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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

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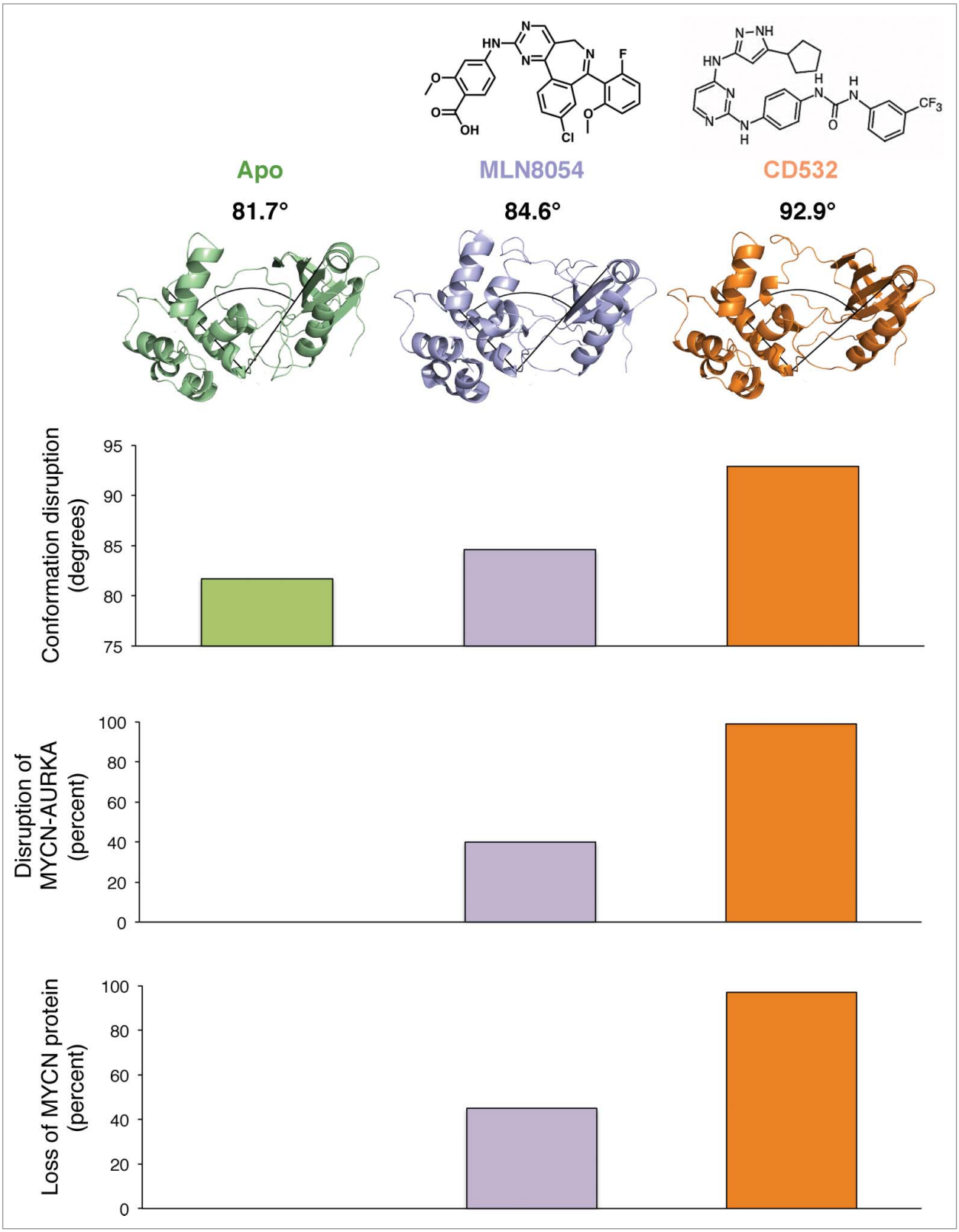

Figure 1. CD532 stabilizes an inactive conformation of AURKA to degrade MYCN. From top: Chemical structure of modest conformation-disrupting compound MLN8237 and the true conformation-disrupting compound CD532. Representation of crystal structures of AURKA Apo (empty binding pocket, 4J8N, sage), AURKA 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 amphosteric activity rather than kinase enzyme inhibition.

MLN8054, the precursor for the clinical AURKA inhibitor MLN8237 (alisertib), 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 saturating concentrations of MLN8237 the AURKA-MYCN interaction is only disrupted by 40%. In contrast, CD532 completely disrupted interactions between MYCN-AURKA, 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 amphosteric MYCN targeting is achieved through inhibition of AURKA kinase function. We found that CD compounds primarily phenocopied MYCN inhibition—characterized by loss of S-phase—rather than the super-G_2 arrest typically seen with AURKA inhibition.[7,8] Interestingly, the latency of super-G_2 arrest due to AURKA inhibition is much longer than the latency for S-phase blockade seen with MYCN knockdown, offering an explanation for how simultaneous blockade of both the kinase and the MYCN-stabilizing functions of AURKA leads to a predominantly MYCN effect.

The above observations suggest that CD compounds would be more selective for MYC-driven cancers than conventional non-CD inhibitors of AURKA. By testing the sensitivity of 169 cell lines to CD532, we found that CD compounds were MYCN-targeted therapies by multiple measures; *MYCN* amplification status, *MYC*/*MYCN* mRNA expression levels, and MYC-like gene signatures all predicted sensitivity to CD532. Since MYC proteins are ubiquitous in dividing cells, yet generally show higher expression in cancer, we assessed whether MYC-directed therapy was a therapeutically viable strategy. CD532 reduced protein levels of MYCN protein in models of both neuroblastoma and medulloblastoma, and extended survival and reduced tumor burden in a p53-null model of MYCN-expressing sonic-hedgehog subtype medulloblastoma, confirming the existence of a therapeutic window for targeting oncogenic levels of MYCN.

The demonstration that altering the conformation of a kinase with a small molecule can have consequences so 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-MYCN 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 amphosterically target these interactions.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Prochownik EV, Vogt PK. Therapeutic Targeting of Myc. 2010; 1:650-9. Available from: http://gan.sagetpub.com/lookup/doi/10.1177/1947601910377494; PMID:21132100
2. Otto T, Horn S, Brockmann M, Eilers U, Schüttrumpf L, Popov N, Kenney AM, Schulte JH, Beijersbergen R, Christiansen H, et al. Stabilization of N-Myc is a critical function of Aurora A in human neuroblastoma. Cancer Cell 2009; 15:67-78; PMID:19111882; http://dx.doi.org/10.1016/j.ccr.2008.12.005
3. Gustafson WC, Meyerowitz JG, Nekritz EA, Chen J, Benes C, Charron E, Simonds EF, Seeger R, Matthay KK, Hertz NT, et al. Drugging MYCN through an allosteric transition in aurora kinase A. Cancer Cell 2014; 26:414-27; PMID:25175806; http://dx.doi.org/10.1016/j.ccr.2014.07.015
4. Jura N, Zhang X, Endres NF, Seeliger MA, Schindler T, Kuriyan J. Catalytic control in the EGF receptor and its connection to general kinase regulatory mechanisms. Molecular Cell 2011; 42:9-22; PMID:21474065; http://dx.doi.org/10.1016/j.molcel.2011.03.004
5. Dodson CA, Kosmopoulou M, Richards MW, Atrash B, Bavetsias V, Blagg J, Bayliss R. Crystal structure of an Aurora-A mutant that mimics aurora-B bound to MLN8054: insights into selectivity and drug design. Biochem J 2010; 427:19-28; PMID:20067443; http://dx.doi.org/10.1042/BJ20091530
6. Brockmann M, Poon E, Berry T, Carstensen A, Deubzer HE, Rycak L, Jamin Y, Thway K, Robinson SP, Roels F, et al. Small molecule inhibitors of aurora-a induce proteasomal degradation of N-Myc in childhood neuroblastoma. Cancer Cell 2013; 24:75-89; PMID:23792191; http://dx.doi.org/10.1016/j.ccr.2013.05.005
7. Manfredi MG, Ecsedy JA, Chakravarty A, Silverman L, Zhang M, Hoar KM, Stroud SG, Chen W, Shinde V, Huck JJ, et al. Characterization of Alisertib (MLN8237), an investigational small-molecule inhibitor of aurora A kinase using novel in vivo pharmacodynamic assays. Clin Cancer Res 2011; 17:7614-24; PMID:22016509; http://dx.doi.org/10.1158/1078-0432.CCR-11-1536
8. Gogolin S, Ehemann V, Becker G, Brueckner LM, Dreidax D, Bannert S, Nolte I, Savelyeva L, Bell E, Westermann F. CDK4 inhibition restores G(1)-S arrest in MYCN-amplified neuroblastoma cells in the context of doxorubicin-induced DNA damage. Cell Cycle 2013; 12:1091-104; PMID:23462184; http://dx.doi.org/10.4161/cc.24091