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PI3King on *MYCN* to Improve Neuroblastoma Therapeutics

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MYCN is an oncogenic driver of childhood neuroblastoma, a frequently lethal pediatric tumor. In a recent paper in *Science Translational Medicine*, Chanthery and colleagues demonstrate that PI3K inhibition leads to the dual therapeutic benefits of enhanced MYCN degradation and loss of a paracrine angiogenic signal mediated by MYCN.

Despite decades of ever-improving outcomes across diverse pediatric cancers, neuroblastoma has remained a frustrating clinical entity. Most children are diagnosed with tumors that harbor genetic and biological features highly correlated with a poor treatment outcome. Current therapy for such high-risk patients includes dose-intensive chemotherapy, radiotherapy, and retinoids. Though there have been recent impressive translational successes for this tumor, such as immunotherapy using an antibody targeting cell-surface GD2 given with immunostimulatory cytokines (Yu et al., 2010), 3 year relapse free survival estimates for high-risk disease remain under 50%.

Further compounding the frustration is the fact that the genome of neuroblastoma is one of the most comprehensively characterized among pediatric cancers, but it has not yet led to more effective treatment. The recent discovery that the *ALK* receptor tyrosine kinase is constitutively activated in ~10% of neuroblastomas (Mossé et al., 2008) provides one such therapeutic opportunity, as *ALK* inhibitors have been in development due to the involvement of this kinase in a subset of non-small-cell lung cancers and anaplastic lymphomas. First generation *ALK* inhibitors such as crizotinib are already in Phase 2 trials for children with relapsed or refractory neuroblastoma and may make their way soon into upfront therapy for those patients with *ALK*-mutated tumors.

Contrast that with *MYCN*, the only other bona fide oncogene yet discovered in neuroblastoma that was initially identified almost 30 years ago (Brodeur et al., 1984). Despite this lead-time and a great deal of effort, no therapeutic has yet emerged to be able to target this clear oncogenic driver of the most aggressive subset of neuroblastomas. *MYCN*, which is a homolog of the *MYC* proto-oncogene, is somatically amplified in the tumor cells of ~20% of neuroblastoma patients (and in ~40% of those with a high-risk phenotype). *MYCN* amplification is independently correlated with advanced stage disease and poor outcome and therefore is used worldwide in risk classification algorithms. Moreover, genetically engineered mouse models with *MYCN* expression targeted to neural crest tissue develop tumors that resemble human neuroblastoma (Weiss et al., 1997). MYC proteins, including MYCN, serve pleiotropic roles in malignancy, such as altering metabolic programs, supporting angiogenesis, promoting self-renewal and "stemness," and driving proliferation while inhibiting differentiation.

ALK as a kinase is a pharmacologically tractable target, and a wealth of experience suggests that inhibition of activated kinases can lead to clinically impressive tumor responses. MYCN, in contrast, has long been seen as a problematic therapeutic target, as inactivating a highly abundant nuclear transcription factor that operates through a network of protein-protein interactions is pharmacologically daunting. Still, tumors are remarkably heterogeneous and cancer cells are remarkably adaptive. Resistance to targeted therapeutics can be efficiently selected for, especially when cells have

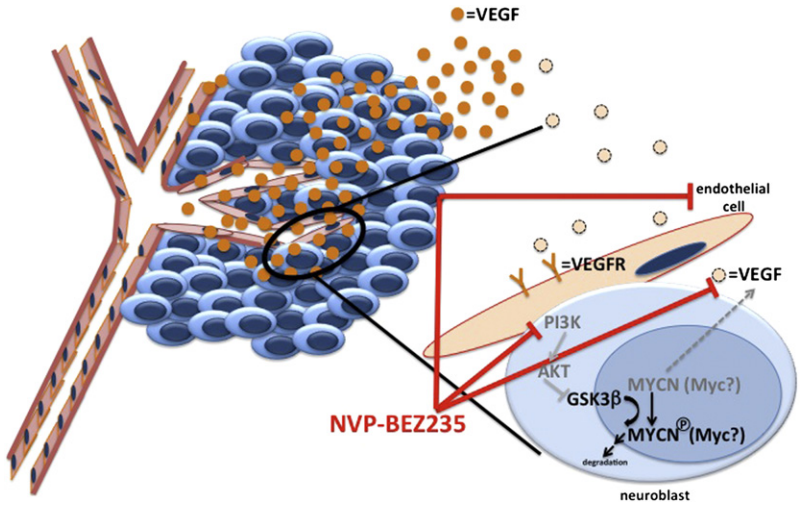

Figure 1. Neuroblast-Intrinsic and -Extrinsic Therapeutic Effects of the Dual PI3K/mTOR Inhibitor NVP-BEZ235

MYCN (and MYC, when similarly dysregulated) induce vasculogenesis via secretion of VEGF and other angiogenic factors. Tumor-associated angiogenesis is similarly supported by paracrine signaling via VEGF downstream of MYCN. NVP-BEZ235 impacts tumorigenesis through inhibition of PI3K and mTOR kinases, through PI3K inhibition-mediated degradation of MYCN, through GSK3β-mediated phosphodegradation pathways, and through direct anti-angiogenic effects on tumor-associated endothelial cells themselves. Not shown are effects attributable to mTOR inhibition, and pleiotropic effects realized by antagonism of MYCN activity in neuroblasts.

functionally redundant or degenerate pathways to compensate for the one being targeted, as is often the case with apical kinases such as ALK. MYC proteins, however, are unique in that their actions are largely non-redundant. In many experimental systems, the only gene products that can substitute for *MYC* are other *MYC* homologs. Emerging data using animal models also suggest that toxicity associating with MYC inhibition might not be as problematic as initially feared (Soucek et al., 2008). So MYC remains a compelling therapeutic target for neuroblastoma and many other cancers (Delmore et al., 2011).

It is therefore of great interest that a recent paper in *Science Translational Medicine* demonstrates the benefits of inhibiting PI3K and mTOR signaling with the small molecule NVP-BEZ235 in complementary models of neuroblastoma (Chanthery et al., 2012). Neuroblastomas are richly vascular tumors, particularly those with *MYCN* amplification, and it has long been inferred that MYCN may modulate tumor angiogenesis via regulation of VEGF expression, as has been more formally demonstrated for MYC (Baudino et al., 2002). Indeed, it has been shown that PI3K/mTOR blockade leads to destabilization of MYCN and commensurate reduction in VEGF secretion, along with inhibition of neuroblastoma progression in murine models (Chesler et al., 2006). One critical component lacking up until now had been the relative contributions of inhibiting PI3K/mTOR in the tumor cells themselves, as opposed to in other cells within the tumor microenvironment. That is, is the observed anti-tumor activity mediated by tumor cell intrinsic or extrinsic mechanisms, or both?

Chanthery et al., (2012) demonstrate that NVP-BEZ235 inhibits neuroblast proliferation in vitro and that this effect is more pronounced in those cell lines with *MYCN* amplification and overexpression. They then corroborate an impact on tumor progression using both a neuroblastoma xenograft model (in which contributions of the immune compartment are lacking) as well as a *MYCN*-dependent transgenic model (recapitulating a *MYCN* "amplified" tumor arising at its autochthonous site). Though tumor regressions were not described, there was inhibition of tumor growth in both models, attributed to both reductions in tumor-associated vascular density and neuroblast proliferation. MYCN was markedly reduced in treated tumors, and evidence for both PI3K and mTOR inhibition was demonstrated, supporting the notion that PI3K inhibition led to derepression of GSK3β with resultant Thr58 phosphorylation and destabilization of MYCN.

The authors used a series of elegant experiments to then decipher the contributions of the tumor compartment by introducing wild-type MYCN or a Thr58 mutant MYCN that is resistant to Thr58-mediated destabilization into a neuroblastoma cell line with undetectable MYCN. These models allow the investigators to attribute tumor-cell autonomous effects of NVP-BEZ235 to MYCN degradation by correlating MYCN stability, VEGF secretion, and recruitment of HUVEC cells in endothelial migration assays. The impact of NVP-BEZ235 on MYCN stability and proliferation was reduced in cells expressing the Thr58 mutant MYCN. Importantly, VEGF secretion and HUVEC migration also was shown to be substantially MYCN-dependent, supporting a paracrine role downstream of MYCN. Indeed, direct knock-down of MYCN or indirect upregulation via knock-down of HUWE1 (an E3-ligase that degrades phosphorylated MYCN) led to commensurate changes in MYCN stability, VEGF secretion, and HUVEC recruitment, underscoring a prominent role for MYCN in this paracrine activity. NVP-BEZ235 clearly had a direct impact on endothelial cells themselves. However, this suggests the anti-angiogenic effects seen in vivo were both tumor cell intrinsic and extrinsic (Figure 1).

It should be noted that the impact of NVP-BEZ235 (via PI3K/mTOR inhibition) was more modest in most of these assays than was knock-down of MYCN, suggesting that its impact on MYCN was suboptimal. Still, an agent that targets the predominant oncogenic driver in this malignancy to impact diverse MYCN-mediated functions and represses tumor-associated angiogenesis directly through effects on endothelial cells and indirectly through paracrine mediated effects of tumor cells is certainly worth exploring clinically. Chanthery et al., (2012) demonstrate compelling indirect evidence that VEGF is the intermediate in this paracrine process but do not directly demonstrate this either by VEGF knock-down or by using available VEGF antagonists in their assays. Might PI3K/mTOR inhibition synergize with VEGF antagonists to potentiate these anti-angiogenic effects? Also, it is fair to

wonder whether these effects are truly restricted to neuroblastomas with *MYCN* amplification, as surmised, or might operate similarly through *MYC* when this homolog is deregulated. In high-risk neuroblastomas that lack *MYCN* amplification, *MYC* is frequently deregulated (Westermann et al., 2008), suggesting some degree of *MYC* or *MYCN* augmentation may be essential for the high-risk phenotype. This was not directly tested because the available transgenic model for this tumor mimics *MYCN* amplification as an oncogenic driver and no *MYCN* non-amplified tumor xenografts were explored. Though elucidation of a novel *MYCN*-directed therapeutic is significant enough, the impact may be further broadened to a greater proportion of patients with high-risk neuroblastoma should *MYC* serve a similar role, which is a worthy pursuit, and may extend the relevance of these findings to all human cancers that usurp MYC signaling as an essential component of sustaining the malignant phenotype.

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The RAF Inhibitor Paradox Revisited

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The success of the RAF protein kinase inhibitor vemurafenib for the treatment of *BRAF* mutant metastatic melanoma has produced another poster child for the promise of personalized medicine. However, the results of a recent study also reveal unexpected pitfalls in the application of signal transduction-targeted therapies.

The era of personalized cancer medicine is upon us. The cancer patient's genome can now be interrogated for specific genetic alterations to guide the application of therapies specifically targeted to those alterations. A dramatic therapeutic advance in this area is the BRAF-selective inhibitor vemurafenib, which has provided a significant improvement in overall survival compared to the previous standard of care for metastatic melanoma (Chapman et al., 2011). However, recent findings with vemurafenib and other protein kinase inhibitors demonstrate that the new era of signal transduction-targeted therapies is handicapped by some of the same issues that have plagued traditional cytotoxic drugs.

One key distinction between targeted versus cytotoxic therapies is decreased normal cell toxicity. Symptoms such as the classic myelosuppression associated with many cytotoxic antineoplastics are not as limiting with targeted agents, whose therapeutic effects are typically achievable at doses lower than those conferring myelosuppression or other dose-limiting toxicities. However, rapidly acquired cancer cell resistance shortens the duration of treatment response. For example, although the initial response to vemurafenib is impressive, with a response rate of ~50% and significant survival benefit, tumor resistance usually occurs within 2–18 months of initial treatment. Multiple mechanisms of resistance have been described, including mutational activation of NRAS or receptor tyrosine kinase-mediated activation of RAS, both leading to CRAF-dependent activation of MEK-ERK signaling (Figure 1) (Johannessen et al., 2010; Nazarian et al., 2010). Thus, as for cytotoxic drugs, combinations of targeted therapies will be needed, both to enhance the initial response and to reduce the subsequent onset of drug resistance. Such combinations may also have advantages in blocking the existing tumor without inducing or allowing new ones to appear.

That chemotherapy can both cure and cause cancer is not a new concept. Conventional cytotoxic chemotherapy has long been known to contribute to the