Editorial

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 shown to improve patients' survival [2]. Unfortunately, almost invariably, patients treated with EGFR blockade drugs develop resistance [3]. From a molecular perspective, acquired resistance to anti-EGFR 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 performed 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 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 circulating tumor DNA (ctDNA), commonly referred to as liquid biopsies, enables non-invasive 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 circulating tumor DNA [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 and colleagues, 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 (yet to be understood) 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 and colleagues analyzed post-progression 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: (1) a validation dataset, with 73 patients showing ctDNA profile suggestive of prior anti-EGFR exposure with serial blood sampling and (2) a separate retrospective dataset with 107 case to evaluate overall response rate and median time to rechallenge therapy.

The results revealed that the relative mutant allele frequency (rMAF) 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. This would ensure the greatest expansion of RAS/EGFR ECD wild-type clones.

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].

How do the current results affect future clinical studies?

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 re-challenge therapies.

How far are we from implementing the above ctDNA measurements in clinical practice?

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 more than 50% from their original levels.

Based on the results of Parseghian and colleagues it is likely that future studies will also need to consider fine tuning of the time for rechallenge, for example by waiting till mutant *RAS* levels minimally drop (become non-detectable) 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.

G. Siravegna[1*] and A. Bardelli[1,2]

[1]Department of Oncology, University of Torino; [2]Candiolo Cancer Institute-FPO, IRCCS, Candiolo, Italy;

(*E-mail: giulia.siravegna@ircc.it)

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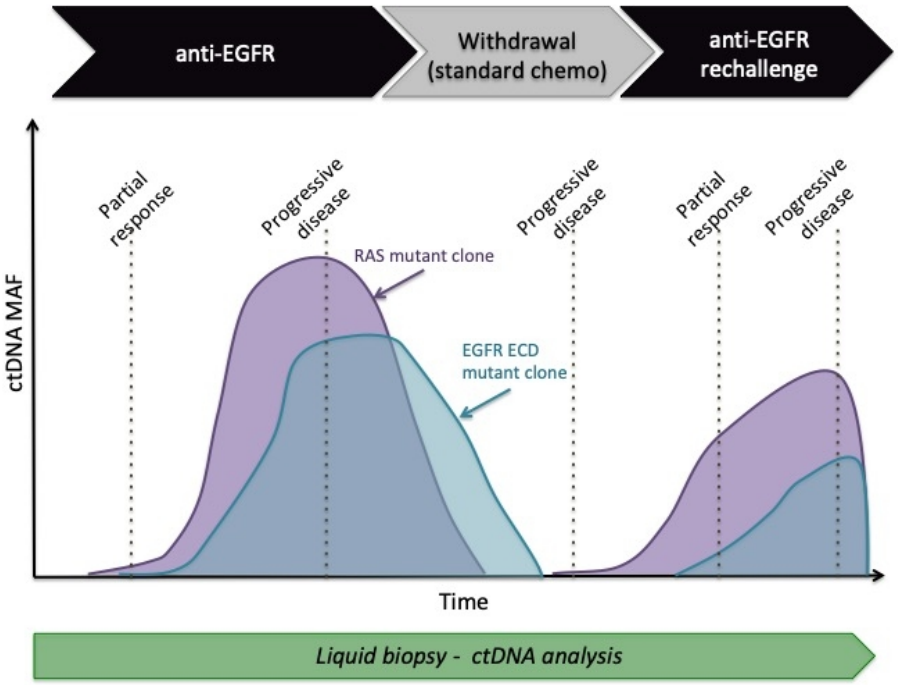
Figure legend

Schematic representation of *RAS* and *EGFR* ECD mutation (purple and blue line, 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.

References

1. Fakih MG. Metastatic colorectal cancer: current state and future directions. J Clin Oncol 2015; 33: 1809-1824.
2. Peeters M, Price T. Biologic therapies in the metastatic colorectal cancer treatment continuum--applying current evidence to clinical practice. Cancer Treat Rev 2012; 38: 397-406.
3. Bardelli A, Siena S. Molecular mechanisms of resistance to cetuximab and panitumumab in colorectal cancer. J Clin Oncol 2010; 28: 1254-1261.
4. Arena S, Bellosillo B, Siravegna G et al. Emergence of multiple EGFR extracellular mutations during cetuximab treatment in colorectal cancer. Clin Cancer Res 2015.
5. Krishnamurthy N, Spencer E, Torkamani A, Nicholson L. Liquid Biopsies for Cancer: Coming to a Patient near You. J Clin Med 2017; 6.
6. McCormick F. K-Ras protein as a drug target. J Mol Med (Berl) 2016; 94: 253-258.
7. Parseghian C, Loree J, Morris V, al. e. Anti-EGFR Resistant Clones Decay Exponentially After Progression: Implications for Anti-EGFR Re-challenge. Ann Oncol 2018; 29: doi.org/10.1093/annonc/mdy509
8. Schwaederle M, Chattopadhyay R, Kato S et al. Genomic Alterations in Circulating Tumor DNA from Diverse Cancer Patients Identified by Next-Generation Sequencing. Cancer Res 2017; 77: 5419-5427.
9. 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: 224ra224.
10. Siravegna G, Marsoni S, Siena S, Bardelli A. Integrating liquid biopsies into the management of cancer. Nature Reviews Clinical Oncology 2017.
11. Siravegna G, Bardelli A. Blood circulating tumor DNA for non-invasive genotyping of colon cancer patients. Mol Oncol 2016; 10: 475-480.
12. 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: 532-536.
13. Diaz LA, Williams RT, Wu J et al. The molecular evolution of acquired resistance to targeted EGFR blockade in colorectal cancers. Nature 2012; 486: 537-540.
14. 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.
15. 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.



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