

Overcoming Adaptive Resistance to KRAS Inhibitors Through Vertical Pathway Targeting

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SUMMARY

KRAS G12C inhibitors have shown promise in KRAS G12C-mutant lung cancer but intrinsic and acquired resistance are common. Cotreatment with inhibitors of the protein phosphatase SHP2 can abrogate the adaptive response of

cancer cells to KRAS inhibitors resulting in greater suppression of MAPK signaling and enhanced tumor growth inhibition.

See related article by Ryan et al., p. 1633

In this issue of *Clinical Cancer Research*, Ryan and colleagues study the adaptive response of KRAS G12C-mutant cells to treatment with KRAS G12C inhibitors (1). The MAPK pathway is a key regulator of cellular proliferation, survival, and differentiation, and alterations in this pathway are present in cancers of nearly all lineages. Despite intense efforts to develop selective inhibitors of MAPK signaling as anticancer drugs, clinical success has been modest to date. Initial drug discovery efforts were focused on inhibiting downstream effectors such as MEK, but clinical efficacy was limited by the narrow therapeutic index of even highly selective allosteric inhibitors. More recent strategies have sought to leverage the biochemical mechanisms through which *RAS* and *BRAF* mutations induce pathway activation to develop mutant-specific inhibitors with less normal tissue toxicity. The greatest clinical impact to date has been observed with RAF inhibitors, such as vemurafenib, that inhibit MAPK signaling only in tumors that express codon 600 *BRAF* mutations. As such these drugs have no antitumor effects in tumors in which RAF signaling is driven by upstream alterations, such as those with *RAS* mutations.

The RAS proteins are guanosine nucleotide binding molecular switches that integrate inputs from cell surface receptors and other intracellular signaling pathways, which are then transmitted to downstream effector cascades such as the MAPK and PI3 kinase pathways. Activating *RAS* mutations result in the accumulation of active GTP-bound RAS. The current generation of KRAS G12C inhibitors are selective, covalent inhibitors of the G12C mutant, and as a result, these drugs do not inhibit wild-type RAS. These compounds function by trapping the KRAS G12C mutant in the GDP-bound state (2). As a result, inhibitors of upstream activators of RAS can, in a cell context-dependent manner, potentiate the activity of KRAS G12C inhibitors by increasing the pool of GDP-bound KRAS G12C. Conversely, as KRAS G12C must cycle into its inactive state for these drugs to function, cellular adaptations that increase the pool of GTP-bound KRAS can confer drug resistance.

Clinical trials of highly selective, covalent KRAS G12C inhibitors are now ongoing. Early results with one such compound, AMG 510, have been promising with 11 of the first 23 patients with KRAS G12C-mutant non-small cell lung cancer achieving a partial response (3). Unfortunately, in some patients, responses were short-lived, and minimal activity was observed in colorectal cancer where only one of the first 26 patients responded. While these results provide proof-of-concept that targeting KRAS G12C is feasible, the data suggest that mechanistically informed combinations will be required to achieve durable responses in most patients. That the clinical activity to AMG 510 has varied as a function of tumor lineage mirrors the experience with the BRAF inhibitor vemurafenib, where single-agent activity was minimal in colorectal cancer due to feedback-induced activation of EGFR signaling.

In this issue of *Clinical Cancer Research*, Ryan and colleagues used the covalent KRAS G12C inhibitors ARS-1620 and AMG 510 to study the adaptive response of KRAS G12C-mutant lung, colon, and pancreatic cancer cell lines to selective KRAS G12C inhibition (1). They found that KRAS G12C inhibitors downregulated MAPK pathway activation in all cell lines as demonstrated by a decrease in the expression of phosphorylated MEK, ERK, and RSK at 4 hours. However, MAPK pathway reactivation was observed in most cell lines by 24–48 hours despite continued suppression of GTP-bound KRAS. By performing isoform-specific pulldown assays, they were able to show that treatment with the KRAS G12C inhibitor resulted in a several fold increase in NRAS-GTP and HRAS-GTP levels. The results suggest that KRAS G12C-mutant cell lines adapt rapidly to selective inhibition of mutant KRAS by activating wild-type RAS and that this oncogenic bypass was sufficient to restore MAPK signaling. Further analysis revealed that the increased activation of wild-type RAS activity was the result of increased receptor tyrosine kinase (RTK) activation. Complicating matters, the specific RTK driving the rebound in MAPK signaling varied among the cell lines in the panel.

As the data suggested that cotargeting a single RTK such as EGFR would be unlikely to maximally suppress MAPK pathway activity, they analyzed whether co-inhibiting the protein tyrosine phosphatase SHP2 could attenuate the activation of wild-type RAS. SHP2 activates RAS downstream of RTKs through several mechanisms, including dephosphorylation of Sprouty proteins. As predicted, cotreatment of cells with ARS-1620 and the SHP2 inhibitor, SHP099, induced more durable ERK inhibition by abrogating RTK-mediated MAPK pathway reactivation. In mice, the combination was well-tolerated and induced sustained MAPK pathway inhibition in xenograft tumors and suppression of tumor growth. On the basis of these compelling preclinical data, we agree with the authors that the KRAS G12C and SHP2 inhibitor combination warrants testing in patients.

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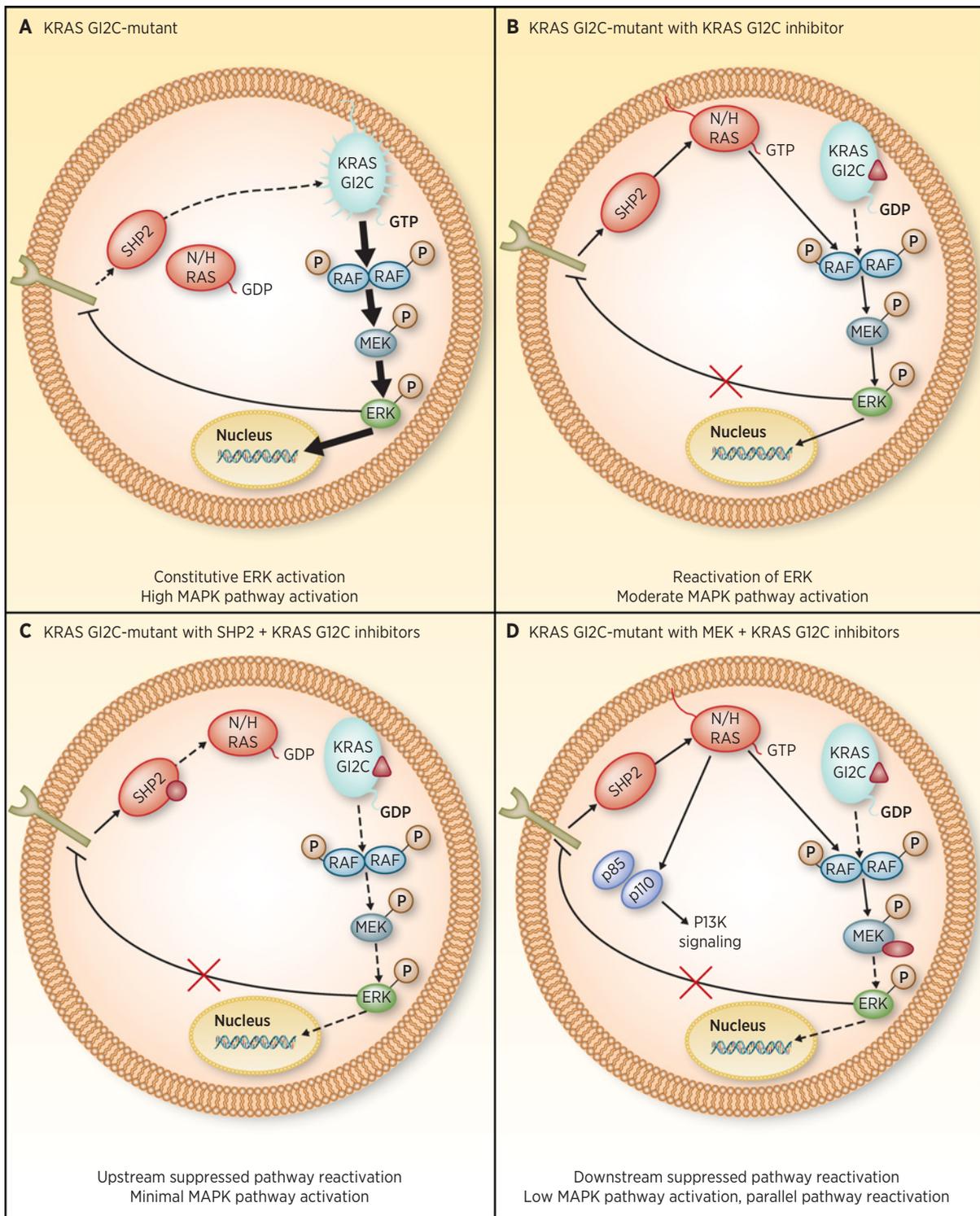


Figure 1. KRAS G12C-mutant cancer cells restore MAPK pathway signaling following selective G12C inhibition through RTK-mediated activation of wild-type RAS. **A**, In KRAS G12C-mutant cancer cells, RTK activation is suppressed by ERK-mediated-negative feedback. **B**, Treatment of KRAS G12C-mutant cells with the covalent G12C inhibitor ARS-1620 results in a release of upstream feedback inhibition of RTKs on the cell surface, which bypass KRAS G12C inhibition by activating wild-type RAS. This results in a rebound in MAPK pathway signaling. **C**, Cotreatment of cells with a SHP2 inhibitor can prevent RTK-mediated activation of wild-type RAS resulting in more durable MAPK pathway inhibition. **D**, While cotreatment with a MEK inhibitor can also prevent rebound in MAPK pathway signaling, the resulting relief of negative feedback inhibition of RTKs and RAS can lead to activation of parallel oncogenic pathways such as the PI3K/AKT pathway.

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The results reported by Ryan and colleagues are highly analogous to the adaptive response of *BRAF* V600E-mutated tumors to treatment with selective RAF inhibitors and suggest that the clinical development of KRAS G12C inhibitors will follow a similar path. Treatment of *BRAF* V600E-mutant cells with RAF inhibitors results in a rapid relief of upstream feedback inhibition of RTKs and RAS. This results in induction of *BRAF* dimers, which are insensitive to RAF inhibitors and reactivation of ERK signaling (4). As even a small rebound in MAPK pathway signaling can attenuate the clinical response to RAF inhibitors, combination therapies that target multiple nodes in the pathway induce more profound responses in preclinical models. Consistent with these results, the combination of RAF and MEK inhibitors is more effective than either alone in *BRAF* V600E-mutant melanoma.

However, MEK inhibitors, by targeting a downstream node in the MAPK pathway, can release upstream RTK and RAS from feedback inhibition which can, in some cellular contexts, result in activation of parallel signaling pathways such as the PI3 kinase pathway. Therefore, cotargeting upstream may prove to be more effective than cotargeting downstream as it would be predicted to not only induce more potent MAPK pathway inhibition, but also prevent reciprocal activation of parallel prooncogenic signaling pathways (Fig. 1). In support of this hypothesis, in colorectal cancer, where EGFR is the dominant RTK contributing to ERK reactivation, EGFR and RAF inhibitor combinations, with or without MEK inhibition, are required to durably inhibit MAPK signaling (5). The data reported by Ryan and colleagues, suggest that similar vertical cotargeting of the MAPK pathway will

be required to achieve the full potential of KRAS G12C inhibitors as was required for RAF inhibitors. Furthermore, their data suggest that in *BRAF* V600E-mutant tumors, cotargeting of SHP2 may be preferred to RTK and RAF inhibitor combinations. It remains unknown, however, whether current SHP2 inhibitors will have an adequate therapeutic index to inhibit SHP2 and RAS activation sufficiently to induce antitumor responses in patients. It is also possible that alternative mechanisms of resistance will quickly emerge that limit the benefit of vertical pathway cotargeting, at least in some patients. Despite these concerns, we eagerly await clinical trials testing the combination of KRAS G12C (and *BRAF*) and SHP2 inhibitors and expect that KRAS G12C will soon join the growing list of oncogenic mutations that can be selectively targeted in patients.

Disclosure of Potential Conflicts of Interest

R. Yaeger is a paid consultant for Array BioPharma, reports receiving commercial research grants from Array BioPharma, Novartis Pharmaceuticals, and Boehringer Ingelheim, and is an unpaid consultant/advisory board member for Array BioPharma. D.B. Solit is a paid consultant for Pfizer, Loxo Oncology, Lilly Oncology, Illumina, QED Therapeutics, and Vividion Therapeutics. No other potential conflicts of interest were disclosed.

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References

- Ryan MB, Fecce de la Cruz F, Phat S, Myers DT, Wong E, Shahzade HA, et al. Vertical pathway inhibition overcomes adaptive feedback resistance to KRASG12C inhibition. *Clin Cancer Res* 2020;26:1633–43.
- Lito P, Solomon M, Li LS, Hansen R, Rosen N. Allele-specific inhibitors inactivate mutant KRAS G12C by a trapping mechanism. *Science* 2016;351:604–8.
- Govindan R, Fakih MG, Price TJ, Falchook GS, Desai J, Kuo JC, et al. Phase 1 study of AMG 510, a novel molecule targeting KRAS G12C mutant solid tumors. *Ann Oncol* 2019;30:v159–v193.
- Lito P, Pratils CA, Joseph EW, Tadi M, Halilovic E, Zubrowski M, et al. Relief of profound feedback inhibition of mitogenic signaling by RAF inhibitors attenuates their activity in BRAFV600E melanomas. *Cancer Cell* 2012;22:668–82.
- Corcoran RB, Andre T, Atreya CE, Schellens JHM, Yoshino T, Bendell JC, et al. Combined BRAF, EGFR, and MEK inhibition in patients with BRAF(V600E)-mutant colorectal cancer. *Cancer Discov* 2018;8:428–43.