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Viewpoint

Selectively Targeting Tropomyosin Receptor Kinase A (TRKA) via PROTACs

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ABSTRACT: Tropomyosin receptor kinases (TRKs) are promising cancer therapeutic targets. Chen et al. (*J. Med. Chem.* 2020, DOI: 10.1021/acs.jmedchem.0c01342) report the discovery of CG416 and CG428 as two potent small-molecule proteolysis-targeting chimera (PROTAC) degraders selective for TRKA over TRKB and TRKC. CG416 and CG428 are valuable research tool compounds for *in vitro* and *in vivo* studies and promising lead compounds for further optimization.

Tropomyosin receptor family kinases (TRKs) comprise three members, namely, TRKA, TRKB, and TRKC, which are encoded by the *NTRK1*, *NTRK2*, and *NTRK3* genes, respectively. Aberrant activation of TRK pathways has been observed in different types of human cancers, with *NTRK* gene chromosomal translocation being the most well studied. Accordingly, targeting TRK fusion proteins in human cancers holds great therapeutic promise. Indeed, the FDA has approved larotrectinib as the first pan TRK inhibitor for the treatment of TRK-fusion positive solid tumors regardless of tissue origin, which has been lauded as a new era of "tissue-agnostic" cancer medicines.[1]

However, larotrectinib and entrectinib, which are considered first generation pan TRK inhibitors, have their own limitations, including CNS-related on-target side effects and emergence of drug resistance due to mutations of TRK proteins. Second generation TRK pan inhibitors such as selitrectinib and repotrectinib are able to overcome some mutation-related resistance and also have reduced CNS-related on-target side effects.[2] However, there is still a need to develop a new therapeutic strategy to overcome drug-resistance to these pan TRK inhibitors and to abolish CNS-related on-target side effects of current approved TRK inhibitors.

The proteolysis-targeting chimera (PROTAC) strategy was proposed in 2001 with the objective to achieve targeted protein degradation.[3] In recent years, induced protein degradation by PROTACs has been pursued as an effective strategy for target validation and for discovery and development of a new type of drugs.[4−8] As compared to traditional small-molecule inhibitors, PROTAC-based small-molecule degraders have a number of major advantages.[4−8] First, due to their catalytic nature, PROTAC degraders can achieve far greater cellular potencies in inhibiting the function(s) of the target protein than their corresponding inhibitors. Second, PROTAC degraders do not need to target a functional site in the proteins of interest, thus expanding the druggable genome. Third, PROTAC degraders can achieve very high selectivity even among closely related homologous proteins. Finally, by removing a protein from disease tissues, PROTAC degraders can overcome resistances associated with traditional small-molecule inhibitors. To date, orally bioavailable PROTAC degraders of the androgen receptor and the estrogen receptor have been advanced into clinical development[9,10] and additional PROTAC degraders are expected to enter into clinical development in the near future.

In this issue of the *Journal of Medicinal Chemistry*, Chen et al. report the design and evaluation of PROTAC TRK degraders.[11] The authors designed a series of TRK PROTAC degraders by attaching a pan-TRK inhibitor GNF-8625 to thalidomide, which is a ligand for cereblon/cullin 4A (Figure 1). The linking point on GNF-8625 was rationally chosen based on a cocrystal structure of a GNF-8625 analogue in complex with TRKA. A medicinal chemistry campaign was launched to identify the optimal linker length comprising either a linear alkyl chain or a polyethylene glycol (PEG) chain, which led to the discovery of CG416 and CG428 as the most promising degraders. CG416 and CG428 reduce TPM3-TRKA fusion protein levels with DC_{50} values of 0.48 nM and 0.36 nM in KM12 cells, respectively, and potently inhibit TRKA downstream signal PLCγ1 phosphorylation. CG416 and CG428 also degrade wild type TRKA in HEL cells with DC_{50} values of 1.26 nM and 2.23 nM. Furthermore, CG416 and CG428 are 3- and 5-fold more potent in inhibition of cell growth than the corresponding pan TRK inhibitor GNF-8625. Of significance, the authors showed that these designed PROTAC degraders using a pan TRK inhibitor can achieve significant selectivity in degradation of TRKA over TRKB and TRKC. For example, CG416 and CG428 exhibit complete degradation TPM3-TRKA at low nanomolar concentrations, while only reducing the levels of AGBL4-TRKB and ETV6-

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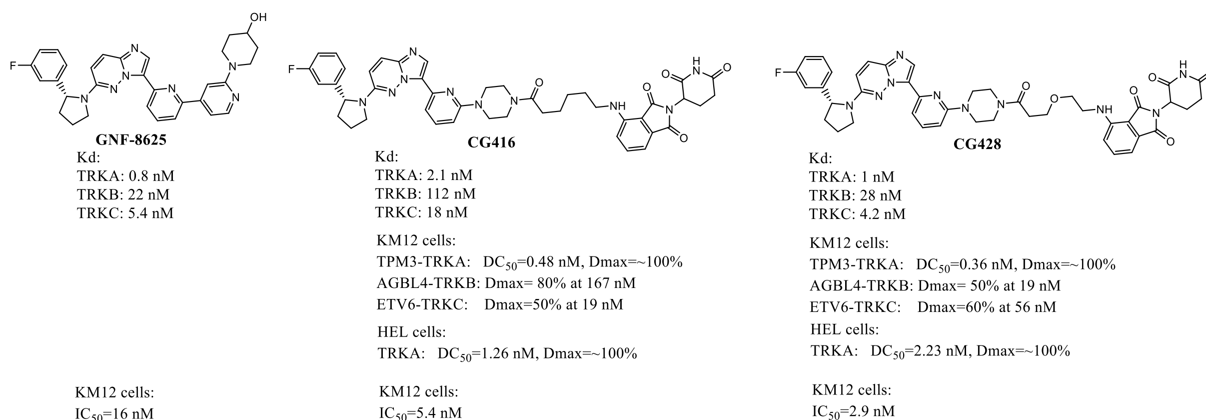

Figure 1. Structures of TRK inhibitor GNF-8625, PROTAC degraders CG416 and CG428, and their K_d, degradation and IC_{50} values.

TRKC fusion proteins in KM12 cells by a maximum of 50−80%. Tandem mass tag-based quantitative proteomics analysis showed that CG416 significantly downregulates only five proteins (TRKA, GDF15, ERRFI1, DUSP4, and AREG) and upregulates two proteins (SOX4 and FZD6), suggesting relatively high selectivity. Mechanistic studies provided convincing data that CG416 and CG428 function as *bone fide* PROTAC degraders. Pharmacokinetic studies showed that CG416 and CG428 have good plasma exposures with intraperitoneal administration in mice, supporting their utilities for both *in vitro* and *in vivo* investigations.

Overall, this study provides another example that the PROTAC strategy can be successfully employed to effectively degrade unwanted proteins. The unexpected selectivity achieved by CG416 and CG428 against TRKA over TRKB and TRKC further illustrates the power of this strategy; however, detailed studies are needed to understand the structural requirements for the selective degradation of TRKA. Although good plasma exposure is achieved by CG416 and CG428 in mice, further studies are needed to demonstrate whether degradation of TRKA can be achieved in tissues. Extensive optimization will be needed if an oral TRK degrader is necessary for ultimate clinical development. Nevertheless, CG416 and CG428 will serve as lead compounds for further optimization and useful research tools to investigate the biology and therapeutic potential of selective TRKA degradation *in vitro* and *in vivo*.

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