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New Strategies in Refractory and Recurrent Neuroblastoma: Translational Opportunities to Impact Patient Outcome

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Abstract

Neuroblastoma remains responsible for a disproportionate amount of childhood cancer morbidity and mortality despite recent significant advances in understanding the genetic basis of tumor initiation and progression. About half of newly diagnosed patients can be reliably identified as having tumors of low malignant potential, and these children have cure rates of greater than 95% with little or no cytotoxic therapy. On the other hand, the other half of neuroblastomas typically present in an explosive fashion with widely metastatic disease, and reliable tumor-specific biomarkers have been defined for this phenotype as well. Empiric approaches to high-risk neuroblastoma therapy have relied on dramatic escalation of chemotherapy dose intensity, and recently the incorporation of targeted immunotherapy, but nearly 50% of children with high-risk disease will be refractory to therapy or suffer a relapse, both of which are invariably fatal. Future improvements in high-risk neuroblastoma outcomes will require the identification of disease and patient-specific oncogenic vulnerabilities that can be leveraged therapeutically. Rational development of novel approaches to neuroblastoma therapy requires forward-thinking strategies to unequivocally prove activity in the relapse setting, and ultimately efficacy in curing patients when integrated into frontline treatment plans.

BACKGROUND

Neuroblastoma is a pediatric cancer typically occurring in young children that arises from the developing sympathetic nervous system¹. Tumors arise in adrenal medullary tissue or paraspinal ganglia and may be localized or widely metastatic at diagnosis. Children with localized neuroblastoma and favorable tumor genomic characteristics have an excellent overall survival probability with little or no cytotoxic therapy. However, approximately 50% of neuroblastoma patients have a clinically aggressive form of the disease with overall survival rates of less than 40%^{1, 2}. While high-risk neuroblastoma accounts for only 4% of all pediatric cancer diagnoses, it is responsible for 12% of pediatric cancer deaths³ and new therapies are clearly needed.

For children with localized disease, the general trend has been on therapy reduction focused on maintaining outstanding cure rates while minimizing treatment related morbidity. On the other hand, there has been a several decades long trend of escalating chemotherapeutic dose intensity for patients with the high-risk form of the disease. For example, neuroblastoma is perhaps the only solid human malignancy where myeloablative chemotherapy followed by autologous stem cell rescue has been proven by several investigators to improve survival^{4–6}. Thus, transplant after intensive chemotherapeutic induction chemotherapy has been standard

practice in this disease. However, the improvements in outcome are modest and associated with significant immediate and late toxicity. For patients who show a good anti-tumor response to upfront chemotherapy, two major advances inform current practice. First, Matthay *et al* showed that patients randomized to treatment with the differentiation agent isotretinoin after myeloablative consolidation had a decreased rate of relapse compared to randomized controls⁴. More recently, after years of preclinical and pilot studies, Yu *et al* published results of a randomized phase 3 trial that was stopped early due to meeting early criteria for efficacy, demonstrating that maintenance immunotherapy of a chimeric monoclonal antibody ch14.18 directed against the GD2 ganglioside combined with cytokines and isotretinoin was superior to treatment with isotretinoin alone (66% +/- 5% vs 46% +/- 5% at 2 years, $p = 0.01$)⁷. Despite these recent advances in treatment, 10–20% of patients with high-risk neuroblastoma will never achieve a remission and be refractory to current treatment and 50–60% of patients who complete treatment will experience a relapse. There are currently no known curative salvage regimens for refractory and recurrent high-risk neuroblastoma. Identifying oncogenic vulnerabilities in children with refractory neuroblastoma that can be leveraged therapeutically have the hope of changing this current reality and ultimately impacting the newly diagnosed patient as well.

ON THE HORIZON

Biomarker discovery

Neuroblastoma has been in the forefront of incorporation of genomics into clinical practice. Shortly after the identification of the *MYCN* oncogene in 1983, it was shown that children with amplification of this gene have a worse clinical outcome, thus establishing *MYCN* as the first DNA-based biomarker for therapy selection in cancer^{8–10}. Other tumor DNA aberrations are likewise prognostic and are currently used clinically such as overall tumor DNA index (ploidy) and copy number status at chromosome arms 1p and 11q². Likewise, RNA signatures appear to provide robust prognostic information^{11–15}, but these have not found their way into clinical practice. International cooperation will be absolutely essential to provide robust validation cohorts in order to establish genomics-based prognostic signatures with high enough sensitivity and specificity for clinical utility. Ultimately, many of these biomarkers will also predict activity of many of the targeted therapeutics discussed below.

Therapeutic target discovery

Several groups are exploring the hypothesis that oncogenic vulnerabilities in neuroblastoma cells can be discovered via highly parallel resequencing of tumor genomes. Data will be emerging shortly that will define the mutational landscape of high-risk neuroblastoma. An early success was the identification of gain of function mutations in the anaplastic lymphoma kinase gene (*ALK*), Figure 1. In addition to being the major familial neuroblastoma predisposition gene, mutations or amplification of *ALK* occur in about 10–15% of high-risk neuroblastoma cases^{16–20}. Mutations result in constitutive activation of this receptor tyrosine kinase, providing an oncogenic driver analogous to activation via translocation events such as *NPM-ALK* in anaplastic large cell lymphoma and *EML4-ALK* in non-small cell lung cancer. The remarkable activity, with minimal toxicity, of the *ALK* inhibitor Crizotinib in *ALK* fusion positive small cell lung cancer²¹, provided additional proof-of-concept for an ongoing pediatric phase I/II clinical trial testing *ALK* inhibition strategies in neuroblastoma and other *ALK* activated pediatric neoplasms such as *ALCL* and inflammatory myofibroblastic tumor.

The aurora kinase A (*AURKA*) gene provides another promising therapeutic target in neuroblastoma. It has been shown that *AURKA* is highly expressed in high-risk tumors, and

in addition to AURKA's expected growth promoting roles, also stabilizes the MYCN protein by direct physical association, preventing MYCN's degradation²². The Pediatric Preclinical Testing Program (PPTP), which tests early phase agents in xenograft models of pediatric cancers, identified the AURKA inhibitor MLN8237 as a potent inhibitor of neuroblastoma – the only potent small molecule activity identified to date in this screening program in the neuroblastoma panel, resulting in fast-tracked development of an ongoing pediatric clinical trial²³.

The insulin-like growth factor (IGF) signaling pathway plays an important role in the development and maintenance of pediatric tumors, including neuroblastoma²⁴. IGF1R signaling has been shown to promote neuroblastoma tumorigenesis, inhibit apoptosis^{24, 25} and disruption of IGF1R signaling by a small molecule inhibitor or antibody inhibits neuroblastoma growth in both *in vitro* and *in vivo* models of the disease^{26, 27}. Inhibitors against IGF1R and its downstream target mTOR, are currently being evaluated in early phase pediatric clinical trials^{27, 28}.

While not yet translated to the clinic, there are several other promising neuroblastoma targets that also have small molecule inhibitors in adult trials. Through an unbiased RNAi screen of the protein kinome in neuroblastoma cell lines, the cell cycle checkpoint kinase CHK1 was identified as uniquely potent in inducing cytotoxicity following protein depletion^{29, 30}. CHK1 is constitutively activated in neuroblastoma cell lines and primary tumor tissues. The unique single agent activity is likely through myc-induced replication stress, a finding that was recently supported in MYC driven lymphoma models³⁰. Other druggable targets include PLK1 which emerged from a small molecule screen in neuroblastoma tumor initiating cells³¹, MEK inhibitors which may reverse hyperactivated ras mediated retinoid resistance in neuroblastoma³² and dual PI3K/mTOR inhibitors which destabilize MYCN and inhibit tumor vasculature³³. Molecularly targeted agents are currently being tested in combination with Irinotecan and Temozolamide because this combination was well tolerated and had modest activity in a phase II clinical trial for children with recurrent and refractory neuroblastoma³⁴, providing a chemotherapeutic backbone for new agent integration into more traditional therapeutic regimens (ClinicalTrials.gov Identifier: NCT01141244)³⁵.

Immunotherapy—A recent randomized phase 3 trial showed a significant improvement in high-risk neuroblastoma patient survival in those treated with the chimeric monoclonal antibody ch14.18 following conventional cytotoxic therapy⁷. This approach is both disease and patient specific as this antibody targets the disialoganglioside GD2 expressed on the surface of most neuroblastoma cells, and the effect is augmented by the addition of the cytokines interleukin-2 (IL-2) and granulocyte-macrophage colony-stimulating factor (GM-CSF) due to antibody-dependent cytotoxicity (ADCC)^{7, 36, 37}. This pivotal trial followed years of development of anti-GD2 approaches in early phase clinical trials that all documented some degree of activity, as well as considerable toxicity such as dose limiting pain, since GD2 is also expressed on pain fibers^{38–41}. Current efforts are underway to potentially improve both the antibody and augmentation of the immune response so that future immunotherapy will be even more effective and less toxic. These strategies include a fully humanized anti-GD2 protein, and physically linking this molecule to IL-2, potentially allowing for ADCC in the proper microenvironment but limiting systemic toxicity such as capillary leak, hypotension and fever^{42, 43}. The results of the phase II study of the hu-14.18-IL2 antibody showed that patients with a low burden of disease (stratum 2) had a 22% complete response rate⁴³. Another humanized antibody, Hu14.18K322A, has modifications that decrease antibody complement activation, which may result in fewer side effects, and alternative post translational modification that may allow higher ADCC^{44, 45}. Most investigators have considered immunotherapy as a post-chemotherapy remission-

consolidation strategy targeting minimal residual disease, but it is also possible to use these anti-GD2 agents with chemotherapy to more effectively induce remission. Future randomized phase III trials of immunotherapeutic strategies will be required to definitively establish which strategy provides the right balance of anti-tumor efficacy with toxicity.

Targeted radiotherapy—Neuroblastoma is an exquisitely radiosensitive neoplasm, but its disseminated nature challenges successful implementation of conventional radiotherapeutic approaches. Since neuroblastoma arises from neural crest progenitors, nearly 85% of tumors have a functional sympathetic norepinephrine transporter (NET) protein on their cell surface. Investigators have taken advantage of this by targeting radiolabeled benzylguanidine (norepinephrine analogue) for diagnostic (low dose) and therapeutic (high dose) purposes. Indeed, a large phase II clinical trial of children with recurrent and refractory neuroblastoma treated with a single dose of ^{131}I -metaiodobenzylguanidine (^{131}I -MIBG) showed an impressive objective response rate (CR and PR) in 36% of patients, with an additional 34% having stable disease for a median time of 6 months, and palliation of symptoms such as pain⁴⁶. There are plans to test this agent in a randomized controlled trial for newly diagnosed neuroblastoma patients in first response to see if this targeted radiotherapeutic can provide improved durable remission rates when integrated into consolidation therapy. Additional studies are aimed at providing enhanced activity of ^{131}I -MIBG by combination with radiosensitization agents such as Irinotecan, histone deacetylase inhibitors (<http://www.nant.org/>), and perhaps targeted agents such as CHK1 inhibitors^{47, 48}. It should be noted that a hypothetical limitation of the ^{131}I -MIBG therapy is that I-131 emits largely beta-energy, which because of its relatively long path-length, the resident cell itself is not targeted. Therefore, efforts are underway to test the α -particle emitting norepinephrine analogue ^{211}At -MABG, which may have superior efficacy against minimal residual disease in neuroblastoma than ^{131}I -MIBG due to its higher energy transfer and much shorter path length⁴⁹.

Epigenetic therapy

An emerging paradigm is that pediatric cancers have relatively few somatic mutations compared to common adult malignancies^{50–52}. In neuroblastoma, this is despite the fact that the genome is highly rearranged, with large chromosomal copy number alterations. Thus, it is likely that defects in DNA methylation and acetylation play a major role in regulating the high-risk neuroblastoma transcriptome. Ongoing efforts in epigenomic profiling are focused on defining the likelihood that any such aberrancies result in targetable vulnerabilities for drug development. Non-specific histone deacetylase inhibitors are currently being tested in the clinic, and investigators must take advantage of any observed anti-tumor activity to determine what defects in the tumor genome and/or epigenome could predict for clinical efficacy^{47, 53}.

Rational Drug Development Strategies

A paradigm that is emerging in cancer therapeutics is that while clearly there is differential sensitivity to therapeutics between tumor types, the same is true within histologies, at diagnosis and at relapse. For any therapeutic, be it a traditional chemotherapy, radiation or a targeted small molecule, by identifying individuals most likely to respond, effective agents will continue in clinical development in the appropriate populations, and more importantly there will be improved efficacy. Our current understanding of the underlying molecular circuitry of neuroblastoma is just emerging, and is years from contributing to a comprehensive personalized approach to therapy, but this is on the horizon. To achieve this goal, investigators must identify biomarkers of therapy response that are useful to the clinician. The ultimate goal will be to have robust makers of specific oncogenic vulnerabilities for each individual patient, and this will require access to tumor material in

real time. This is especially critical in the traditional phase 1 setting, as most molecular data is derived from diagnostic specimens and relapsed tumors are rarely biopsied. In order to achieve this goal, several milestones must be reached. First, ongoing genomic profiling and systems biology approaches must deliver on the accurate and complete characterization of all mutant pathways in this disease, at diagnosis and at relapse. Second, investigators must utilize large and well-annotated collections of neuroblastoma-derived cell lines and transgenic preclinical models to test the hypotheses that specific biomarkers indeed predict response to a therapy under development. Finally, pediatric oncologists will need to reconsider traditional drug development paradigms, and seek to design early phase clinical trials that select for patients most likely to show signs of anti-tumor efficacy but understanding the tumor at the time of trial entry in order to support rapid development in the refractory disease setting, allowing for early testing for impact on survival in newly diagnosed high-risk neuroblastoma patients.

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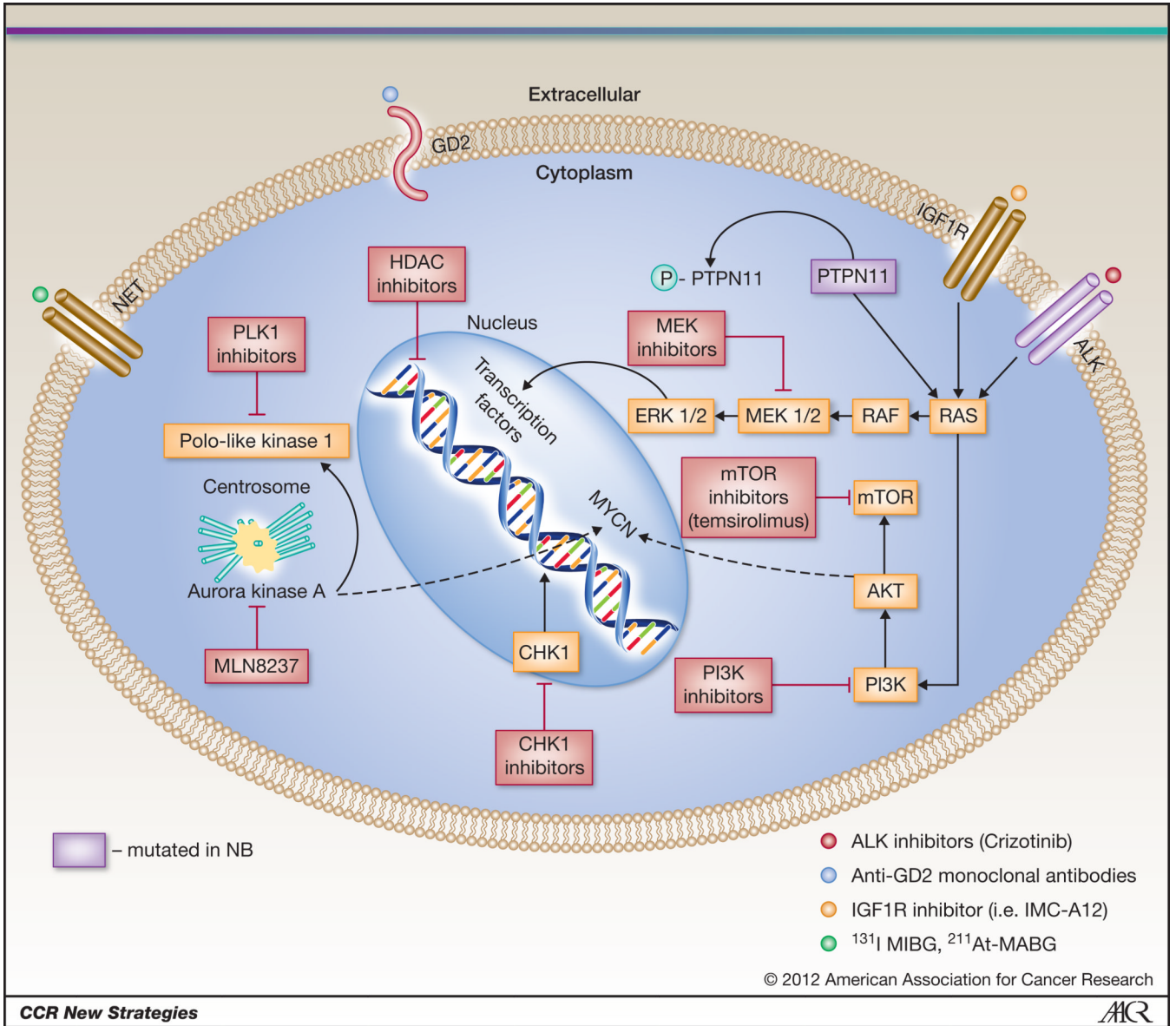


Figure 1. Targeted therapy in neuroblastoma: Current approaches to targeted therapy for neuroblastoma involve several modalities including 1. small molecular inhibitors of activated signaling pathways (ALK, IGF1R, MEK, PLK1, AURKA, PI3K and mTOR inhibitors) 2. radiopharmaceuticals targeting the NET receptor (¹³¹I-MIBG, ²¹¹At-MABG) and 3. immunotherapy (anti-GD2 antibodies).

Neuroblastoma: Approaches to Targeted Therapy

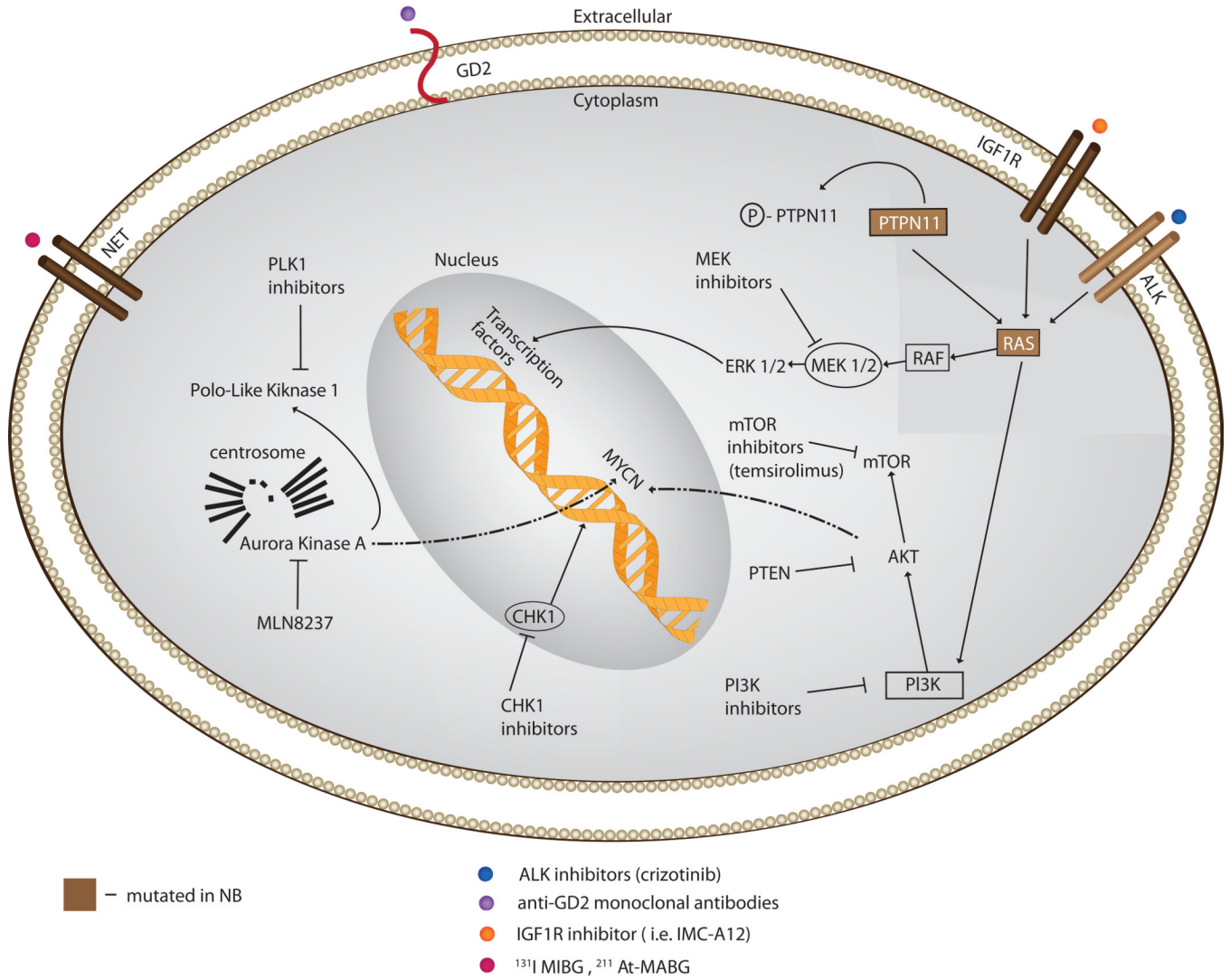


Figure 2.