Plasticity of Resistance and Sensitivity to Anti-Epidermal Growth Factor Receptor Inhibitors in Metastatic Colorectal Cancer

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Contents

Abstract

Colorectal cancer (CRC) is one of the most prevalent cancers and the second leading cause of cancer mortality worldwide. Survival in the metastatic setting has been gradually improved by the addition to cytotoxic chemotherapy of agents targeting the vascular endothelial growth factor (VEGF) and epidermal growth factor receptor (EGFR). Considerable heterogeneity exists within CRC due to the varied genetic and epigenetic mechanisms involved in differing pathways of carcinogenesis. The knowledge of molecular abnormalities underlying colorectal tumourigenesis and the progression of dysplastic precursors to invasive and ultimately metastatic lesions has advanced in recent years by comprehensive sequencing studies. From these genome-scale analyses, we know that a handful of genes are commonly affected by somatic mutations,

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whereas recurrent copy-number alterations and chromosomal translocations are rarer in this disease. Even though some of these molecular abnormalities make genes acting as drivers of cancer progression, translation of this recognition for therapeutic purposes is still limited, encompassing only as standard of care the exclusion of *RAS*-mutated cancers for better selecting patients to candidate to EGFR-targeted therapy with monoclonal antibodies. However, the effort of ameliorating molecular selection should not be considered exhausted by demonstration of *RAS* and *BRAF*-induced resistance, as the genomic landscape of response to EGFR blockade has been demonstrated to be wider and dynamically multifaceted. In this chapter we will review main molecular biomarkers of de novo (primary) and acquired (secondary) resistance to EGFR-targeted monoclonal antibodies in metastatic CRC and discuss therapeutic implications.

Keywords
Cetuximab • Colorectal cancer • EGFR • Liquid biopsy • Panitumumab • RAS

1 Introduction

Colorectal cancer (CRC) is one of the most prevalent cancers and the second leading cause of cancer mortality worldwide (Siegel et al. 2017). Survival in the metastatic setting has been gradually improved by the use of fluorouracil/leucovorin in doublet or triplet combinations with oxaliplatin (FOLFOX) and/or irinotecan (FOLFIRI) together with agents targeting the vascular endothelial growth factor (VEGF) and epidermal growth factor receptor (EGFR) (Ciombor et al. 2015). Nowadays, precision oncology significantly influences current and emerging therapies for metastatic CRC patients by the demonstration that the molecular refinement has led to substantial improvements in clinical outcomes (overall survival, OS and progression-free survival, PFS) (Douillard et al. 2013). This approach, together with advancement in surgical resection for selected patients with limited liver and/or lung involvement, has indeed significantly improved median overall survival to over 40 months from diagnosis (Heinemann et al. 2014; Schwartzberg et al. 2014; Van Cutsem et al. 2011; Venook et al. 2014).

Considerable heterogeneity exists within colorectal tumours due to the varied genetic and epigenetic mechanisms involved in differing pathways of carcinogenesis. The knowledge of molecular abnormalities underlying colorectal tumourigenesis and the progression of dysplastic precursors to invasive and ultimately metastatic lesions has advanced in recent years by comprehensive sequencing studies (Cancer Genome Atlas Network 2012). From these genome-scale analyses, we know that a handful of genes are commonly affected by somatic mutations, whereas recurrent copy-number alterations and chromosomal translocations are rarer in this disease (Vogelstein et al. 2013). Even though some of these molecular abnormalities make genes acting as drivers of cancer progression, translation of this recognition for therapeutic purposes is still limited, encompassing only as standard of care the exclusion of *RAS*-mutated cancers for better selecting patients to candidate to EGFR-targeted therapy with monoclonal antibodies. It should be acknowledged

that the process of refining molecular selection for these therapeutics has paralleled, and in some instances enhanced the quest for targets actionable at the clinical level. In particular, well-known oncogenes such as *BRAF* and *ERBB2*, that are now among most promising targets in this tumour (Corcoran et al. 2014; Sartore-Bianchi et al. 2016b), have been studied as biomarkers of resistance to anti-EGFR therapies. On the other hand, the effort of ameliorating molecular selection should not be considered exhausted by demonstration of *RAS* and *BRAF*-induced resistance, as the genomic landscape of response to EGFR blockade has been demonstrated to be wider (Bertotti et al. 2015) and dynamically multifaceted (Siravegna et al. 2015). In this chapter we will review main molecular biomarkers of de novo (primary) and acquired (secondary) resistance to EGFR-targeted monoclonal antibodies in metastatic CRC and discuss therapeutic implications.

2 Primary Resistance

Primary resistance is defined as ab initio refractoriness to anticancer treatment. It could be explained by resistance-conferring factors pre-existing in the bulk of tumour cells (Leto and Trusolino 2014) that we may not recognize due to tumour heterogeneity (Tannock and Hickman 2016). Intratumour heterogeneity is present early in cancer development and cancer treatment selects for resistant subclones. The main therapeutic implication is that a single drug may not be adequate enough to treat a genetically heterogeneous tumour, since a pre-treatment cancer cell population harbouring resistance genetic alteration, even if present at a low frequency, can contribute to therapeutic failure and poor outcome in a Darwinian fashion (Fisher et al. 2013; Tannock and Hickman 2016; Misale et al. 2014; Sartore-Bianchi et al. 2016a). Among molecular biomarkers of resistance to EGFR-targeted therapies in CRC, alterations in the *RAS/RAF/MAPK* pathway have been the most consistently shown to predict resistance, and *RAS* mutations have been the only reaching clinical grade. The landscape of molecular alterations impacting on sensitivity or resistance to these therapeutics is still interspersed with many other biomarkers, however they should be considered only in the context of translational research.

RAS In the EGFR signalling pathway a dominant downstream direction involves the activation of the G-protein intermediate *RAS*, and subsequent signalling through *BRAF*, *MEK*, and *ERK* (the *MAP* kinase pathway). Mutations in the *RAS* family of proto-oncogenes (*KRAS*, *NRAS*, *HRAS*) result in constitutive activation of *MAP* kinase pathway signalling that is independent of activation of receptor tyrosine kinases such as EGFR. In CRC *KRAS* is the predominantly mutated isoform, whereas *NRAS* mutations are found more rarely and the *HRAS* mutated isoform is extremely uncommon and therefore not tested by routine (Prior et al. 2012). As a consequence, the upstream pharmacological blockade of the receptor can be circumvented for cancer progression by constitutive signalling of activated GTP-bound *RAS* forms (Bardelli and Siena 2010). This discovery by our group and others has been acknowledged as a landmark step for the evolution of precision

medicine in the field of CRC (Benvenuti et al. 2007; Lièvre et al. 2006; Ushijima and Yoshino 2016). The most common *RAS* mutations in colon cancer occur at exon-2 (codons 12 and 13) of *KRAS*, and are present in about 42% of cases (Peeters et al. 2015). It is estimated that among tumours classified as *KRAS* exon 2 wild type, about one out of five carries other mutations in *KRAS* exon 3 (3.8–4.3%), *KRAS* exon 4 (6.2–6.7%), *NRAS* exon 2 (2.9–3.8%), *NRAS* exon 3 (4.2–4.8%) or *NRAS* exon 4 (0.3–0.5%), overall accounting for an additional total 11% of the so-called extended *RAS* or pan-*RAS* mutated CRCs (Peeters et al. 2015; Sorich et al. 2015). Initially, resistance to anti-EGFR mAbs was reported as associated with mutations confined to those occurring in codons 12 and 13 of exon 2 of the *KRAS* gene (Benvenuti et al. 2007; Lièvre et al. 2006). These findings were subsequently confirmed in retrospective analyses of large clinical trials (Amado et al. 2008; Van Cutsem et al. 2011) and reached clinical grade (Schmoll et al. 2012). However, retrospective analyses of multiple trials demonstrated that also additional mutations in exons 2, 3 and 4 of *KRAS*/*NRAS* exerted a similar predictive negative effect (De Roock et al. 2010), and this has been confirmed by analyses of phase III pivotal studies for development of anti-EGFR moAbs (Douillard et al. 2013; Van Cutsem et al. 2015). A meta-analysis of nine randomized controlled trials also confirmed that the treatment with both cetuximab and panitumumab had superior efficacy in terms of PFS and OS for extended *RAS* WT (i.e. *KRAS* exons 3 and 4 and *NRAS* exons 2, 3 and 4) compared with the expanded *RAS* mutant subgroup, and the efficacy was not significantly different between the expanded *RAS* mutant and *KRAS* exon 2 mutant subgroups (Sorich et al. 2015). Based on these studies, the screening of expanded *RAS* mutations for patients with metastatic CRC is currently recommended by principal treatment guidelines and included in the license for panitumumab and cetuximab for metastatic CRC (Van Cutsem et al. 2016) (Fig. 1). However, even though the presence of *RAS* mutations is a prerequisite for anti-EGFR moAbs, efficacy, its absence does not warrant response, as only 40–50% of patients with *RAS* WT tumours achieve objective response to treatment (Misale et al. 2014). Finally, the *KRAS* gene has been found not only to be mutated but also amplified, although in a very small percentage of CRC patients (0.7%), and this amplification has been observed as a mechanism in both primary and acquired resistance to EGFR inhibitors (Valtorta et al. 2013).

BRAF *BRAF* is an oncogene that encodes a downstream effector of *KRAS* in the *MAPK* pathway. In CRC tumours, mutations leading to constitutive *BRAF* activation have been reported in 47% of hyper-mutated tumours and 3% of non-hypermutated tumours (Cancer Genome Atlas Network 2012), and approximately 5–10% of CRC tumours overall (De Roock et al. 2010). Of note, *KRAS* and *BRAF* mutations are mainly mutually exclusive in CRC (Richman et al. 2009). The presence of an activating mutation in *BRAF* conveys a strong prognostic significance, with mutated tumours conferring a poor prognosis with aggressive tumour biology and shorter OS, regardless of the treatment regimen (Safaee Ardekani et al. 2012). It should be noted that this prognostic impact should be regarded as confined to the *BRAF* V600E mutation, whereas *BRAF* mutations affecting codons 594 and

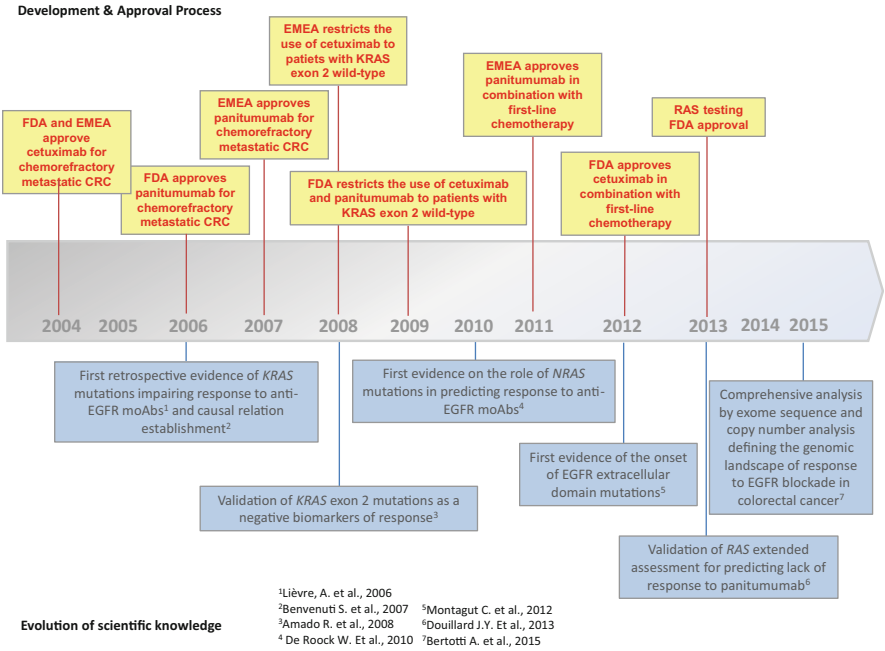

Fig. 1 Parallel development of approval processes and evolution of the knowledge of molecular mechanisms of resistance to EGFR-targeted monoclonal antibodies in metastatic colorectal cancer

596, occurring in <1% of CRCs, display differences in terms of clinicopathologic features and less adverse clinical outcome (Cremolini et al. 2015).

Many different retrospective studies and meta-analyses by our group and others have suggested that the presence of a *BRAF* mutation also confers a weaker benefit from anti-EGFR monoclonal antibodies, negatively interfering with EGFR blockade (Benvenuti et al. 2007; Bertotti et al. 2015; Di Nicolantonio et al. 2008; Yuan et al. 2013). For these patients, even though there is not a formal demonstration by first-line combination trials that cetuximab or panitumumab are not effective (Douillard et al. 2013; Van Cutsem et al. 2015; Rowland et al. 2015), a general consensus exists for using an ab initio more intense chemotherapy regimens or enrolling in clinical studies with *BRAF*-directed strategies (Sartore-Bianchi et al. 2016a) to counteract the poor prognosis, making anti-EGFR treatment a less preferable option.

Family of HER *HER2* – the human epidermal growth factor receptor 2 (*HER2*/neu) is a well-established oncogenic driver in breast and gastric cancer, and recent data are highlighting a renewed role for this molecular target in CRC (Sartore-Bianchi et al. 2016a, c). Expression rates in historical series for CRC range widely from 1.6% (Ingold Heppner et al. 2014) to 47.4% (Park et al. 2007), but the sample

size inclusion of distinct subgroups and the use of different diagnostic methods and scoring systems may account for this variability. In three of the most recent series, the rate of *HER2* positivity (immunohistochemistry [IHC] score of 2+/3+, or *HER2* gene amplification by in-situ hybridization) ranged from 1.6 to 6.3% (Ingold Heppner et al. 2014; Seo et al. 2014; Richman et al. 2016). In a consensus study aimed at defining CRC-specific criteria for *HER2* positivity (Valtorta et al. 2015), we demonstrated that there is a clinically sizeable 5% fraction of *KRAS* wild-type CRC patients harbouring *HER2*-positive tumours, and this knowledge paralleled translational research with demonstration of *HER2* as a therapeutic target (Sartore-Bianchi et al. 2016c).

On the other hand, *HER2* amplification has been proposed as a biomarker of resistance to anti-EGFR antibodies. In 2011, we recognized *HER2* amplification as a potential mechanism of primary resistance to cetuximab within a "quadruple wild type" population (*KRAS*, *NRAS*, *BRAF* and *PIK3CA* wild type) of immune-compromised mice harbouring CRC xenograft (patient-derived xenografts, PDX, a.k.a. "xenopatients") (Bertotti et al. 2011). The same adverse effect was shown by retrospective analyses of patients treated with cetuximab (Yonesaka et al. 2011) or panitumumab (Martin et al. 2013; Sartore-Bianchi et al. 2016c). Interestingly, this effect has been demonstrated to be dependent on the level of amplification (Martin et al. 2013) and to contribute to both de novo and acquired drug resistance (Yonesaka et al. 2011). These results altogether suggest that patients with *HER2*-amplified CRC should be enrolled in clinical trials with *HER2*-targeted therapies regardless of having received a previous anti-EGFR inhibitor (Sartore-Bianchi et al. 2016c).

Together with amplification, also *HER2* somatic mutations have been reported to occur in CRC at a frequency of about 3%, independently or concomitantly with amplification (Cancer Genome Atlas Network 2012). *HER2* activating mutations tend to fall in several hotspots (residues 309–310 in the extracellular domain and residues 755–781 and 842 in the kinase domain), and cause oncogenic transformation of colon epithelial cells (Kavuri et al. 2015). It has been shown that these mutations produce resistance to cetuximab and panitumumab in preclinical models of CRC including PDXs and that dual *HER2*-targeted therapy with either trastuzumab plus neratinib or trastuzumab plus lapatinib can induce tumour regression (Kavuri et al. 2015).

HER3 also has been described to have a role as a potential biomarker of resistance to anti-EGFR treatments. In a cohort of metastatic CRC patients treated in second- or third-line therapy with irinotecan and cetuximab, *HER3* overexpression was associated with shorter PFS and OS (Scartozzi et al. 2011). *HER3* has been found to be also mutated in approximately 11% of CRC patients (Jaiswal et al. 2013), even though further studies are needed to elucidate the predictive role in conferring primary or acquired resistance to EGFR inhibitors.

cMET The mesenchymal-epithelial transition (*MET*) protooncogene encodes for *c-MET*, a receptor with tyrosine kinase activity targeting hepatocyte growth factor (HGF) (Trusolino et al. 2010). Activation of this pathway by gene amplification has

been implicated in metastatic progression of CRC, since *MET* has been historically reported to be overexpressed in 50% and amplified in 2–10% of primary CRCs, with the rate of amplification increasing up to 18–89% in metastases (Di Renzo et al. 1995; Zeng et al. 2008). However, more recent studies adopting ISH technologies together with newer NGS approaches clearly indicate that *MET* amplification ranges between 0.4 and 2.2% of cases (Cancer Genome Atlas Network 2012; Raghav et al. 2016), in both primary tumours and metastatic deposits (Raghav et al. 2016). As far as resistance to anti-EGFR therapies is concerned, in a recent complete exome sequence and copy number analyses of 129 PDX and targeted genomic analyses of 55 patient tumours, *MET* amplification was identified as a cause of primary resistance to cetuximab in 2.3% of cases (Bertotti et al. 2015). All in all, *MET* amplification rarely occurs and accounts for primary resistance in CRC, even though it is among alterations predominantly involved in acquired resistance (see below section on acquired resistance).

3 Biomarkers of Sensitivity

EGFR Gene Copy Number Gain During the initial development of EGFR antibodies in metastatic CRC, it was predicted that EGFR protein expression would be required for therapeutic efficacy. Therefore, initially only EGFR-expressing CRCs were allowed into clinical trials (Saltz et al. 2004; Cunningham et al. 2004). However, subsequent analyses demonstrated a lack of association between EGFR expression and response (Chung et al. 2005; Moroni et al. 2008), eventually leading to decline of this restriction in label. On the other hand, an association was found with an *EGFR* gene copy number increase (Moroni et al. 2005) and this was originally postulated by our group as a biomarker of sensitivity to anti-EGFR therapy (Moroni et al. 2005). This finding was confirmed in preclinical experiments (Bertotti et al. 2015) as well as clinical cohorts (Personeni et al. 2008; Sartore-Bianchi et al. 2007; Scartozzi et al. 2009), however overt EGFR gene amplification is rarely observed in CRC, and correlation with response has been mainly based on balanced chromosome 7 polysomy rather than amplification, even though it is unknown whether the former could have an equivalent biologic effect in driving cancer progression and predicting response to EGFR-targeted agents. Also, detection by ISH methods of copy number gain suffers from issues of standardization, as it was shown by an interlaboratory reproducibility ring study conducted by our group (Sartore-Bianchi et al. 2012). In the end, even though an association between an increase in EGFR gene dosage and response has been established and confirmed especially in case of amplification (Bertotti et al. 2015), still this is not a validated biomarker for EGFR-targeted agents.

IRS2 The insulin receptor substrate (IRS) family of proteins are cytoplasmic adaptor that mediate through phosphorylation signalling between receptor tyrosine kinases of IGF1R and downstream effectors with roles in normal growth, metabolism and differentiation such as PI3K activation. IRS2 over-expression has been

reported in 6–7% of MSS CRCs (Cancer Genome Atlas Network 2012; Nunes et al. 2015). A preclinical study has shown that IRS2 over-expression in the absence of an upstream activator leads to AKT phosphorylation and also increases CRC cell adhesion (Day et al. 2013). Amplifications and sequence alterations in the tyrosine kinase receptor adaptor gene IRS2 have been identified also in tumours with increased sensitivity to anti-EGFR therapy. Expression analyses of 100 CRC PDXs with wild-type *KRAS*, *NRAS*, *BRAF* and *PIK3CA* showed increased IRS2 levels as a significant predictor of cetuximab sensitivity in cases without other mechanisms of resistance to EGFR therapy (Bertotti et al. 2015).

4 Acquired (Secondary) Resistance

Acquired (or secondary) resistance refers to disease progression during an ongoing treatment that was initially effective. This occurs eventually in all metastatic CRCs and can be caused by gene mutations of the molecular target arising during treatment, expansion of resistant subclones in the context of intratumour heterogeneity selected under the pressure of cancer treatment, upregulation of a partially inhibited pathway or activation of alternative pathways. Even in patients with a refined *RAS* extended wild-type status, the tumour becomes refractory after a median of 5.2 months by developing secondary resistance (Kim et al. 2016). Liquid biopsy for monitoring of circulating tumour DNA (ctDNA) has been demonstrated by our group and others to be a powerful diagnostic tool for understanding dynamic mechanisms of tumour evolution in CRC (Misale et al. 2014; Van Emburgh et al. 2014). Molecular analysis performed on a tissue biopsy from a tumour is indeed a single snapshot in time subjected to selection bias due to spatial tumour heterogeneity, whereas analyses performed by liquid biopsy overcome this limitation and unveiled main mechanisms of acquired resistance to EGFR inhibitors.

RAS and BRAF Various studies from our group and others have already demonstrated concordance between liquid biopsy and tumour-tissue biopsy for molecular characterization of clinically validated biomarkers for CRC such as *KRAS* and *BRAF* mutations (Thierry et al. 2014; Siravegna et al. 2015). At the same time, experiments in preclinical models (Misale et al. 2012) and translational studies with longitudinal monitoring by liquid biopsy have revealed clonal evolution during therapies with anti-EGFR antibodies showing that mutant *RAS* clones rise in blood during EGFR blockade (Misale et al. 2012) and decline upon withdrawal of treatment (Siravegna et al. 2015). It is conceivable that these subclones are less fit in the untreated tumour and acquire fitness as a consequence of adaptation to the perturbation induced by the treatment itself. Further, anti-EGFR pressure gives rise to multiple emergent circulating mutations of *MAPK* pathway within the same patient, with and individual average of almost three mutations (Bettegowda et al. 2014), in what has been called a “war of clones”. Interestingly, the relative frequency of individual *KRAS* alleles is similar but not identical in primary and acquired resistance: we firstly reported the secondary occurrence of codon

61 mutations (Misale et al. 2012) that rarely occur in anti-EGFR naïve patients, and now it is established that these mutations in either the *KRAS* or *NRAS* genes are more prevalent in the acquired than in the primary resistance setting (Bettegowda et al. 2014).

Family of HER *HER2* amplification has been associated also to acquired resistance to anti-EGFR moAbs. Yonesaka et al. showed that patients with acquired resistance to cetuximab had an increased percentage of *HER2* amplification in post-treatment samples compared to the proportion present in pretreatment tumour cells (Yonesaka et al. 2011). These authors reported that in this context hyper-activation of *HER2* signalling is triggered not only by *HER2* amplification but also by overproduction of heregulin, a *HER3* ligand.

MET HGF-induced *MET* activation has been proposed as a mechanism of cetuximab resistance in CRC (Liska et al. 2011). We firstly reported by tissue analysis and longitudinal ctDNA monitoring that *MET* amplification is also associated with secondary resistance to anti-EGFR monoclonal antibodies (Bardelli et al. 2013). This observation was paralleled by functional analysis in CRC preclinical models indicating that HGF plays an important role in driving *MET*-mediated resistance to anti-EGFR monoclonal antibodies. HGF stimulation was demonstrated to be sufficient to confer cetuximab and panitumumab resistance both in vitro and in vivo, thus supporting the possibility that HGF overexpression by cancer cells or the surrounding stroma might be an independent mechanism of acquired (or primary) resistance to cetuximab (Bardelli et al. 2013). Further, we recently showed that *MET* amplification can simultaneously arise together with *KRAS* amplification within the same patient after initial response to EGFR inhibition in a context of substantial intrapatient heterogeneity (Sartore-Bianchi et al. 2016d).

EGFR External Domain Mutations Mutations affecting the extracellular domain of the EGFR have not been reported in the absence of treatment with EGFR inhibitors in metastatic CRC (Esposito et al. 2013; Montagut et al. 2012). In 2012 it was firstly demonstrated that that cell lines with acquired resistance to cetuximab showed a mutation of the extracellular domain of the EGFR, 1476C>A, leading to a substitution of serine to arginine at amino acid 492 (S492R) (Montagut et al. 2012). This mutation interferes with binding to cetuximab but not to panitumumab in preclinical models and parallel observations in patients with acquired resistance to cetuximab. These findings have been confirmed subsequently in retrospective cohorts (Arena et al. 2015) and at the clinical level in the ASPECCT trial of cetuximab versus panitumumab for chemorefractory metastatic CRC, where the EGFR S492R was detected in 1% of patients in the panitumumab arm and 16% in the cetuximab arm in post-treatment plasma ctDNA samples (Newhall et al. 2014). It has been subsequently discovered that several other mutations in the EGFR extracellular domain can occur in preclinical models of cetuximab resistance (S464L, G465R and I491M) and in patients (R451C and K467T), mainly located in the cetuximab-binding region, except for the R451C mutant (Arena et al. 2015).

From a clinical standpoint there are important therapeutic implications, since *EGFR* ectodomain mutations prevent binding to cetuximab but a subset is permissive for interaction with panitumumab (Arena et al. 2015), and it has been shown that new generation EGFR inhibitors such as the anti-EGFR antibody mixture Sym004 that bound and abrogated ligand-induced phosphorylation of EGFR mutants can overcome cetuximab/panitumumab resistance mediated by EGFR mutations (Sánchez-Martín et al. 2016). Interestingly, emerging knowledge is also indicating that there is a differential kinetic in the appearance of *EGFR* versus *RAS* mutated alleles during EGFR-targeted treatment, since *RAS* mutations emerge earlier than *EGFR* ECD variants (Van Emburgh et al. 2014). Subclonal *RAS*, but not *EGFR* extracellular domain mutations, have been shown indeed to be present in CRC samples obtained before exposure to EGFR blockade. Finally, retrospective analysis in a clinical cohort indicates that patients who experience greater and longer responses to EGFR blockade preferentially develop EGFR extracellular domain mutations, while *RAS* mutations emerge more frequently in patients with smaller tumour shrinkage and shorter progression-free survival (Van Emburgh et al. 2016).

5 Conclusions

Approved anti-EGFR antibodies cetuximab and panitumumab provide significant clinical benefit for the treatment of CRC, with patients in the metastatic setting now reaching an OS of more than 30 months. These advances have been made thanks to evolution of surgical techniques for metastasectomy, introduction of new agents, but to an important extent also to a refinement of molecular selection based on newer pharmacogenomics strategies. In this regard, the discovery of mechanisms of resistance to anti-EGFR monoclonal antibodies has enhanced clinical results, paving the way for a precision medicine approach in this disease. This effort should not be considered exhausted, as the genomic landscape of response to EGFR blockade has been demonstrated to be starred by a myriad of molecular abnormalities and, thanks to application of liquid biopsy for longitudinal analysis of the instable tumour genome, dynamically multifaceted (Fig. 2). Next challenges in this field will include the translation of current knowledge of tumour evolution mechanisms in the clinic for preventing or overcoming resistance at the individual patient level.

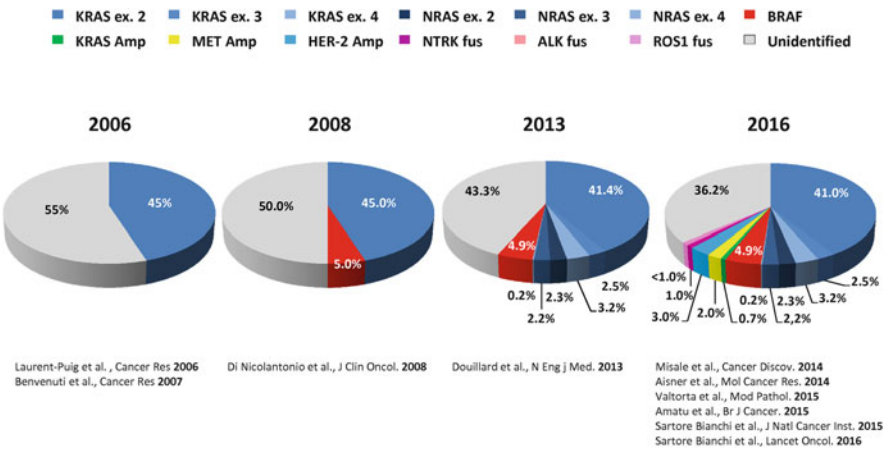

Fig. 2 Biomarkers of resistance to EGFR-targeted monoclonal antibodies and known actionable targets for therapy in metastatic colorectal cancer. Evolution from 2006 until today

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