Minireview
Molecular determinants of anti-EGFR sensitivity and resistance in metastatic colorectal cancer

Since 2004, the clinical impact of monoclonal antibodies (mAbs) targeting the epidermal growth factor receptor (EGFR) on patients with metastatic colorectal cancer (MCRC) has been clearly established. The combination of these biological agents with conventional chemotherapy has led to a significant improvement in response rate, progression-free survival and overall survival in first-line as well as in second- or third-line treatment of MCRC. However, the high variability of response and outcome in MCRC patients treated with these anti-EGFR mAbs has highlighted the need of identifying clinical and/or molecular predictive markers to ensure appropriate use of targeted therapies. The presence of somatic KRAS mutations has been clearly identified as a predictive marker of resistance to anti-EGFR in MCRC, and the use of anti-EGFR mAbs is now restricted to patients with no detectable KRAS mutation. Several studies have indicated that amplification of EGFR, overexpression of the EGFR ligands and inactivation of the anti-oncogene TP53 are associated with sensitivity to anti-EGFR mAbs, whereas mutations of BRAF and PIK3CA and loss of PTEN expression are associated with resistance. Besides these somatic variations, germline polymorphisms such as those affecting genes involved in the EGFR pathway are associated with sensitivity to anti-EGFR mAbs, whereas mutations of IRAK4, IRAK1 and IRAK2 in patients with metastatic colorectal cancer (MCRC). Cetuximab corresponds to a chimeric mouse human IgG1 mAb, and panitumumab is a fully human IgG2 mAb.

Indeed, several randomised trials conducted in chemorefractory as well as in chemonaive MCRC patients have reported significant results for cetuximab and panitumumab monotherapy or chemotherapy-based (CT) regimens (Cunningham et al., 2004; Jonker et al., 2007; Van Cutsem et al., 2007, 2009a; Bokemeyer et al., 2009). In a randomised phase II trial including irinotecan-refractory MCRC patients, Cunningham et al. (2004) reported that cetuximab plus irinotecan significantly improved the response rate and progression-free survival (PFS) when compared with cetuximab alone (22.9 vs 10.8% and 4.1 vs 1.5 months, respectively). Recently, a phase III randomised trial conducted by Van Cutsem et al. (2009a,b) showed that in chemonaive MCRC patients, the addition of anti-EGFR to irinotecan-based CT lead to an 8.2% increase in the objective response (46.8 vs 38.4%), a 0.9-month increase in the PFS (8.9 vs 8 months) and a 1.3-month increase in the overall survival (OS) (19.9 vs 18.6 months). Although the response to anti-EGFR mAbs observed in some patients has confirmed that EGFR activation is oncogenic, as predicted by cellular and animal models, the molecular mechanisms underlying EGFR activation in colorectal cancer remain obscure and are probably heterogeneous. This situation contrasts with in lung adenocarcinoma in which the key mechanism of EGFR activation underlying sensitivity to EGFR inhibitors corresponds to activating mutations within the EGFR tyrosine kinase domain. Although the use of anti-EGFR mAbs was initially restricted to MCRC patients with a detectable expression of EGFR by immunochemistry (IHC), the lack of IHC predictive value and the heterogeneous clinical response have highlighted the need to identify reliable markers predictive of response to anti-EGFR mAbs (Chung et al., 2005).

Two types of molecular predictive markers have been investigated. The majority of studies published so far have analysed somatic alterations affecting effectors of EGFR pathways, such as the receptor–ligand complex, the RAS–RAF–mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3 kinase (PI3K)–Akt–PTEN transduction cascades and p53 (Moroni et al., 2005; Lièvre et al., 2006, 2008; Benvenuti et al., 2007; Di Fiore et al., 2007; Frattini et al., 2007; Khambata-Ford et al., 2007; Amado et al., 2008; De Roock et al., 2008; Di Nicolantonio et al., 2008; Oden-Gangloff et al., 2009; Van Cutsem et al., 2009a). Studies have indicated that amplification of EGFR and overexpression of the EGFR ligands are associated to sensitivity to anti-EGFR mAbs,
whereas mutations of BRAF, of PIK3CA and loss of PTEN expression are associated with resistance (Figure 1, bottom panel). The second type of predictive markers investigated are germline polymorphisms of genes involved in the EGFR pathway (Khambata-Ford et al, 2007; Graziano et al, 2008; Garm Spindler et al, 2009) or within the immunoglobulin receptors, considering the potential role of antibody-dependent cellular cytotoxicity (ADCC) in the action of anti-EGFR mAbs (Zhang et al, 2007; Bibeau et al, 2009). At the present time, the only molecular marker predictive of the response to anti-EGFR mAbs, which has been unambiguously validated in MCRC by numerous studies, is the somatic mutation of KRAS as a marker of resistance to anti-EGFR (Lie`vre et al, 2006, 2008; Di Fiore et al, 2007; Frattini et al, 2007; Amado et al, 2008; De Roock et al, 2008; Van Cutsem et al, 2009a). This has led to systematic KRAS genotyping in MCRC patients and to the restriction of anti-EGFR mAbs to patients with no detectable KRAS mutation. Nevertheless, KRAS mutations are obviously not the only determinants of the clinical response to anti-EGFR.

**OVERVIEW OF THE EGFR PATHWAY**

The receptors of EGF are composed of homodimers or heterodimers of four related glycoproteins: HER1 (or EGFR), HER2 (or Erbb2), HER3 and HER4 (Figure 1, top panel). These receptors are composed of an extracellular ligand-binding domain, a transmembrane segment and an intracellular protein tyrosine kinase domain. In a normal cell, activation of EGFR is induced by the binding of the ligands to the ectodomain (Ciardiello and Tortora, 2001, 2008). Approximately ten ligands can activate the EGFR pathway. The ligands for HER1/EGFR are EGF, TGF-α, HR-EGF, amphiregulin, epiregulin and VGF; hereregulins are the ligands of Her-3; NRG2, NRG3, heregulins and β-cellulin are the ligands of ErbB-4, and Her-2 is an orphan receptor. Binding of the ligands will induce EGFR homodimerisation (EGFR-EGFR) or heterodimerisation (ERBB2-HER2; EGFR-HER3 or EGFR-HER4) and then ATP-dependent phosphorylation of tyrosine residues located within the intracellular domain. The phosphorylated EGFR will then lead to the activation of the RAS–RAF–MAPK kinase and the PI3K–Akt transduction cascades. The RAS–RAF–MAPK kinase is the major downstream signalling route of the EGFR pathway and it controls cell-cycle progression, differentiation, survival and in particular, the G1/S-phase transition (Ciardiello and Tortora, 2008). The RAS proteins are encoded by three genes, which are mutually exclusive.

**KRAS MUTATION: A VALIDATED PREDICTIVE MARKER OF RESISTANCE TO ANTI-EGFR**

Since 2004, the predictive value of somatic KRAS mutation, in terms of resistance to anti-EGFR mAbs, has been established by numerous studies. These studies (see Table 1), mainly focusing on mutations of codons 12 and 13, and more recently on codon 61, have been based on molecular analyses of tumour-extracted DNAs from patients included in retrospective studies, as well as in prospective randomised trials (Lie`vre et al, 2006, 2008; Benvenuti et al, 2007; Di Fiore et al, 2007; Frattini et al, 2007; Amado et al, 2008; De Roock et al, 2008; Bokemeyer et al, 2009; Loupakis et al, 2009a; Van Cutsem et al, 2009a). The first line of evidence that KRAS mutation was a strong predictor of resistance to anti-EGFR mAbs was the observation that in chemorefractory patients treated with cetuximab-based CT, <2% of patients with detectable KRAS mutation exhibited an objective response, whereas ~40% of patients with no detectable KRAS mutation showed a clinical response (Lie`vre et al, 2006, 2008; Benvenuti et al, 2007; Di Fiore et al, 2007, 2008a, b; Frattini et al, 2007; De Roock et al, 2008; Loupakis et al, 2009a). The impact of KRAS status in response to anti-EGFR mAbs was also documented by trials in chemorefractory patients comparing cetuximab or panitumumab monotherapy vs best supportive care, with an increase in the response rate in patients with no KRAS mutation from 8 to 12.8% and from 10 to 17%, respectively (Karapetis et al, 2008).

In chemonaive patients with no detectable KRAS mutation, the addition of anti-EGFR antibodies to CT increased the objective response from approximately 46 to 60% when compared with CT.
alone (Douillard et al., 2008, 2009; Bokemeyer et al., 2009; Van Cutsem et al., 2009a). Except in the recent COIN randomised trial (Maughan et al., 2009), the addition of anti-EGFR mAbs to CT has also been shown to lead to a significant increase in the median PFS when compared with irinotecan- or oxaliplatin-based CT alone in chemonaive as well as in chemorefractory patients. In contrast, in mutant KRAS patients, no clinical benefit was observed from the combination of anti-EGFR plus CT. The effect of PFS when compared with irinotecan- or oxaliplatin-based CT.

PIK3CA and PTEN alterations

The demonstration that in MCRC patients KRAS mutations confer resistance to anti-EGFR has validated the hypothesis that targeting EGFR will be inefficient if activation of the EGFR transduction cascade results from somatic alterations affecting downstream effectors (Figure 1, bottom panel). This led to an investigation of the predictive value of somatic alterations affecting other effectors. In colorectal cancer, activation of the PIK3CA pathway can result from inactivation of PTEN expression, detected in 19.9% of patients using another IHC scoring system (Moroni et al., 2009b). PTEN is a tumour suppressor that interferes with the activation of PI3K and PI3KCA, phosphorylating the plasma membrane lipid second messenger PIP-3 to generate PIP-3. The loss of PTEN function induces an increase in PIP-3 concentration and PIK3CA pathway activation.

Nevertheless, these results were not confirmed by the recent study of Prenen et al. (2009), in which PIK3CA mutations were identified in 5 of 39 (13%) responders vs 18 of 160 (11%) nonresponders (P = 0.781). These contradictory results highlight the fact that the association between PIK3CA mutations and resistance to anti-EGFR is not yet validated. 

**PTEN** exerts an effect as a tumour suppressor by dephosphorylating the plasma membrane lipid second messenger PIP-3 generated by the action of PI3KCA. The loss of PTEN function induces an increase in PIP-3 concentration and PIK3CA pathway activation. IHC and FISH analyses are the two main methods that have been used to evaluate the PTEN status in studies focused on anti-EGFR resistance. However, the molecular mechanisms leading to PTEN inactivation are heterogeneous and include genomic deletions, inactivating mutations and promoter hypermethylation, indicating that PTEN integrity cannot be explored by a simple method. In a series of 27 MCRC patients, Frattini et al. (2007) reported that PTEN alteration detected by IHC in primary tumours was significantly associated with cetuximab resistance. More recently, Loupakis et al. (2009b) have analysed by IHC the impact of PTEN cytoplasmic staining intensity in 85 primary tumours and 55 related metastases from patients treated with cetuximab plus irinotecan. First, using a specific scoring system for PTEN expression, these authors found that IHC staining was concordant in 5 of 39 (13%) responders vs 23% in patients with no PIK3CA mutation and to 31% in patients with no KRAS/PIK3CA mutations (Sartore-Bianchi et al., 2009). Nevertheless, these results were not confirmed by the recent study of Prenen et al. (2009), in which PIK3CA mutations were identified in 5 of 39 (13%) responders vs 18 of 160 (11%) nonresponders (P = 0.781). These contradictory results highlight the fact that the association between PIK3CA mutations and resistance to anti-EGFR is not yet validated.

**OTHER SOMATIC PREDICTIVE MARKERS OF RESPONSE TO ANTI-EGFR**

PIK3CA and PTEN alterations

The demonstration that in MCRC patients KRAS mutations confer resistance to anti-EGFR has validated the hypothesis that targeting EGFR will be inefficient if activation of the EGFR transduction cascade results from somatic alterations affecting downstream effectors (Figure 1, bottom panel). This led to an investigation of the predictive value of somatic alterations affecting other effectors. In colorectal cancer, activation of the PIK3CA pathway can result either from activating PIK3CA mutations or from inactivation of the PIK3CA protein. Since 2005, the impact of PIK3CA mutations on MCRC patients treated with anti-EGFR has been investigated by several studies that have reported a PIK3CA mutation frequency ranging from 6 to 13% (Moroni et al., 2005; Liévre et al., 2006; Perrone et al., 2009; Prenen et al., 2009; Sartore-Bianchi et al., 2009).

In the studies of Moroni et al. (2005), Liévre et al. (2006) and Perrone et al. (2009), none of the responders to anti-EGFR therapy had a PIK3CA mutation, but this association was not statistically significant, given the limited number of patients. In contrast, a significant association between the presence of PIK3CA mutation and response to anti-EGFR has been recently reported by Sartore-Bianchi et al. (2009) in a study including 110 patients treated with anti-EGFR mAbs. A total of 32 (29%) patients had a KRAS mutation and 15 (13%) had a PIK3CA mutation. When compared with the unselected population, the response rate to anti-EGFR increased from 20 to 26% in patients with no KRAS mutation, to 23% in patients with no PIK3CA mutation and to 31% in patients with no KRAS/PIK3CA mutations (Sartore-Bianchi et al., 2009).

Nevertheless, these results were not confirmed by the recent study of Prenen et al. (2009), in which PIK3CA mutations were identified in 5 of 39 (13%) responders vs 18 of 160 (11%) nonresponders (P = 0.781). These contradictory results highlight the fact that the association between PIK3CA mutations and resistance to anti-EGFR is not yet validated.
correlation of PTEN gene copy number and sensitivity to anti-EGFR. The absence of PTEN gene dosage alteration was significantly associated with response rate, whereas PTEN gene deletion was correlated with worse PFS. No significant association was found using PTEN IHC expression. All these results clearly indicate that no obvious association has yet been established between PTEN alterations and anti-EGFR therapy in MCRC patients. It should also be noted that commercially available anti-PTEN antibodies do not always provide reproducible results in IHC. Further studies are needed to determine the most efficient and reproducible methods for PI3KCA and PTEN analysis in order to clearly evaluate the predictive value of each marker separately or in combination with KRAS status.

**BRAF mutation**

Two studies support that V600E mutation, resulting in strong activation of the BRAF protein downstream to KRAS, was associated with shorter PFS and OS in MCRC chemorefractory patients treated with anti-EGFR mAbs (Di Nicolantonio et al, 2008; Laurent-Puig et al, 2009). Nevertheless, it seems that whatever the treatment, this mutation in MCRC is mainly associated with poor prognosis, which may interfere with its predictive value regardless of anti-EGFR mAbs (Tol et al, 2010; Van Cutsem et al, 2010).

**TP53 mutations**

In a normal cell, the p53 protein exerts an effect not only as a guardian of the genome, which is activated when DNA damage occurs, but also as a policeman of oncogenes, which become active when oncogenes are inappropriately activated, thus inducing apoptosis and/or senescence (Efeyan and Serrano, 2007; Halazonetis et al, 2008). Alteration of the p53 pathway has been reported to be systematically observed in non-small cell lung cancer with activating EGFR mutations, suggesting that p53 inactivation is required to allow expansion of a cell with EGFR pathway activation (Mounavar et al, 2007). On the other hand, it has been shown that PI3K signalling activates p53-mediated growth suppression, suggesting that p53 exerts an effect as a brake for the activated PI3K transduction cascade (Kim et al, 2002). Taken together, these data led us to formulate the hypothesis that activation of the EGFR pathway should be oncogenic, and therefore anti-EGFR antibodies should only be efficient in tumours, only if p53 is inactivated. We have recently analysed the impact of TP53 mutations on 64 MCRC patients treated with cetuximab plus CT (Oden-Gangloff et al, 2009). In this series, TP53 mutations were found in 41 of 64 patients and were significantly associated with controlled disease (P = 0.037) and higher PFS (20 vs 12 weeks, P = 0.004). In the subgroup of patients without KRAS detectable mutation, we have also found that controlled disease and PFS were significantly improved in TP53 mutated patients when compared with TP53 nonmutated patients. These results might be explained not only by the fact that EGFR activation is oncogenic only if TP53 is inactivated, but also by the fact that inactivation of TP53 could be one of the mechanisms leading to EGFR activation. Cellular models and studies on larger MCRC series are necessary to clarify the relationship between TP53 status and sensitivity to anti-EGFR.

**EGFR gene copy number and expression of its ligands**

EGFR gene copy number A common mechanism of EGFR activation is the increase in the EGFR copy number corresponding to the gain of copies or amplification of the chromosome 7 region on which EGFR is located. A copy number increase of EGFR resulting from chromosome 7 polysomy probably does not have the same biological significance than amplification of the EGFR locus. In contrast to breast cancer in which tumour cells exhibit HER2 amplification in homogeneous regions with a relationship between expression and gene amplification, colorectal cancer is characterised by a heterogeneous EGFR gene copy number pattern and, furthermore, no correlation has been established between the EGFR copy number and the EGFR IHC staining. Based on the results obtained in patients with metastatic breast cancer treated with trastuzumab, in which ERBB2 amplification correlates with sensitivity to mAb therapy, Moroni et al (2005) investigated the impact of the EGFR gene copy number, assessed by FISH, on the anti-EGFR mAb response in a series of 30 MCRC patients. This study reported a positive significant association between an increased EGFR gene copy number and the response rate to cetuximab or panitumumab. Since 2005, many reports have investigated the relationship between EGFR copy number and response to mAb therapy in MCRC, but only five of them restricted the analysis to the subgroup of wild-type KRAS patients. In the study by Cappuzzo et al (2008), in a series of 85 chemorefractory patients treated with cetuximab, determined that the best cutoff significantly associated with the response rate and PFS was 2.92 EGFR gene copy number per cell. Interestingly, these authors further analysed their population according to the EGFR copy number cutoff previously reported by Santore-Bianchi et al (2009) and observed a significant association for response rate but not for PFS. It can be concluded from the different studies

### Table 2 Influence of EGFR status (gene copy number) on subject response in KRAS wild-type MCRC patients treated with anti-EGFR mAb-based CT

| Study            | N   | EGFR cutoff | PFS (months) | OS (months) |
|------------------|-----|-------------|--------------|-------------|
|                  |     |             | Responders   | Nonresponders |
| Moroni et al (2005) | 20  | 3+          | 6            | 1           |
|                  |     | <3          | —            | —           |
| Personeni et al (2008) | 58  | 2.83+       | 10           | —           |
|                  |     | 2.83        | —            | 12          |
| Laurent-Puig et al (2009) | 116 | 2+          | 20           | 13 — NA     |
|                  |     | 2+          | 80           | 10          |
| Scartozzi et al (2009) | 44  | 2.6+        | 14           | 29          |
|                  |     | 2.6        | 16           | 9           |
|                  |     | <2.6       | 29           | 50          |
|                  |     | 2.12       | 10.6         | 18          |
|                  |     | <2.12      | 10.3         | 15          |
| Tol et al (2010) | 155 | 3+          | 21.9         | 19.2        |
|                  |     | 3+          | 23.7         | 25.4        |

Abbreviations: CI = confidence interval; CISH = chromogenic in situ hybridisation; CR = complete response; CT = chemotherapy; EGFR = epidermal growth factor receptor; FISH = fluorescence in situ hybridisation; mAb = monoclonal antibody; MCRC = metastatic colorectal cancer; NA = not assessable; OS = overall survival; PFS = progression-free survival; PR = partial response; PD = progressive disease; SD = stable disease. *FISH. **CISH.
published so far that only a high EGFR gene copy number, resulting from gene amplification and observed in a small fraction of patients, is probably predictive of anti-EGFR sensitivity in MCRC patients.

Expression of EGFR ligands Few studies have investigated the predictive role of EGFR ligand expression on anti-EGFR response in MCRC. A transcriptome analysis of 164 CRCs, using a 640 probe set, showed that the EGFR ligands, epiregulin (EREG) and amphiregulin (AREG), were highly expressed in 25% of the samples (Khambata-Ford et al, 2007). In this study, analysis of metastatic frozen samples from a population of 80 MCRC patients treated with cetuximab monotherapy showed that patients with disease control expressed the EGFR ligands at a higher level than nonresponders. Moreover, patients with tumours exhibiting higher EREG or AREG expression had significantly longer PFS, suggesting that these tumours were EGFR dependent and were therefore particularly sensitive to the inhibition of the ligand–receptor interaction by anti-EGFR mAb (Khambata-Ford et al, 2007).

In a recent study performed on paraffin-embedded samples in refractory MCRC patients treated with cetuximab and irinotecan, the KRAS status and the EREG and AREG mRNA expression were first determined on 220 primary samples and the results were then validated on a series of 67 patients (Jacobs et al, 2009). The mRNA levels of EREG and AREG significantly correlated with the absence of detectable KRAS mutation. When ligand expression level was combined with the KRAS status, the median OS was 65 weeks in patients with no detectable KRAS mutation and a high EREG expression vs 31 weeks (P = 0.01) in patients without detectable KRAS mutation and low EREG expression (Jacobs et al, 2009).

GERMLINE POLYMORPHISMS ASSOCIATED WITH CLINICAL RESPONSE TO ANTI-EGFR MABS
Three types of germline polymorphisms suspected of modulating the response to anti-EGFR have been investigated. The first corresponds to a CA repeat present within the EGF intron 1, which has been shown to modulate EGFR transcription efficiency, the longer (L) repeats being associated with a reduced transcription when compared with the shorter (S) ones. The second, located within the EGF gene, corresponds to a SNP (G > A, c.444G > A). In 110 chemotherapy-refractory MCRC patients treated with cetuximab–irinotecan, Graziano et al (2008) showed that EGF intron-1 variant (SIS vs L/L and L/S) and EGF61 variant (G/G vs A/A and A/G) were significantly associated with a better OS. Similar results were obtained on EGFR61 genotypes in a study on chemoresistant MCRC patients treated with cetuximab plus irinotecan CT (Garm Spindler et al, 2009). In patients with no detectable KRAS mutation, homozygous phenotypes (A/A and G/G) were associated with a lower progression rate (19 vs 60%) than EGFR61 A/G patients (P = 0.006) and a significant increase in OS (17.1 vs 5.9 months). The third type of germline polymorphism investigated, in the context of anti-EGFR therapies in MCRC, corresponds to polymorphic amino-acid substitutions within the receptors of the Fc fragment of the immunoglobulins (Igs). The H131R and V158F variations within the FC\gamma RIa and FC\gamma RIla have been shown to modify the affinity of the Ig receptors and are therefore predicted to modulate the ADCC in patients receiving anti-EGFR IgG1 mAb. To date, only four studies have found significant association between these polymorphisms and the clinical outcome. In a first study performed in a series of 39 chemoresistant patients treated with cetuximab monotherapy, Zhang et al (2007) found that the presence of the FCGR2A 131 H allele and of the FCGR3A 158 F allele were significantly associated with better PFS; however, these results could not be replicated on a larger series including 130 subjects (Lurje et al, 2008). In 64 chemoresistant MCRC patients treated with cetuximab-based CT, Bibeau et al (2009) found that the FCGR2A 131 H/H and FCGR3A 158 V/V genotypes, predicted to result in a higher affinity of the Ig to the receptor, were significantly associated with better PFS. In this study, analysis of these germline polymorphisms in the subgroup of patients with no detectable KRAS mutation showed that the median PFS was 9.6 months in the 131 H/H or 158 V/V patients vs 4.6 months in the subgroup of patients harbouring the other genotypes (P = 0.015).

In contrast, a more recent study (Pander et al, 2010), performed on KRAS wild-type patients receiving chemotherapy plus cetuximab, showed that subjects homozygous for the FCGR3A 158 F allele have a better PFS than those bearing other allelic combinations. Several factors may account for these discrepancies; although ethnic origin is unlikely to be involved, previous treatment regimens and association of irinotecan with cetuximab could modify patients’ response. Further studies involving testing integrity of the EGFR pathway are clearly needed to clarify this point.

MUTATIONAL STATUS IN PRIMARY COLORECTAL ADENOCARCINOMAS AND RELATED METASTASES
One of the main subjects of debate is the relevance of KRAS genotyping on primary tumours, whereas anti-EGFR mAbs are used to treat a metastatic disease. Indeed, some publications have indicated that concordance of mutational status of KRAS and other EGFR downstream effectors between primary and metastases is not absolute. In a series of 48 CRC patients, an overall concordance of 92% was observed between primary tumours and metastases for the presence of mutations within KRAS exon 2 and BRAF exon 5 (Arite et al, 2008); however, in the specific subgroup of patients with KRAS mutation, 23% of discordance was observed between the two tumour sites. In a study including 38 MCRCs, Molinari et al (2009) reported a concordance for KRAS and BRAF mutational status of 92% and 100%, respectively. More recently, the analysis of KRAS, BRAF and PIK3CA in tumour invasion fronts, lymph nodes and distant metastases revealed a discordance between primary tumours and lymph node metastases of 31, 4 and 13%, respectively (Baldus et al, 2010). A discordance between primary tumours and distant metastases was observed for KRAS in 10% of the cases and for PIK3CA in 5% of the cases (Baldus et al, 2010). In a large series of > 800 samples tested for KRAS, using a highly sensitive SNaPshot-based method (Di Fiore et al, 2007), we observed a discordant KRAS mutational status between tumours and metastases in 10% of the paired samples (Lamy et al, 2010). These studies clearly indicate that given the genetic heterogeneity and genetic evolution of CRC under treatment, the most appropriate issue on which molecular profiling should be performed remains to be determined.

CONCLUSION AND PERSPECTIVES
In conclusion, the numerous studies published since 2006 on the markers of sensitivity and resistance to anti-EGFR mAbs in MCRC have clearly shown that KRAS, BRAF, PIK3CA and other genetic predictive markers, using either candidate gene approaches, global analysis based on pangenomic/transcriptomic/proteomic portraits or cellular or animal models. Some technical and conceptual questions concerning KRAS genotyping remain unsolved. One important question under debate is to define in MCRC patients, the most appropriate tissue on which DNA
analysis should be performed in the context of targeted therapies. Indeed, most studies published so far are based on the analysis of genetic material extracted from primary tumours, whereas targeted therapies are mainly directed at metastatic disease treatment. As highlighted by several recent studies, this approach may have limitations: (1) the primary tumour may not always be available; (2) as indicated above, several genetic changes may occur between the primary tumour and the corresponding metastases; and (3) genetic analyses performed on paraffin-embedded tissues may generate artefacts because of fragmentation and chemical modification of DNA. Therefore, we think, as previously shown, that genetic analysis based on highly sensitive detection from bloodstream of circulating mutant DNA might be of critical interest (Di Fiore et al., 2008a; Yen et al., 2009).

As the new markers will have to be validated by independent studies, the challenge for the clinician, before treating patients, will be the progressive integration of these markers in the routine and the development of a decisional algorithm, which should probably be stratified first by KRAS genotyping. In patients with KRAS mutations, the choice of the most appropriate treatment is problematic. Although the use of anti-EGFR mAbs is not recommended in these patients, it should be underlined that disease stability can be observed in up to 50% of chemorefractory patients treated with anti-EGFR alone in association with CT (Di Fiore et al., 2008b). In these patients, targeted therapies will probably be based, in the future, on molecules able to inhibit effectors of the EGFR transduction cascade and located downstream from the KRAS protein.

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