Failure is not final: ctDNA-guided rechallenge therapy in colorectal cancer

Medical treatment of metastatic colorectal cancer (mCRC), the second tumor type for incidence, relies primarily on chemotherapies [1]. The addition of targeted agents such as the anti-EGFR antibodies cetuximab and panitutumab has improved patients' survival [2]. Unfortunately, almost invariably, patients treated with EGFR blockade drugs develop resistance [3]. From a molecular perspective, acquired resistance to anti-EGFR (epidermal growth factor receptor) treatment is associated with two main mechanisms: the first involves the emergence of activating mutations in EGFR downstream effectors (primarily KRAS, NRAS and BRAF), while the second relies on mutations in the EGFR extracellular domain (ECD) that impair antibody binding to its target [4].

Treatment of patients who respond and then relapse to EGFR blockade drugs remains an unmet clinical need for at least two reasons: first, the molecular bases of relapse are patient-specific and difficult to define as tissue biopsies are not systematically carried out in this setting and have intrinsic risks [5]; secondly, *KRAS* and *NRAS* secondary mutations, which occur in about 30%–40% of the patients, are presently undruggable as the corresponding proteins are recalcitrant to pharmacological blockade [6]. As a result, upon failure to chemo plus anti-EGFR therapy, mCRC patients usually undergo additional lines of standard chemotherapy (irinotecan and/or oxaliplatin-based regimens) together with antiangiogenic drugs. None of these treatments is currently based on a molecular rationale.

In this issue of *Annals of Oncology*, Parseghian et al. [7] provide evidence that monitoring levels of *KRAS* and *EGFR* ECD mutations in circulating tumor DNA (ctDNA) of patients has relevance in this setting. Specifically, their findings suggest that tracking kinetics of resistance mutations in blood can be used to guide additional rounds of anti-EGFR therapy.

This study builds on the extraordinary progress made in the ability to measure tumor-derived somatic variants in the blood of patients with solid cancers [8, 9]. The analysis of ctDNA, commonly referred to as liquid biopsies, enables noninvasive identification of molecular alterations emerging during treatment evolution, allowing real time genetic profiling of the overall disease [10, 11].

The results presented by Parseghian et al. rely on previous evidence that mutations associated with resistance to anti-EGFR therapy can be detected in ctDNA [12, 13]. Furthermore, in 2015 we and others reported that mutant KRAS clones, which emerge in blood during EGFR blockade, decline upon withdrawal of anti-EGFR antibodies, indicating that clonal evolution continues beyond clinical progression [14, 15].

The decay of *KRAS* mutations in blood upon anti-EGFR therapy withdrawal is an indication of clonal evolution during therapy. However, the exact molecular bases of this process have not yet been elucidated. The most intuitive hypothesis, which is also supported by Parseghian et al., is that following the Darwinian evolutionary theory, cells with the highest fitness (KRAS and EGFR mutant) are able to survive, ultimately leading to therapeutic failure. The fitness of this resistant population may be, however, innately limited, allowing for the rapid (re)growth of the remaining (sensitive/wild-type) cell population. While this could indeed be the case for KRAS activating variants (which renders CRC cells independent from upstream EGFR inhibition), how EGFR ECD mutants negatively affect the fitness of CRC cells in the absence of an EGFR blockade and, as a result, decay in blood, is less intuitive. These questions should be further addressed as they could reveal important cellular mechanisms responsible for clonal competition and fitness in patients receiving treatment with anti-EGFR antibodies.

The most important finding of the current study is the accurate determination of the kinetics of the *KRAS* and *EGFR* decay in blood, which had so far remained poorly defined. To address this, Parseghian et al. analyzed postprogression ctDNA samples obtained from 135 RAS/BRAF wild-type mCRC patients who underwent EGFR blockade therapy and acquired *RAS* and/or *EGFR* mutations during treatment. Two additional cohorts where included (i) a validation dataset, with 73 patients showing ctDNA profile suggestive of prior anti-EGFR exposure with serial blood sampling and (ii) a separate retrospective dataset with 107 cases to evaluate overall response rate and median time to rechallenge therapy.

The results revealed that the relative mutant allele frequency of RAS and EGFR mutant clones, decays exponentially within a cumulative half-life of 4.4 months (Figure 1). Interestingly, when individual mutations were analyzed separately, RAS mutant clones were found to decay faster (half-life of 3.7 months), while EGFR ones dropped within 4.7 months.

Most importantly, in the retrospective cohort, the longer the time intervals from EGFR blockade discontinuation and time to rechallenge therapy, the higher was the overall response rate. This is relevant as it suggests that future studies exploiting ctDNA kinetics to guide therapy in this setting should be aimed at maximizing *RAS*/*EGFR* mutant levels decay before anti-EGFR therapy is reinitiated.

Furthermore, the interval between two exposures to EGFR blockade should be taken carefully into account as it impacts the efficacy of the rechallenge therapy. This is also consistent with previous evidence that upon a second round of therapy, resistant clones that had decayed in blood raise again [14].

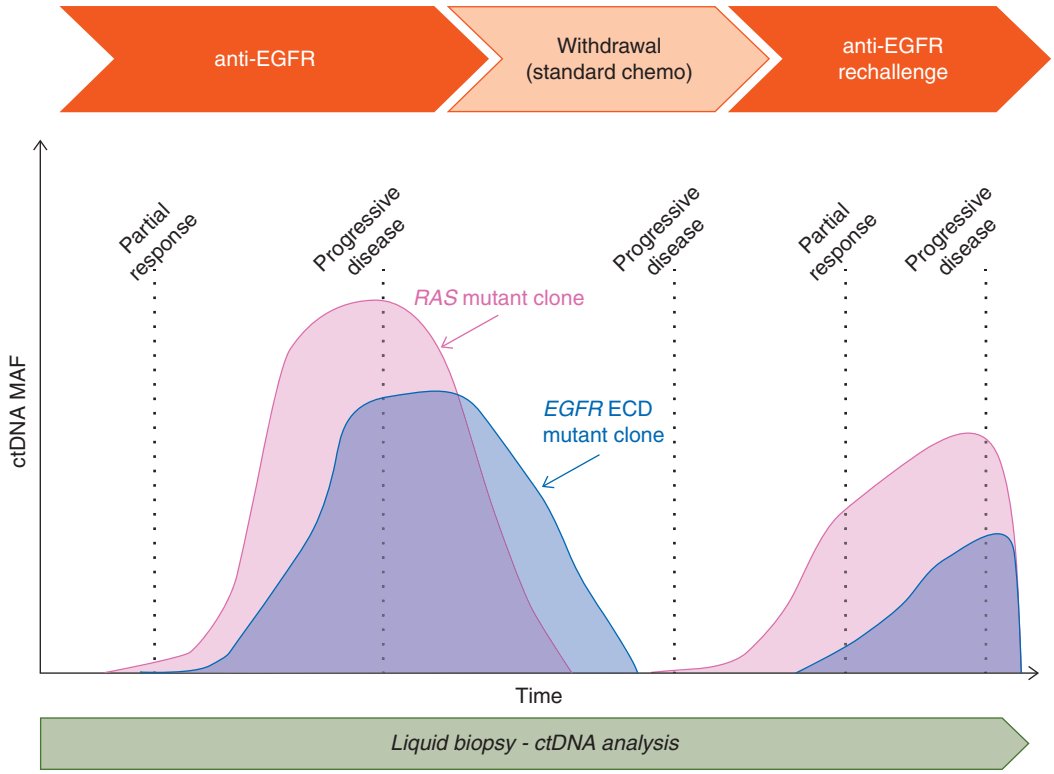

Figure 1. Schematic representation of *RAS* and *EGFR* ECD mutation (purple and blue lines, respectively) dynamics monitored through treatment by liquid biopsy-based, ctDNA analyses. Mutant clones, emerge at acquisition of resistance, but decay in the circulation upon anti-EGFR drugs withdrawal, although with different timings. When rechallenge therapy is administered, mutant clones raise again in the blood.

Firstly, they further encourage delivering multiple rounds of anti-EGFR rechallenge therapies for mCRC cases who initially respond to this regimen. Secondly, they offer additional support to the use of liquid biopsies (in this case measuring *KRAS* and *EGFR* mutant clones), to monitoring tumor kinetics (clones' half-life) and to guide the timing of rechallenge therapies.

Notably, clinical experimentations, which build upon clonal kinetics, are already ongoing in this clinical setting. For example, CHRONOS (the Greek God of time; NCT03227926), is aimed at using liquid biopsies to identify mCRC patients originally responsive to anti-EGFR therapy who can then benefit from rechallenge. In CHRONOS patients receive a second round of EGFR blockade drugs based on *RAS/BRAF* ctDNA kinetics; specifically patients are being rechallenged when *RAS/BRAF* levels drop by >50% from their original levels.

Based on the results of Parseghian et al. it is likely that future studies will also need to consider fine tuning of the time for rechallenge, e.g. by waiting till mutant *RAS* levels minimally drop (become nondetectable) in blood before reinitiating treatment. Future studies should also include measurement of *EGFR* ECD mutations given that, as shown in the current manuscript, they decay when EGFR therapy is suspended.

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Disclosure

The authors have declared no conflicts of interest.

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Is front-line checkpoint blockade ATTRACTIVE in advanced gastric cancer?

Immune checkpoint inhibitors are transforming oncological practice. Approvals in advanced disease have signified a landmark shift in the treatment paradigms of many cancers. Anti-programmed cell death protein 1 (PD-1) monoclonal antibodies are now approved in the chemorefractory setting for Asian patients and a subset of PD-L1-positive patients with advanced gastro-oesophageal adenocarcinoma [1, 2]. Responses in gastro-oesophageal adenocarcinoma have, however, been mixed. Anti-PD-1 and anti-PD-L1 antibodies in the second and third lines, respectively, versus chemotherapy, have failed to show an overall survival (OS) benefit [3, 4] and therefore outside clinical trials, the role of checkpoint inhibitors is currently limited to PD-1 inhibition for selected patients in the third or subsequent line with the exception of mismatch repair (MMR)-deficient disease [5].

Globally, gastric cancer (cardia and non-cardia) is the fifth most common cancer and third leading cause of cancer-related mortality [6]. Survival for patients with advanced disease is poor and treatment options beyond first line are limited. Only trastuzumab, for human epidermal growth factor receptor 2 (HER2)-positive cancers in the first-line advanced setting [7] and ramucirumab in the second-line [8], has shown significant benefit in addition to standard chemotherapy, underlining the need for novel therapies and the unmet need in this area, especially given the lack of progress in recent years in the front-line setting [9–13].

Safety and efficacy of nivolumab in combination with S-1/capecitabine plus oxaliplatin in patients with previously untreated, unresectable, advanced, or recurrent gastric/gastro-esophageal junction cancer: interim results of a randomized phase 2 trial (ATTRACTION-4) is a two-part study designed to evaluate nivolumab in combination with chemotherapy. In this issue, Boku et al. report results of the safety and efficacy of a randomised phase II study of nivolumab (360 mg q3w) in combination with S1(S)/capecitabine(Cape) plus oxaliplatin (OX) in patients with untreated, unresectable, advanced or recurrent HER2-negative gastric/gastroesophageal junction adenocarcinoma, part 1 [14]. Safety was the primary end point and upon condition that the combination was safe and tolerable and $\geq$2 of 15 patients achieved a complete or partial response the phase III study, part 2 was initiated. In part 1, both chemotherapy backbones were found to be safe with objective response rates (ORR) of 57.1% [95% confidence interval (CI) 34.0%–78.2%) and 76.5% (95% CI 50.1%–93.2%) observed for nivolumab plus SOX and nivolumab plus CapeOX, respectively. Median progression-free survival was 9.7 months (5.8 to not reached) and 10.6 months (5.6–12.5) in the nivolumab plus SOX and nivolumab plus Cape/OX groups, respectively. Median OS was not reached with a median follow-up of 13.2 months. No significant differences in safety or efficacy were seen between the two groups.

These results merit validation in the ongoing phase III ATTRACTION-4 study (part 2) comparing investigators choice SOX/CapeOX plus nivolumab versus SOX/CapeOX plus placebo in chemotherapy naive patients. There is sound rationale to evaluating immune checkpoint inhibition in earlier lines of therapy. Response rates to monotherapy PD-1 inhibition in chemorefractory disease are modest with 11%–12% objective response reported [1, 15]. Beyond the cytotoxic effects of oxaliplatin-based chemotherapy, induced effects on the immune stimulation have the potential to be beneficial in combination with immune checkpoint blockade [16]. In preclinical lung adenocarcinoma mouse models, immunogenic chemotherapy (oxaliplatin combined with cyclophosphamide) has been shown to sensitise tumours lacking T-cell infiltrates to PD-1 antibodies [17].

In ATTRACTION-4, part 1, the combination of oxaliplatin/fluoropyrimidine chemotherapy together with nivolumab was considered to have a manageable toxicity profile. It is worth commenting, however, that all patients in the safety population ($n = 39$) experienced treatment related adverse event (TRAE) leading to dose delay or reduction in 94.9% and 15.4% developed grade $\geq$3 treatment-related serious adverse events according to the National Cancer Institute Common Terminology Criteria for Adverse Events, Version 4.0 [18]. Most frequently reported TRAEs in both groups were expected adverse events associated with oxaliplatin and/or fluoropyrimidines. TRAEs led to treatment discontinuation for three patients (14.3%) in the nivolumab plus SOX group and two patients (11.1%) in the nivolumab