A new “angle” on kinase inhibitor design: Prioritizing amphosteric activity above kinase inhibition

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Abbreviations: MYC, v-myc avian myelocytomatosis viral oncogene homolog; MYCN, v-myc avian myelocytomatosis viral oncogene neuroblastoma-derived homolog; AURKA, Aurora kinase A; CD, conformation-disrupting.

The MYCN oncoprotein has remained an elusive target for decades. We recently reported a new class of kinase inhibitors designed to disrupt the conformation of Aurora kinase A enough to block its kinase-independent interaction with MYCN, resulting in potent degradation of MYCN. These studies provide proof-of-principle for a new method of targeting enzyme activity-independent functions of kinases and other enzymes.

As critical drivers of many cancers, members of the MYC family of oncoproteins have long been desirable therapeutic targets. MYC is a transcription factor with no easily druggable ligand-binding sites and thousands of downstream transcriptional targets. Thus, targeting MYC proteins, at least with current chemical techniques and drug-like molecules, has been challenging.¹ We recently reported the development of a novel class of inhibitors that act by binding the active site of Aurora kinase A (AURKA) to effect a conformational change that prevents protein–protein interaction and thereby potently induces degradation of the prominent MYC family protein, MYCN.

MYCN, the neuroblastoma-derived MYC family member, drives aggressive subsets of several cancers including high-risk neuroblastoma, high-risk medulloblastoma, as well as neuroendocrine lung, prostate, and pancreatic tumors. A shRNA screen performed by Otto et al. in MYCN-amplified neuroblastoma cells demonstrated degradation of MYCN that was not observed in response to kinase inhibition. Further studies revealed that AURKA physically associated with MYCN, and that this interaction was critical for stabilizing MYCN and for high levels of expression of the oncoprotein. Moreover, loss of AURKA protein—but not simple inhibition of its kinase activity—led to sufficient loss of MYCN protein to effect cell death.²

To capitalize on these findings we designed a panel of compounds with a core that would bind in the ATP binding pocket of AURKA and provide a handle from which additional chemical moieties could extend to produce a conformational change in the kinase. Such inhibitors would inhibit AURKA orthostERICALLY, because they are ATP-competitive, as well as allosterically, because they would proHIBIT protein–protein interaction. We therefore refer to this class of dual-functioning inhibitors as amphosteric inhibitors. We screened this panel of inhibitors in neuroblastoma cell lines and found that some compounds induced rapid and profound degradation of MYCN.³ Loss of MYCN by these conformation-disrupting (CD) compounds required proteasomal degradation of MYCN and occurred in both a time-dependent and dose-dependent manner. Co-crystallization of AURKA with our lead compound, CD532, demonstrated that these CD inhibitors drastically altered the conformation of AURKA compared to either Apo (empty binding pocket) or ATP-bound protein, or AURKA complexed with the conventional non-CD inhibitors VX-680 or MLN8237 (Fig. 1).

The most surprising feature of the co-crystal structure was the mechanism by which CD inhibitors induced this conformational change. Typically, the newer class of kinase inhibitors known as Type II inhibitors stabilize the inactive state of a kinase by inducing a flip in the DFG motif in the ATP binding pocket of a kinase.⁴ Based on chemical similarity with Type II inhibitors of other kinases, we predicted that our CD compounds would act as Type II inhibitors. However, crystallographic studies demonstrated that CD532 did not induce a Type II flip in the DFG motif, but instead formed a
Figure 1. CD532 stabilizes an inactive conformation of AURKA to degrade MYCN. From top: Chemical structure of modest conformation-disrupting compound MLN8237 and the true conformation-disrupting compound CD532. Representation of crystal structures of AURKA Apo (empty binding pocket, 4J8N, sage), AURKA with MLN8054 (2WTV, lavender), and AURKA with CD532 (4J8M, orange); the angle is between α-carbons of V324, E308, and A172. Columns represent the extent of conformation disruption, the extent of disruption of the MYCN-AURKA interaction as measured by co-immunoprecipitation, and the extent of MYCN loss as measured by western blot.
series of unique interactions that produced a conformational change distinct from any known physiologic state. This might be because our screen was designed to select for ampho steric activity rather than kinase enzyme inhibition.

MLN8054, the precursor for the clinical AURKA inhibitor MLN8237 (alsertib), induces a modest conformational change in AURKA, ~25–40% of the magnitude effected by CD compounds, and has a correspondingly modest effect on degradation of MYCN.\(^5,6\) At saturation and has a correspondingly modest effect magnitude effected by CD compounds, suggesting that the magnitude of conformational disruption directly influenced the equilibrium between unbound MYCN and MYCN bound to AURKA. These observations also suggest that the view of kinases existing in an “active state” versus an “inactive state” is overly simplistic—stabilization of alternative conformations, some perhaps non-physiological, can have profound consequences for manipulation of kinase function.

Although degradation of MYCN was a principal goal, disruption of conformation using an ATP-competitive strategy necessitates that ampho steric MYCN targeting is achieved through inhibition of AURKA kinase function. We found that CD compounds primarily phenocopied MYCN inhibition—characterized by loss of S-

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Divergent from simple inhibition of kinase activity has broad implications for the manipulation of biological systems in general. In particular, we should be more hesitant to apply the term “undruggable” as it is unlikely that the AURKA-MYC interaction is the only oncogenic enzyme–protein interaction that can be disrupted by a small molecule. However, this in itself poses challenges unique from the relatively simple ways in which we conceptualize signaling pathways. More work is required to identify such protein–protein interactions, to characterize the roles of these interactions in cellular physiology and pathophysiology, and to develop a new generation of kinase inhibitors to ampho sterically target these interactions.

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No potential conflicts of interest were disclosed.

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