More than four decades since their first identification, small monomeric guanosine triphosphatases (GTPases) remain among the most studied oncogenic proteins. Three GTPases in the rat sarcoma viral oncogene (RAS) family, KRAS, NRAS, and HRAS, are activated by "gain-of-function" mutations in up to 25% of all cancers, driving proliferative signaling to support tumor growth. Their flat topology and lack of a clear ligand binding site (with the exception of the undruggable GTP pocket) has given rise to a wide range of indirect strategies to target RAS proteins with varying degrees of success (Figure 1A). Mutant KRAS remains among the hottest targets in oncology, and concerted efforts to target oncogenic KRAS<sub>G12C</sub> culminated in 2019–2020 with the discovery of allosteric covalent inhibitors that attack the nucleophilic cysteine residue, which is present uniquely in this specific KRAS mutant. The work of numerous academic and industry teams ultimately delivered four KRAS<sub>G12C</sub> covalent inhibitors currently in clinical trials for cancer. Among these, Mirati Therapeutics’ drug candidate MRTX849 (Figure 1A) has shown promising results and tolerability in patients affected by nonsmall cell lung cancer (NSCLC) and colorectal cancer (CRC) driven by KRAS<sub>G12C</sub> mutant. Nevertheless, the search for improved strategies continues, and in the current issue, the Crews laboratory has used MRTX849 as a warhead in the first cell-active PROteolysis TARgeting Chimera (PROTAC) against KRAS<sub>G12C</sub>. This work elegantly illustrates the differentiating opportunities and rational design challenges of covalent PROTACs, and delivers striking insights into the trade-offs of inhibition versus degradation.

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to be critical to achieve target degradation, and LC-2 was identified as the most potent KRAS degrader among a small library of PROTACs featuring covalent KRAS<sup>G12C</sup> inhibitor MRTX849 linked to a VHL ligand. In a thorough screen using different cell lines bearing hetero- and homozygous KRAS<sup>G12C</sup> mutations, LC-2 engaged and induced degradation of KRAS with DC<sub>50</sub> (concentration required to achieve 50% degradation) values between 0.25 and 0.76 μM and D<sub>max</sub> (maximum degradation) from 75−90% of total KRAS. Neither engagement nor degradation was observed in cell lines harboring other KRAS mutations (e.g., G13D), providing evidence for the selectivity of LC-2 toward KRAS<sup>G12C</sup>. Furthermore, the authors rigorously showed, using conventional competition and cotreatment experiments, that LC-2 acts via a bona fide PROTAC mechanism since its degradation activity was found to be dependent on both E3 ligase and proteasome activity. Interestingly, the authors found that LC-2-mediated KRAS<sup>G12C</sup> degradation kinetics and maximal degradation were cell-line dependent, and further investigation will be required to understand whether these differences depend on kinetics of the LC-2-mediated ternary complex or on features of UPS regulation intrinsic to each cell line.

It is known that covalent inhibition of KRAS leads to an increase in KRAS expression; however, LC-2 achieved maximal degradation within 24 h, persisting up to 72 h, suggesting that KRAS degradation may be a more effective strategy than KRAS inhibition for prolonged attenuation of

Figure 1. (A) Prominent approaches targeting oncogenic RAS, including recent success with covalent inhibitors; (B) covalent KRAS<sup>G12C</sup> PROTAC mechanism of action.
KRAS signaling. LC-2 impaired phosphorylation down-stream of KRAS, but signaling inhibition kinetics appeared to correlate with target engagement suggesting that KRAS inhibition rather than degradation was the major trigger of the observed phenotypic effects. Furthermore, cell viability experiments showed no marked improvement compared to MRTX849 treatment, likely due to the noncatalytic nature of PROTAC LC-2. Nevertheless, LC-2 represents a valuable high-quality tool for KRAS chemical biology and a starting point for the development of improved KRAS degraders.

These results are particularly remarkable in contrast to previous attempts to degrade endogenous KRASG12C, which were proven unsuccessful. The PROTACs in this prior work relied on a different KRAS warhead, recruited Cereblon E3 ligase instead of VHL, and induced degradation of overexpressed GFP-KRASG12C, but not the endogenous protein. These results sit alongside similarly contrasting outcomes for covalent PROTACs targeting Bruton’s tyrosine kinase (BTK) for which very similar designs from different research groups yielded polar opposite degradation profiles. These findings suggest that a covalent linkage between the POI and the PROTAC might additionally restrict the conformational space of the ternary complex, thereby limiting the poses that lead to effective protein ubiquitination. Together, these data highlight that PROTAC design is nontrivial, and the varied factors of warhead, E3 ligase, and linker length demand careful selection to yield an effective degrader. This ephemeral structure—activity relationship has so far precluded a fully rational approach to PROTAC design and most degraders including those described by Crews et al. are designed on an empirical case-by-case basis. The future of covalent PROTACs may instead lie with development of covalent E3 ligase ligands, which could effectively convert an E3 ligase into a targeted degrader for the lifetime of the protein.

The present work marks an important milestone in the protein degradation field and suggests that KRAS degradation may yet hold promise. A key challenge will be the development of noncovalent KRAS degraders that can exploit a catalytic mode of action and thus enhance the effects of KRAS inhibition while improving the therapeutic window. Moreover, while the formation of a covalent bond

Figure 2. (A) Chemical structure of PROTAC LC-2; (B) concentration-dependent degradation of KRASG12C by LC-2 and effects on ERK phosphorylation in NCI-H2030 cells. Reproduced from ref 4. Copyright 2020 American Chemical Society.
with KRAS\textsuperscript{G12C} is required to drive high occupancy and cellular potency for conventional small molecule inhibitors, the event-driven nature of targeted degradation offers the tantalizing possibility of KRAS PROTACs based on readily identified reversible weak binders as well as the advantage of targeting diverse mutant forms of RAS or even specific RAS complexes. However, relying on a low-affinity warhead also risks myriad off-target events, and it remains to be seen whether promiscuous degradation could undermine the outstanding therapeutic index offered by targeting oncogenic KRAS mutants.

The event-driven nature of targeted degradation offers the tantalizing possibility of KRAS PROTACs based on readily identified reversible weak binders as well as the advantage of targeting diverse mutant forms of RAS or even specific RAS complexes.

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Notes
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