IN THE SPOTLIGHT

Progress on Covalent Inhibition of KRAS<sup>G12C</sup>

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**Summary:** Recent reports of small-molecule approaches to directly inhibit oncogenic KRAS<sup>G12C</sup> have invigorated the RAS research community by raising the possibility of drugging a protein that was long considered “undruggable.” A new iteration of covalent compounds targeting the allosteric switch II pocket of KRAS<sup>G12C</sup> showed improved potency and selectivity and enabled studies demonstrating that KRAS<sup>G12C</sup> rapidly cycles its nucleotide substrate. This report illustrates the value of chemical probes in dissecting RAS biology and raises additional hope for development of viable pharmacologic strategies for directly targeting KRAS<sup>G12C</sup>. *Cancer Discov; 6(3): 233–4. ©2016 AACR.*

See related article by Patricelli et al., p. 316 (7).

A circuitous path that started with farnesyl transferase inhibitors has, after several decades of investigation of multiple indirect approaches, returned to focus on directly targeting KRAS for cancer therapy. Several groups have now advanced unique direct targeting strategies to modulate the output of RAS signaling, including GTP-competitive inhibitors and molecules that prevent interactions between KRAS and several of its effectors (1–6). At the forefront of these has been an approach targeting a novel binding pocket in KRAS that forms when RAS is GDP bound. This pocket, referred to as the Switch II pocket (SIIP), sits adjacent to the location of the residue encoded by the most common activating KRAS mutation, KRAS<sup>G12C</sup>. In this issue of *Cancer Discovery*, Patricelli and colleagues (7) report a new iteration of covalent SIIP binders that improves on compounds initially reported by Shokat and Wells (4).

Large-scale tumor sequencing has shown that the activating KRAS<sup>G12C</sup> mutation occurs in 20% of non–small cell lung cancers (NSCLC) and is the most common RAS mutation, accounting for one-half of all RAS mutations in NSCLC overall. Codon 12 is located at the edge of the GTP-binding site of RAS and is also adjacent to a dynamic element called Switch II that participates in physical interactions with some RAS effectors and is critical for GAP-mediated GTP hydrolysis. Influenced by a recent trend within medicinal chemistry toward development of covalent inhibitors that target solvent accessible cysteines, it was hypothesized that the KRAS<sup>G12C</sup> mutant might be irreversibly targeted with compounds bearing an electrophile. For KRAS<sup>G12C</sup>, this approach is particularly attractive because it requires the existence of the very mutation associated with oncogenesis, which in theory should provide a degree of selectivity over normal tissues that do not contain KRAS<sup>G12C</sup>. This could therefore widen the therapeutic window of these covalent compounds. Several years ago, the group led by Shokat and Wells successfully completed a screen to identify chemical fragments that covalently attach to KRAS<sup>G12C</sup> and serendipitously identified the SIIP. The resulting first-generation SIIP covalent compounds were notable for their preference for GDP-bound KRAS (4).

In this issue of *Cancer Discovery*, Patricelli and colleagues build upon prior work by developing a new, but related, covalent SIIP compound, ARS-853 (7). ARS-853 is directly evolved from first-generation compounds that showed exciting biochemical activity but modest cellular activity. Optimization of electrophile positioning and modification of a portion of the scaffold that interacts with a hydrophobic portion of the SIIP produced clear improvements in the ability of this compound class to engage KRAS<sup>G12C</sup> in cells. Additionally, ARS-853 showed excellent selectivity in reacting with KRAS<sup>G12C</sup> protein over a large segment of cysteine-containing cellular proteins as measured by a comprehensive unbiased mass spectrometry analysis. ARS-853 also showed improved activity against cancer cell lines containing KRAS<sup>G12C</sup> with IC<sub>50</sub> values in the low micromolar range. Furthermore, the authors were able to use ARS-853 to make several important observations about RAS biology, including (i) that KRAS<sup>G12C</sup> rapidly cycles its nucleotide substrate, (ii) that signaling by KRAS<sup>G12C</sup> can be modulated by upstream effectors, and (iii) that even within KRAS<sup>G12C</sup>-cancer cell lines, the degree of dependence on KRAS<sup>G12C</sup> for cell viability and growth varies.

Rapid nucleotide exchange as a general property of KRAS<sup>G12C</sup> (see Fig. 1) has important strategic implications for targeting RAS<sup>G12C</sup>. It should be noted that rapid exchange contrasts with the prevailing dogma in the field that activated KRAS proteins tend to be “locked” in a constitutively active GTP-bound form, which appears to be the case for KRAS<sup>G12D</sup> and KRAS<sup>G12V</sup>, KRAS mutations dominant in pancreatic and colorectal cancers. Not only does rapid nucleotide exchange open the possibility of autoactivation of KRAS by virtue of the 10-fold higher concentrations of GTP over GDP in the cell, it also rationalizes mechanisms by which RAS effectors, including upstream actors such as EGFR, may modulate RAS<sup>G12C</sup>...
signaling activity. Indeed, Patricelli and colleagues found that combinations of ARS-853 with EGFR-directed tyrosine kinase inhibitors (TKI) produced the most profound effects on cell death and were required to completely disable signaling through the PI3K pathway. In some respects, this may seem surprising given that KRAS and EGFR mutations rarely co-occur and, in fact, appear to be synthetically lethal (8). Furthermore, although EGFR TKIs are not used clinically in KRAS-mutant cancers due to their lack of single-agent activity, the findings from Patricelli and colleagues suggest that in some instances they may in fact be additive if not synergistic with ARS-853 in KRASG12C lung cancers. These findings open new and previously unanticipated therapeutic opportunities. It will be important to determine if the observations in the H358 cell line are more broadly applicable to other KRASG12C-positive cancers. It is possible that multiple different RTKs can provide upstream signaling inputs and the specific RTK may depend on the genomic context of the KRASG12C-mutant cancer and/or tissue of origin. From a RAS biology perspective, these observations add to the ever-growing theme that RAS signaling is extremely complex and must be delicately balanced for compatibility with life.

Clearly, additional benchmarks must be met, such as testing in in vivo systems, before we speculate too much that SIIP inhibitors might be useful in clinical settings. Nevertheless, these molecular tools have already proven valuable in furthering our understanding of RAS biology and will likely enable many more experiments that would not be possible otherwise. A somewhat related but larger question remains: Does this work have implications for targeting other KRAS mutations? The evidence suggesting that some KRAS mutations have unique biology and should therefore be considered independently continues to grow (9, 10). Should the SIIP approach be attempted for other common mutations like KRASG12D or KRASG12V? It is tempting to conclude that SIIP compounds rely heavily on covalent interactions and therefore require G12C, but in reality the answer is unknown, because neither affinity measurements for noncovalent versions of SIIP binders nor classic kinetic measures such as $K_{on}$/K for covalent versions are available. If sufficiently potent reversible SIIP binders for other mutants could be developed, it may be possible to apply reversible versions of SIIP binders to other mutant forms of RAS. Regardless, this work takes an important step toward drugging one of the most important targets in cancer.

Disclosure of Potential Conflicts of Interest

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