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Optimising the use of cetuximab in the continuum of care for patients with metastatic colorectal cancer

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ABSTRACT

The anti-epidermal growth factor receptor (EGFR) monoclonal antibody cetuximab in combination with chemotherapy is a standard of care in the first-line treatment of *RAS* wild-type (wt) metastatic colorectal cancer (mCRC) and has demonstrated efficacy in later lines. Progressive disease (PD) occurs when tumours develop resistance to a therapy, although controversy remains about whether PD on a combination of chemotherapy and targeted agents implies resistance to both components. Here, we propose that some patients may gain additional clinical benefit from the reuse of cetuximab after having PD on regimens including cetuximab in an earlier treatment line. We conducted a non-systematic literature search in PubMed and reviewed published and ongoing clinical trials, focusing on later-line cetuximab reuse in patients with mCRC. Evidence from multiple studies suggests that cetuximab can be an efficacious and tolerable treatment when continued or when fit patients with mCRC are retreated with it after a break from anti-EGFR therapy. Furthermore, on the basis of available preclinical and clinical evidence, we propose that longitudinal monitoring of *RAS* status may identify patients suitable for such a strategy. Patients who experience progression on cetuximab plus chemotherapy but have maintained *RAS* wt tumour status may benefit from continuation of cetuximab with a chemotherapy backbone switch because they have probably developed resistance to the chemotherapeutic agents rather than the biologic component of the regimen. Conversely, patients whose disease progresses on cetuximab-based therapy due to drug-selected clonal expansion of *RAS*-mutant tumour cells may regain sensitivity to cetuximab following a defined break from anti-EGFR therapy. Looking to the future, we propose that *RAS* status determination at disease progression by liquid, needle or excisional biopsy may identify patients eligible for cetuximab continuation and rechallenge. With this approach, treatment benefit can be extended, adding to established continuum-of-care strategies in patients with mCRC.

INTRODUCTION

Colorectal cancer is the fourth most prevalent cancer type in the world and causes nearly 700 000 deaths per year worldwide.[1] Patients diagnosed with metastatic colorectal cancer (mCRC) have a limited number of systemic therapeutic options available as well as local therapies, such as resection, ablation or transhepatic irradiation via the injection of yttrium 90. Encouragingly, the median overall survival (OS) in recent phase III first-line trials in *RAS* wild-type (wt) mCRC now exceeds 30 months.[2–5]

The available biologic agents usually planned for first-line therapy include the epidermal growth factor receptor (EGFR) monoclonal antibodies (mAbs) cetuximab or panitumumab and the anti–vascular endothelial growth factor (VEGF) agent bevacizumab. All three agents, plus additional targeted drugs such as aflibercept (VEGF inhibitor), regorafenib (receptor tyrosine kinase inhibitor) and ramucirumab (anti-VEGF receptor 2), have also shown efficacy in the second and later lines of therapy.[6–12] Mounting evidence from randomised, phase III trials and meta-analyses suggests that many patients with *RAS* wt tumours may experience improved survival outcomes when chemotherapy is combined with cetuximab compared with combination with bevacizumab in the first-line setting.[3 5 13–15] Retrospective analyses and meta-analyses have shown that the survival benefit is especially pronounced in patients with *RAS* wt, left-sided primary tumours.[16–19] Although patients with right-sided tumours appear to derive less significant benefits from all available therapies for mCRC in terms of overall response rate (ORR), progression-free survival (PFS) and overall survival (OS) than do those with tumours originating in the left side of the colon,[18–20] some patients may benefit from cetuximab-based therapy if the goal of treatment is response and subsequent cytoreduction.[18 19] Indeed, cetuximab has been shown to yield a very good ORR, early tumour shrinkage (ETS) and depth of response in patients with *RAS* wt tumours. Such alternative metrics of response have been shown to be associated with improvements in

survival[5] compared with bevacizumab in the randomised, phase III, first-line FIRE-3 trial (5-FU, Folinic Acid and Irinotecan (FOLFIRI) Plus Cetuximab Versus FOLFIRI Plus Bevacizumab in First Line Treatment Colorectal Cancer), which evaluated patients with *RAS* wt mCRC.[5 21]

Currently, amplifications and mutations in several genes other than *RAS*, detected pretherapy, are also being investigated as potential predictive biomarkers of response to anti-EGFR therapy. The *BRAF* V600E variant[22 23] appears to be a negative prognostic marker, but due to the relatively low number of colorectal cancers harbouring *BRAF* mutations (and as not all *BRAF* mutations confer resistance), controversy remains regarding the impact of this finding.[24] Other markers of interest include alterations in the EGFR pathway, mutations in the phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α gene (*PIK3CA*) and amplification of the human epidermal growth factor receptor 2 gene, *HER2* (mostly in patients with anti-EGFR-refractory, *RAS* wt disease).[25–33] Furthermore, hypermethylation of tumour DNA appears to be potentially predictive of poorer clinical outcomes in patients receiving anti-EGFR therapy.[34 35] The individual frequencies of all of these mutations and amplifications are low,[25 27 33 36 37] and conflicting data exist regarding whether several of these genomic alterations are true biomarkers for cetuximab resistance. Additional biomarkers that are currently being explored as predictive of cetuximab response include VEGF2, *MET* (mesenchymal-epithelial transition factor mutations), fibroblast growth factor receptor mutations, platelet-derived growth factor, epiregulin, amphiregulin, mIR 31–3p and hepatocyte growth factor.[29 30 38–46] Presently, however, *RAS* mutations and tumour sidedness remain the only robust factors when selecting patients for anti-EGFR therapy in accordance with the drug labels for both cetuximab and panitumumab and the established international guidelines[10 47] and are the most informative for treatment decisions in mCRC.

Colorectal tumours that are *RAS* wt at baseline can evolve resistance to anti-EGFR therapy via a *RAS* status 'shift' to mutated status to escape the drug's effects.[39] It is now known that this change occurs when the *RAS* wt tumour cell population is diminished during anti-EGFR therapy, while pre-existing or newly evolving *RAS*-mutant subclones can proliferate and become detectable by DNA testing.[27 29 48] Other gene mutations, including in *BRAF*, may be subject to the same tumour heterogeneity principles and drug selection dynamics. Another key mechanism of acquired resistance that occurs in approximately 25% of patients treated with anti-EGFR therapy are mutations in the extracellular domain (ECD) of EGFR that prevent further binding of anti-EGFR mAbs to EGFR. Importantly, EGFR ECD alterations emerge as a means for cancer cells to circumvent EGFR blockade—acquired resistance—and do not apparently exist as a mechanism of primary resistance in anti-EGFR-naïve patients. Interestingly, the frequency and type of EGFR ECD mutation varies, depending on previous treatment with cetuximab or panitumumab, and each different EGFR ECD mutation may potentially confer resistance to cetuximab only, panitumumab only or both mAbs.[28 49–51] Finally, these mutations frequently coexist in the same patient at the moment of disease progression, as multiple *RAS*, *BRAF* and EGFR clones are detectable in cell-free circulating tumour DNA (ctDNA) by liquid biopsy.[29 52]

Extending the continuum of care through the delivery of as many lines of efficacious therapy as possible is desirable so that patients with progressive disease (PD) have multiple potentially beneficial treatment options to explore before transitioning into palliative care. For mCRC, however, patients with *RAS* wt tumours are generally assigned two lines of intensive therapy. They receive a first-line anti-EGFR mAb (cetuximab or panitumumab) or bevacizumab plus doublet/triplet chemotherapy (5-fluorouracil, leucovorin and oxaliplatin (FOLFOX), 5-fluorouracil, leucovorin and irinotecan (FOLFIRI), 5-fluorouracil, leucovorin, oxaliplatin and irinotecan (FOLFOXIRI)). This is followed by second-line 'switch' of either the biologic, the chemotherapy backbone or both on PD. For patients who respond to a therapy and whose disease does not progress but require an interruption due to their preference or recovery from drug toxicity or surgery, a pause-and-resume (commonly referred to as stop-and-go or reintroduction/re-exposition) schedule of a biologic with or without chemotherapy can help them to complete the full planned treatment and maintenance period.[53–55]

Multiple studies with dedicated second-line, third-line or mixed second-line and further-line patient populations have indicated improved survival and ORR with cetuximab monotherapy or cetuximab in combination with chemotherapy[56–62] and with panitumumab monotherapy or panitumumab plus chemotherapy.[57 63–67] Overall, all current guidelines conclude that patients with *RAS* wt mCRC who do not receive a biologic in the first line (but are fit enough to receive it in later lines) should be considered candidates for an anti-EGFR therapy in the next available line. Then, in the third line and beyond, patients are usually offered other targeted or chemotherapy-based interventions, often with limited expectations of clinical benefit. This is because, currently, it is widely accepted that after patients with left-sided, *RAS* wt mCRC have received an anti-EGFR antibody and bevacizumab sequentially (in either order) and experience progression, their disease is permanently resistant to both the biologic and cytotoxic agents that have been administered. However, this conclusion may not be accurate for all patients as some tumours may retain sensitivity to the targeted agent (although becoming resistant to the cytotoxic agents in the chemotherapy programme) or regain sensitivity after a 'treatment break' from those specific agents.[68–73] Thus, many patients exhaust standard treatment options while maintaining a good performance status and are therefore not ready to transition to a solely palliative-care approach. Here, we review the evidence for continuation or reuse strategies beyond the first line with

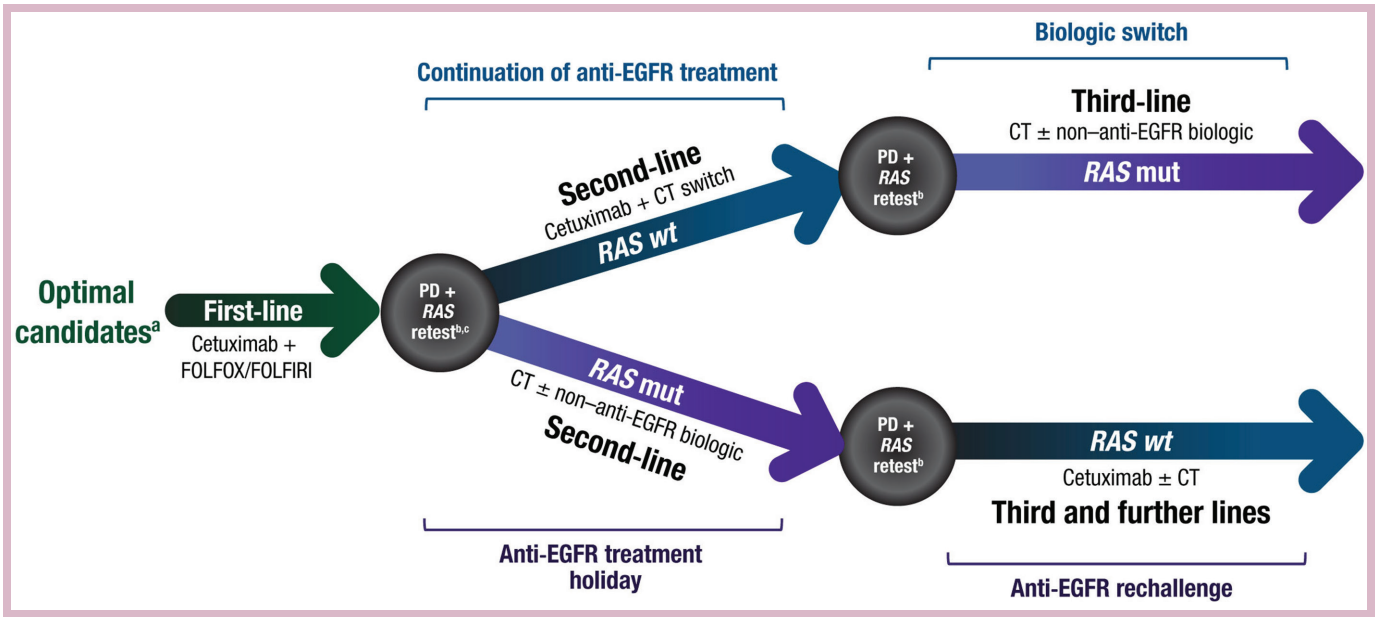

Figure 1 A proposed treatment model for the decision-making process when choosing between cetuximab continuation vs rechallenge. [a]Typically patients with left-sided, *RAS* wt mCRC or those with right-sided, *RAS* wt mCRC in need of rapid tumour shrinkage. [b]By liquid biopsy. [c]Other evidence-based biomarkers can also be included in the panel of tests when feasible. EGFR, epidermal growth factor receptor; FOLFIRI, 5-fluorouracil, leucovorin and irinotecan; FOLFOX, 5-fluorouracil, leucovorin and oxaliplatin; mut, mutant; PD, progressive disease; wt, wild-type.

cetuximab, a well-established agent for *RAS* wt mCRC, as a method of expanding the therapeutic options available to patients and maximising the number of (potentially curative) therapeutic lines before the initiation of palliative care (figure 1).

Notably, the bulk of available evidence for anti-EGFR retreatment stems from trials that use cetuximab rather than panitumumab. Furthermore, the two mAbs are known to behave somewhat differently in the context of treatment sequencing.[74–81]

Selecting cetuximab-eligible patients by liquid biopsy testing

Because the predominance of specific tumour cell subclones is dictated by selection pressures such as targeted therapy, retreatment strategies with cetuximab could be individualised with longitudinal tracking of detectable *RAS* mutations (and any future confirmed predictive biomarker of response) as a measure of potential sensitivity to cetuximab.[27 29 30 39 51] Depending on the location of metastatic disease in some patients, traditional tumour needle or excisional biopsies may be invasive and risky. Thus, a less invasive method is desirable (eg, liver needle aspirates or plasma sampling). This 'liquid' biopsy can fulfil the retesting requirement by providing a non-invasive method for the detection and analysis of ctDNA. Of note, >75% of patients with advanced CRC have been shown to have detectable ctDNA (79.8% in the RASANC study (*RAS* Mutation Testing in the Circulating Blood of Patients With Metastatic Colorectal Cancer)),[82 83] and multiple useful platforms currently exist to allow the identification of tumour mutations via PCR of ctDNA: digital PCR[84]; digital next-generation sequencing[85]; BEAMing (beads, emulsion, amplification and magnetics)[86]; pyrophosphorolysis-activated polymerisation[87]; personalised profiling by deep sequencing[88] and tagged-amplicon deep sequencing.[89] Liquid biopsies have proven to be clinically robust. In a cohort of 98 patients with mCRC, Schmiegel *et al*[90] used BEAMing to test for *RAS* tumour mutation status and found 91.8% concordance between ctDNA and tissue-based testing. Similarly, Vidal *et al*[91] detected a 93% concordance rate in a cohort of 115 patients with mCRC. In the prospective, multicentre RASANC study (n=425), the concordance rate was 83.7% in the overall population and was even higher in patients with their primary tumour in place, liver metastasis and synchronous disease.[83]

Overall, *RAS* is the only widely recognised predictive molecular biomarker of cetuximab response and the only biomarker with extensive evidence of a high concordance between tissue-based and blood-based testing. However, we will refer to patients under consideration in this article more generally as 'cetuximab-eligible patients', for whom this definition is subject to change as additional research is published in the coming years. Furthermore, no subgroup analyses by tumour location are available for the studies on treatment beyond progression discussed here. However, we use the term 'cetuximab-eligible' to encompass optimal candidates for cetuximab-based therapy, including those with left-sided, *RAS* wt mCRC, and those with right-sided, *RAS* wt mCRC for whom cytoreduction is a key treatment goal, or who have previously responded to cetuximab-based therapy.

Continuation treatment strategy

Patients who received and progressed on cetuximab-containing regimens in the preceding line may retain cetuximab sensitivity,[68–70 92] having instead become refractory to the chemotherapy backbone (table 1). In a study conducted by Feng *et al*,[69] patients with *RAS* wt tumours whose disease progressed during first-line cetuximab plus chemotherapy were randomised to receive a different chemotherapy backbone with or without cetuximab as second-line treatment. The cetuximab-continuation group demonstrated better PFS, OS and disease control rates and a potentially better ORR than did the chemotherapy-only (no cetuximab continuation) group. Extended *RAS* analysis for the retrospective study revealed that baseline *RAS* wt status correlated with response to continuation of cetuximab; ETS during first-line cetuximab-based treatment also correlated with improved efficacy outcomes during second-line cetuximab continuation. Additionally, Ciardiello *et al*[68] found evidence of a survival benefit from continued cetuximab plus FOLFOX in patients whose disease progressed during first-line cetuximab plus FOLFIRI and had *KRAS/NRAS/BRAF/PIK3CA* wt tumours. Both Feng *et al* and Ciardiello *et al* switched chemotherapy backbones when going from first-line to second-line treatment and used a chemotherapy switch alone as a comparator arm. Therefore, if sensitivity to cetuximab was preserved, switching to a non-cross-resistant chemotherapy backbone after PD could reinvigorate responsiveness. No new safety findings were identified from cetuximab continuation in any of these studies.[68 69 72]

By contrast, the majority of panitumumab retreatment trials have followed the stop-and-go (re-exposition) model rather than continuation (ie, patients did not experience progression on panitumumab immediately before retreatment).[93 94] For example, a phase II Japanese study examining the administration of panitumumab plus chemotherapy following six cycles of first-line panitumumab plus FOLFOX is currently ongoing (SAPPHIRE (Safety and Efficacy Study of mFOLFOX6 + Panitumumab Combination Therapy and 5-FU/LV + Panitumumab Combination Therapy in Patients With Chemotherapy-naïve Unresectable Advanced Recurrent Colorectal Carcinoma); NCT02337946); therapy prolongation appears to be planned in the absence of PD, and the focus of the study is the feasibility of oxaliplatin treatment extension rather than panitumumab continuation.[95]

As has been demonstrated, deriving maximum benefit from cetuximab before introducing a biologic switch could extend the number of potentially efficacious lines of therapy for fit patients and thereby optimise the continuum of care.[69] We advance the hypothesis that using ETS during first-line cetuximab and extended *RAS* analysis at PD can enable effective patient selection for this therapeutic strategy (figure 1).[69] For patients whose tumours transition from cetuximab sensitive to insensitive (eg, by converting to *RAS* mutant), a treatment break

Table 1 Evidence for cetuximab continuation plus chemotherapy backbone switch in patients with *KRAS* wt (or retrospectively evaluated *RAS* wt) mCRC whose disease has progressed on cetuximab plus chemotherapy treatment

Line of treatment	Study	Previous regimen	Arm A			Arm B		
			PFS, mo	OS, mo	ORR, %	PFS, mo	OS, mo	ORR, %
Dedicated second line	Feng *et al*[69] (2016; retrospective study)	Cetuximab+mFOLFOX6 or FOLFIRI	Cetuximab+CT switch (n=102)			CT switch (n=96)		
			6.3*	17.3*	18.6	4.5	14.0	9.4
	Ciardiello *et al*[68] (2015; CAPRI-GOIM†)	Cetuximab+FOLFIRI	Cetuximab+FOLFOX (n=34)			CT switch (n=32)		
			6.9*	23.7	29.4	5.3	19.8	9.4
	Vladimirova *et al*[72] (2016)	Cetuximab+FOLFOX	Cetuximab+FOLFIRI (n=20)					
			6.5‡	–	20.0			
Mixed third and further lines	Fora *et al*[70] (2013)	Standard-dose cetuximab+irinotecan	High-dose cetuximab+irinotecan					
			2.8‡	6.6‡	(DCR=45)			

All studies included a *KRAS* wt population, except where indicated.
*P<0.05.
†Retrospective extended *RAS/BRAF/PIK3CA* wt.
‡OS2 and PFS2 shown instead of all OS and PFS.
CT, chemotherapy; DCR, disease control rate; FOLFIRI, 5-fluorouracil, leucovorin and irinotecan; FOLFOX, 5-fluorouracil, leucovorin and oxaliplatin; mCRC, metastatic colorectal cancer; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; wt, wild-type.

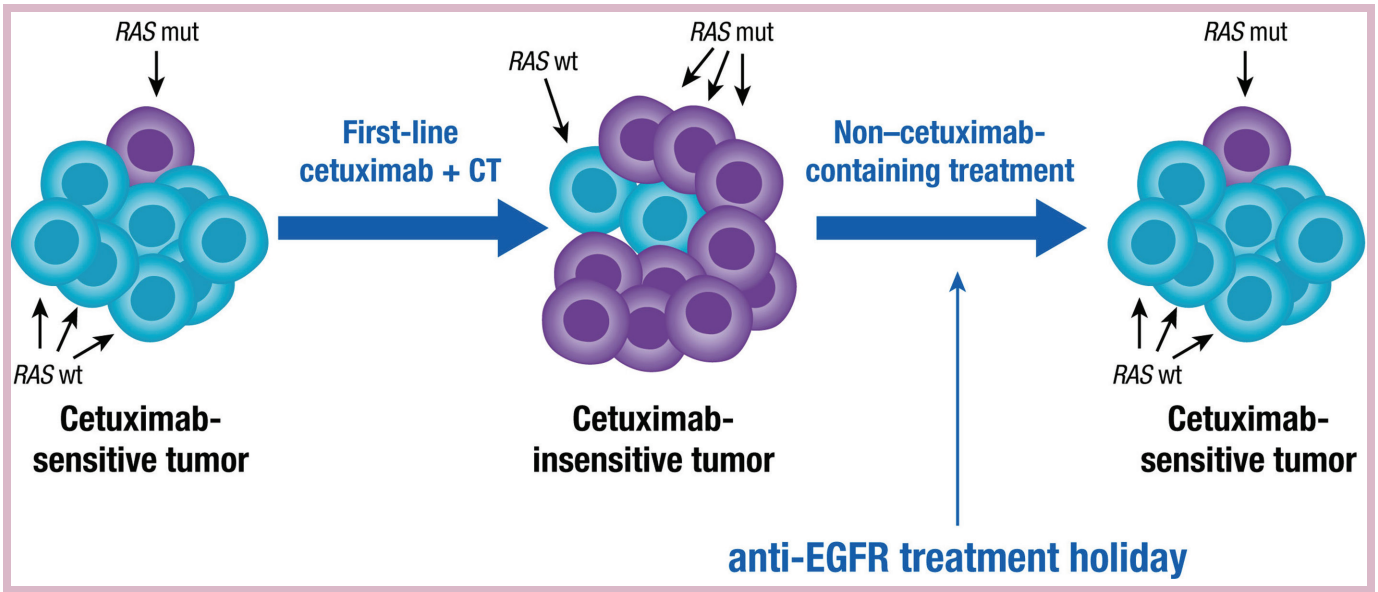

Figure 2 A model for the biological rationale for rechallenge therapy: clonal selection in heterogeneous baseline *RAS*[a] wt tumours during anti-EGFR therapy. [a]Additional or secondary acquired mechanisms of resistance can also be driven by mutations in the extracellular domain of the EGFR, and other potential biomarkers continue to be investigated. EGFR, epidermal growth factor receptor; mut, mutant; wt, wild-type.

followed by cetuximab rechallenge may allow another means of extending the continuum of care. Here, again, liquid biopsy for *RAS* status may prove highly informative.

Rechallenge treatment strategy

Evidence of rechallenge therapy in multiple tumour types has been published.[96–98] Because no conclusive randomised trials have been completed to test whether longitudinal *RAS* status monitoring can identify patients who regain cetuximab sensitivity, we will outline the available preliminary preclinical (biological) and clinical support for this treatment approach.

Biological evidence

Tumour cells in a patient who is receiving treatment are constantly under selection pressures from the therapy being administered. Studies have suggested that mutations conferring resistance to therapy can arise during treatment. For example, when retested for *(K)RAS* status, >50% of patients with acquired resistance to first-line anti-EGFR therapy demonstrate a 'switched' status from *(K)RAS* wt to mutant,[27 29–31 39 51 82] and the opposite observation (tumours switching from mutant *KRAS* to undetectable mutant status) has been made during bevacizumab treatment.[99] Importantly, from studies monitoring plasma levels of *RAS*-mutant ctDNA, even short 'holidays' off anti-EGFR therapy may restore tumours to a cetuximab-sensitive state.[39 68 100] Intratumour heterogeneity and drug-selected clonal evolution can account for these 'switches' (figure 2). The suggested mechanism for such a switch is the constant presence of a small number of *RAS*-mutant cell subclones persisting in the tumours, rather than de novo *RAS* mutations appearing in previously *RAS* wt tumour cells.[73] Thus, a tumour can contain predominantly *RAS* wt clones, test as *RAS* wt and respond to cetuximab treatment until the *RAS* wt clones are depleted. In this environment, the *RAS*-mutant clones have the opportunity to continue proliferating and surviving and thus come to represent the dominant tumour subclonal population.[51 100] Indeed, an in vitro study of two separate *KRAS* wt colorectal cancer cell populations showed that, under selection pressure from cetuximab treatment, the surviving population developed *KRAS* amplifications; when allowed to proliferate for 160 days without additional cetuximab treatment, the population of cells achieved significantly lower levels of *KRAS* amplification.[39]

The principles of clonal selection likely also apply to other mechanisms of resistance to anti-EGFR therapy.[29] Indeed, a number of genetic modifications beyond *RAS* have been related to acquired drug resistance to anti-EGFR therapy. Interestingly, most of these different genetic aberrations are related to or have influence on MEK-ERK pathway activity.[101] The reversion of these additional resistance mechanisms during the anti-EGFR therapy break is controversial and not yet clearly demonstrated. Finally, it should be noted that even if arising on-treatment mutations have been identified, the exact threshold associated with resistance to anti-EGFR therapy has not been established, and some patients continue to experience disease control for several months after *RAS*-mutant clones emerge.[51 102] Overall, there is a strong biological rationale for cetuximab rechallenge.

Clinical evidence

There is currently abundant clinical evidence for *RAS* status switch[103 104] but little evidence analysing the direct relationship between a return to undetectable *RAS*-mutant status and a resensitisation to cetuximab.[39] However, there exists clinical evidence for the feasibility of cetuximab rechallenge in patients with baseline *(K)RAS* wt mCRC whose disease

Table 2 Evidence for cetuximab rechallenge in patients with *KRAS* wt mCRC whose disease has previously progressed on cetuximab plus chemotherapy treatment and received who have received at least one line of additional, non–anti-EGFR therapy

Study	Previous regimen
Liu *et al*[71] (2015; retrospective study)	Summary: ► Patients with *KRAS* wt mCRC received anti-EGFR therapy (cetuximab or panitumumab) based treatment – When PD occurred, patients received a break from anti-EGFR therapy (median duration, 4.6 months) – 80 patients were retreated with cetuximab ± CT ± other targeted agents Results: ► 58.0% (CR/PR/SD) ► Patients who responded to prior anti-EGFR therapy were more likely to obtain clinical benefit upon rechallenge (PFS, 4.9 vs 2.5 months; P=0.007) ► The clinical benefit rate on rechallenge showed a marginally significant association with interval time between the two anti-EGFR based therapies (P=0.053)
Santini *et al*[73] (2012)	Summary: ► Patients with *KRAS* wt mCRC received anti-EGFR therapy (cetuximab) + (FOLF)IRI – When PD occurred, patients received a break from anti-EGFR therapy (median duration, 6.0 months) – 39 patients were retreated with cetuximab + (FOLF)IRI Results: ► ORR, 53.8% (plus 35.9% SD) ► Median PFS, 6.6 months ► Interval effect not discussed
Rossini *et al*[105] (2017; CRICKET)	Summary: ► Patients with *RAS*/*BRAF* wt mCRC who became resistant to first-line cetuximab + irinotecan ► Treated with third-line cetuximab + irinotecan ► N=26 Results: ► ORR, 23% (plus 31% SD)

CR, complete response; CT, chemotherapy; EGFR, epidermal growth factor receptor; FOLFIRI, 5-fluorouracil, leucovorin, and irinotecan; IRI, irinotecan; mCRC, metastatic colorectal cancer; ORR, overall response rate; PD, progressive disease; PFS, progression-free survival; PR, partial response; SD, stable disease; wt, wild-type.

progresses on first-line anti-EGFR therapy plus chemotherapy; such patients are usually rechallenged with cetuximab with or without chemotherapy in the later-line setting after ≥3 months of therapy that does not contain an anti-EGFR targeted agent. In a study by Santini *et al*[73] (table 2), 39 patients whose disease had previously progressed on cetuximab plus irinotecan-based therapy were rechallenged with cetuximab plus irinotecan after a treatment break during which they received non-irinotecan-based chemotherapy alone. The median treatment break for these patients was 6 months, and patients had received a median of four lines of therapy prior to study enrolment. Nevertheless, median PFS was 6.6 months, and the ORR with cetuximab plus irinotecan rechallenge was 53.8% and included two complete responses. Stable disease was achieved by a further 35.9%, for a total disease control rate of 89.7%. Finally, in a recent prospective study by Gruppo Oncologico del Nord Ovest in Italy, the CRICKET study (Cetuximab Rechallenge in Irinotecan-pretreated mCRC, *KRAS*, *NRAS* and *BRAF* Wild-type Treated in 1st Line With Anti-EGFR Therapy), 27 patients with mCRC were to be treated with cetuximab plus FOLFIRI or FOLFOXIRI in the first line followed by bevacizumab plus oxaliplatin-based chemotherapy in the second line and cetuximab plus irinotecan rechallenge in the third line. The third-line ORR was 23%, with 54% of patients experiencing disease control, and the study met its primary end point.[105] Rechallenge treatment was well tolerated in all studies. The prospective biological determinations of ctDNA are ongoing.

Also noteworthy is a retrospective study by Liu *et al*[71] analysing patients with baseline *KRAS* wt status who received cetuximab (n=76) or cetuximab plus erlotinib (n=13) after a median of 4.57 months of treatment break from anti-EGFR therapy. Patients who had previously responded to anti-EGFR therapy achieved a median PFS of nearly 5 months.[71] Rechallenge treatment was found to be well tolerated. Additionally, Liu *et al*[71] suggested that the length of the anti-EGFR treatment interval may be related to the responsiveness of patients to rechallenge, on the basis of the observation that patients whose break from anti-EGFR therapy was longer than the median appeared more likely to respond. Overall, these studies suggest that good ORR and PFS can be achieved with cetuximab rechallenge in a patient population that has experienced progression on many prior lines of therapy, and these observations underscore the importance of optimising and extending the continuum of care.

One limitation of both the retrospective trial by Liu *et al* and prospective trial by Santini *et al* (except the CRICKET study) is that enrolment occurred prior to the standardisation of extended *RAS* analysis; therefore, data are not yet available for patients with *RAS* wt tumours. Furthermore, no retest was conducted for *RAS* status as liquid biopsy had not yet been validated. However, the fact that a patient population with previous progression on cetuximab-based therapy can achieve a median PFS of 5–6 months with rechallenge suggests that sensitivity to cetuximab, at least in some patients, can be meaningfully regained. However, certain selection criteria could be recommended here, such as establishing a minimum recommended treatment break length or selecting patients who had a durable response or disease stabilisation during the last cetuximab-based treatment.

Additional evidence for cetuximab rechallenge therapy can be found in case studies.[103 106 107] Of note, Siravegna *et al*[39] identified a patient with mCRC who initially achieved stable disease for 6 months with cetuximab plus irinotecan; on disease progression, the patient received XELOX (capecitabine and oxaliplatin) and experienced progression again after 3 months. The patient was then rechallenged with cetuximab plus irinotecan and achieved a partial response.

Notably, anti-EGFR rechallenge strategy data are available primarily for cetuximab but not for panitumumab. The limited data on panitumumab rechallenge come from two case reports by Siravegna *et al*,[39] in which the patients achieved a partial response and stable disease when retreated with panitumumab following *RAS* mutational status switch and reversal. Additionally, Hata *et al*[108] describe two patients with good outcomes when retreated with panitumumab after a line of non-anti-EGFR therapy, although neither patient had experienced PD during prior panitumumab-based therapy (in the earlier line, one stopped panitumumab treatment due to toxicity, and the other had completed the determined number of panitumumab cycles). Finally, some reports have described responses to panitumumab treatment after failure of cetuximab, primarily in heavily pretreated patients.[109 110]

Ongoing studies, including additional analyses of the CRICKET study (NCT02296203), a study in Japan (UMIN000016439), and a similar study in Israel (trial identification number not yet available), will help determine whether this treatment strategy is feasible and which patients are most suitable for this approach. Although no completed trial to date has tracked *RAS* mutational status longitudinally throughout treatment, progression and rechallenge, a highly relevant phase III trial (FIRE-4; NCT02934529) is currently being conducted. This study has a planned enrolment of 550 patients with *RAS* wt mCRC who will receive first-line cetuximab-based therapy and third-line cetuximab rechallenge. This trial specifically includes non-invasive (liquid biopsy) *RAS* status assessment on PD and will thus directly test our proposal that longitudinal *RAS* status monitoring can be a tool in selecting patients suitable for cetuximab rechallenge after a treatment break. As of this writing, the FIRE-4 study is actively recruiting patients and has an estimated completion date of March 2022.

Although the studies we have reviewed have reported good clinical efficacy with cetuximab continuation and rechallenge, and evidence of a possible connection to *RAS* status switch, the treatment strategies outlined in figure 1 are not considered routine practice at this time. As new evidence emerges and patient selection becomes more refined, these strategies are likely to become valuable for oncologists looking to extend the continuum of potentially curative therapy for patients with a good performance status. Finally, it is also possible that other mechanisms of acquired resistance to cetuximab (eg, *EGFR*,[27 51] *HER2/MET* amplifications,[104] *KRAS/NRAS, BRAF, PIK3CA*[111 112]) arise in the same way and coexist in the same tumour. Some may be subject to similar dynamics of clonal selection under pressure, and, therefore, return to predominantly wt status after the anti-EGFR treatment is halted (except for EGFR ECD mutations, which do not pre-exist in untreated tumours but emerge de novo under anti-EGFR treatment and persist in the tumour).[51]

CONCLUSION

Anti-EGFR mAb plus chemotherapy is a standard of care in the first-line setting for patients with *RAS* wt mCRC, with a particular benefit for patients with left-sided tumours.[10 11] Importantly, cetuximab can also be a significant component in second-line and later-line treatments, thereby affording additional treatment opportunities to suitable patients and optimising and extending the continuum of care. In cetuximab-naïve patients, later-line treatment with cetuximab plus chemotherapy generally results in efficacy benefits over chemotherapy alone.[56 59] Use of cetuximab monotherapy or cetuximab plus chemotherapy in second-line and later-line mCRC does not yield any new safety signals, thereby further supporting its utility.[56 57 59 113 114]

Notably, patients do not have to be cetuximab naïve to extract benefits from second-line and later-line use of cetuximab.[68–70 72] Treatment with cetuximab beyond progression in conjunction with a different chemotherapy backbone results in efficacy benefits, although this approach still needs to be confirmed in a large-scale clinical study. Evaluation of tumour *RAS* status on progression on first-line cetuximab plus chemotherapy may be informative in identifying candidates for continuation of cetuximab after the *RAS* threshold relevant for clinical resistance has been determined. Additionally, patients whose tumours 'switched' to *RAS*-mutant status following previous cetuximab-based therapy can regain cetuximab sensitivity and be rechallenged with cetuximab after a 'holiday' of several months, with renewed efficacy benefits.[71 73] Liquid biopsy-based *RAS* testing will be needed to select for patients who can receive continuation cetuximab in the second line versus a treatment break (from anti-EGFR therapy) followed by rechallenge in third or further lines. We anticipate the results of trials such as FIRE-4, which may solidify the concept of cetuximab rechallenge as a routine therapeutic strategy

to optimise and extend the continuum of care in patients with mCRC. To this end, further research into additional biomarkers of response to anti-EGFR agents will ensure definition of the optimal patient populations for these strategies, thereby maximising the continuum of care.

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REFERENCES

1. GLOBOCAN. Estimated cancer incidence, mortality and prevalence worldwide in 2012. 2012.
2. Van Cutsem E, Lenz H-J, Köhne C-H, *et al*. Fluorouracil, leucovorin, and irinotecan plus cetuximab treatment and *RAS* mutations in colorectal cancer. *J Clin Oncol* 2015;33:692–700.
3. Lenz H, Niedzwiecki D, Innocenti F, *et al*. CALGB/SWOG 80405: phase III trial of FOLFIRI or mFOLFOX6 with bevacizumab or cetuximab for patients with expanded RAS analyses in untreated metastatic adenocarcinoma of the colon or rectum. *ESMO* 2014 Abstract 501O.
4. Falcone A, Cremolini C, Masi G, *et al*. FOLFOXIRI/bevacizumab (bev) versus FOLFIRI/bev as first-line treatment in unresectable metastatic colorectal cancer (mCRC) patients (pts): results of the phase III TRIBE trial by GONO group. *J Clin Oncol* 2013;31. Abstract 3505.
5. Stintzing S, Modest DP, Rossius L, *et al*. FOLFIRI plus cetuximab versus FOLFIRI plus bevacizumab for metastatic colorectal cancer (FIRE-3): a post-hoc analysis of tumour dynamics in the final RAS wild-type subgroup of this randomised open-label phase 3 trial. *Lancet Oncol* 2016;17:1426–34.
6. Bennouna J, Phelip J-M, André T, *et al*. Observational cohort of patients with metastatic colorectal cancer initiating chemotherapy in combination with bevacizumab (CONCERT). *Clin Colorectal Cancer* 2017;16:129–40.
7. Hasegawa H, Taniguchi H, Mitani S, *et al*. Efficacy of second-line bevacizumab-containing chemotherapy for patients with metastatic colorectal cancer following first-line treatment with an anti-epidermal growth factor receptor antibody. *Oncology* 2017;92:205–12.
8. Kuramochi H, Ando M, Itabashi M, *et al*. Phase II study of bevacizumab and irinotecan as second-line therapy for patients with metastatic colorectal cancer previously treated with fluoropyrimidines, oxaliplatin, and bevacizumab. *Cancer Chemother Pharmacol* 2017;79:579–85.
9. Tabernero J, Van Cutsem E, Lakomý R, *et al*. Aflibercept versus placebo in combination with fluorouracil, leucovorin and irinotecan in the treatment of previously treated metastatic colorectal cancer: prespecified subgroup analyses from the VELOUR trial. *Eur J Cancer* 2014;50:320–31.
10. Van Cutsem E, Cervantes A, Adam R, *et al*. ESMO consensus guidelines for the management of patients with metastatic colorectal cancer. *Ann Oncol* 2016;27:1386–422.
11. Tabernero J, Yoshino T, Cohn AL, *et al*. Ramucirumab versus placebo in combination with second-line FOLFIRI in patients with metastatic colorectal carcinoma that progressed during or after first-line therapy with bevacizumab, oxaliplatin, and a fluoropyrimidine (RAISE): a randomised, double-blind, multicentre, phase 3 study. *Lancet Oncol* 2015;16:499–508.
12. Wang Y, Liu S, Liu S, *et al*. Regorafenib treatment for management of colorectal cancer: a systematic review and meta-analysis of 6 randomized controlled trials. *Am J Ther* 2018;25:e276–8.
13. Heinemann V, Rivera F, O'Neil BH, *et al*. A study-level meta-analysis of efficacy data from head-to-head first-line trials of epidermal growth factor receptor inhibitors versus bevacizumab in patients with RAS wild-type metastatic colorectal cancer. *Eur J Cancer* 2016;67:11–20.
14. Khattak MA, Martin H, Davidson A, *et al*. Role of first-line anti–epidermal growth factor receptor therapy compared with anti–vascular endothelial growth factor therapy in advanced colorectal cancer: a meta-analysis of randomized clinical trials. *Clin Colorectal Cancer* 2015;14:81–90.
15. Kumachev A, Yan M, Berry S, *et al*. A systematic review and network meta-analysis of biologic agents in the first line setting for advanced colorectal cancer. *PLoS One* 2015;10:e0140187.
16. Holch JW, Ricard I, Stintzing S, *et al*. The relevance of primary tumour location in patients with metastatic colorectal cancer: a meta-analysis of first-line clinical trials. *Eur J Cancer* 2017;70:87–98.
17. Arnold D, Lueza B, Douillard JY, *et al*. Prognostic and predictive value of primary tumour side in patients with RAS wild-type metastatic colorectal cancer treated with chemotherapy and EGFR directed antibodies in six randomized trials. *Ann Oncol* 2017;28:1713–29.
18. Tejpar S, Stintzing S, Ciardiello F, *et al*. Prognostic and predictive relevance of primary tumor location in patients with RAS wild-type metastatic colorectal cancer: retrospective analyses of the CRYSTAL and FIRE-3 Trials. *JAMA Oncol* 2016. (Epub ahead of print 10 Oct 2016).
19. Venook AP, Neidzwiecki D, Innocenti F, *et al*. Impact of primary (1°) tumor location on overall survival (OS) and progression-free survival (PFS) in patients (pts) with metastatic colorectal cancer (mCRC): analysis of CALGB/SWOG 80405 (Alliance). *J Clin Oncol* 2016;34:Abstract 3504.
20. Sagawa T, Hamaguchi K, Sakurada A, *et al*. Primary tumor location as a prognostic and predictive factor in metastatic colorectal cancer (mCRC) treated with chemotherapy plus cetuximab: a retrospective analysis. *J Clin Oncol* 2017;35:711.
21. Modest DP, Stintzing S, Fischer von Weikersthal L, *et al*. Relation of early tumor shrinkage (ETS) observed in first-line treatment to efficacy parameters of subsequent treatment in FIRE-3 (AIOKRK0306). *Int J Cancer* 2017;140:1918–25.
22. Pietrantonio F, Petrelli F, Coinu A, *et al*. Predictive role of BRAF mutations in patients with advanced colorectal cancer receiving cetuximab and panitumumab: a meta-analysis. *Eur J Cancer* 2015;51:587–94.
23. Rowland A, Dias MM, Wiese MD, *et al*. Meta-analysis of BRAF mutation as a predictive biomarker of benefit from anti-EGFR monoclonal antibody therapy for RAS wild-type metastatic colorectal cancer. *Br J Cancer* 2015;112:1888–94.
24. Summers MG, Smith CG, Maughan TS, *et al*. *BRAF* and *NRAS* locus-specific variants have different outcomes on survival to colorectal cancer. *Clin Cancer Res* 2017;23:2742–9.
25. Foltran L, Maglio GD, Pella N, *et al*. Prognostic role of *KRAS, NRAS, BRAF* and *PIK3CA* mutations in advanced colorectal cancer. *Future Oncol* 2015;11:629–40.
26. Misale S, Di Nicolantonio F, Sartore-Bianchi A, *et al*. Resistance to anti-EGFR therapy in colorectal cancer: from heterogeneity to convergent evolution. *Cancer Discov* 2014;4:1269–80.
27. Morelli MP, Overman MJ, Dasari A, *et al*. Characterizing the patterns of clonal selection in circulating tumor DNA from patients with colorectal cancer refractory to anti-EGFR treatment. *Ann Oncol* 2015;26:731–6.
28. Montagut C, Dalmases A, Bellosillo B, *et al*. Identification of a mutation in the extracellular domain of the Epidermal Growth Factor Receptor conferring cetuximab resistance in colorectal cancer. *Nat Med* 2012;18:221–3.

29. Sartore-Bianchi A, Siena S, Tonini G, *et al*. Overcoming dynamic molecular heterogeneity in metastatic colorectal cancer: multikinase inhibition with regorafenib and the case of rechallenge with anti-EGFR. *Cancer Treat Rev* 2016;51:54–62.
30. Sartore-Bianchi A, Siena S. Plasticity of resistance and sensitivity to anti-epidermal growth factor receptor inhibitors in metastatic colorectal cancer. *Handb Exp Pharmacol* 2017. (Epub ahead of print 6 Apr 2017).
31. Siena S, Sartore-Bianchi A, Di Nicolantonio F, *et al*. Biomarkers predicting clinical outcome of epidermal growth factor receptor–targeted therapy in metastatic colorectal cancer. *J Natl Cancer Inst* 2009;101:1308–24.
32. Sartore-Bianchi A, Trusolino L, Martino C, *et al*. Dual-targeted therapy with trastuzumab and lapatinib in treatment-refractory, KRAS codon 12/13 wild-type, HER2-positive metastatic colorectal cancer (HERACLES): a proof-of-concept, multicentre, open-label, phase 2 trial. *Lancet Oncol* 2016;17:738–46.
33. Jeong JH, Kim J, Hong YS, *et al*. HER2 Amplification and cetuximab efficacy in patients with metastatic colorectal cancer harboring wild-type RAS and BRAF. *Clin Colorectal Cancer* 2017;16:e147–52.
34. Ouchi K, Takahashi S, Yamada Y, *et al*. DNA methylation status as a biomarker of anti-epidermal growth factor receptor treatment for metastatic colorectal cancer. *Cancer Sci* 2015;106:1722–9.
35. Scartozzi M, Bearzi I, Mandolesi A, *et al*. Epidermal growth factor receptor (EGFR) gene promoter methylation and cetuximab treatment in colorectal cancer patients. *Br J Cancer* 2011;104:1786–90.
36. De Roock W, Claes B, Bernasconi D, *et al*. Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. *Lancet Oncol* 2010;11:753–62.
37. Tabernero J, Lenz H-J, Siena S, *et al*. Analysis of circulating DNA and protein biomarkers to predict the clinical activity of regorafenib and assess prognosis in patients with metastatic colorectal cancer: a retrospective, exploratory analysis of the CORRECT trial. *Lancet Oncol* 2015;16:937–48.
38. Vallböhmer D, Zhang W, Gordon M, *et al*. Molecular determinants of cetuximab efficacy. *J Clin Oncol* 2005;23:3536–44.
39. Siravegna G, Mussolin B, Buscarino M, *et al*. Clonal evolution and resistance to EGFR blockade in the blood of colorectal cancer patients. *Nat Med* 2015;21:795–801.
40. Liska D, Chen C-T, Bachleitner-Hofmann T, *et al*. HGF rescues colorectal cancer cells from EGFR Inhibition via MET activation. *Clin Cancer Res* 2011;17:472–82.
41. Pugh S, Thiébaut R, Bridgewater J, *et al*. Association between miR-31-3p expression and cetuximab efficacy in patients with KRAS wild-type metastatic colorectal cancer: a post-hoc analysis of the New EPOC trial. *Oncotarget* 2017;8:93856–66.
42. Sunakawa Y, Yang D, Moran M, *et al*. Combined assessment of EGFR-related molecules to predict outcome of 1st-line cetuximab-containing chemotherapy for metastatic colorectal cancer. *Cancer Biol Ther* 2016;17:751–9.
43. Saridaki Z, Tzardi M, Papadaki C, *et al*. Impact of KRAS, BRAF, PIK3CA mutations, PTEN, AREG, EREG expression and skin rash in ≥2nd line cetuximab-based therapy of colorectal cancer patients. *PLoS One* 2011;6:e15980.
44. Igarashi H, Kurihara H, Mitsuhashi K, *et al*. Association of microRNA-31-5p with clinical efficacy of anti-EGFR therapy in patients with metastatic colorectal cancer. *Ann Surg Oncol* 2015;22:2640–8.
45. Chung S, Dwabe S, Elshimali Y, *et al*. Identification of novel biomarkers for metastatic colorectal cancer using angiogenesis-antibody array and intracellular signaling array. *PLoS One* 2015;10:e0134948.
46. Mizukami T, Togashi Y, Naruki S, *et al*. Significance of *FGF9* gene in resistance to anti-EGFR therapies targeting colorectal cancer: A subset of colorectal cancer patients with *FGF9* upregulation may be resistant to anti-EGFR therapies. *Mol Carcinog* 2017;56:106–17.
47. National Comprehensive Cancer Network. Colon cancer: NCCN Clinical practice guidelines in oncology. 2016. https://www.nccn.org/professionals/physician_gls/default.aspx.
48. Lunke S, Lee B, Kranz S, *et al*. Intratumorous heterogeneity for RAS mutations in a treatment-naïve colorectal tumour. *J Clin Pathol* 2017;70:720–3.
49. Sanchez-Martin FJ, Bellosillo B, Gelabert-Baldrich M, *et al*. The first-in-class anti-EGFR antibody mixture sym004 overcomes cetuximab resistance mediated by EGFR extracellular domain mutations in colorectal cancer. *Clin Cancer Res* 2016;22:3260–7.
50. Arena S, Bellosillo B, Siravegna G, *et al*. Emergence of multiple EGFR Extracellular mutations during cetuximab treatment in colorectal cancer. *Clin Cancer Res* 2015;21:2157–66.
51. Van Emburgh BO, Arena S, Siravegna G, *et al*. Acquired RAS or EGFR mutations and duration of response to EGFR blockade in colorectal cancer. *Nat Commun* 2016;7:13665.
52. Strickler JH, Loree JM, Ahronian LG, *et al*. Genomic landscape of cell-free DNA in patients with colorectal cancer. *Cancer Discov* 2017.
53. Chang PH, Huang JS. Successful rechallenge of cetuximab following severe infusion-related reactions: a case report. *Chin J Cancer Res* 2014;26:E10–2.
54. George TJ, Laplant KD, Walden EO, *et al*. Managing cetuximab hypersensitivity-infusion reactions: incidence, risk factors, prevention, and retreatment. *J Support Oncol* 2010;8:72–7.
55. Nielsen DL, Pfeiffer P, Jensen BV. Re-treatment with cetuximab in patients with severe hypersensitivity reactions to cetuximab. Two case reports. *Acta Oncol* 2006;45:1137–8.
56. Hong YS, Kim HJ, Park SJ, *et al*. Second-line cetuximab/irinotecan versus oxaliplatin/fluoropyrimidines for metastatic colorectal cancer with wild-type *KRAS*. *Cancer Sci* 2013;104:473–80.
57. Price TJ, Peeters M, Kim TW, *et al*. Panitumumab versus cetuximab in patients with chemotherapy-refractory wild-type KRAS exon 2 metastatic colorectal cancer (ASPECCT): a randomised, multicentre, open-label, non-inferiority phase 3 study. *Lancet Oncol* 2014;15:569–79.
58. Pantelic A, Markovic M, Pavlovic M, *et al*. Cetuximab in third-line therapy of patients with metastatic colorectal cancer: a single institution experience. *J BUON* 2016;21:70–9.
59. Gil Delgado M, Spano J-P, Khayat D. Cetuximab plus irinotecan in refractory colorectal cancer patients. *Expert Rev Anticancer Ther* 2007;7:407–13.
60. Lim R, Sun Y, Im SA, *et al*. Cetuximab plus irinotecan in pretreated metastatic colorectal cancer patients: the ELSIE study. *World J Gastroenterol* 2011;17:1879–88.
61. Buzaid AC, de Cerqueira Mathias C, Perazzo F, *et al*. Cetuximab plus irinotecan in pretreated metastatic colorectal cancer progressing on irinotecan: the LABEL study. *Clin Colorectal Cancer* 2010;9:282–9.
62. Wilke H, Glynne-Jones R, Thaler J, *et al*. Cetuximab plus irinotecan in heavily pretreated metastatic colorectal cancer progressing on irinotecan: MABEL Study. *J Clin Oncol* 2008;26:5335–43.
63. Shitara K, Yonesaka K, Denda T, *et al*. Randomized study of FOLFIRI plus either panitumumab or bevacizumab for wild-type KRAS colorectal cancer-WJOG 6210G. *Cancer Sci* 2016;107:1843–50.
64. Peeters M, Oliner KS, Price TJ, *et al*. Analysis of KRAS/NRAS mutations in a phase III study of panitumumab with FOLFIRI compared with FOLFIRI alone as second-line treatment for metastatic colorectal cancer. *Clin Cancer Res* 2015;21:5469–79.
65. Peeters M, Price TJ, Cervantes A, *et al*. Final results from a randomized phase 3 study of FOLFIRI ± panitumumab for second-line treatment of metastatic colorectal cancer. *Ann Oncol* 2014;25:107–16.
66. Peeters M, Price TJ, Cervantes A, *et al*. Randomized phase III study of panitumumab with fluorouracil, leucovorin, and irinotecan (FOLFIRI) compared with FOLFIRI alone as second-line treatment in patients with metastatic colorectal cancer. *J Clin Oncol* 2010;28:4706–13.
67. Gasparini G, Buttitta F, D'Andrea MR, *et al*. Optimizing single agent panitumumab therapy in pre-treated advanced colorectal cancer. *Neoplasia* 2014;16:751–6.
68. Ciardiello F, Normanno N, Martinelli E, *et al*. Cetuximab beyond progression in RAS wild type (WT) metastatic colorectal cancer (mCRC): the CAPRI-GOIM randomized phase II study of FOLFOX versus FOLFOX plus cetuximab. *Ann Oncol* 2015;26:iv120–1.
69. Feng Q, Wei Y, Ren L, *et al*. Efficacy of continued cetuximab for unresectable metastatic colorectal cancer after disease progression during first-line cetuximab-based chemotherapy: a retrospective cohort study. *Oncotarget* 2016;7:11380–96.
70. Fora AA, McMahon JA, Wilding G, *et al*. A phase II study of high-dose cetuximab plus irinotecan in colorectal cancer patients with KRAS wild-type tumors who progressed after standard dose of cetuximab plus irinotecan. *Oncology* 2013;84:210–3.
71. Liu X, George GC, Tsimberidou AM, *et al*. Retreatment with anti-EGFR based therapies in metastatic colorectal cancer: impact of intervening time interval and prior anti-EGFR response. *BMC Cancer* 2015;15:713.
72. Vladimirova LY, Abramova NA, Kit OI. Treatment for RAS wild-type (wt) metastatic colorectal cancer (mCRC): continuation of anti-

EGFR therapy while switching chemotherapy regimen. *ASCO* 2016. Abstract 744.
73. Santini D, Vincenzi B, Addeo R, *et al*. Cetuximab rechallenge in metastatic colorectal cancer patients: how to come away from acquired resistance? *Ann Oncol* 2012;23:2313–8.
74. Modest D, Stintzing S, Fischer von Weikersthal L, *et al*. 2nd-line therapies after 1st-line therapy with FOLFIRI in combination with cetuximab or bevacizumab in patients with KRAS wild-type metastatic colorectal cancer (mCRC) - analysis of the AIO KRK 0306 (FIRE 3) trial. *ESMO GI* 2014. Abstract 0-0018.
75. Lam KO, Lee VH, Liu RK, *et al*. Bevacizumab-containing regimens after cetuximab failure in Kras wild-type metastatic colorectal carcinoma. *Oncol Lett* 2013;5:637–40.
76. Norguet E, Dahan L, Gaudart J, *et al*. Cetuximab after bevacizumab in metastatic colorectal cancer: Is it the best sequence? *Digestive and Liver Disease* 2011;43:917–9.
77. Bianco R, Rosa R, Damiano V, *et al*. Vascular endothelial growth factor receptor-1 contributes to resistance to anti-epidermal growth factor receptor drugs in human cancer cells. *Clin Cancer Res* 2008;14:5069–80.
78. Cascinu S, Rosati G, Nasti G, *et al*. Treatment sequence with either irinotecan/cetuximab followed by FOLFOX-4 or the reverse strategy in metastatic colorectal cancer patients progressing after first-line FOLFIRI/bevacizumab: an Italian Group for the Study of Gastrointestinal Cancer phase III, randomised trial comparing two sequences of therapy in colorectal metastatic patients. *Eur J Cancer* 2017;83:106–15.
79. Bennouna J, Hiret S, Borg C, *et al*. Bevacizumab (Bev) or cetuximab (Cet) plus chemotherapy after progression with bevacizumab plus chemotherapy in patients with wild-type (WT) KRAS metastatic colorectal cancer (mCRC): final analysis of a French randomized, multicenter, phase II study (PRODIGE 18). *Ann Oncol* 2017;28:v158–208.
80. Rosati G, Nasti G, Lonardi S, *et al*. A phase III multicenter trial comparing two different sequences of second/third line therapy (irinotecan/cetuximab followed by FOLFOX-4 vs. FOLFOX-4 followed by irinotecan/cetuximab in K-RAS wt metastatic colorectal cancer (mCC) patients refractory to FOLFIRI/bevacizumab. *Ann Oncol* 2015;26:vi2.
81. Hecht JR, Cohn A, Dakhil S, *et al*. SPIRITT: a randomized, multicenter, phase II study of panitumumab with FOLFIRI and bevacizumab with FOLFIRI as second-line treatment in patients with unresectable wild type KRAS metastatic colorectal cancer. *Clin Colorectal Cancer* 2015;14:72–80.
82. Bettegowda C, Sausen M, Leary RJ, *et al*. Detection of circulating tumor DNA in early- and late-stage human malignancies. *Sci Transl Med* 2014;6:224ra24.
83. Bachet JB, Bouche O, Taieb J, *et al*. RAS mutations concordance in circulating tumor DNA (ctDNA) and tissue in metastatic colorectal cancer (mCRC): RASANC, an AGEO prospective multicenter study. *J Clin Oncol* 2017;35. Abstract 11509.
84. Vogelstein B, Kinzler KW. Digital PCR. *Proc Natl Acad Sci U S A* 1999;96:9236–41.
85. Lanman RB, Mortimer SA, Zill OA, *et al*. Analytical and clinical validation of a digital sequencing panel for quantitative, highly accurate evaluation of cell-free circulating tumor DNA. *PLoS One* 2015;10:e0140712.
86. Dressman D, Yan H, Traverso G, *et al*. Transforming single DNA molecules into fluorescent magnetic particles for detection and enumeration of genetic variations. *Proc Natl Acad Sci U S A* 2003;100:8817–22.
87. Liu Q, Sommer SS. Pyrophosphorolysis-activated polymerization (PAP): application to allele-specific amplification. *BioTechniques* 2000;29:1072–6.
88. Newman AM, Bratman SV, To J, *et al*. An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage. *Nat Med* 2014;20:548–54.
89. Forshew T, Murtaza M, Parkinson C, *et al*. Noninvasive identification and monitoring of cancer mutations by targeted deep sequencing of plasma DNA. *Sci Transl Med* 2012;4:136ra68.
90. Schmiegel W, Scott RJ, Dooley S, *et al*. Blood-based detection of *RAS* mutations to guide anti-EGFR therapy in colorectal cancer patients: concordance of results from circulating tumor DNA and tissue-based *RAS* testing. *Mol Oncol* 2017;11:208–19.
91. Vidal J, Muinelo L, Dalmases A, *et al*. Plasma ctDNA RAS mutation analysis for the diagnosis and treatment monitoring of metastatic colorectal cancer patients. *Ann Oncol* 2017;28:1325–32.
92. Vladimirova LY, Agieva AA, Engibaryan MA. Anti-EGFR monoclonal antibodies in locally advanced head and neck squamous cell cancer. *Vopr Onkol* 2015;61:580–2.
93. Metges J, Raoul J, Achour N, *et al*. PANERB study: panitumumab after cetuximab-based regimen failure. *J Clin Oncol* 2010;28:Abstract e14000.
94. Pietrantonio F, Perrone F, Biondani P, *et al*. Single agent panitumumab in KRAS wild-type metastatic colorectal cancer patients following cetuximab-based regimens: clinical outcome and biomarkers of efficacy. *Cancer Biol Ther* 2013;14:1098–103.
95. Nagata N, Mishima H, Kurosawa S, *et al*. mFOLFOX6 plus panitumumab versus 5-FU/LV plus panitumumab after six cycles of frontline mFOLFOX6 plus panitumumab: a randomized phase II study of patients with unresectable or advanced/recurrent, RAS wild-type colorectal carcinoma (SAPPHIRE) — study design and rationale. *Clin Colorectal Cancer* 2017;16:154–7.
96. Araki K, Fukada I, Horii R, *et al*. Trastuzumab rechallenge after lapatinib- and trastuzumab-resistant disease progression in HER2-positive breast cancer. *Clin Breast Cancer* 2015;15:432–9.
97. Barbieri E, Rubino D, Hakim R, *et al*. Eribulin long-term response and rechallenge: report of two clinical cases. *Future Oncol* 2017;13:35–43.
98. Cappuzzo F, Morabito A, Normanno N, *et al*. Efficacy and safety of rechallenge treatment with gefitinib in patients with advanced non-small cell lung cancer. *Lung Cancer* 2016;99:31–7.
99. Gazzaniga P, Raimondi C, Nicolazzo C, *et al*. ctDNA might expand therapeutic options for second line treatment of KRAS mutant mCRC. 2017;28:v573–94.
100. Tonini G, Imperatori M, Vincenzi B, *et al*. Rechallenge therapy and treatment holiday: different strategies in management of metastatic colorectal cancer. *J Exp Clin Cancer Res* 2013;32:92.
101. Sforza V, Martinelli E, Ciardiello F, *et al*. Mechanisms of resistance to anti-epidermal growth factor receptor inhibitors in metastatic colorectal cancer. *World J Gastroenterol* 2016;22:6345–61.
102. Misale S, Yaeger R, Hobor S, *et al*. Emergence of KRAS mutations and acquired resistance to anti-EGFR therapy in colorectal cancer. *Nature* 2012;486:532–6.
103. Bouchahda M, Karaboué A, Saffroy R, *et al*. Acquired KRAS mutations during progression of colorectal cancer metastases: possible implications for therapy and prognosis. *Cancer Chemother Pharmacol* 2010;66:605–9.
104. Pietrantonio F, Vernieri C, Siravegna G, *et al*. Heterogeneity of acquired resistance to anti-EGFR monoclonal antibodies in patients with metastatic colorectal cancer. *Clin Cancer Res* 2017;23:2414–22.
105. Rossini D, Santini D, Cremolini C, *et al*. Rechallenge with cetuximab + irinotecan in 3rd-line in RAS and BRAF wild-type metastatic colorectal cancer (mCRC) patients with acquired resistance to 1st-line cetuximab+irinotecan: the phase II CRICKET study by GONO. *Ann Oncol* 2017;28:iii1–12.
106. Hata A, Katakami N, Kitajima N. Successful cetuximab therapy after failure of panitumumab rechallenge in a patient with metastatic colorectal cancer: restoration of drug sensitivity after anti-EGFR monoclonal antibody-free interval. *J Gastrointest Cancer* 2014;45:506–7.
107. Ma J, Yang QL, Ling Y. Rechallenge and maintenance therapy using cetuximab and chemotherapy administered to a patient with metastatic colorectal cancer. *BMC Cancer* 2017;17:132.
108. Hata A, Katakami N, Fujita S, *et al*. Panitumumab rechallenge in chemorefractory patients with metastatic colorectal cancer. *J Gastrointest Cancer* 2013;44:456–9.
109. Wadlow RC, Hezel AF, Abrams TA, *et al*. Panitumumab in patients with KRAS wild-type colorectal cancer after progression on cetuximab. *Oncologist* 2012;17:14–34.
110. Kajitani T, Makiyama A, Arita S, *et al*. Anti-epidermal growth factor receptor antibody readministration in chemorefractory metastatic colorectal cancer. *Anticancer Res* 2017;37:6459–68.
111. Xu JM, Wang Y, Wang YL, *et al*. PIK3CA mutations contribute to acquired cetuximab resistance in metastatic colorectal cancer patients. *Clin Cancer Res* 2017;23:4602–16.
112. Toledo RA, Cubillo A, Vega E, *et al*. Clinical validation of prospective liquid biopsy monitoring in patients with wild-type RAS metastatic colorectal cancer treated with FOLFIRI-cetuximab. *Oncotarget* 2017;8:35289–300.
113. Sobrero AF, Maurel J, Fehrenbacher L, *et al*. EPIC: phase III trial of cetuximab plus irinotecan after fluoropyrimidine and oxaliplatin failure in patients with metastatic colorectal cancer. *J Clin Oncol* 2008;26:2311–9.
114. Harbison CT, Horak CE, Ledeine JM, *et al*. Validation of companion diagnostic for detection of mutations in codons 12 and 13 of the KRAS gene in patients with metastatic colorectal cancer: analysis of the NCIC CTG CO.17 trial. *Arch Pathol Lab Med* 2013;137:820–7.