experimental research study showed that activating mutations in NRAS robustly stimulates tumorigenesis by suppressing apoptosis in the condition of inflammation. Thus, NRAS mutations might be predictors of treatment effect as well as a treatment target. Since KRAS and NRAS mutations were completely exclusive, NRAS mutational status might be a valuable predictor to wild-type KRAS patients receiving anti-EGFR therapy. Similar to KRAS, the common mutational sites of NRAS were codons 12, 13, and 61, and the mutational frequency was approximately 3% in wild-type KRAS populations. The data from two small-sample-size studies showed that wild-type KRAS patients carrying NRAS mutations, had lower response rates for anti-EGFR therapy compared with those with dual wild-type genes. A poor prognostic effect was observed in patients with NRAS mutations in the COIN trial. Moreover, in a retrospective randomized Phase III study evaluating response to panitumumab, treatment with panitumumab resulted in improved PFS in patients with wild-type KRAS/NRAS rather than those with wild-type KRAS/mutational NRAS. Although the mutational frequency of NRAS is very low, there is a strong trend toward a negative response to anti-EGFR antibodies therapy in populations for wild-type KRAS/mutational NRAS.

**PIK3CA mutations and PTEN-null**

In addition to the RAS/RAF/MEK/MAPK signaling pathway, the PI3K/AKT/mTOR pathway also plays an important role in the development of malignant tumors. The most common mutation sites of phosphoinositide 3-kinase (PI3K) are located on exons 9 and 20. It was observed that cell lines carrying wild-type PI3CA/PTEN expression were more sensitive to cetuximab than cell lines with PIK3CA mutations or loss of PTEN expression. This implied the potential correlation between cell response to anti-EGFR antibody therapy and the two genes level, in vitro. However, clinical results were not consistent. Before this study, in a small-sample-size clinical study, the association between PIK3CA mutations and response to cetuximab was not identified for unselected patients that was in agreement with another report. Subsequently, Sartore-Bianchi et al indicated PIK3CA mutations were independently associated with a poorer clinical outcome of anti-EGFR antibodies therapy in mCRC patients, and this effect was enlarged in the wild-type KRAS subgroup. No conclusions were made regarding the predictive value of mutations at PI3K in the PICCOLO trial comparing panitumumab plus irinotecan, versus only irinotecan for patients with wild-type KRAS. Simultaneously, a retrospective analysis also did not find any association in terms of PIK3CA mutations and the efficacy of cetuximab. It was difficult to draw conclusions from these conflicting findings due to small-sample-size and very few mutational PI3K cases. Thus, to further explore the relationship between PIK3CA mutations and efficacy of anti-EGFR antibodies therapy in colorectal cancer patients, exons 9 and 20 of PI3K should be analyzed respectively. A large cohort study containing 1,022 mCRC patients treated with cetuximab revealed a poorer clinical outcome for patients with exon 20 mutations rather than exon 9 mutations in wild-type KRAS populations (Table 1). It was likely that similar findings were observed in other tumors found in, for example, breast cancer patients.

![Table 1](https://www.dovepress.com/)

**Table 1** PI3K exon 20 mutations and clinical outcomes of panitumumab- or cetuximab-based treatment in wild-type KRAS patients with metastatic colorectal cancer

<table>
<thead>
<tr>
<th>References</th>
<th>Year</th>
<th>Regimen</th>
<th>Design</th>
<th>Mutant/wild-type</th>
<th>OS</th>
<th>PFS</th>
<th>OR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moroni et al.</td>
<td>2005</td>
<td>CTX/Pani ± chemotherapy</td>
<td>RCo</td>
<td>2/21</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Perron et al.</td>
<td>2009</td>
<td>CTX/Pani ± chemotherapy</td>
<td>RCo</td>
<td>1/17</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>De Roock et al.</td>
<td>2010</td>
<td>CTX/Pani ± chemotherapy</td>
<td>RCo</td>
<td>9/329</td>
<td>34 ± 51 w</td>
<td>11.5 ± 24 w</td>
<td>0% vs 36.8%</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HR=3.29</td>
<td>HR=2.52</td>
<td>OR=0.00</td>
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<td></td>
<td></td>
<td></td>
<td>P=0.0057</td>
<td>P=0.013</td>
<td>P=0.029</td>
</tr>
</tbody>
</table>

**Abbreviations:** CTX, cetuximab; Pani, panitumumab; RCo, retrospective cohort study; NA, date not available; PFS, progression-free survival; OS, overall survival; w, weeks; OR, odds ratio; HR, hazard ratio.

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**Biomarkers predicting resistance to anti-EGFR therapy in wild-type KRAS mCRC**
Because the negative effect of PI3K exon 9 mutations was not observed in this meta-analysis, the whole effect largely depended on exon 20 mutations. Hence, PI3K exon 20 mutations are of more predictive value for anti-EGFR therapy.

The loss of the PTEN is widespread in human tumors. The absence of PTEN function could constitutively activate downstream effectors of the PI3K signaling pathway in cancer cells.52 This suggested dysfunction of PTEN might be related to the reduced efficacy of antitumor therapies and the shorter survival rate of patients. The PTEN loss detected by immunohistochemistry decreased OS instead of PFS in wild-type KRAS patients treated with cetuximab.9 On the contrary, a cohort study has reported wild-type KRAS patients with PTEN-positive on metastatic, rather than primary tumors, had longer PFS.26 This interesting finding implied that biological characteristics of primary tumors and metastases was to some extent different, which is contradictory to the result found in another study.53 Two other retrospective studies have revealed that PTEN expression was related to longer time to progression (TTP) in patients carrying wild-type KRAS.24,55 PTEN expression loss was found to cause poor PFS and OS in wild-type KRAS colorectal cancer patients undergoing anti-EGFR antibodies therapy (Table 2),56 which was in accordance with the results found in a meta-analysis study.57 Nevertheless, there was no statistical significance with respect to the association between PTEN-null and the clinical outcome of cetuximab in the NCIC CTG/AGITG CO.17 trial.23 An important problem that influenced the results of the clinical trials was that interpretation of immunohistochemistry results may be determined by multiple factors, such as the academic level of analysts and/or different scoring systems. Another question that remained uncertain was whether PTEN protein expression of primary tumors and metastases was different. If these problems are resolved, the combination of PTEN expression and KRAS mutational status might be a better predictor of the efficacy of anti-EGFR antibodies therapy.

In addition, several researchers found that PI3K mutations and PTEN expression were joint predictors of the clinical outcome of anti-EGFR therapy.31–33 The wild-type KRAS patients with PTEN-null/PI3K mutations had a significantly shorter OS, and there was very strong trend toward the decrease in PFS.32 However, the data were limited for the combination of PI3K mutations and PTEN loss as predictive markers.

### EGFR and its ligands

Activation mutations of the kinase domain in EGFR, such as mutations of exons 18, 19, and 21, have been reported to be associated with the response to tyrosine kinase inhibitors (TKIs) (gefitinib or erlotinib) in lung cancer,58,59 but such mutations were rare in colorectal cancer.60 In contrast, the relationship between EGFR gene copy number as well as expression, and anti-EGFR antibodies therapy in colorectal cancer, has been largely concerned with. Unselected patients’ tumors which had an increased EGFR gene copy

<table>
<thead>
<tr>
<th>References</th>
<th>Year</th>
<th>Regimen</th>
<th>Design</th>
<th>Expression/loss</th>
<th>OS</th>
<th>PFS/TTP</th>
<th>OR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laurent-Puig et al</td>
<td>2009</td>
<td>CTX + chemotherapy</td>
<td>RCo</td>
<td>89/22</td>
<td>16.2 vs 11.8 m</td>
<td>PFS: 3.14 vs 3.0</td>
<td>NA</td>
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<td></td>
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<td></td>
<td>P=0.013*</td>
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<td>15.1 vs 13.1 m</td>
<td>HR=0.50</td>
<td>NA</td>
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<td>P=0.127*</td>
<td>PFS: 5.3 vs 3.7 m</td>
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<td>14.5 vs 13.5 m</td>
<td>HR=0.45</td>
<td>NA</td>
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<td></td>
<td>P=0.026*</td>
<td>TTP: 5.0 vs 3.7 m</td>
<td></td>
</tr>
<tr>
<td>Loupakis et al</td>
<td>2009</td>
<td>CTX + chemotherapy</td>
<td>RCo</td>
<td>17/10</td>
<td>6.9 vs 3.9 m</td>
<td>PFS: 2.44 vs 1.5</td>
<td>NA</td>
</tr>
<tr>
<td>Baridaki et al</td>
<td>2011</td>
<td>CTX + chemotherapy</td>
<td>RCo</td>
<td>64/14</td>
<td>1.1 vs 1.0 m</td>
<td>HR=2.7</td>
<td>NA</td>
</tr>
<tr>
<td>Sood et al</td>
<td>2012</td>
<td>CTX/Pani ± chemotherapy</td>
<td>RCo</td>
<td>NA</td>
<td>0.33 vs 0.32 m</td>
<td>HR=0.65</td>
<td>NA</td>
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<td></td>
<td></td>
<td>P=0.0015</td>
<td>PFS: 1.4 vs 1.2</td>
<td></td>
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<tr>
<td>Tural et al</td>
<td>2014</td>
<td>CTX + chemotherapy</td>
<td>RCo</td>
<td>26/15</td>
<td>0.34 vs 0.32 m</td>
<td>HR=0.4</td>
<td>42% vs 12%</td>
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<tr>
<td></td>
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<td></td>
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<td></td>
<td>P=0.008</td>
<td>PFS: 9.5 vs 6.1 m</td>
<td></td>
</tr>
<tr>
<td>Razis et al</td>
<td>2014</td>
<td>CTX ± chemotherapy</td>
<td>RCo</td>
<td>59/80</td>
<td>0.34 vs 0.32 m</td>
<td>HR=0.4</td>
<td>42% vs 12%</td>
</tr>
</tbody>
</table>

Note: *=Log-rank test.
Abbreviations: CTX, cetuximab; Pani, panitumumab; RCo, retrospective cohort study; NA, date not available; HR, hazard ratio; PFS, progression-free survival; TTP, time to progression; m, months; w, weeks; OS, overall survival; OR, odds ratio.
MET as a proto-oncogene, is activated by its ligand, hepa-
eres in wild-type KRAS patients still requires more reliable
produced gratifying results, their role as predictive biomark-
and OS benefit in the presence of high expressed epiregulin
from a Phase III clinical trial of cetuximab and best support-
might be involved. Moreover, in 2014 a retrospective study
expression, which suggested other signaling pathways
in primary tumors were significantly associated with better
outcomes in wild-type KRAS mCRC patients treated with
epitaxins and trastuzumab, which was supported by another
results. Objective responses were also observed in low EGFR
expression, which suggested other signaling pathways
and MET gene as proto-oncogenes, have recently been given
a Phase II clinical trial of cetuximab and best support-
caret 

Malignant tissue may also stimulate tumor growth and promote
tumor cell survival in vitro, but in vivo, increased MET expres-
sion could restore sensitivity to cetuximab in cetuximab-resistant
and EGFR-negative cases. Therefore, MET expression could restore sensitivity to cetuximab in metastatic colorectal cancer cells.

The Src gene family includes several kinases that function as signaling intermediaries for growth factor receptors. MET might mediate secondary resistance to anti-EGFR antibodies by promoting Src family kinase activity. This suggests that targeting Src family kinases could be a rational therapy strategy for reversing drug resistance. Current in vitro results support the potential of using Src family kinase inhibitors to restore sensitivity to anti-EGFR therapy in cetuximab-resistant cells.

In conclusion, the limited efficacy of anti-EGFR therapy is due to acquired resistance, which is often mediated by MET overexpression. Further studies are needed to clarify the role of MET in anti-EGFR resistance and to develop strategies to overcome this resistance.
Other potential biomarkers
Tyrosine kinase domain of each member of the HER family is highly conserved, and the structure and function is of high homology, which underlies the molecular basis of both the interaction of the receptors and cross-activation. HER2 amplification was prospectively identified as a predictive marker of resistance to anti-EGFR antibodies therapy, and in response to combination therapy, against HER2 and EGFR from mCRC xenografts. Subsequently, this phenomenon also existed among mCRC patients in a small-sample clinical study. In addition, wild-type KRAS colorectal cancer patients with positive HER3, detected by immunohistochemistry, had poor clinical outcomes in two retrospective studies. It would seem that HER2 and HER3 are possible biomarkers for anti-EGFR antibodies therapy, but these studies were also limited due to a small sample size.

Integrins can affect cell growth and repair via their receptors. The integrin pathway is activated in the absence of HER3 activation, implying that it may have a role in HER3 negative tumors. Upon univariate or multivariate analysis, integrin β4 rs8669 genotyping might be superior in selecting colorectal cancer patients who are more likely to benefit from anti-EGFR therapies in the HER3-negative/wild-type KRAS subgroup.

Other downstream effectors of EGFR have also been recently evaluated. For example, phosphorylated protein kinase B and MAPK expression in metastatic tumors, were related to poorer clinical outcomes in 72 mCRC patients receiving irinotecan-cetuximab. Mitogen-activated protein kinase phosphatases (MKPs) can inhibit MAPK activity via crosstalk between distinct MAPK pathways, or between MAPK signaling and other intracellular signaling modules. MKP1 is the best-characterized member of MKP family. In vitro, ectopic expression of MKP1 could inhibit the anti-EGFR drug. AG1478 induced apoptosis is activated via suppressing JNK (a MAPK) activation in the lung cancer cell PC-9. Thus, MKP1 over-expression may be a potential biomarker of resistance to anti-EGFR agents. In a small-sample-size clinical study, including 48 mCRC patients, patients with MKP1 over-expression in the wild-type KRAS subgroup had a low response (7% vs 44%) and poor PFS (13 vs 32 weeks).

Insulin-like growth factor 1 (IGF1) is closely related to cell growth and proliferation. The expression of IGF1 and its receptor may play a significant role in both the occurrence and growth of colorectal cancer. The downstream pathway of IGF1 and EGFR was completely overlapped, which implied that tumor cell growth might be stimulated by the activation of each of IGF1 and EGFR. Hence, IGF1 expression may induce resistance to anti-EGFR therapy in colorectal cancer. For example, polymorphisms of genes in the IGF1 pathway may act as potential biomarkers for cetuximab efficacy in mCRC patients with wild-type KRAS. However, the evidence regarding these predictive markers was insufficient and limited.

Antibody-dependent cell-mediated cytotoxicity (ADCC) is an immune mechanism in which specific antibodies are directed against a targeted antigen on tumor cells, causing their lysis via innate immune effector cells. The Fragment c (Fc) domain of the IgG1 monoclonal antibody was shown to induce ADCC. The Fc portion of cetuximab can activate ADCC through interactions with the Fc receptors (FcR) on the effector immune cells. Bibeau et al discovered combined FcRIIa/FcRIIIa polymorphisms are prognostic factors for disease progression in mCRC patients treated with cetuximab and irinotecan beyond KRAS mutation status. This implies that ADCC might play an important role in cetuximab efficacy.

Conclusion and prospection
The advent of anti-EGFR monoclonal antibodies has far-reaching implications for the treatment of refractory colorectal cancer. However, KRAS mutation status has been defined as a negative predictive factor for this therapy, a considerable proportion of patients with wild-type KRAS have no response to the treatment. This indicates that there are other factors affecting the efficacy of anti-EGFR monoclonal antibodies. Thus, it is necessary to further improve predictive accuracy and avoid futile treatment. Combination with other biomarkers is a rational strategy for further selecting appropriate populations. In recent years, more evidence reveals that KRAS and NRAS, as a whole, are more predictive of the efficacy of anti-EGFR monoclonal antibodies therapy. BRAF, which is prone to be a prognostic instead of predictive factor, is not very significant in terms of identifying whether anti-EGFR monoclonal antibodies are added in colorectal treatment. There is strong evidence that combined detection of NRAS, PI3K (especially exon 20), and the PTEN gene status in wild-type KRAS colorectal cancer patients, can identify more patients unlikely to respond to anti-EGFR monoclonal antibodies. It would seem that over-expression of EGFR ligands predict superior clinical outcomes for anti-EGFR antibodies therapy in mCRC patients with wild-type KRAS. The predictive evidence of other potential biomarkers such as MET, HER2, HER3, MKP1, and IGF1 is both limited and inadequate.

There are still some problems that will need to be solved, before implementing these predictive markers in clinics. Firstly, lower variant frequency of these markers limits the
clinical application and requires more reliable evidence from prospective studies with larger sample-sizes. Secondly, different detection methods and cutoff values used to detect the same markers in some of the studies may interfere with the comparability of these findings. Hence, establishing standardized methods and unifying the optimal cutoff value are rather necessary. Thirdly, immunohistochemistry as a semiquantitative method is affected by multiple factors and should therefore have standardized criteria, or be replaced by more sensitive and specific detection methods. Finally, most evidence on the acquired resistance is limited to preclinical studies.

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Disclosure
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