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The Stromal and Immune Landscape of Colorectal Cancer Progression during Anti-EGFR Therapy

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In this issue of *Cancer Cell*, Woolston et al. show that colorectal cancers that become refractory to initially effective anti-EGFR therapy contain an abundance of stromal and immune cells, irrespective of the contextual presence of resistance-conferring mutations. This reconfiguration puts forward therapeutic opportunities for patients who relapse on EGFR-targeting treatment.

The EGFR antibodies cetuximab and panitumumab are used in patients with EGFR-expressing, *KRAS*, or *NRAS* wild-type metastatic colorectal cancer (mCRC) either in combination with standard chemotherapy, for first-line treatment, or as single agents when tumors become resistant to prior cytotoxic regimens. However, only 20% of individuals experience tumor regressions, and only an additional 30% have some extent of clinical benefit in terms of disease stabilization (Douillard et al., 2013). This relatively low response rate is compounded by the dismal reality that subjects who initially respond typically become refractory to treatment in a period of months. In this issue of *Cancer Cell*, Woolston et al. (2019) offer a comprehensive picture of the identifying traits of primary and acquired resistance to cetuximab in a cohort of 35 mCRC patients (Figure 1). In a difference from previous studies, mostly conducted in a retrospective manner and focused on a small number of candidate biomarkers, here the authors embarked on a prospective trial whereby biopsies collected before initiation of single-agent cetuximab and at the time of disease progression were subjected to whole-exome and RNA-sequencing analyses and immunophenotyping.

Lack of response to antibody treatment *ab initio* has been partly ascribed to the occurrence of mutations or amplifications in genes encoding other tyrosine kinase receptors or RAS downstream effectors, which, similar to mutationally activated RAS, trigger compensatory pathways sustaining EGFR-independent tumor growth (Bertotti et al., 2015). Since these genetic abnormalities occur individually at very low frequency, their catalog has not yet saturated the space of mCRC with primary resistance to EGFR antibodies. Indeed, when Woolston et al. (2019) stratified global genomic data onto response annotation, they pinpointed previously unrecognized alterations, including biallelic inactivation of *NF1* (encoding a GTPase-activating protein that antagonizes RAS function) and *KRAS* and *BRAF* mutations endowed with attenuated enzymatic and transforming activity. Interestingly, hypomorphic *BRAF* and *KRAS* mutations co-existed in the same tumor or, when present singly, were associated with polysomy of the corresponding chromosome. This suggests that their individual contribution to cetuximab resistance is suboptimal and requires either a cooperative or a dosage effect for complete manifestation.

Secondary resistance is often propelled by the clonal expansion of the same alterations responsible for primary resistance, with a preponderance of RAS pathway mutations. Such alterations may arise *de novo* on a stochastic basis, as a consequence of tumor genetic instability, or may pre-exist as minor subclones in the original tumor population because of genetic heterogeneity and become positively selected under drug pressure (Khan et al., 2018). Genetic instability and heterogeneity explain why acquired resistance mutations are usually polyclonal and can be more accurately grasped by analysis of circulating tumor DNA (ctDNA)—which incorporates DNA fragments shed by the whole tumor—than by examination of solid biopsies—which, by definition, are subspatial snapshots of the entire lesion (Khan et al., 2018). Woolston et al. (2019) report a number of genetic alterations of acquired resistance, including already-characterized mutations in components of the RAS pathway (Khan et al., 2018) and a hitherto-unidentified amplification of *FGF10* (encoding a ligand of the FGFR2 tyrosine kinase receptor). These genetic aberrations were detected in only a limited number of post-treatment biopsies but were mostly captured in ctDNA samples, further attesting to the pervasiveness of tissue-sampling bias. Of note, resistance mutations in ctDNA were calculated to occur in a minority of cells, in keeping with previous reports demonstrating the presence of recurrent but subclonal RAS pathway mutations in the blood of cetuximab-refractory mCRC patients (Bettegowda et al., 2014; Khan et al., 2018). Altogether, these findings suggest that tumor relapse is engendered by polyclonal mutuality, with an ecosystem of different subclones contributing to therapeutic resistance. However, we cannot exclude that when subclonal alterations are present at a very low allele frequency the impact on resistance may remain sub-threshold, and other (non-genetic) determinants could subsidize or substitute for DNA mutations to reduce responsiveness to EGFR inhibition.

Woolston et al. (2019) strongly embrace the assumption that progression on cetuximab can be also fostered by non-mutational mechanisms and extend their

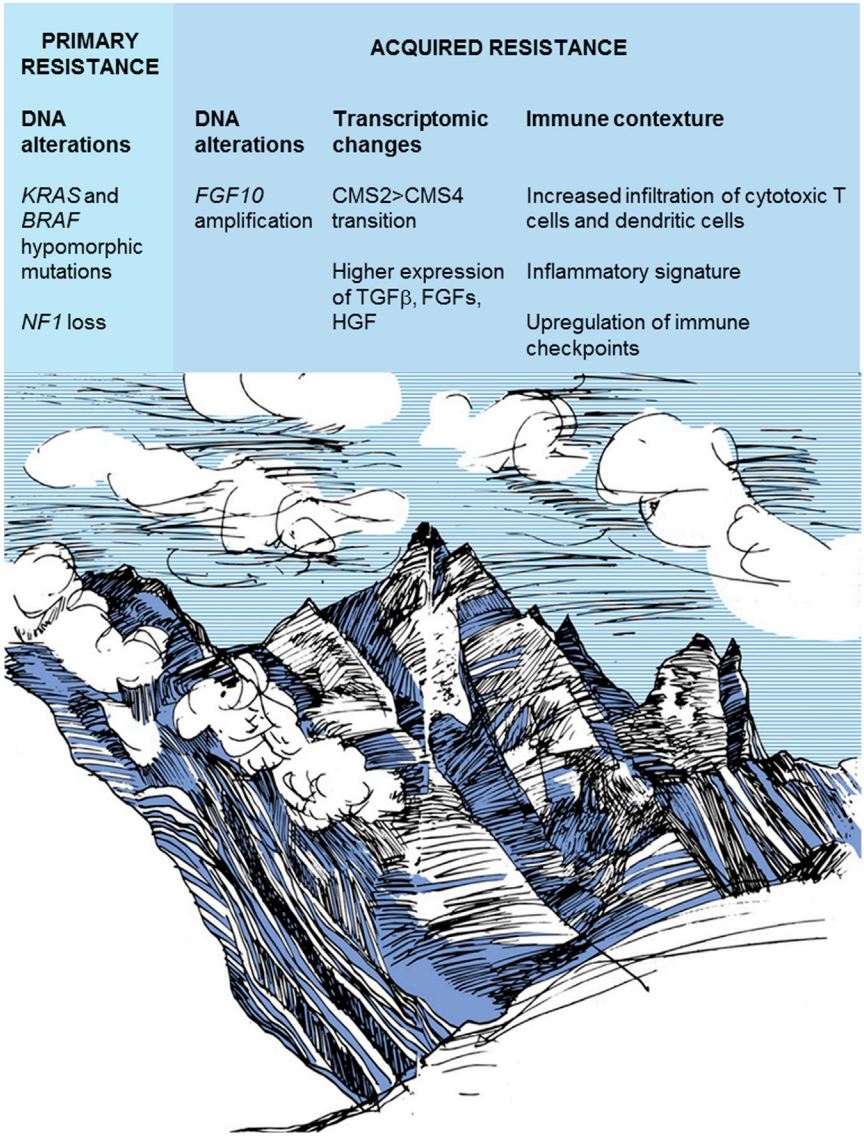

Figure 1. New Genomic, Transcriptomic, and Immune Elements in the Landscape of Cetuximab Resistance in mCRC

Novel genetic alterations are found to be associated with, and causally responsible for, treatment failure in tumors that are or become insensitive to cetuximab therapy. Furthermore, tumors with acquired resistance undergo a transcriptomic shift from the CMS2 subtype, which features a predominantly epithelial phenotype, to the CMS4 subtype, characterized by a high content of stromal growth factors that are shown to protect cancer cells from the antiproliferative effects of EGFR inhibition. Finally, tumors from patients who progress on cetuximab are more infiltrated by immune cells and have higher expression levels of immune checkpoints than tumors from cetuximab-naive patients.

investigation by delineating the transcriptomic profiles of matched sensitive and post-therapy resistant tumors. First, they confirm that a subgroup of tumors with gene expression traits reminiscent of those portrayed by the transient-amplifying precursors of the normal intestine (assigned to the so-called CMS2 consensus transcriptional subtype) were enriched for cetuximab-responsive cases. Then, the authors show that the majority of tumors with acquired resistance to cetuximab (including some harboring subclonal mutations) underwent a gene expression transition toward a stroma-rich (CMS4) phenotype featuring high content of carcinoma-associated fibroblasts (CAFs) and increased expression of CAF-derived growth factors such as TGF-β, HGF, and FGF family ligands. Consistent with the observed association between stromal abundance and drug resistance, the CAF secretome was found to exert a protective activity against cetuximab. These results highlight a key role for transcriptionally regulated growth factors in conveying survival cues that safeguard CRC tumors from the effects of EGFR blockade, in agreement with previous findings (Zanella et al., 2015).

Intriguingly, Woolston et al. (2019) describe a more copious representation of cytotoxic T lymphocytes and dendritic cells, increased expression of a T cell-associated inflammatory signature, and upregulation of immune checkpoints in CMS4-like, TGF-β-high resistant tumors. This result corroborates a retrospective study documenting heightened infiltration of cytotoxic, effector memory, and regulatory T cells in CRC tumors treated with cetuximab and chemotherapy (Van den Eynde et al., 2018). However, the coexistence of elevated TGF-β activity and immune inflammation is unexpected, as TGF-β is known to impair T cell function and promote T cell physical exclusion (Tauriello et al., 2018). How can cetuximab-resistant tumors concomitantly display high levels of immune suppressive TGF-β and an active immune microenvironment? One possibility is that increased immune infiltration precedes the CMS2-to-CMS4 transition. This would be coherent with the notion that cetuximab triggers IgG1 antibody-mediated immunogenic cell death (Pozzi et al., 2016) and with the observation that EGFR pathway activity in lung cancer prompts immune escape, which is counteracted by EGFR inhibition (Akbay et al., 2013). If this is the case, immune stimulation would be a direct consequence of productive cetuximab treatment rather than a hallmark of cetuximab resistance, and it would be interesting to see whether cetuximab-sensitive tumors at maximal response to EGFR blockade have already undergone the inflammatory shift shown by resistant tumors. One could even push this reasoning to the extreme: strengthened TGF-β activity might be a delayed adaptive mechanism to contrast cetuximab-induced immune cell deployment. In this scenario, immunotherapy is expected to synergize with cetuximab in the early phases of response rather than after the emergence of resistance.

The application of genomic technologies has enabled the identification of

clinically actionable DNA alterations in RAS wild-type mCRC tumors that fail or cease to respond to EGFR antibodies, including those illustrated in this study. By providing fresh evidence that acquired resistance to cetuximab also entails a stromagenic and immune-inflamed phenotype, results from Woolston et al. (2019) have important ramifications for the biological understanding of CRC evolution under EGFR blockade and introduce potential opportunities for targeting a novel repertoire of non-mutational vulnerabilities.

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M-TAP Dance: Targeting PRMT1 and PRMT5 Family Members to Push Cancer Cells Over the Edge

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In this issue of *Cancer Cell*, Fedoriw and colleagues characterize a potent reversible inhibitor of type I PRMTs, GSK3368715, with anti-proliferative effects on numerous cancer types. Using a combination of GSK3368715 with PRMT5 inhibitors, the authors show that a threshold of overall arginine methylation reduction needs to be achieved for synergistic anti-tumor activity.

Arginine methylation is a post-translational modification that occurs on many proteins, such as histones and RNA binding proteins (RBPs). In mammalian cells, nine protein arginine methyltransferases (PRMTs) contribute to catalyze three major forms of arginine methylation on proteins using *S*-adenosylmethionine (SAM) as the methyl donor: monomethylarginine (MMA), asymmetric dimethylarginine (aDMA), and symmetric dimethylarginine (sDMA) (Blanc and Richard, 2017).

Type I PRMT1 and type II PRMT5 are the main enzymes responsible for generating most of aDMA and sDMA, respectively. Both enzymes share common substrates with a preference for histone 4 arginine 3 and RGG/RG motifs, most