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## Mechanisms of Innate and Acquired Resistance to anti-EGFR therapy: A Review of Current Knowledge with a Focus on Rechallenge Therapies

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### Abstract

Innate and acquired resistance to anti-EGFR therapy (EGFRi) is a major limitation in the treatment of metastatic colorectal cancer (mCRC). Although *RAS* genes are the most commonly mutated innate and acquired oncogenes in cancer, there are a number of other mechanisms which limit the effectiveness of EGFRi. Patients with innate resistance have been found to contain *BRAFV600E* mutations, and possibly *MET*, *MEK*, *PIK3CA*, *PTEN* and *HER2* alterations. Meanwhile, *BRAFV600E* mutations may also be involved in acquired resistance to EGFRi, in addition to EGFR ectodomain mutations, *MET* alterations, and possibly *HER2* amplification. In addition, paracrine effects and cell fate mechanisms of resistance are being increasingly described as contributing to acquired resistance. Utilization of circulating tumor DNA has been paramount in monitoring the dynamic nature of acquired resistance, and has helped to guide treatment decisions, particularly in the EGFRi rechallenge setting. Herein, we provide an in-depth review of EGFRi resistance mechanisms and describe the current therapeutic landscape in the hopes of identifying effective rechallenge strategies.

### Introduction

Colorectal cancer (CRC) represents a heterogeneous group of dynamic biologic phenomena with differing sets of genetic events, accompanying immune responses, and influences of exogenous factors, providing a challenge for personalized therapeutic approaches. These personalized treatments most often involve kinase inhibitors or monoclonal antibodies (moAbs) that target specific alterations known to drive the proliferation and survival of cancer cells.(1) In this scenario, the epidermal growth factor receptor (EGFR) family plays a key role in tumor growth and progression by promoting a variety of functions including proliferation, survival, invasion, and immune evasion.(2) These therapies have improved patient outcomes, however despite significant progress in strategies for cancer treatment,

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their use is limited by the presence of pre-existing innate resistance mechanisms or by the ability of cancer cells to acquire resistance to therapy.(2,3)

## Innate Resistance to EGFRi

Patients with *KRAS/NRAS (RAS)* wildtype metastatic colorectal cancer (mCRC) have improved survival when treated with anti-EGFR (EGFRi) moAbs.(4,5) However, these agents do not benefit mCRC patients with oncogenic *RAS* mutations (Figure 1).(6–9) The *KRAS* mutation was initially identified in codons 12 and 13 of exon 2 which result in constitutive activation of the RAS-RAF-MEK-ERK (MAPK) pathway.(8–11) Activating mutations in *KRAS* are detected in approximately 40% of mCRC,(2,3) with good concordance between primary tumors and distant metastases.(12) Expanded *RAS* mutations in *KRAS* exon 3 or 4, or in *NRAS* exon 2, 3, or 4 have also been noted to predict a lack of benefit from EGFRi, increasing the prevalence of all innate *RAS* mutations to 50–55%. (8,13,14)

Though less common, alterations in *BRAFV600E* result in constitutive activation of the MAPK pathway and may predict lack of benefit from EGFRi.(15) In a retrospective analysis of patients with EGFRi-based chemotherapy, the progression-free survival (PFS) (median 8 vs 26 weeks, HR 3.74, 95% CI 2.44–5.75) and overall survival (OS) (median 26 vs 54 weeks, HR 3.03, 95% CI 1.98–4.63) was shorter in patients with *BRAF* mutated tumors than those with *BRAF* wildtype mCRC.(16) In a study evaluating 5-fluorouracil (5-FU) plus irinotecan (FOLFIRI) with panitumumab in the second-line setting, patients with *BRAF* mutated mCRC had a median PFS of 2.5 months and an OS of 4.7 months, compared with a PFS and OS of 6.9 and 18.7 months, respectively, in patients with *BRAF* wildtype mCRC. (17) Indeed, a large meta-analysis found limited benefit of adding EGFRi to standard regimens in *RAS* wildtype/*BRAF* mutated mCRC patients, however it was not definitively predictive.(18) Non-V600E *BRAF* mutations have also been identified in mCRC, however appear associated with a much better prognosis than V600E mutations, which some attribute to a lack constitutive activation of MAPK signaling.(19,20) However, in our review of over 2000 patients with mCRC, we identified only 11 non-V600E mutated *BRAF* tumors and none of these responded to EGFRi.(21) Others have shown mixed effect of EGFRis and interpretation of reports in these non-V600E mutated tumors is difficult given small samples sizes and differences in downstream activity of various *BRAF* mutations.(22)

*PIK3CA* mutations may also contribute to innate resistance to EGFRi. Mutations occur at a prevalence of 10%–20%, and are mainly located at hot spots in exons 9 and 20, resulting in constitutive activity of PI3K- activating downstream AKT/mTOR signaling.(23)

Retrospective studies have demonstrated that *PIK3CA* mutations in exon 20 may be associated with lack of response to EGFRi in mCRC.(16,24) However, *PIK3CA* and *RAS* mutations co-exist and may have confounded the initial studies. Subsequent studies have failed to definitively demonstrate a role of *PIK3CA* in EGFRi resistance. In the same pathway, loss of function of PTEN that occurs through mutations, deletions or transcriptional silencing is present in 30 % of sporadic CRCs.(25) PTEN inactivation has been associated with non-responsiveness to anti-EGFR moAbs in mCRC patients in several studies, however other studies have failed to show that loss is anything more than

prognostic(25–27) PTEN has been shown to play a role in modulating EGFR intracellular trafficking and degradation suggesting a plausible mechanism for inducing resistance.(28)

Further, innate resistance to EGFRi may be secondary to amplification of HER2 found in 3–5% of *RAS* wildtype mCRC patients.(29,30) Retrospective and in-vitro analyses have suggested limited activity of anti-EGFR in HER2-amplified tumors, although not universally demonstrated.(30,31) A recent review of the PFS of patients on the HERACLES trial during their EGFRi by Bregni et al. showed contrary evidence refuting the use of HER2 amplification as a biomarker and highlights the controversy and challenges in interpreting information on rare subtypes.(32) Several ongoing (NCT03384940, NCT03365882) and completed clinical studies with *HER2* targeted agents after failure of EGFRi will hopefully provide patient cohorts to validate these findings.(33) *HER2* and *ERBB3* mutations have also been noted in CRC, however there is still limited data regarding their implications.(34) *HER2* but not *ERBB3* mutations appear associated with a negative prognosis, and unclear association EGFRi benefit, however unlike breast cancer where HER kinase inhibition has therapeutic benefit, the SUMMIT trial failed to show any utility of neratinib in *HER2/ERBB3* mutated mCRC, suggesting a distinct biology from breast cancer.(35,36) These *HER2*-activating mutations produced resistance to the EGFR moAbs, cetuximab or panitumumab, when transfected into two cetuximab-sensitive CRC cell lines 57 providing pre-clinical rationale that they may be associated with EGFRi resistance.

The *MET* gene, which encodes the tyrosine kinase receptor for Hepatocyte Growth Factor (HGF), has been found to have a role in several tumor types. Gene amplification, overexpression, activating mutations or autocrine stimulation cause constitutive activation of *MET*.(37) *MET*(38) amplification has been proposed as an additional biomarker of innate resistance to EGFRi. However, these mechanisms may not abrogate all benefit from EGFRi as is seen with the absolute resistance noted from *RAS* mutations. *MET* amplifications have only been identified in approximately 1% of untreated mCRC.(38) Preclinical data has shown that xenograft tumors carrying *MET* amplification did not respond to EGFRi, and this alteration was mutually exclusive with *KRAS/BRAF/NRAS*, and *PIK3CA* mutations, as well as *HER2* amplification.(38) However, the prevalence is very rare, precluding more definitive assessment of its role in innate resistance.(39)

In addition, important biologic differences based on primary tumor location have been identified. Right-sided tumors, even when controlling for stage and tumor size, have a worse prognosis.(40) Recently, retrospective reviews of multiple trials utilizing EGFRi in various lines of therapy have demonstrated that right-sided tumors appear less responsive to EGFRi.(41) This has changed clinical decision making, as planned post-hoc analyses of the CALGB 80405 and FIRE-3 studies, among others, demonstrated significant decreases in OS between patients with right-or left-sided *RAS*<sup>WT</sup> tumors depending on which biologic was received in the first-line setting.(41–43) Patients with right-sided tumors had improved OS if bevacizumab was combined with a doublet, while patients with left-sided tumors derived more benefit from doublet plus EGFRi. The mechanism of this distinction is not well defined, but right-sided primary CRCs are known to have distinct pathobiology and characteristics, including higher rates of *BRAF* mutation, microsatellite instability (MSI-high), and high CpG island methylator phenotype (CIMP-high).(44) While there are

multiple potential mechanisms, it is of note that expression of EGFR ligands and AREG and EREG differ between right- and left-sided tumors, in part due to their epigenetic regulation and association with CIMP-high tumors.(45) Further prospective studies are warranted with retrospective analyses ongoing to further characterize the key biological variables responsible for differences in response to EGFRi based on anatomy.

### Acquired Resistance to EGFRi

Among patients who initially respond to EGFRi, acquired mutations ultimately develop and result in secondary resistance (Figure 1). Growing utilization of plasma circulating tumor DNA (ctDNA) testing has allowed for the non-invasive detection of heterogeneous molecular abnormalities which result in the evolution of resistance to targeted therapies in mCRC(5,46–48) EGFR blockade results in evolutionary pressure and the outgrowth of subclonal populations of cells with resistance mechanisms.(49) Concurrent selection and expansion of multiple tumor subclones results in polyclonal mechanisms of resistance that drive tumor progression (Figure 2).(50,51) These data suggest that overcoming resistance will require a strategy capable of surmounting multiple heterogeneous resistance mechanisms between different tumor subclones in the same patient. The mechanisms by which tumors develop acquired resistance to therapy can typically be subcategorized into mechanisms which include activation of bypass signaling pathways, secondary alterations in the EGFR receptor, and adaptive or cell fate changes.(1,3)

### MAP-Kinase Pathway Alterations as Mechanisms of Acquired Resistance to EGFRi

The most common mechanism of acquired resistance to EGFRi results from the activation of signaling pathways that “bypass” the drug target to maintain survival and proliferation.(16) Among these, *RAS* mutations, in addition to being a key driver of innate resistance to EGFRi antibodies in mCRC, also play a vital role in acquired resistance.(16,52–54) An estimated 50% of patients with acquired resistance will have a detectable secondary *RAS* mutation.(16,52,54) Acquired mutations in *RAS* most commonly occur in exons 3–4 (67% of *NRAS* and 50% of *KRAS* mutations versus 3.4% and 8.2%, respectively, in innate resistance).(55,56) An additional critical feature is that greater than one alteration in *RAS* has been frequently identified in the same patient after treatment with EGFRi.(47,48) Modeling suggests that some *RAS* mutations are pre-existing in the tumor but below the threshold of detection by standard assays.(47,48,57) By ctDNA, rare *RAS* clones detectable prior to EGFRi are present in upwards of 38% of patients, however clinical responses are still seen, reiterating the fact that these subclones may not be clinically significant in the untreated tumor.(58,59)

Another mechanism implicated in activation of EGFR downstream pathway and in consequently EGFRi resistance is promoter-specific DNA methylation. While methylation of direct effectors of this signaling pathway has not been reported, promoter methylation of *RASSF1* and *RASSF2* occurs in as many as 80% of CRC tumors.(60,61) *RASSF1* and *RASSF2* are modulators of the growth inhibitory and pro-apoptotic effects of active *RAS*, and consequently their inactivation is thought to promote *RAS*-driven tumorigenesis and

could have an impact on EGFRi response. However, additional studies are still required to definitively establish its tumor-suppressor activity in CRC. Other epigenetic phenomena may also be important predictors for resistance to EGFR. Many studies have identified a subset of microRNAs (miRNAs) associated with a lack of benefit from anti-EGFR therapy, however few have robust external validation.(62,63) None of these miRNAs have been applied clinically to date, however overexpression of several, such as miR-31-5p, have been reproducibly associated with a lack of benefit from EGFRi in several studies.(64,65)

Another mechanism of acquired resistance includes mutations in the EGFR receptor(55,66) or activation of parallel receptor tyrosine kinases (RTKs).(24,38) An acquired mutation in the extracellular domain of EGFR (S492R) has been showed to cause acquired resistance to cetuximab in mCRC.(66) This mutation reduces the affinity of the ligand to the receptor and interferes with binding to cetuximab. Subsequent work defined a number of other ECD mutations, many of which appear to result in cross- resistance to both EGFRis.(46,47,67,68) The role of *EGFR* ECD mutations in driving resistance to EGFRi has now been documented through ctDNA analysis by several groups, and continues to be an active area of investigation in rechallenge studies.(46,47,55,66–69) In contrast to *RAS* mutations, ectodomain (ECD) mutations are almost never seen in untreated tumors.(47,66,68)

There is emerging evidence to suggest that alterations affecting the extracellular domain of EGFR could be also due to different mechanisms besides phosphorylation and glycosylation that need to be better explored. Liao HW discovered in their work that specific extracellular Arg methylations of EGFR render cancer cells resistant to cetuximab antibody therapy.(70) In particular, they reported a post-translational arginine methylation on the extracellular domain of EGFR by protein arginine methyltransferase (PRMT) 1 at R198 and R200 that resulted in increased ligand binding to promote EGFR receptor dimerization and activation, and alters EGFR signaling.(70)

Growth-factor signaling pathway upregulation of alternative and compensatory signaling cascades through receptors other than EGFR has also been noted to cause resistance to EGFRi. In particular, *HER2* amplification has been suggested as both an intrinsic, as well as an acquired mechanism of resistance.(31,71) One explanation may be that pre-existing infrequent *HER2*-amplified clones may expand under the selective pressure of EGFRi, leading to disease progression. Similarly, MEK overexpression has been associated with reduced benefit from anti-EGFR therapy and *in-vitro* experiments demonstrate that combined EGFRi with MEKi can abrogate this resistance.(72,73) *MEK* mutations have been identified in colorectal cancer occurring at a low frequency (2.5% in TCGA) and may be a further mechanism of resistance, though evidence for this is weak to date.(74–76) These mutations also develop in *BRAF* mutant mCRC treated with targeted therapy, providing further evidence that downstream mutations are a plausible mechanism of acquired resistance.(77,78)

*MET* and its ligand HGF have been implicated in acquired resistance to EGFRi.(38,79) Further, in mCRC and other tumor types, *MET* amplification has been found to arise in tumors with pre-existing clones of *MET*-amplified cells which undergo positive selection.(38,80) During treatment with EGFRi, the *MET*-amplified cells have then been shown to

become a dominant population of cells, ultimately decreasing the efficacy of further EGFRi. (38)

Activation of the PIK3CA/AKT/mTOR signaling pathway has also been implicated as a potential mechanism of acquired resistance to EGFRi.(46) However, unlike in metastatic melanoma patients who develop acquired resistance to BRAF inhibitors,(81) functionally relevant variants that cause EGFRi resistance via this pathway are relatively rare in CRC. (46) This mechanism has more definitely been implicated as a cause of acquired resistance to HER2 inhibition.

Single nucleotide polymorphisms (SNPs) in candidate genes associated with regulating the EGFR pathway communication also appeared to be of predictive value in several reports. (82) In particular, Stintzing et al have identified SNPs in genes involved in EGFR turnover that predicted clinical outcome in cetuximab-treated mCRC patients.(82) EGFR recycling could be an interesting mechanism of secondary resistance to cetuximab in mCRC and is also a putative mechanism for PTEN induced EGFRi resistance.(28,82) Further studies are needed to validate these preliminary data.

## Paracrine Effects

Several groups have found that paracrine mechanisms arising from resistant clones can propagate resistance in neighboring cells without genetic drivers of resistance. In several solid tumors, cancer cells produce growth factors that sustain resistance to targeted therapies.(83) In mCRC, overexpression of a TGF- $\alpha$  induced EGFR–MET interaction, with subsequent MET phosphorylation and activation of downstream effectors in an EGFR-independent manner is a known acquired resistance mechanism.(84) These growth factors may have cell-specific effects or may cause effects on nearby cells. In preclinical work, tumor cells initially sensitive to cetuximab react to EGFR blockade by increasing secretion of EGFR ligands such as TGF- $\alpha$  and amphiregulin.(85) Consistent with this, increased levels of circulating EGFR ligands in the plasma of patients at the time of radiologic progression on cetuximab and irinotecan, suggest their potential role as a mechanism of acquired resistance to treatment.(86) This suggests that increased production of EGFR ligands by cetuximab-resistant derivatives can maintain protection of sensitive cells, thus sustaining their growth.(85) This paracrine protective mechanism may be therapeutically exploitable but demonstrates the challenges of tumor heterogeneity stemming not only from adaptive genetic events, but also microenvironmental interactions.

## Cell Fate Mechanisms of Resistance

In addition to these mechanisms, adaptive changes in differentiation status and cell fate are widely associated with resistance in cancer cells. Epithelial-to-mesenchymal transition (EMT) is a complex biological process wherein epithelial cells procedurally lose their original morphology and simultaneously acquire mesenchymal characteristics.(87) It remains unclear whether EMT is a driver of EGFRi resistance.(87) This data needs additional evaluation in future studies in order to further understand the correlation between EMT and acquired resistance to EGFRi.

## Dynamic Nature of Acquired Resistance

Growing utilization of ctDNA testing(48) has allowed for the non-invasive detection of heterogeneous molecular alterations which drive the evolution of resistance to targeted therapies in mCRC.(5,48,49,55,57,68,88) Moreover, because ctDNA measures fragments of DNA shed by malignant cells throughout the body, it allows detection of resistance mechanisms emerging concurrently in distinct metastatic lesions and tumor subclones.(89,90) This reassessment for resistance can be repeated easily with serial lab draws.

Khan and colleagues have demonstrated that serial ctDNA analysis can predict the time to treatment failure using mathematical modeling.(91) They noted that resistance mutations were detected in the blood up to several months prior to the identification of radiographic progression.(54,57,58,91) Based on these findings, they developed a mathematical model to identify the emergence of resistance, knowing that the tumor is composed of two separate populations of cells; those that are treatment-sensitive, and those that are treatment-resistant, both of which have determined growth and death rates. Using serial ctDNA assessments, this allowed for the prediction of time until radiographic relapse with reasonable accuracy.

Serial ctDNA analysis may also be used to predict the loss of resistance mechanisms; an important determinant of potential future therapeutic options. Our group has previously shown that in the absence of continued selective pressure from EGFRi, the relative prevalence of *RAS* mutated and *EGFR* mutated clones declines.(92) *RAS*<sup>MT</sup> and *EGFR*<sup>MT</sup> alleles exponentially decay with a cumulative half-life of approximately 4 months.(5) These data are consistent with prior reports of clinical benefit with EGFRi rechallenge(93,94) and will help guide the timing of rechallenge therapies in the future. These findings provide strong support for the feasibility and validity of genomic profiling of known acquired resistance mutations to EGFRi by ctDNA to predict clonal decay, and provide strong support for rechallenge with EGFRi.

## Clinical Results from EGFRi Rechallenge Studies to Date

Several prospective clinical trials examining EGFRi rechallenge are currently underway. Some results, albeit with small sample sizes, have been published to date, however results have been inconsistent. These varied results may be due to the heterogeneous prognosis of patients who are able to proceed through several lines of standard therapy and still be well enough to enroll on clinical trials. Another explanation for the discrepant results arises from the rules each trial uses to identify the population to rechallenge. Trials have used a mixture of time criteria and ctDNA to select patients for inclusion, which creates a challenge in interpreting results (see Table 1). In one trial, after an EGFRi treatment-free interval of 6 months, one group identified a response rate (RR), stable disease (SD) and disease control rate of 54%, 36%, and 90%, respectively with EGFRi rechallenge. All patients were *KRAS*<sup>WT</sup> (codons 12 and 13), with prior response to EGFRi defined as a clinical benefit (confirmed stable disease (SD) for at least 6 months or clinical response) after a line of cetuximab plus irinotecan-based therapy followed by progressive disease. ctDNA was not tested in this study.(94) Meanwhile, in the recent CRICKET single arm phase 2 study, ctDNA was retrospectively evaluated at enrollment. Patients with tissue-based *RAS*<sup>WT</sup> and

*BRAF*<sup>WT</sup> with at least a partial response (PR) and progression-free survival (PFS) of at least 6 months to first-line cetuximab plus irinotecan were studied and found to have a RR, SD, and DCR rate of 21%, 32%, and 54%, respectively to EGFRi rechallenge. There was a statistically significant correlation between benefit from the first EGFRi and rechallenge. (95) All patients achieving a PR were ctDNA *RAS*<sup>WT</sup> prior to rechallenge with EGFRi and these patients experienced a significantly longer PFS compared to ctDNA *RAS*<sup>MT</sup> patients (4 vs 1.9 months). In other abstracts, EGFRi rechallenge strategies had RR ranging from 8–43% in these preliminary reports, and DCR ranging from 40–53% (Table 1).(96–100) Notably, a dynamic evolution of *KRAS* alterations between first administration and rechallenge with EGFRi was demonstrated by ctDNA in only one of the above mentioned trials.(57) Thus, there is marked heterogeneity in terms of efficacy across different trials exploring rechallenge strategies, and further large scale trials such as FIRE-4 (NCT02934529), and PULSE (NCT03765736) with serial ctDNA testing are needed to identify the best treatment strategies for these patients.

## Future Goals and Remaining Questions

ctDNA testing(48) has allowed for the detection of heterogeneous molecular alterations which underlie the evolution of resistance to targeted therapies in mCRC.(5,46–48,71) Such analyses have uncovered the role of acquired *RAS* and other subclonal mutations in the development of resistance to EGFRi. These findings have paved the way for ongoing clinical efforts to define the efficacy of rechallenge therapies with EGFRi. ctDNA analysis may correlate better with sensitivity of an individual patient and act as a rapid surrogate of tumor response, allowing testing to be timed with the need for an alternate treatment regimen.(101) Finally, it may allow for higher utilization and earlier access to clinical trials.

However, limitations of ctDNA testing remain. Cost is a concern, in addition to the imprecise ability to understand subclonality and trends with a single assay. Optimally, serial testing would be done. Further, the threshold for positivity of a resistance clone and treatment is not yet established and likely not an absolute, but rather an indication of gradation of benefit with reduction in subclonal burden. Premature treatment discontinuation of EGFRi based on ctDNA may sacrifice duration of tumor control, especially where available therapies are limited.

Thus, there are many remaining questions. First, is there a better way to intermittently dose EGFRi to prevent or mitigate resistance? In the frontline setting, the Phase 2 COIN-B study found no significant difference in 10 month or median failure free survival with intermittent versus continuous dosing of cetuximab.(102) However, a similar analysis has not been done in the rechallenge setting. Further, paracrine protective mechanisms could explain why, in the complex scenario of a heterogeneous disease where different subclones exist in the same patient, previously sensitive CRC cells can successfully grow despite EGFRi when they are present with their resistant derivatives.(31,38,47,55,68,92) In fact, one study observed that patients previously treated with EGFRi had an average of 5 different resistant alterations. This emphasizes the limitations of single drug therapy to overcome a broad array of resistance mechanisms, particularly as these mutations often occur in multiple functionally distinct targets in individual patients.(47) These findings support the use of ctDNA analysis



to define the genomic landscape of patients with mCRC and to guide more targeted therapies in the setting of therapeutic resistance. However, much of the data to date is based on preclinical work and is yet to be examined in prospective studies.

Finally, there is a great deal of interest in methods of intercepting acquired resistance to EGFRi. The MAPK pathway is a key regulator of cellular proliferation and survival. Extracellular signal regulated kinase (ERK) is a downstream member of this pathway and plays a central role in transmitting extracellular signals from activated RTKs such as EGFR/FGFR/PDGFR/VEGFR. The feasibility of pharmacologic inhibition of the MAPK pathway in cancer has been shown through the success of BRAF and MEK inhibitors in the treatment of various cancer subtypes, especially patients who harbor *BRAF*<sup>MT</sup>. In mCRC, one group demonstrated that regardless of the mutation that confers resistance to EGFRi, the outcome is always sustained activation of MEK and ERK.(103) More recently, post-progression ctDNA from patients with mCRC treated with a BRAF inhibitor and EGFRi demonstrated clones that reactivated MAPK.(78,104) These data have provided a rationale for the use of combined MAPK inhibition and EGFRi to overcome resistance to EGFR antibodies. Blockade of ERK1/2 directly is postulated to overcome many of the current limitations of EGFRi in patients with acquired *RAS* mutations, and such studies are planned. Thus, these mechanisms may be partially analogous but further clinical data is needed. There are several ongoing phase 1/2 trials examining the role of MEK inhibition with EGFRi (NCT03087071, NCT02857270).

Several randomized clinical trials are currently ongoing to further clarify acquired resistance mechanisms to EGFRi, and will certainly assist clinicians in timing of rechallenge therapies, as well as in the discovery of therapeutic efforts to reverse resistance to EGFRi(5). Moreover, data to date support the use of ctDNA profiling to guide treatment of patients with mCRC and to track clonal evolution. Further large-scale prospective trials, in addition to education and dissemination of existing best practices is critical.

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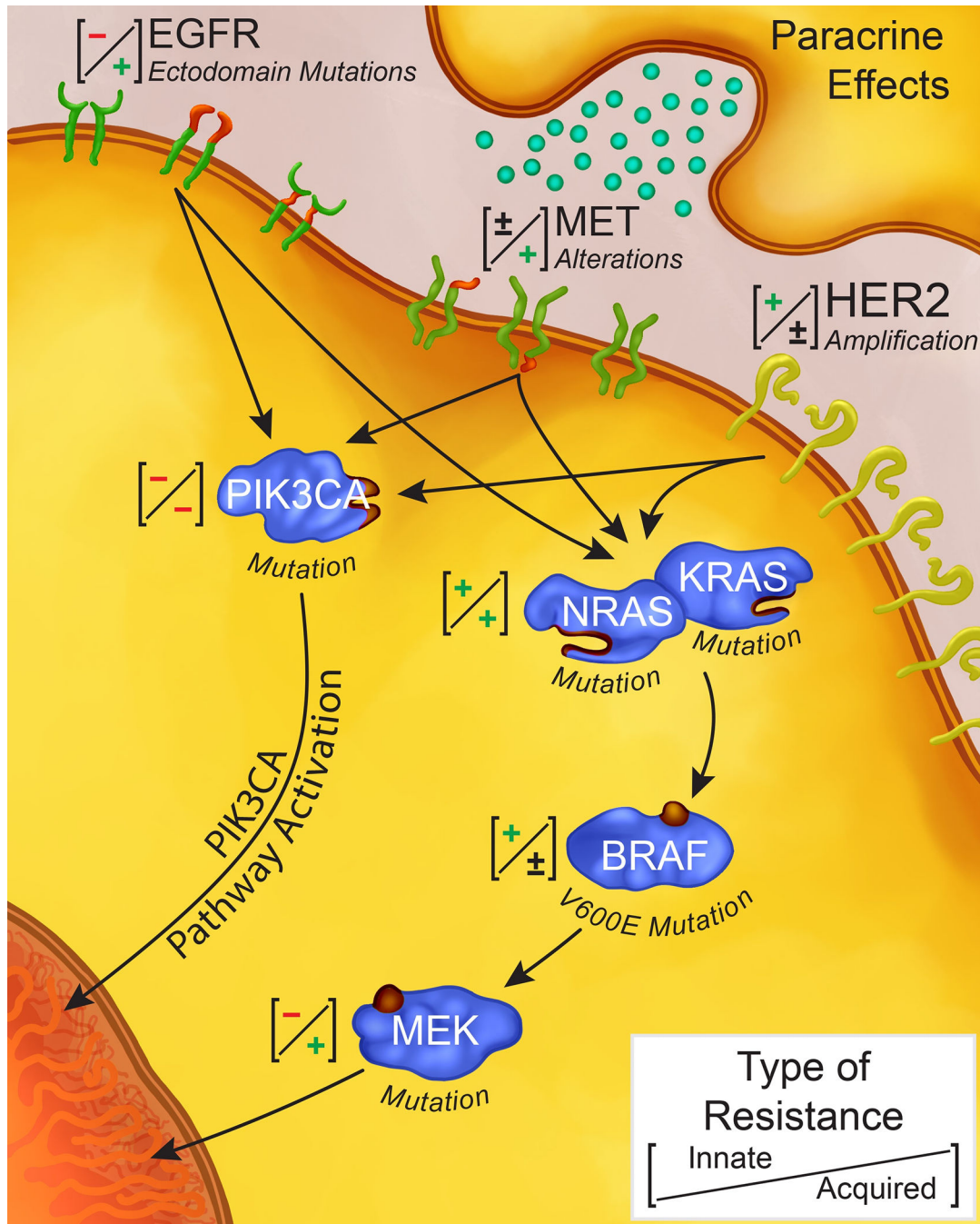
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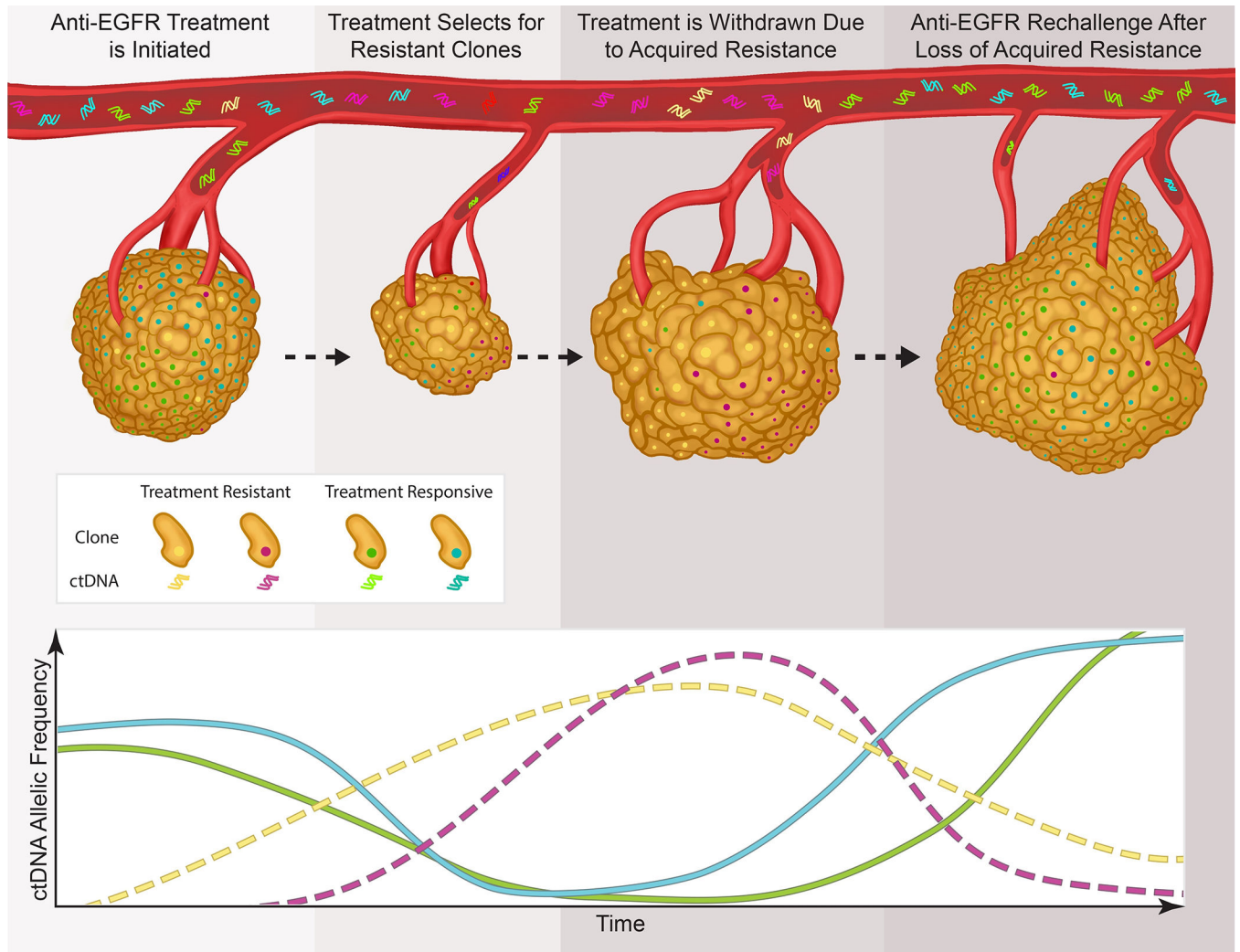
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**Figure 1.** Forms of innate and acquired resistance to anti-EGFR agents in metastatic colorectal cancer. (+) denotes the presence of a resistance mechanism (either innate or acquired). (-) denotes the absence of a resistance mechanism (either innate or acquired).



**Figure 2.** Selection, expansion, and loss of multiple tumor subclones at time of treatment and withdrawal of anti-EGFR highlights potential use of anti-EGFR re-challenge strategies.

**Table 1.**

Summary and Clinical Results from Anti-EGFR Rechallenge Studies to Date

Study	NCTN ID	N	Population	ctDNA	Treatment	ORR/DCR
Wadlow et al.(105)	NCT00842257	20	Prospective <i>KRAS</i> wild type with prior failure of cetuximab	Not assessed	panitumumab	0%/45%
Pietrantonio et al. (106)	n/a	30	Retrospective <i>KRAS</i> wild type with prior failure of cetuximab	Not assessed	Panitumumab	30%/67%
Saif et al.(107)	n/a	15	Retrospective <i>KRAS</i> wild type with prior failure of cetuximab	Not assessed	Panitumumab	27%/55%
Santini et al.(94)	n/a	39	Prospective Prior EGFRi PFS > 6 months and EGFRi free > 6 months	Not assessed	cetuximab + irinotecan	54%/90%
CRICKET Cremolini et al. (95)	NCT02296203	28	Prospective Prior EGFRi partial response AND PFS > 6 months with an intervening line of therapy	Integrated	cetuximab + irinotecan	21%/54%
Tsuji et al.(96)	n/a	36	Prospective Prior EGFRi PFS > 6 months with an intervening line of therapy	Not assessed	cetuximab + irinotecan	3%/56%
Nogueira et al.(96)	n/a	15	Retrospective Prior EGFRi with an intervening line of therapy	Not assessed	EGFRi +/- chemotherapy	13%/40%
Osawa et al.(98)	n/a	33	Prospective Refractory to 5-FU, irinotecan, oxaliplatin, with last EGFRi > 16 weeks prior	Integrated	cetuximab + irinotecan	16%/56%
TRECC Karani et al.(100)	n/a	68	Retrospective Prior EGFRi with intervening line of therapy	Not assessed	EGFRi +/- chemotherapy	43%/ unknown
FIRE-4 Heinemann et al.	NCT02934529	550	Prospective Prior FOLFIRI plus cetuximab PFS > 6 months and intervening line of treatment	Integrated	Randomized: cetuximab + irinotecan vs standard of care	Not yet reported
PULSE Strickler et al.	NCT03765736	500	Prospective Umbrella protocol for patients refractory to 5-FU, irinotecan, oxaliplatin, EGFRi, and immunotherapy if MSI-H	Integral	Multi-armed but rechallenge arm is panitumumab	Not yet reported
Parseghian et al.	NCT03087071	84	Prospective Refractory to anti-EGFR with 3 cohorts: ctDNA detected EGFR S492R mutation OR <i>RAS/BRAF</i> mutation OR no ctDNA mechanism of resistance	Integral	Panitumumab +/- trametinib if ctDNA <i>RAS/BRAF</i> mutation	Not yet reported