The gRASs Is Greener: Potential New Therapies in Lung Cancer with Acquired Resistance to KRASG12C Inhibitors

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Inhibitors of KRAS^{G12C} that bind the target in its inactive conformation and lock it in off-mode have shown early signs of clinical activity in patients with *KRAS*^{G12C}-mutant lung cancer, but responses tend to be short-lived and invariably prelude the development of acquired resistance through largely unexplored mechanisms. A new study describes the emergence of RAS-MAPK heterogeneous subclonal alterations in a patient relapsed on a KRAS^{G12C} inactive-state inhibitor and identifies a novel KRAS^{Y96D} resistant variant that is druggable by a next-generation compound capable of associating with KRAS^{G12C} in its active configuration.

KRAS and the structurally related NRAS and HRAS GTPases relay mitogenic stimuli from the extracellular environment to the cell nucleus by stimulating a series of cytoplasmic kinases (RAF, MEK, and ERK, collectively defining the MAP kinase cascade) that culminates in the stabilization and activation of transcription factors driving cell-cycle progression (1). For reversible implementation of this pathway, RAS proteins oscillate between an active, GTP-bound and an inactive, GDP-bound state at rates controlled by upstream growth factor-dependent signals. RAS family oncogenic mutations are common in tumors and typically result in single amino acid substitutions that constitutively activate the encoded enzymes by thwarting their ability to hydrolyze GTP, hence compromising catalytic autoinhibition. *KRAS* mutations, in particular, are found in lung, pancreatic, and colorectal cancers at frequencies of about 30%, 90%, and 40%, respectively (2).

Effective targeting of KRAS has proven daunting due to its high affinity for GTP and lack of sufficiently large pockets that enable accommodation of allosteric inhibitors. Moreover, pharmacologic interception of KRAS downstream effectors – namely, the MAP kinase cascade – is usually counteracted by feedback signal compensation (3). These limitations notwithstanding, the promise of KRAS inactivation has been recently revived by the discovery of inhibitors that selectively target KRAS proteins harboring a glycine-to-cysteine mutation at position 12 (G12C). Such small molecules covalently bind the mutated cysteine and occupy a pocket in the so-called switch-II region when KRAS^{G12C} is in its inactive, GDP-bound conformation, thus abrogating RAS-dependent signaling. Inactive-state KRAS^{G12C} inhibitors can do so because the mutant protein, although mostly engaged in its active conformation, still undergoes nucleotide cycling and experiences periods of inactivity, which allows for drug trapping and covalent attack (4).

Findings from recently completed and ongoing phase I/II trials are a testimony to the merits – but also a warning of the shortcomings – of targeting KRAS^{G12C}. When tested in patients with *KRAS*^{G12C} mutant metastatic non-small cell lung cancer (NSCLC), the KRAS^{G12C} inhibitor sotorasib (AMG 510) was efficacious, with an overall response rate of 32.2% and a median progression-free survival of 6.3 months (5). Likewise, the objective response rate of patients with advanced or metastatic NSCLC treated with adagrasib (MRTX849, another inactive-state KRAS^{G12C} inhibitor

characterized by a long half-life that equals the 24-hour synthesis rate of the KRAS protein) was 45% (6). Although disease control was remarkable in both studies, a relatively large fraction of patients responded suboptimally to either therapy, and many of those who had received some benefit relapsed quickly. According to preclinical experiments in isogenic cell lines, poor response *ab initio* (known as primary resistance) might be explained with a rapid process of nonuniform adaptation whereby some cells escape inhibition by producing new $KRAS^{G12C}$ (which is promptly converted to the active, drug-refractory state) whilst others without sufficient expression of newly synthetized $KRAS^{G12C}$ are eliminated by treatment (7). Less is known about the mechanisms underlying acquired resistance, and whether they mainly involve selection of genetically resistant subclones or plastic fitness variations.

In this issue of *Cancer Discovery*, Tanaka and colleagues (8) begin to delineate genetic alterations that may be responsible for the acquisition of secondary resistance in the clinic and illustrate potential therapeutic opportunities to target some of them. They describe a patient with metastatic NSCLC positive for the $KRAS^{G12C}$ mutation who was treated with adagrasib. The patient had an initial objective response (32% reduction in tumor size) but showed evidence of progressive disease after approximately 4 months of treatment. Comparative analysis of cell-free DNA (cfDNA) before treatment and at the onset of resistance revealed the persistence of the $KRAS^{G12C}$ mutation and the appearance of many distinct new mutations, all giving rise to protein products that are not druggable by inactive-state $KRAS^{G12C}$ inhibitors. These alterations are predicted to converge on the reactivation of the RAS-MAPK pathway and include gain-of-function mutations in *KRAS* (which likely originated *in trans* in the remaining wild-type gene copy), *NRAS*, *BRAF*, and *MAP2K1* (encoding the MEK1 protein) (Figure 1).

An interesting piece of information is the discovery of a novel, previously unidentified tyrosine-to-aspartate mutation at position 96 of KRAS ($KRAS^{Y96D}$) (Figure 1). Based on the crystal structure of different inactive-state $KRAS^{G12C}$ inhibitors bound to $KRAS^{G12C}$, the Y96D substitution appears to disrupt a critical hydrogen bond between the hydroxyl group of tyrosine 96 and the pyrimidine ring of adagrasib. More in general, the amino acid change at the tyrosine 96 locus is thought to weaken drug-target chemical interactions by making the switch-II pocket of the mutant enzyme more hydrophilic. This modification affects target occupancy also by $KRAS^{G12C}$ inhibitors other than adagrasib, thus representing a shared liability of currently available compounds. Consistent with a functional role of $KRAS^{Y96D}$, ectopic introduction of the mutant gene into $KRAS^{G12C}$-addicted cancer cell lines attenuated the growth-suppressing effect of inactive-state $KRAS^{G12C}$ inhibitors and enhanced RAS signaling, indicating that $KRAS^{Y96D}$ is an oncogenic mutation that leads to constitutive RAS activation and imparts resistance to $KRAS^{G12C}$ blockade.

Of note, the allele frequency of the $KRAS^{G12C}$ mutation in the post-treatment cfDNA was much higher than that of the newly emerging alterations, pointing to $KRAS^{G12C}$ as a truncal mutation that

is not extinguished by treatment and dominates over minor subclonal branches harboring the putative resistance alterations. Given the very low prevalence (also cumulatively) of the identified mutations, their causal role in establishing tumor progression and clinical relapse is not immediately evident. However, cfDNA values hardly allow for inferring the relative contribution of the different subclonal mutations to the genomic architecture of the tumor, and it may well be that the representation of mutant DNA was more prominent – therefore, more pervasive in dictating resistance – in the lesions carried by the patient than in blood. It may also be that paracrine growth-factors secreted by the tiny portion of resistant cells protected the surrounding arrays of sensitive cells from the therapeutic insult. Finally, in the absence of ultradeep multiregion sequencing data on the pre-treatment tumor tissues, it remains unclear whether the mutant subpopulations preexisted at very low frequency in the original tumor or materialized *de novo* during treatment.

Can one envisage therapeutic options to overcome acquired resistance to inactive-state $KRAS^{G12C}$ inhibitors? Importantly, Tanaka and colleagues (8) show that a new compound targeting active, GTP-bound $KRAS^{G12C}$ retains potency against $KRAS^{Y96D}$ (Figure 1). This drug, called RM-018, has affinity for the chaperone protein cyclophilin-A. The resulting complex facilitates the formation of extensive protein-protein surface interactions that sterically occlude $KRAS^{G12C}$ in its active state and preclude KRAS association with downstream signaling effectors. When tested in $KRAS^{G12C}$ mutant cell lines with exogenous expression of $KRAS^{Y96D}$, RM-108 markedly impaired cell proliferation and reduced RAS signaling. This is welcome evidence that at least one mechanism of therapeutic resistance could be tamed pharmacologically, although it will be crucial to extend these initial observations from engineered cells to *in vitro* and *in vivo* models in which *KRAS* G12C and Y96D mutations spontaneously arise during the tumor natural history.

Tracking down an individual therapy covering the plethora of heterogeneous mutant proteins documented in the study by Tanaka and colleagues (8) will likely be problematic, especially when considering that the identified mutations in *KRAS* and *NRAS* (with the exception of $KRAS^{Y96D}$) are not actionable. Nonetheless, some of the reported mutations (specifically, those detected in the *BRAF* and *MAP2K1* genes) result in proteins that are vulnerable to pharmacologic neutralization, which bodes well for dual therapies against inactive or active $KRAS^{G12C}$ together with BRAF or MEK inhibitors (Figure 1). Fortunately enough, inactive-state $KRAS^{G12C}$ drugs are well tolerated (5,6), with no dose-limiting toxicities or grade 4 therapy-related adverse events. Therefore, a further opportunity could be the design of multiple combination therapies in which a common anti-$KRAS^{G12C}$ backbone is combined with treatments that impact the MAP kinase cascade more profoundly than single-agent BRAF or MEK blockade, for example through vertical inactivation of both BRAF and MEK or by including ERK inhibitors (Figure 1). At least in principle, concomitant shrinkage of the dominant bulk of $KRAS^{G12C}$ mutant cells together with the *MAP2K1* and *BRAF* mutant minor subclones might engender a “cascade effect” on the growth dynamics of other mutant

subclones present in the tumor ecosystem, potentially leading to extinction or at least contraction of drug-resistant foci fueled by currently undruggable non-G12C *KRAS* or *NRAS* mutations (9). Clonal variations in the genetic composition of treated tumors may also modify the synthetic rate of newly produced $KRAS^{G12C}$ and the ratio between active and inactive RAS in functionally heterogeneous tumor subpopulations, which may influence adaptive fitness and susceptibility to inactive-state inhibitors. As done with other targeted therapies in different tumor contexts, preemptive strategies aimed at using inhibitors against the resistance oncoproteins as upfront therapies, before clinical manifestation of the corresponding mutations, should be considered as well (10).

More work is needed to better understand the population prevalence, biological relevance, and therapeutic exploitability of the proposed resistance mechanisms, and it is difficult to anticipate whether laboratory results will be successfully translated into clinical benefit for patients with $KRAS^{G12C}$ mutant cancer. At the same time, much work has also already been done. Until only a couple of years ago, the land of opportunities for effective and durable treatment of *KRAS* mutant tumors was inaccessible and desolate. With a growing body of knowledge on the genetic determinants of acquired resistance to inactive-state $KRAS^{G12C}$ inhibitors and an expanding arsenal of different classes of $KRAS^{G12C}$-targeting agents, this land is more fecund now, and yields blades of greener grass.

Authors' Disclosures: L. Trusolino reports grants from Symphogen, Servier, Pfizer, Menarini, Merck KGaA, and Merus, and personal fees from Eli Lilly, AstraZeneca, and Merck KGaA outside the submitted work. No disclosures were reported by the other author.

Acknowledgments: This study was supported by Associazione Italiana per la Ricerca sul Cancro (AIRC) Investigator Grant 22802, AIRC 5 × 1000 grant 21091, AIRC/Cancer Research UK/FC AECC Accelerator Award 22795, H2020 grant agreement no. 754923 COLOSSUS, and Fondazione Piemontese per la Ricerca sul Cancro-ONLUS 5 × 1000 Ministero della Salute 2016. L. Trusolino is a member of the EurOPDX Consortium.

Figure legend

Figure 1. Potential therapeutic options for $KRAS^{G12C}$ mutant NSCLC with acquired resistance to inactive-state $KRAS^{G12C}$ inhibitors, tailored around the genomic characteristics of resistance

mutations. Illustration adapted and modified from Creative Commons under a Creative Commons Attribution CC0 2.0 (https://creativecommons.org/publicdomain/zero/2.0/).

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KRAS G12F
KRAS G12V
KRAS G13D
?
?
NRAS Q61K
NRAS Q61R
NRAS Q61L
MEK/ERK
inhibitors
MAP2K1 K57N
MAP2K1 Q56P
MAP2K1 E102_I103 del
KRAS G12C
active state
inhibitors
KRAS Y96D
BRAF
inhibitors
BRAF V600E