The "Neuro" of Neuroblastoma: Neuroblastoma as a Neurodevelopmental Disorder

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Neuroblastoma is a childhood cancer derived from cells of neural crest origin. The hallmarks of its enigmatic character include its propensity for spontaneous regression under some circumstances and its association with paraneoplastic opsoclonus, myoclonus, and ataxia. The neurodevelopmental underpinnings of its origins may provide important clues for development of novel therapeutic and preventive agents for this frequently fatal malignancy and for the associated paraneoplastic syndromes.

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Neuroblastoma as an Aberration of Neural Crest Development

Neuroblastoma is a malignancy of the sympathetic ganglia and adrenal medulla, structures derived from the embryonic neural crest. Over one third of neuroblastomas are diagnosed in infants, and 75% are diagnosed by 5 years of age. It is important to improve neuroblastoma treatment because neuroblastomas account for 15% of all pediatric cancer deaths, despite representing only 8% of new diagnoses and affecting only 1 in every 8,000 live births. Neuroblastoma can be viewed as resulting from a failure of neural crest cell differentiation, so understanding normal neural crest differentiation may help identify novel targets for neuroblastoma therapy and, potentially, prevention.[1]

Normal trunk neural crest cells leave the dorsal aspect of the neural tube, migrate ventrally, close to the neural tube, and begin to differentiate in response to local cues (Fig 1). Some of them differentiate into neurons of the sympathetic ganglia or sympathetic neuron-like chromaffin cells of the adrenal medulla in response to such cues as bone morphogenetic protein from the nearby dorsal aorta.[2] Neurons promote the differentiation of adjacent neural crest-derived cells into Schwann cells (associated with neural axons), and satellite cells (associated with neural cell bodies).[3]

Neuroblastomas and neuroblastoma cell lines contain cells that resemble immature sympathetic neurons (sometimes called N-type cells) and Schwann cells (S-type cells). Because patients whose tumors have higher proportions of Schwann cells have better outcomes, the relationship between the cell subtypes has been a source of some discussion. *MYCN* gene amplification occurs in approximately 22% of neuroblastoma, and it is a hallmark of the most aggressive tumors. This is not to say that *MYCN* does not play any role in non-*MYCN*-amplified neuroblastomas; even in normal neural crest, *MYCN* functions to maintain the pluripotent, proliferative state of, prevent differentiation of, and, ironically and in the right microenvironment, promote apoptosis in neuroblasts.[4] *MYCN* amplification is present in immature neuronal neuroblastoma cells, but it is rarely found in Schwann-like neuroblastoma cells, which have less malignant potential than neuron-like cells.[5] X-inactivation studies showed that neuroblastoma neuron- and Schwann cell–like cells share a common progenitor,

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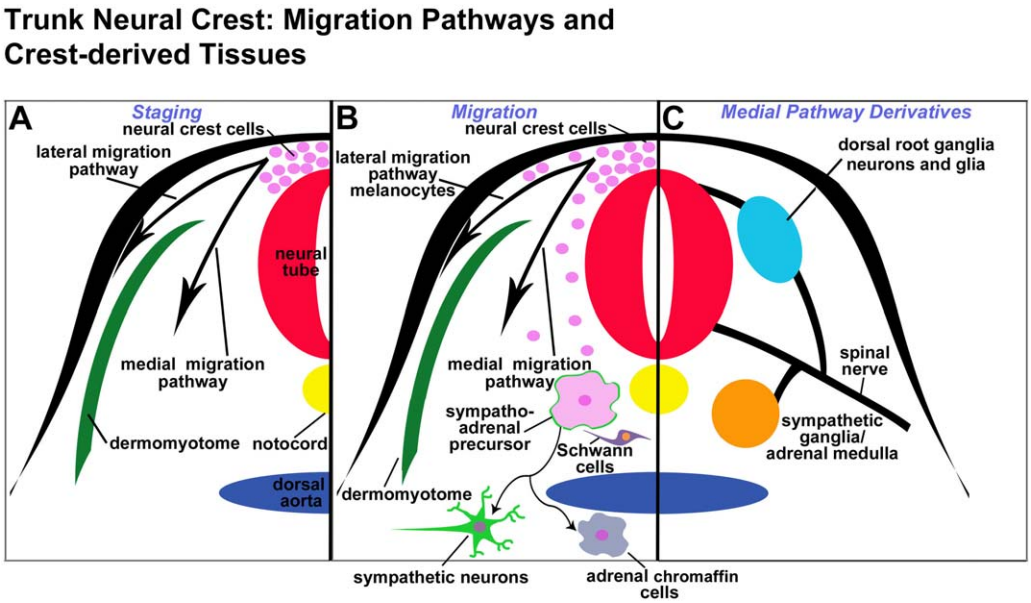

FIGURE 1: Trunk neural crest cells: formation, migration pathways, and medial pathway derivatives. (A) Early in vertebrate development, neural crest cells (pink) form at the dorsal aspect of the neural tube (red) and migrate laterally or medially (black arrows). (B) Slightly later in development, some of the medially migrating neural crest cells differentiate into sympathoadrenal precursor cells (pink with green outline). These can give rise to mature sympathetic neurons (green), adrenal chromaffin cells (gray), and Schwann cells (purple). (C) In mature vertebrates, the sympathetic ganglia contain sympathetic neurons. The adrenal medulla, a sympathetic ganglion-like structure, contains adrenal chromaffin cells. Schwann cells reside in sympathetic ganglia and dorsal root ganglia, and populate all spinal nerves. Schwann-like satellite cells associate with sympathetic and dorsal root ganglia. The adrenal medulla also contains Schwann-like cells.

but that only neuron-like cells show chromosomal changes.[6] Dialog between neuroblastoma cells and Schwann cells can be driven by increased neuroblastoma cell expression of neuregulin-1, a major Schwann cell mitogen.[7] Recruitment of normal Schwann cells by mutant neurons may account for most of the mixed cellular phenotype characteristic of neuroblastomas. Alternatively, the cell of origin of the Schwannian cells in undifferentiated neuroblastomas may differ from that in mature ganglioneuromas; that is, Schwannian neuroblastoma cells may derive from the malignant clone, whereas Schwann cells in mature ganglioneuromas may be normal Schwann cells.

Normal neural crest cells are characterized by their ability to self-renew and differentiate into multiple cell types.[8] Importantly, stem cell properties can be reacquired by differentiated neural crest derivatives.[9] The ability of cells to self-renew and differentiate along multiple lineages is also a feature of the stem/precursor-like cells found in cancers. Tumor stem/progenitor cells may occur when mature cells dedifferentiate in response to genetic and epigenetic alterations and/or if developing cells accumulate genetic and epigenetic alterations. It remains to be determined which is true of neuroblastoma, but the fact that neuroblastoma is a disease of childhood, and the maintenance of protein expression and post-translational modification patterns characteristic of embryonal neural crest in neuroblastomas[10] support the latter model. Neuroblastoma tumors and neuroblastoma cell lines, in addition to neuron- and Schwann cell–like cells, may also contain stem-like cells (I-type cells). These stem-like cells express stem cell markers, are more tumorigenic than other neuron-like cells on xenotransplantation, and can develop into immature neurons with elevated expression of sympathetic/adrenal markers, such as tyrosine hydroxylase and dopamine-β-hydroxylase, on exposure to retinoic acid (RA[11]). Other culture conditions can induce Schwann-like phenotypes.[12] They can also form secondary spheres in culture (self-renew). In neuroblastoma cell lines, miR-101 and one of its targets, LaminA, may control neuroblastoma stem-like cell self-renewal and tumor-propagating properties.[13] Therefore, neuroblastoma stem-like cells meet criteria for stem-like or progenitor cells (multilineage differentiation) and tumor-initiating cells, and ongoing efforts are aimed at ablating these cells, and/or forcing their differentiation.

The precise cell of origin of neuroblastoma remains unknown. Neural crest cell-derived neuroblasts may be one cell of origin. *MYCN* amplification is characteristic of a subset of human neuroblastomas. When a tyrosine hydroylase promoter drives expression of *MYCN* in mouse neuroblasts, mature sympathetic neurons, and cells of the adrenal medulla, neuroblastomas develop.[14] With *MYCN* expression in multipotent neural crest-derived cells of the adrenal gland (sympathoadrenal progenitor cells; SAPs) in vitro,

cells demonstrate increased cell proliferation, self-renewal, and a bias toward neuronal differentiation, but do not form tumors on xenotransplantation.[15] Additional genetic alterations in SAPs may be required to drive tumorigenesis. Alternatively, it remains possible that driver mutations must occur in neuroblasts for neuroblastoma to form in vivo.

The shared plasticity of neuroblastoma cells and developing neural crest stem cells inspired the idea that cell differentiation might be an effective neuroblastoma therapy. The possibility that neuroblastoma cells could be differentiated into Schwann cells has not yet been studied therapeutically. RA treatment causes differentiation of immature neuron-like cells into more mature neurons in vitro; its effectiveness in patients remains uncertain. However, neuroblastoma cells with mutations in *NF1*, a negative regulator of Ras signaling, fail to differentiate in response to RA, and blocking mitogen-activated protein kinase (MAPK) kinase (MEK) signaling downstream of *NF1* mutation enhances effects of RA.[16] Indeed, mutations in Ras-MAPK signaling pathway genes are found in 78% of relapsed neuroblastoma.[17] Combined MEK inhibition and RA therapy has not yet been tested in patients, but it might be considered as a rational approach to inducing neuronal differentiation.

Understanding the Phenomenon of Spontaneous Regression

Clinically, neuroblastoma is known for heterogeneous clinical behavior, from spontaneous regression or differentiation, to relentless progression despite intensive, multimodality therapy. However, it is the propensity for spontaneous regression that makes this tumor so fascinating. Therefore, a better understanding of the mechanisms responsible for spontaneous regression might help to identify alternative therapeutic approaches. Several mechanisms have been proposed to explain the phenomenon of spontaneous regression of neuroblastomas, including neurotrophin deprivation, humoral or cellular immune response, loss of telomerase activity, or alterations in epigenetic regulation.[18]

Neuroblastoma has the highest rate of spontaneous regression of any human cancer.[19,20] Clinically, spontaneous regression of neuroblastoma was codified as a specific stage of disseminated neuroblastoma in infants with a generally good prognosis, known as IVS (now 4S or MS). However, this was just the most dramatic example of spontaneous regression, which can be observed in infants with any stage of disease if they have biologically favorable tumors.[21,22] This conclusion is further supported by mass screening studies, in which screened populations demonstrated a doubled or tripled neuroblastoma prevalence and no change in overall mortality from neuroblastoma.[23,24] The increased prevalence of neuroblastoma observed in the screened populations was likely attributable to detection of tumors (of all stages) that would otherwise have regressed spontaneously, and indeed the prevalence of spontaneous regression was at least as high as the prevalence of clinically detected neuroblastomas.[23,24] Furthermore, almost all tumors detected by mass screening were biologically favorable, suggesting that biologically favorable tumors rarely evolve into biologically unfavorable tumors.[25]

The tyrosine receptor kinase (Trk) neurotrophin receptors have critical roles in the development and maintenance of the central and peripheral nervous systems, and they also have important roles in neuroblastoma pathogenesis.[26,27] High TrkA expression is associated with favorable clinical and biological features, such as younger patient age, lower tumor stage, and absence of *MYCN* amplification, and these patients have an excellent outcome.[28,29] In contrast, TrkB is coexpressed at high levels with its ligand, brain-derived neurotrophic factor (BDNF), in clinically and biologically unfavorable tumors, especially those with *MYCN* amplification.[30] Activation of the TrkB-BDNF autocrine pathway can lead to invasion, metastasis, angiogenesis, and drug resistance.[30–32] Tumors expressing high levels of TrkA undergo neuronal differentiation in vitro when exposed to nerve growth factor (NGF), but the same cells die within a week if deprived of NGF.[29,33] Thus, culture conditions recapitulate the behavior of TrkA-expressing neuroblastomas, which undergo neuronal differentiation or spontaneous regression, depending on the presence or absence of NGF in their microenvironment.

Migrating neural crest precursors (and favorable neuroblastomas that express TrkA) survive despite a lack of available NGF in their microenvironment. This could result from expression of TrkAIII, which is a constitutively active, alternatively spliced isoform.[34] Thus, the conversion from a TrkA-expressing, NGF-independent neuroblastoma to a NGF-dependent tumor could be the consequence of a developmentally programmed isoform switch from TrkAIII to TrkA. Alternatively, NGF-independent neuroblastomas could depend on another receptor or pathway for survival, and then switch dependence to TrkA, only to undergo apoptosis and regress in the absence of ligand (see Fig 2). However, the available data are most consistent with a TrkA dependence mechanism accounting for spontaneous regression of neuroblastomas.

Spontaneous regression of neuroblastomas and other types of cancer can sometimes follow an acute infection. Furthermore, tumor-infiltrating lymphocytes have been observed in neuroblastomas, and there is evidence for the presence of both tumor-targeted T cells and antineural

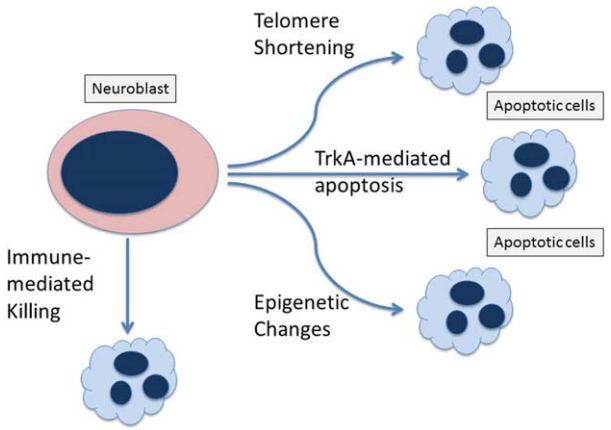

FIGURE 2: Mechanisms of neuroblastoma regression. Shown are the major mechanisms that have been proposed to explain the phenomenon of neuroblastoma regression in infants. Three of the mechanisms (telomere shortening, TrkA-mediated apoptosis in the absence of NGF, and epigenetic changes) likely lead to apoptosis and programmed cell death. However, cellular or humoral immune response to tumor antigens would likely lead to direct, immune-mediated killing of neuroblastoma cells. NGF = nerve growth factor; TrkA = Trk receptor tyrosine kinase A.

antibodies in patients with neuroblastoma.[35] Thus, one possible mechanism of spontaneous regression could be a consequence of a host-mediated immune response. Interestingly, the paraneoplastic opsoclonus myoclonus ataxia syndrome (OMAS) is associated with the presence of antineural antibodies and a favorable outcome in patients with neuroblastoma (see section on "Neuroimmunology of OMAS" below).[36,37] Around 40% of patients with OMAS have neuroblastoma, which suggests that the other 60% either had a neuroblastoma that regressed, or they have a de novo autoimmune disease. Neuroblastoma cells from patients with high-risk disease may evade immune destruction by downregulating human leukocyte antigen (HLA) class I molecules.[38] However, most tumors from patients with stage 4S neuroblastoma express normal levels of HLA class I antigens.[39] Upregulation of class I expression in neuroblastoma cells can be induced by interferon-γ,[38] which suggests that upregulation of HLA class I in vivo might be used to augment immune surveillance and promote tumor regression.

Telomeres are specialized structures at the ends of chromosomes that are important for the replication and stability of chromosomes. The regulation of the telomere length is controlled, in part, by expression of the enzyme telomerase, which is frequently high in cancer and immortalized cells, but low in most normal and senescent cells.[4] Hiyama et al[40] studied the regulation of telomere length and activity of the telomerase in 100 samples of neuroblastomas. Most of the tumors that exhibited a high level of telomerase activity were associated with a poor prognosis, and all tumors with *MYCN* amplification had high telomerase activity. Interestingly, most of the tumor samples from 4S neuroblastoma had low telomerase activity or short telomeres, a pattern that is usually associated with senescent cells.[41] These data suggest that loss of telomerase activity is a biologically plausible mechanism to explain spontaneous regression of neuroblastoma and, possibly, of other tumors. However, low telomerase activity is also associated with other biologically favorable features of predominantly low-risk tumors, so the independent role of telomerase is not clear.

Changes in gene expression related to alterations in promoter DNA methylation, histone modification, chromatin remodeling, or other mechanisms might also impact the survival in neuroblastoma cells. Epigenetic changes affecting expression of genes relevant to neuroblastoma development were initially reported more than a decade ago, and several studies have suggested that alterations in gene methylation and histone modification are related to patient outcome.[42] The correlation between epigenetic changes and neuroblastoma behavior has been increasingly studied, particularly because next-generation sequencing analyses of neuroblastoma have reported a very limited number of previously unrecognized recurrent somatic mutations.[43] Therefore, epigenetic changes might help to explain poorly understood aspects of disease presentation and clinical behavior.[44]

Thus, there are several possible mechanisms that could explain the phenomenon of spontaneous regression of neuroblastomas, including neurotrophin deprivation, immune-mediated destruction, loss of telomerase causing telomere shortening, developmentally regulated epigenetic changes, or other mechanisms. Indeed, it is possible that different mechanisms are responsible for spontaneous regression in individual cases.[18] There are currently no animal models of neuroblastoma that mimic the spontaneous regression observed in 4S disease.[45] Our challenge will be to better understand what triggers regression and develop therapeutic strategies that take advantage of this understanding to treat infants with biologically targeted therapies.

Neuroimmunology of OMAS

OMAS is a rare, enigmatic brain syndrome that can occur from infancy to adulthood, but the median age of onset in pediatric cohorts is in the second year of life. In its full form, the clinical syndrome is highly recognizable with multidirectional fast saccadic bursts of conjugate eye movements (opsoclonus), arrhythmic random jerks of limbs, face, and trunk (myoclonus), and ataxia. Irritability, sleep disturbance, and behavioral and cognitive regression are common accompaniments. OMAS has a

paraneoplastic association with neural crest tumors (neuroblastoma, neuroganglioma, etc.) in approximately 40% of cases.[46,47] Other cases of OMAS occur as an infectious process or, more commonly, a para- or postinfectious process. In some patients, there is evidence of tumor and infection. OMAS complicates only 2% to 3% of all neuroblastoma cases.[37] Searching for a neural crest tumor in OMAS patients typically involves imaging and metabolic studies (metaiodobenzylguanidine [MIBG] imaging, urine catecholamines), although computed tomography (CT) or magnetic resonance imaging is considered superior to metabolic studies given that some of the tumors appear to have low metabolic activity.[48] The tumor is abdominal/pelvic in approximately two thirds of cases and thoracic in the remainder.

Patients with OMAS have better outcomes from a tumor perspective than neuroblastoma patients without OMAS. Rudnick et al showed that metastatic disease occurs in only 2 of 21 (10%) of neuroblastoma patients with OMAS, compared to 229 of 654 (35%) of neuroblastoma patients without OMAS.[16] Likewise, survival is better in OMAS-neuroblastoma patients compared to neuroblastoma patients without OMAS.[37] Pathological examination has shown that in OMAS patients with neuroblastoma, the tumor typically has a dense inflammatory cell infiltrate (CD20 B cells and CD4 and CD8 T cells), whereas inflammatory cell infiltrate is less common in neuroblastoma tumors uncomplicated by OMAS.[49] These tumor data support the hypothesis that OMAS is associated with a pronounced autoreactive immune response against the tumor.

The immunology of OMAS has been studied in surprising detail for a rare disease,[50–61] although no unifying immune biomarker has yet been identified and the syndrome remains a clinical diagnosis. Because of the presence of the key features of opsoclonus, myoclonus, and ataxia, the brainstem and cerebellum have been considered to be the primary sites of immunological attack; this is supported by imaging studies.[50] Cerebrospinal fluid neurofilament is elevated in acute OMAS patients, suggesting that neurons are the primary cellular target.[51] Autoantibodies have been a focus of interest in OMAS, and the recent discoveries of cell surface autoantibodies associated with autoimmune encephalitis syndromes has led to the hypothesis that OMAS could be a "cell surface autoantibody syndrome"; however, there is evidence for a cell surface antibody in only a minority of patients with OMAS.[53] Children with OMAS are typically negative for classic onconeuronal antibodies (such as anti-Hu, Ri, and Yo), and using immunhistochemistry against brain tissue sections,[54] OMAS patients often are found to have immunoglobulin G binding to neuronal tissue without a common binding pattern to suggest a unifying autoantibody biomarker.[53] The role of B cells and humoral immunity has been suggested,[55–58] as have T-cell mechanisms.[59–61] A cartoon summarizing the basic immunological mechanisms thought to be involved is shown in Figure 3.

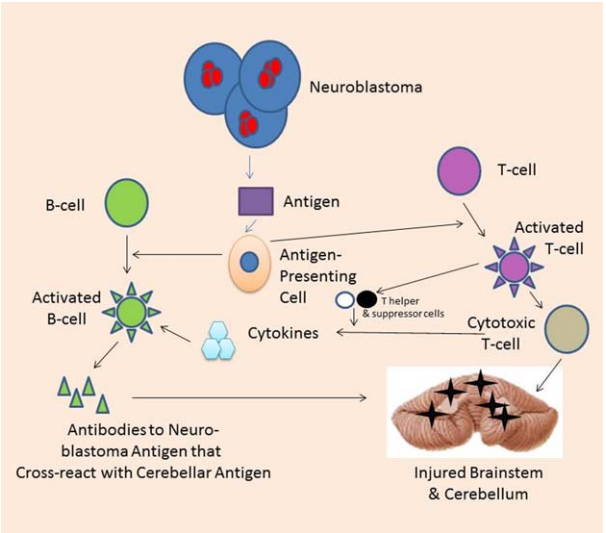

FIGURE 3: B-cell and T-cell involvement in the pathogenesis of opsoclonus-myoclonus-ataxia syndrome (OMAS) in neuroblastoma. OMAS is thought to be a consequence of the cross-reaction of a neuroblastoma antigen with proteins in the cerebellum and brainstem. Both cytokine-stimulated B-cell generation of antibodies and T-cell cytotoxicity are thought to be involved.

Historical outcome data have shown that OMAS has the potential to be a devastating disease, with permanent motor and cognitive disability a common outcome.[47] Patients with relapsing disease do worse than patients with monophasic disease, emphasizing the therapeutic principle of inducing remission and trying to prevent relapses with chronic immune suppression in patients with severe disease.[47] Although there are no randomized, controlled trials in OMAS, there are reasonable cohort studies (retrospective and prospective) to show that for patients with severe disease, multimodal therapy (adrenocorticotrophic hormone or corticosteroids *plus* rituximab, cyclophosphamide, or steroid sparing immune suppression) do better than patients given corticosteroids and intravenous immunoglobulin alone.[62,63] More-modern aggressive immune suppression appears to convey better outcomes in cognition and motor scores compared to historical treatments,[63] and early treatment with rituximab appears to improve outcomes compared to later treatment[64]; however, it should be emphasized that none of these studies were randomized or controlled.

In summary, OMAS is a paraneoplastic or infection-associated, immune-mediated central nervous system (CNS) syndrome, which is probably immunologically complex and possibly heterogeneous. Clinical and histological tumor data

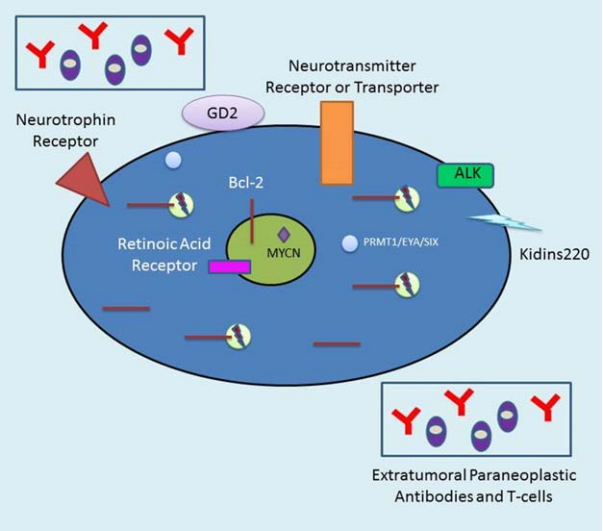

FIGURE 4: Proposed targets for neuroblastoma therapy. Proposed and experimental targeted therapy for neuroblastoma has been aimed at compounds on the cell surface (GD2; ALK; neurotrophin receptors; neurotransmitter uptake systems; Kidins220), in nuclear and mitochondrial membranes (retinoic acid receptor; Bcl-2), in the cytoplasm (PRMT1/EYA/SIX), and in the nucleus (MYCN). Therapy for opsoclonus-myoclonus-ataxia syndrome is aimed at the immune mechanisms through which it is thought to arise. ALK = anaplastic lymphoma kinase; Bcl-2 = B-cell lymphoma 2; EYA = eyes absent; GD2 = disialganglioside 2; MYCN = N-Myc; PRMT1 = protein arginine methyl-transferase type I; SIX = sine oculis.

suggest that there is a robust immune response against the tumor in OMAS patients, and this immune response may confer tumor outcome benefits at the expense of an "autoreactive" immune response against the brain. As is true for many immune-mediated CNS disorders, early recognition and early treatment improve outcomes.

Targeted Therapy for Neuroblastoma: A Developmental Neurobiological Approach

Targeted therapy for cancer aims to attack cancer cells while leaving normal cells unscathed. In the case of developmental neoplasms, this may mean identifying therapeutic targets that are expressed during embryogenesis or early postnatal life but are not expressed, sequestered in a drug-inaccessible intracellular compartment, or downregulated in more mature stages. As such, efforts to identify targets uniquely found in neural crest cells or uniquely expressed in neoplastic neural crest cell derivatives have been vigorous, but such targets are neither plentiful nor easy to attack.[1] To date, clinically or preclinically developed approaches have been aimed at outfacing targets on the exterior cell surface; transmembrane targets that bridge between the extracellular and intracellular compartments; intracellular targets; and, in the case of paraneoplastic effects of neuroblastoma, systemic targets extrinsic to the neuroblastoma cell itself (Fig 4).

The best-studied target on the exterior surface of the neuroblastoma cell is the ganglioside, disialganglioside 2 (GD2). Preclinically studied anti-GD2 therapeutics use GD2 as a cellular address and depend on conjugation of the GD2-binding agent to a toxin or drug to trigger a cytolytic cascade.[65] Proposed anti-GD2 antibodies have been conjugated to ^{131}I, DNA-damaging drugs, microtubule disrupting drugs, diphtheria or Pseudomonas toxin, and nanoparticles loaded with toxic drugs in an attempt to target cytolysis to neuroblastoma cells. A recent phase II study of consolidation therapy with anti-GD2 antibodies plus isotretinoin demonstrated enhanced rate and duration of complete or very good partial response in patients with high-risk neuroblastoma who relapsed after first remission.[66] Unfortunately, treatment of neuroblastoma with targeted immunotherapy using chimeric anti-GD2 monoclonal antibodies also attacks pain fibers and is associated with significant pain, requiring management with a high-dose opioid infusion.[66]

Transmembrane targets include neurotransmitter receptors and the monoamine uptake system. Whereas these are neither required for cell survival nor unique to neuroblastoma cells, neuroblastoma is a peripheral nervous system tumor, and toxic or toxin-conjugated neurotransmitter analogues can at least be made sufficiently polar as not to be CNS permeant.[68,69] One of the first toxic neurotransmitter analogues proposed for use in targeted therapy of neuroblastoma was 6-hydroxydopamine (6-OHDA). 6-OHDA is not distributed centrally after peripheral administration; it enters neurons through the monoamine uptake system and generates toxic reactive oxygen species in situ, thereby killing the cells. In vitro and preclinical studies showed the promise of 6-OHDA alone[68,70] and in combination with normal cell-selective free radical scavengers.[71] But the cumulative toxicity of 6-OHDA and the selective free radical scavengers precluded their utility as adjunctive agents for neuroblastoma therapy. Another strategy, using 6-mercaptodopamine to selectively reduce reduction-activated antimitotic prodrugs in neuroblastoma cells,[69] was hampered by the limited shelf-life of this catecholamine analogue.

But neurotransmitter analogues turn out to be useful both as therapeutic agents and as diagnostic aids in imaging of neuroblastoma. MIBG is a catecholamine analogue that can be labeled with ^{123}I for scintigraphy and with ^{131}I for focused radiotherapy for neuroblastoma. Recently, ^{124}I-MIBG positron emission tomography/CT has also been proposed for detection of neuroblastoma.[72] The sensitivity of ^{123}I-MIBG scintigraphy ranges from 67% to 100% in studies of biopsy-proven neuroblastoma and approximately 10% of neuroblastomas do not take up MIBG.[73] The response rate to

TABLE 1. Examples of Targeted Therapy Clinical Trials for Neuroblastoma

Target	Targeting Agent	Nature of Targeting Agent	Clinical Trial
Catecholamine transporter	^{131}I-MIBG	Radiolabeled ligand of transporter	NANT-2011-01: ^{131}I-MIBG alone vs. in combination with other chemotherapeutic agents
Ubiquitin E3 ligase	Lenalidomide	Antiangiogenic and apoptosis-inducing coenzyme; activates degradation of Ikaros transcription factors	NANT-2011-04: lenalidomide ± anti-GD2 antibody ± cis-retinoic acid
GD2	Mab Ch14.18	Monoclonal antibody to the disialoganglioside, GD2	NANT-2011-04: lenalidomide ± anti-GD2 antibody ± cis-retinoic acid
Retinoic acid receptor	13-cis-retinoic acid	Differentiation inducer	NANT-2011-04: lenalidomide ± anti-GD2 antibody ± cis-retinoic acid
Ornithine decarboxylase + COX-2	difluoromethylornithine (DMFO) + celecoxib	Synergistic inhibitors of MYC-dependent generation of polyamines	NANT-2012-01: (DMFO + celecoxib) ± other chemotherapeutic agents
Tyrosine and Raf kinases	Sorafenib	Autophagy inducer; angiogenesis inhibitor	NANT-2013-02: sorafenib ± other chemotherapeutic agents
Phosphoinositol-3-kinase (PI3K)	SF1126	Inhibitor of PI3K and the mTOR signaling pathway; putative MYCN inhibitor	NANT-2014-01: phase 1 SF1126 trial
ALK	Crizotinib	Inhibitor of anaplastic lymphoma kinase (ALK) and c-ros oncogene 1 (ROS1)	Children's Oncology Group study, NCT00939770
ALK, ROS1, tyrosine receptor kinase (Trk)	Entrectinib	Oral pan-Trk, ROS1, and ALK inhibitor	STARTRK-1: phase 1/2a study in patients with advanced solid tumors with relevant molecular alterations
TrkB	Lestaurtinib	Multi-Trk inhibitor	Phase 1 consortium study completed with encouraging results; phase 2 in planning

COX-2 = cyclooxygenase-2; GD2 = disialganglioside 2; ^{131}I-MIBG = iodine-131 metaiodobenzylguanidine; mTOR = mammalian target of rapamycin.

^{131}I-MIBG therapy in patients with refractory or relapsed neuroblastoma is 27%, with refractory patients demonstrating higher 24-month overall survival than relapsed patients.[74]

As neural crest-selective arbiters of life-death decision making, the NGF receptors, TrkA and p75NTR, would seem to be the perfect transmembrane targets for neuroblastoma therapy. Studies aimed at blocking protective signaling and potentiating death signaling in neuroblastoma cells have informed much of our understanding of NGF signaling and its downstream effects. But, unfortunately, depending upon the degree to which and the milieu in which it is expressed, either receptor can be pro- or antiapoptotic. Furthermore, from cell to cell in a

single tumor at a single time, the expression and downstream effects of TrkA and p75NTR vary widely.[75–77] This between- and within-tumor variability would make the design of drugs with predictable effects on neuroblastoma near impossible.

The B-cell lymphoma 2 (Bcl-2) family of proteins are also arbiters of life-death decision making and are both membrane bound and cytoplasmic in localization. In view of the expression of and signaling through antiapoptotic Bcl-2 family members by neuroblastoma[78,79] and the redox effects of Bcl-2 on neural cells,[80] the antineuroblastoma efficacy of the reduction-activated chemotherapeutic agent, neocarzinostatin, was hypothesized to be enhanced by overexpression of Bcl-2. This is indeed the case,[81,82] raising the prospect of using neocarzinostatin and other enediyne prodrugs as chemotherapy for neuroblastoma. In fact, treatment of neuroblastoma cells with neocarzinostatin in vitro also led to caspase 3–mediated cleavage of Bcl-2 to a proapoptotic fragment,[83–85] and tumor xenografts that expressed both Bcl-2 and caspase 3, but not one or the other, demonstrated enhanced sensitivity to neocarzinostatin in a nude mouse model.[83] This finding has been extrapolated to other tumors of the nervous system as well.[86]

The pathogenesis of neuroblastoma has been hypothesized to include maturational arrest of neural crest precursor cells.[10] As such, intracellular targets for neuroblastoma therapy have included proteins involved in induction and enactment of differentiation. The furthest along the therapeutic pipeline of these is the family of RA receptors, most commonly targeted with 13-cis-RA. Studies of 13-cis-RA as postconsolidation therapy after bone marrow transplantation are promising, but lack statistical power to definitively say whether or not it is effective in high-risk neuroblastoma.[87]

Downregulation of another potential target (Kidins220) induced Schwannian differentiation in neuroblastoma cells in vitro, a finding that is of interest because Schwann cell–like neuroblastomas are thought to be less aggressive clinically than neural or stem cell–like neuroblastomas. This raises the possibility of altering the malignant character, rather than the survival, of neuroblastoma cells as a therapeutic adjunct.[88]

One characteristic of the high-risk neuroblastoma is amplification and overexpression of the oncogene, *MYCN*. *ALK* is a target gene of N-Myc (MYCN) protein, and mutations of *ALK* underlie many cases of hereditary neuroblastoma.[89] However, MYCN and anaplastic lymphoma kinase (ALK) have proven to be difficult targets, both because of their promiscuous signaling roles in normal developing cells and because of the development of resistance to drugs aimed at them. A search is ongoing for downstream effectors of MYCN and ALK in neuroblastoma cells that might serve as effective therapeutic targets. Eyes absent homolog 1 (EYA1) and sine oculis-related homeobox 3 (SIX3), two proteins important in normal eye and ear development, appear promising in this regard.[90]

In contrast to the targeting of markers of malignancy of neural crest cells in neuroblastoma itself, the biology targeted for treatment of neuroblastoma-related OMAS is systemic immunoreactivity that may be triggered by the tumor, but does not depend on its persistence (see above section on "Neuroimmunology of OMAS"). In recent years, as biological response modifiers and monoclonal antibody therapies have matured, so has the antiparaneoplastic syndrome armamentarium. There is now evidence for efficacy of rituximab, ofatumumab, and mycophenolate mofetil in children with OMAS associated with a history of neuroblastoma.[63,91,92]

Although neuroblastoma is generally the clinical province of pediatric oncologists, the developmental origins, oncogenic events, and potential therapeutic targets are processes and molecular entities familiar to developmental and molecular neurobiologists. Many contributions have already been made by the neurology and neurobiology communities to our understanding of and clinical approach to neuroblastoma,[1,4,9,93] and ongoing clinical trials for neuroblastoma reflect the work of multidisciplinary consortia (Table). We anticipate that the interactions and collaborations among pediatric neurologists, neurobiologists, and pediatric oncologists will only become stronger as our understanding of neural crest development continues to grow.

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Author Contributions

N.R. was primary author on the section on "Neuroblastoma as an Aberration of Neural Crest Development"; G.B. on "Understanding the Phenomenon of Spontaneous Regression"; RD on "Neuroimmunology of

Opsoclonus Myoclonus Ataxia Syndrome"; and RS on "Targeted Therapy for Neuroblastoma."

Potential Conflicts of Interest

Nothing to report.

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