

Neuroblastoma: Current Imaging and Therapeutics

Abstract

Further study of promising therapies is needed for neuroblastoma, the most common extracranial solid tumor in children under the age of 2 years that accounts for approximately 15% of childhood mortality due to cancer. Exploration into new treatments must address the tumor's characteristic marked variability, ranging from spontaneous regression to differentiation into benign tumors to rapid fatal progression in addition to the fact that 50% of patients already have metastases at presentation. Once neuroblastoma disseminates to the bone, the survival rate is less than 7%. Critical to making progress in the management of neuroblastoma as determined by age, disease stage and biological/biochemical markers is the improvement of imaging modalities for initial staging and follow up for response to treatment. Also important to progress in the management of neuroblastoma is the general furthering of drug development since the current treatment regimens (surgical resection, chemotherapy, radiotherapy and immunotherapy) have proven to be inadequate for treating advanced disease and frequently cause patients to experience severe side effects that make them intolerable for many children. Promising therapies include immunotherapy, using antibodies to trigger a patient's own immune system to destroy neuroblastoma cells and anti-adhesion. Of particular interest in the realm of anti-adhesion is the concept of attenuation of NF- κ B over-activation as increased NF- κ B activity is thought to play a role in the development of tumor resistance to chemotherapeutic agents and radiation. Novel drugs to attenuate NF- κ B activity may potentially revolutionize treatment by allowing reduced drug and radiation doses for dose-sensitive patients as well as increased overall treatment effectiveness.

Key Words: *neuroblastoma, immunotherapy, anti-adhesion, NF- κ B activity, neuroblastoma imaging, neuroblastoma therapeutics*

1. INTRODUCTION

Neuroblastoma, a tumor that derives from the sympathetic nervous system and most often manifests as an abdominal mass originating from the adrenal glands, is the most common extracranial solid tumor of childhood and accounts for approximately 15% of childhood mortality due to cancer. New treatments for neuroblastoma are needed; current treatments are limited due to side effects and an increasing lack of effectiveness when disease is advanced. As part of disease management, patients receive an extensive workup involving multiple imaging modalities and biochemical testing to categorize them as high-risk, medium-risk, or low-risk. Although strides have been made in optimizing the use of imaging modalities and therapies for neuroblastoma, high-risk patients continue to exhibit a mere survival rate of less than 40%. [1] Part of the complexity of managing neuroblastoma patients is that the clinical behavior of the tumor is variable, ranging from spontaneous regression to differentiation into benign tumors to rapidly progressive metastatic disease. This review paper details the complexity of this disease, including current guidelines on its management as well as a call to action regarding the need to further develop new therapies that address the challenges of advanced disease and side effects, including immunotherapy and anti-adhesion.

2. CHARACTERISTICS OF NEUROBLASTOMA PATHOLOGY

An embryonal malignancy of the sympathetic nervous system, neuroblastoma is the most common extracranial solid tumor presenting in children under the age of 2 years; 90% of cases occur before the age of five. In fact, neuroblastoma accounts for approximately 8% to 10% of pediatric malignancies and accounts for 15% of all childhood cancer-related deaths. [1-4]

Neuroblastoma can arise from any neural crest element of the sympathetic nervous system, a branch of the autonomic nervous system that is primarily responsible for the fight-or-flight response and the production of the hormone epinephrine. Solid tumors often begin in the form of a lump or mass in one of the adrenal glands and present clinically as an abdominal mass but can also develop in nerve tissues of the neck, chest, abdomen or pelvis [5-7]. Not be confused with neuroblastoma, esthesioneuroblastoma, also known as olfactory neuroblastoma, is believed to arise from the olfactory epithelium; its classification remains controversial, given that it is not a sympathetic nervous system malignancy. [8,9]

2.1 Clinical Presentation

The most common clinical symptoms of neuroblastoma include pain (34%), fever (28%), and weight loss (21%), depending on the size of the tumor mass and, if it exists, the extent of metastasis. Rare but highly indicative characteristics of neuroblastoma include lower limb paresis due to intraspinal epidural extension of a primary paraspinal tumor (4%), severe diarrhea refractory to standard treatment due to excess production of vasoactive intestinal peptide (VIP) by tumor cells (4%), acute cerebellar encephalopathy manifesting as cerebellar ataxia, rapid and random eye movements (opsoclonus), myoclonic jerks due to unknown metabolic disturbances (2%-8%), Horner syndrome especially in patients with lesions in the cervical or upper thoracic sympathetic ganglia (1%-7%) and hypertension, flushing and periods of excessive sweating due to the increased concentrations of catecholamines (0%-2%). [4]

2.2 Etiology

The cause of neuroblastoma is largely unknown, although most physicians believe it is due to accidental cell growth that occurs during normal development of the adrenal glands. Many factors have been identified as potentially being linked to this including familial inheritance and environmental factors such as assisted pregnancies, paternal exposure to electromagnetic fields and prenatal exposure to alcohol, pesticides, or phenobarbital. However, independent studies have not yet confirmed the association of these factors with the development of neuroblastoma. [10-12] In addition, no significant variation in incidence has been noted between races or geographic area, namely North America and Europe. [12]

2.3 Clinical Behavior & Prognostic Factors

The hallmark feature of neuroblastoma is that it is one of the rare human malignancies known to demonstrate contrasting patterns of clinical behavior; it can exhibit spontaneous regression from an undifferentiated state to a benign form of maturation to ganglioneuroma to life-threatening progression to metastatic disease. No insight on this clinical behavior has yet come from microscopic investigation. Only "age" and "stage", to some extent, predict the course of disease. For instance, poor survival even with intensive therapy is associated with metastatic disease in 55% of patients with neuroblastoma who are older than 1 year and 40% of patients with neuroblastoma at all ages. [4] Around 10% of tumors undergo spontaneous regression in the absence of or with minimum therapeutic intervention. Interestingly, with neuroblastoma, spontaneous regression occurs between 10 and 100 times more frequently than it does in any other form of human cancer. [13]; a situation usually associated with a clinically recognizable syndrome called 4S as defined within the International Neuroblastoma Staging System (INSS) which is the most commonly used staging system in Europe and North America. INSS was developed by an expert panel to facilitate comparisons between trials. It was first established in 1998 and was substantially revised in 1993. It differentiates between stages based on surgical excision, lymph node involvement, and metastatic sites. In stage 4S, neuroblastoma manifests as a small primary tumor in the abdomen or thoracic cavity accompanied by metastasis in the liver or bone marrow and/or skin but not in the cortical bone. Although spontaneous regression is most commonly observed in stage 4S, it is also well described in children and adults with disease in stages 1-3. [14,15] Spontaneous maturation to a benign state, i.e. ganglioneuroma, is much rarer than spontaneous regression. However, little is known on the incidence and development of ganglioneuromas because they are not often reported in worldwide tumor registries. [16]

2.4 Diagnostic Testing

The work-up for neuroblastoma is quite extensive. Although the incidence of patients with low-stage disease has increased due to implementation of screening infants at the age of 6 months by checking 24

hour urine collections for elevated levels of homovanillic acid (HMA) and vanillyl mandelic acid (VMA), this practice has not decreased the diagnosis of high-stage neuroblastoma in patients over the age of 1 year. Therefore, it remains unclear as to whether neuroblastoma rapidly progresses to high-stage disease after the age of 1 year or whether a significant portion of advanced stage tumors develop from early infancy. [4] Imaging plays an integral role in the investigation of patients with neuroblastoma through initial staging, assessing response to therapy and surveillance for recurrence. [17,18] The current criteria for the diagnosis and staging of neuroblastoma are based on the INSS [19] or another staging system often employed at the time of diagnosis called the International Neuroblastoma Risk Group Staging System (INRGSS) that it is based more on clinical criteria and image-defined risk factors. [20] The diagnosis of neuroblastoma is defined as pathologic conformation of tumor tissue or neuroblastoma tumor cells in a bone marrow sample in the setting of increased urine or serum catecholamines or catecholamine metabolites dopamine, VMA and HMA. [3] To diagnose neuroblastoma according to the INRGSS, three dimensional measurements of the primary tumor and assessment of image-defined risk factors are mandatory as opposed to INSS which focuses more on surgical excision [20] INRGSS is designed for use primarily at the time of diagnosis and requires the use of CT or MRI in addition to 123-iodine (¹²³I) metaiodobenzylguanidine (MIBG) scintigraphy. INRGSS also does not take into account midline and lymph node involvement as does INSS. It is likely that both staging systems will be used in parallel in future group cooperative studies. [1]

2.5 Treatment Options

Decisions around treatments options which include surgical resection, chemotherapy, radiotherapy and immunotherapy, are based on several criteria such as age, stage of disease and biological and biochemical markers.[2] Patients with localized neuroblastoma tend to have good outcomes. Those presenting before the age of 12 months have better outcomes even with metastatic disease than those presenting after the age of 12 months. Overwhelmingly, patients die following presentation with metastatic disease after the age of 12 months. [21-23] After dissemination to the bone, the survival rate is known to be less than 7% [24]. Therefore, particularly for patients with metastases, a group that constitutes 50% of patients at presentation, an undeniable need exists to develop new therapies [18,24-26].

3.0 IMAGING MODALITIES FOR ASSESSING NEUROBLASTOMA PATIENTS

Standard imaging modalities for assessing patients with neuroblastoma, CT or MRI, are part of the initial diagnostic testing to evaluate primary tumor size and regional extent as well as distant spread to the neck, thorax, abdomen or pelvic sites. However, these scans are recommended as part of the work-up only if the patient is having neurologic symptoms or there is a clinical indication based on a physical exam. In the event that brain imaging is indicated, MRI is the radiographic study of choice due to its superior capabilities in elucidating spinal cord involvement and extension into the epidural space. [27-32] With gadolinium enhancement, the primary tumor often appears heterogeneous. Compared to CT, MRI is superior in assessing for leptomeningeal or epidural extension, extent of cortical bone destruction and tumor invasion of the kidneys, liver, bone marrow and diaphragm. [17] Furthermore, one prospective multi-institutional study that compared the accuracy of CT or MRI alone in conjunction with bone scintigraphy for staging neuroblastoma patients showed that MRI was a much more sensitive test (83%) compared to CT (43%). [33] Other advantages of MRI include lack of ionizing radiation, superb definition of primary tumor with high intrinsic soft-tissue contrast resolution and determination of internal structures. For these reasons, MRI has begun to replace CT scans in many centers. While calcification is not readily identified on MRI and small lymph nodes less than 13 mm are difficult to ascertain, necrosis, cystic areas and hemorrhages are readily seen. Bone marrow and cortical involvement can be successfully assessed via MRI but false positives for bone marrow involvement have been noted after treatment in some cases. [34] To exclude bone marrow involvement, bilateral posterior iliac crest marrow aspirates and core biopsies must be performed. [19,35] CT is used to define the site/extent of tumor and assess for evidence of regional invasion, vascular encasement and adenopathy. It is also used to easily detect characteristic radiographic findings such as soft tissue calcifications which are highly suggestive of neuroblastoma and occur in 80% to 90% of patients. [25,33,36] [17] Furthermore, CT is integral in differentiating

neuroblastoma from Wilms' tumor as these are the two leading causes of an abdominal mass presenting in childhood. [37,38]

3.1 Magnetic Resonance Spectroscopy

While structural MRI is a crucial tool in the assessment of neuroblastomas, it typically provides very little physiological information. The ability of magnetic resonance spectroscopy (MRS) to probe tissue biochemistry is a powerful tool that adds to the information obtained by conventional MRI.

Proton magnetic resonance spectroscopy (1H MRS) offers the unique ability to measure metabolite levels in a non-invasive manner. To date, most studies employing 1H MRS have been performed in the CNS. The resonances seen in the brain by 1H MRS are typically low weight molecules. In the normal brain, the most prominent peak arises from N-acetylaspartate (NAA) at 2.02 ppm. NAA serves as a marker of neuronal density and viability and is typically decreased or completely absent as neurons are replaced by neoplastic tissue. The other major peaks include total creatine [creatine (Cr) + phosphocreatine (PCr)] a marker for brain energy, as well as choline containing compounds (Cho) which are observed at 3.03 and 3.2 ppm, respectively. The choline (Cho) resonance arises from signals of several soluble components of brain myelin and fluid-cell membranes including glycerophosphocholine (GPC), phosphocholine (PCh) and Cho that are normally not soluble. However, pathological alterations in membrane turnover or inflammatory and gliotic processes result in a massive increase in MRS-visible Cho[39]. Thus, elevated choline is the hallmark of brain cancers as well as cancers in other tissue. Furthermore, it has been established that an increase in Cho is directly related to tumor malignancy [40].

Under normal conditions, one should not be able to observe lactate (Lac) as the Lac concentration is very low in the adult brain. Lactate is produced by anaerobic glycolysis. Accelerated anaerobic glycolysis occurs in highly cellular metabolic lesions which have begun to outgrow their blood supply. Consequently, the presence of Lac may indicate high level of malignancy [41,42]. This resonance (observed as a doublet) occurs at 1.32ppm. MR spectra acquired with short echo times are characterized by additional resonances including lipids (Lip) at 0.9 ppm (methyl resonance) and 1.3 ppm (methylene resonance).

Other metabolites detected by 1H MRS include myo-Inositol (ml, 3.5 and 3.6 ppm), glycine (Gly, 3.56 ppm), taurine (Tau, 3.26 ppm) and glutamine/glutamate (Gln/Glu, 2.1-2.5 ppm and 3.75 ppm). The resonance of glycine (Gly) overlaps with those of ml, however, Gly can be unambiguous detected with the choice of longer echo times due to the cancellation of the inositol multiplet. High concentrations of Gly are observed in high-grade gliomas compared to low-grade gliomas [43,44]. Pediatric patients with untreated primitive neuroectodermal tumors (PNETs) demonstrated elevated taurine concentrations; here MRS proved to be useful in the differentiation of PNETs from other tumors including astrocytoma, pilocytic astrocytoma and ependymoma [45].

Using 1H MRS on experimental models of neuroblastoma, e.g. cell lines and xerografts Lindskog et al. detected prominent Cho levels and low lipids contributions in viable tumor. On the other hand, necrotic neuroblastoma tissue is dominated by mobile lipids, polyunsaturated fatty acid and almost undetectable Cho [46,47]. Moreover, the same group showed that response or resistance to chemotherapy was accurately predicted by 1H MRS [48]. A treatment response to chemotherapy was characterized by a significant increase in mobile lipid/choline and mobile lipid/methyl ratios, increased levels of polyunsaturated fatty acids and a significantly decreased Cho resonance in a dose and time dependent matter. This metabolic response precedes effects on tumor volume and may accurately predict tumor regression [47].

In addition, 1H high-resolution magic angle spinning (HR-MAS) MR spectroscopy has been used to identify specific metabolic profiles associated with MYCN-amplified versus non-amplified subtypes of neuroblastoms. The presence of MYCN oncogene has been found to result in a poor prognosis. In vitro HR-MAS MRS performed on cell suspensions with either MYCN-amplified or non-amplified cell lines form separate classes according to their metabolite profiles. PCh/total-Choline (tCho) and taurine/tCho were found to be significantly elevated and GPC/tCho significantly reduced in the MYCN-amplified compared with the MYCN non-amplified cell lines.

Furthermore, ¹H HR-MRS MR spectroscopy was able to characterize the metabolic content of intact biopsy samples obtained from children suffering from neuroblastoma. E.g. Imperiale et al. found higher levels of Cr, Gln/Glu, acetate and Gly in neuroblastoma biopsies compared to healthy adrenal medulla tissue which predominantly contained adrenaline and a large amount of ascorbic acid [49]. Lastly, they explored the potential relationships between the metabolite profile and the patients' clinical data such as the age, disease stage and survival [49].

³¹P MRS is informative on cellular bioenergetics because it reveals concentrations of phosphorous containing intermediates of energy metabolism including nucleoside triphosphates, NTP (α , β and γ), inorganic phosphorous (Pi) and PCr. The chemical shift of the Pi peak is pH dependent and is commonly used for in vivo measurement of intracellular pH.

³¹P MRS is also be used to measure phosphodiester compounds (PDE) and phosphomonoester compounds (PME). The levels of PDE and PME are important markers for the implications in tumors. These compounds are bound in membranes, and are typically not "MR visible". In tumors, due to rapid membrane synthesis, PDE and PME are altered, thus ³¹P MRS spectra of tumors are typically characterized by elevated PME and PDE [50].

In neuroblastomas MR spectra showed substantially elevated PME/ β -NTP ratios compared with a spectrum from a normal control [51].

3.2 MIBG Scans

Since neuroblastoma cells express the norepinephrine transporter (NET) that facilitates uptake of the norepinephrine analog metaiodobenzylguanidine (MIBG), an aralkylguanidine analog of catecholamine precursors concentrated selectively within the secretory granules of catecholamine-producing cells in the sympathetic nervous tissue, MIBG scans used with radioactive iodine provide disease staging and response-to-treatment information. MIBG remains in both the cytoplasm and norepinephrine storage granules of the neuroblastoma cells. The normal physiological biodistribution of MIBG includes the liver, myocardium, salivary glands, intestines, kidneys and thyroid; mild uptake is seen in the adrenal glands. [52-55] The uptake mechanism into the adrenomedullary tissue is accomplished through the type I amine uptake mechanism. Uptake will be seen in 90% to 95% of patients with neuroblastoma at the site of the primary tumor and sites of metastases such as bone, bone marrow and lymph nodes. While metastases to the central nervous system are uncommon, when they do appear MIBG is not as accurate in their evaluation as ¹⁸F-fluorodeoxyglucose positron emission tomography (FDG-PET). This is true because tumors that are MIBG negative have very poorly differentiated cellularity i.e. exhibit de-differentiation and are unable to metabolize the MIBG. [56] When MIBG is used, ¹²³I MIBG is generally preferred to ¹³¹I MIBG due to its sensitivity that translates to higher image quality and fewer scans required with less risk of damage to the thyroid gland. [57-63] The size of the tumor may reflect whether the uptake is uniform or irregular with focal areas of reduced uptake indicating central necrosis versus de-differentiation as discussed above, which is characteristic of more malignant clones of neuroblastoma. Although it has not been confirmed, it is a commonly held belief that well differentiated tumors show a lower MIBG in vivo. Because the uptake of MIBG is directly dependent on catecholamine transporters, patients on medications such as TCAs, phenylpropanolamine, pseudoephedrine and labetalol will show reduced uptake because the mechanism of action of these drugs is to block catecholamine uptake. [64-68] If the MIBG scan is negative, an ¹⁸F-fluorodeoxyglucose positron emission (FDG-PET) scan should be done as should a technetium bone scan to evaluate a patient for cortical bone disease. [69-71]

In addition to use in staging, MIBG scans can also serve as a prognostic indicator and even as therapy. In terms of prognosis, a scoring system for the MIBG scan has been described by Suc and colleagues in which patients with MIBG scores lower than 4 have a higher probability of achieving complete response after induction therapy. Furthermore, Katzenstein and colleagues reported that MIBG scores >3 correlate to ultra high-risk patients who are more likely to relapse after therapy. A strong correlation exists between event-free survival and post-induction MIBG score. [72-77] In terms of therapy, ¹³¹I MIBG has been used in

the form of radiotherapy but its use has remained controversial because of the variation in response rates (ranging from 20% to 60%) and potential side effects. [78]

3.3 Bone Scintigraphy

Bone scintigraphy is another form of imaging that is used at the time of diagnosis with neuroblastoma for staging purposes. Total body radionuclide bone scintigraphy uses ^{99m}Tc -disphosphonate compounds to detect cortical skeletal metastases in a process that is more sensitive than conventional skeletal radiography. The metastatic pattern seen in bone in neuroblastoma patients is typically a symmetrical pattern in the metaphyseal pattern of long bones. Often it is difficult to ascertain metastases in metaphyseal regions of long bone due to frequently normal epiphyseal uptake on bone scintigraphy. On the blood pool phase of bone scintigraphy is the normal growth plate showing a well-defined linear appearance with clear demarcation between plate and metaphysis and symmetrical flaring and blurring of the growth plate with extension into the metaphysis that is suggestive of metastasis. Focal abnormalities commonly found in patients with neuroblastoma include the orbits, the parasagittal areas of the skull and multiple "hot" and "cold" lesions in the spine. Bone scintigraphy can also be used to differentiate cortical metastases from bone marrow metastases, an important distinction since cortical metastases carry a much worse prognosis. The usefulness of bone scintigraphy is more controversial for follow up of patients; conflicting evidence exists as to whether it adds any valuable information to findings on MIBG studies when assessing response to treatment. [79-83]

3.4 FDG-PET

While data on FDG-PET scanning as it relates to neuroblastoma is limited, Kushner et al. found that FDG-PET scanning correlates well with disease status and findings on MIBG scans, though the detection rate of abnormal areas is increased [67]. Even though today's consensus is still that MIBG is superior to FDG-PET, certain organs such as the liver, bone, and bone marrow are better visualized on FDG-PET as opposed to MIBG. [84,85] FDG reflects the increased glycolytic rate of tumor cells; uptake is therefore proportional to tumor cell burden and tumor cell proliferation. [86] In a study conducted by Shulkin, 16 out of 17 neuroblastoma patients exhibited FDG uptake by primary tumors and metastases which led to the conclusion that the majority of neuroblastomas were metabolically active and PET detectable. However FDG-PET is not useful for identifying metastases to the brain because the brain has increased (high normal) FDG uptake at baseline. FDG-PET has high utility in patients with a negative MIBG and in patients with lesions in the neck region where the resolution and co-registration of the FDG study with CT allows for better evaluation of the lesion and response to treatment. [87] 11-C-hydroxyephedrine (HED) is another agent that is useful with PET because it reaches a sensitivity of 99% for detecting lesions even with a shorter half-life. Overall, while false positives have been noted with PET scanning, it is useful to identify physiological variants. [85,88]

3.5 Somatostatin Receptor Scintigraphy

Another useful imaging modality is somatostatin receptor scintigraphy given that neuroblastoma cell lines and tumors express somatostatin receptors. A number of studies employing scintigraphy with ^{111}In -pentetreotide which is a radioactive form of the somatostatin analog octreotide showed that the sensitivity of such imaging is only 55% to 70% when compared to 83% to 94% for MIBG. This difference in sensitivities most likely has to do with the fact that only planar imaging is used in octreotide studies. A few studies using SPECT have shown mildly improved sensitivities. Patients with positive octreotide studies tend to have better outcomes because they also tend to have more favorable biological factors such as nonamplified MYCN, hyperdiploid and intact chromosomal 1p36. In addition, somatostatin receptors are often downregulated in more aggressive tumors and may even vary within a tumor. Therefore, given variability in these receptors among tumors, somatostatin scintigraphy is not routinely performed because it does not provide any more valuable information over MIBG that would alter patient management. [89-91]

3.6 Radiolabeled Antibodies

Radiolabeled antibodies, namely IgG1 monoclonal antibody and anti GD2 antibodies have shown higher sensitivities than even MRI and MIBG but are not readily available. These antibodies are able to bind to specific cell proteins that are expressed on both mature and immature tumor cells and are thereby useful in detecting neuroblastoma lesions. They are especially useful in detecting local tumor recurrences and skeletal metastases earlier than MIBG. Given their complexity, however, these antibodies cannot be routinely employed even though they provide valuable additional information. [92,93]

4.0 MANAGEMENT & THERAPIES FOR NEUROBLASTOMA PATIENTS

Before any decisions are made in terms of management, patients diagnosed with neuroblastoma need to be characterized as low-risk disease, intermediate-risk, or high-risk. Therapies are then tailored to patients based on this classification system; surgical resection, chemotherapy and/or radiotherapy are the available options. Typically, chemotherapy is limited to patients with regional or advanced stage disease, while radiotherapy is reserved for those who have advanced disease and unfavorable biologic characteristics. [3]

4.1 Low-risk Disease

The management of low-risk disease generally consists of surgery alone with a 4-year survival rate of >95% for those with stage I disease. Those patients with stage I disease who relapse after surgery are treated effectively with salvage chemotherapy. In those patients with biologically favorable disease that is not completely resectable (INSS stage 2A, and 2B), chemotherapy is generally not necessary. Many studies have shown that a reduction in chemotherapy is acceptable in these groups if in fact chemotherapy is used. [94] In patients with stage I, 2A, or 2B disease, chemotherapy is only used initially if the patient is experiencing life threatening or organ threatening symptoms i.e. hepatomegaly-causing obstruction, liver dysfunction, or respiratory insufficiency at the time of diagnosis. Infants with 4S disease without MYCN amplification receive supportive care since these tumors often undergo spontaneous regression. [95-101]

4.2 Intermediate-risk Disease

In intermediate-risk neuroblastoma, the main therapies consist of surgical resection and moderate-dose, multi-agent chemotherapy. For stage 3 and stage 4 disease, histologic and biological factors have a huge influence on prognosis. For patients with favorable tumor characteristics, the survival rate is 95% after surgical resection and moderate-dose chemotherapy (agents include cisplatin, doxorubicin, etoposide, and cyclophosphamide). In an effort to address short- and long-term toxicity from chemotherapy, cumulative exposure has been minimized and carboplatin has been substituted for cisplatin due to its improved side effect profile. The goal, if possible, is to eliminate the use of chemotherapy in these patients; many studies are evaluating if this may be an option by studying other molecular genetic variables and using them to identify those patients who require chemotherapy. [102-105]

4.3 High-risk Disease

The management of high-risk neuroblastoma includes induction chemotherapy, local control, consolidation, and treatment of minimal disease with biologic agents. Guidelines for this treatment have largely been established by the Pediatric Oncology Group and the Children's Cancer Group as they have conducted extensive research in this area. Although high-risk neuroblastoma is chemotherapy responsive, only 30% to 40% of patients survive long term. [3]

4.4 Chemotherapy

Overall, the general treatment regime for neuroblastoma includes chemotherapy, surgery, radiation therapy (radiotherapy) and immunotherapy. The first step in the treatment process is generally induction chemotherapy consisting of a combination of anthracyclines, alkylators, platinum compounds and topoisomerase II inhibitors. A direct correlation exists between achieving complete tumor response after

induction therapy and survival. [106] Also, increasing chemotherapy doses may improve initial tumor response rate. [107] In an attempt to achieve maximal tumor reduction before surgical resection is attempted, induction chemotherapy is often a necessary process before surgery. To have any chance at cure, resection of the primary tumor and bulky metastases is necessary. Delayed surgical resection, generally after three rounds of induction chemotherapy, may improve outcome in the resection of the primary tumor and overall survival; administering induction chemotherapy can also help minimize acute complications of surgical resection. [108] In some cases, surgery may be more appropriate at diagnosis rather than after induction chemotherapy.

4.5 Radiation Therapy

With neuroblastoma being one of the most radiosensitive tumors of childhood [5], radiation can be used to enhance the effect of chemotherapy at metastatic sites in some patients [109,110]. In high risk disease, radiotherapy is also used with surgical resection to achieve maximal local control. Shown to reduce local recurrence after surgery regardless of response to induction chemotherapy, application of external beam radiotherapy in doses of 2160 cGy daily in 180 cGy fractions can be applied to the primary tumor site. To prevent relapse, radiotherapy can also be applied to other sites at risk for relapse. If disease advances despite all treatment efforts so far discussed, radiation therapy may also be used for palliative purposes to treat cancer symptoms such as pain and discomfort due to tumor burden. In one retrospective study that evaluated the use of palliative radiotherapy at the Institut Curie in Paris, France, a response rate of 82.4 % was noted with response being defined as more than 25% reduction in tumor mass or any reduction in pain. Also determined was that increasing the radiation dosage to greater than 15 Gy increases the response rate to 100%, suggesting a dose-response relationship. However, actual survival for these patients remained dismal, ranging only from 27 to 43 days [111]

4.6 Myeloablative Consolidation Therapy

A promising treatment for high-risk neuroblastoma, according to a number of studies, is myeloablative consolidation therapy. The CCG-3891 study, for example, demonstrated that myeloablative therapy with purged bone marrow transplantation improved outcomes in this patient population. [106] Other trials in Germany and Europe also showed improved outcomes after myeloablative therapy compared with maintenance chemotherapy [112] or observation alone [113]. Thus, neuroblastoma seems to be one of the few human cancers in which relapse rates are reduced by myeloablative consolidation in first remission. This suggests that with further intensification of consolidation therapy, outcomes may improve. An ongoing randomized trial comparing single versus tandem transplant consolidation for high-risk neuroblastoma is being conducted to further investigate the work of George and colleagues who reported a 3-year, event-free survival of 55% after rapid sequential tandem transplant consolidation therapy [114].

Standard of care for minimal residual disease is post-remission induction maintenance therapy employing a type of synthetic retinoid, such as isotretinoin (cis-RA); it is effective yet non-cytotoxic.

4.7 Immunotherapy

Immunotherapy is an emerging promising therapy in the face of significant limitations of other current options in terms of effectiveness in advanced disease and side effect profile. While still in the experimental stage, immunotherapy is used for minimal disease, after patients have undergone chemotherapy and tumors have been surgically removed. In this type of therapy, monoclonal antibodies directed against neuroblastoma-specific antigens i.e. gangliosidase, GD2 will kill residual neuroblastoma cells via antibody-dependent cellular cytotoxicity. A number of preclinical and clinical trials have also suggested that lymphocyte-, neutrophil- or natural killer cell-mediated antibody-dependent cellular cytotoxicity can be enhanced by co-administration of the cytokines granulocyte/macrophage colony-stimulating factor and interleukin-2. [115,116] 3F8 is one particular IgG3 murine monoclonal antibody directed against the ganglioside GD2 that has shown promise in a number of studies. Essentially, immunotherapy is designed to train the body's own immune system to detect and destroy neuroblastoma

cells that have survived chemotherapy and/or radiation therapy by marking them with these antibodies; it has shown excellent targeting in neuroblastoma. Logistically, the treatment involves the injection of monoclonal antibody 3F8 into the bloodstream along with a protein called GM-CSF (granulocyte-macrophage colony stimulating factor) that stimulates myeloid white cells (neutrophils and macrophages) to make use of the antibodies via a cytotoxic killing mechanism. GM-CSF also plays a major role in mediating the common side effect of severe myelosuppression. [117]

Advancing immunotherapy continues to be investigated in a number of studies and shows considerable promise. In one phase II study conducted through Memorial Sloan-Kettering Cancer Center, 3F8 was administered to 16 patients with stage 4 neuroblastoma with response seen in 2/7 patients with bony lesions and 3/8 patients with bone marrow lesions. Three patients were reported to be long-term survivors (70-130+ months) after treatment without any further systemic therapy and no delayed neurological problems. This suggests that 3F8 has significant treatment potential. [118] Another study determined that long-term remission can be achieved without autologous bone marrow transplant (aBMT) with 3F8 treatment in patients with minimal residual disease stage 4 neuroblastoma diagnosed at more than 1 year of age. In this study, 14 out of 29 patients survived and 13 out of the 14 remained progression free; the side effect profile was noted to be tolerable. It was also determined that if human anti-mouse antibody (HAMA) forms as a result of 3F8 treatment, the lysis of neuroblastoma is neutralized. [119] A study was conducted in which the use of high-dose cyclophosphamide was studied to investigate its effect on inhibiting the humoral immune response to 3F8 that produces HAMA. The study concluded that high-dose cyclophosphamide did in fact block the humoral response to the murine antibody, indicating that high-dose cyclophosphamide does have the potential to prevent host rejection of foreign or not fully humanized proteins to improve the efficacy of 3F8 treatments. [120] Another study investigated the use of radioimmunotherapy in neuroblastoma patients using ¹³¹I-3F8. Overall survival at 18 months post-treatment was found to be 40% and the antibody was seen in 42 patients to be localized in the primary tumor and metastatic sites which included lymph nodes, bone marrow and bone. [121]

4.8 Anti-Adhesion

As discussed above, the standard of care for treatment of metastatic neuroblastoma includes chemotherapy and radiotherapy, both of which have a number of serious side effects. Chemotherapy often causes leucopenia, resulting in increased susceptibility to infection, thrombocytopenia, nausea, vomiting and anemia; radiation treatment can damage vital organs wrapped with neuroblastoma and results in serious long-term side effects particularly in children. Treatment options, therefore, can quickly lack effectiveness once patients develop drug or radiation resistance. This affects particularly high-risk patients and leads to decreased survival rates. "Anti-adhesion" is another promising area of study in novel therapy development which addresses this concept as cell adhesion has been found to mediate development of drug and radiation resistance.

In human cancer, all five classes of cell adhesion molecules (CAM): integrins, cadherins, immunoglobulin-like CAMs, selectins and CD44s are dysregulated. Recently, a number of cell adhesion-mediated survival pathways have been identified with key mediators being LFA-1, VLA-4, FAK, ILK, Src, PI3K, Akt, Ras, MEK, Erk, HMG-CoA reductase, Rho, Rho Kinase, PKC and NF-κB. Thus, a drug could intervene by attenuating the over-activation of those of these key mediators that up-regulate the formation of cell adhesion molecules. Such therapies may reduce the required drug and radiation doses for dose-sensitive pediatric patients, thereby decreasing the amount and severity of side effects of treatments. Anti-adhesion strategies include targeting surface antigens and inhibiting cell adhesion-associated pathways through drug delivery to inhibit CAM-DR. Today, the preclinical and clinical knowledge about the role of cell adhesion molecules greatly exceeds the number of related drugs developed. [122]

Today's anti-adhesion efforts are focusing on NF-κB, a particular transcription factor that enhances the production of inflammatory mediators and serves as a key mediator in a number of signaling pathways involving cell adhesion. Chemotherapy and radiotherapy both appear to induce NF-κB over-expression in tumors and through this mechanism reduce tumor drug and radiation sensitivity. [123] Thus, there is also some suggestion that these aggressive conventional therapies can be avoided in certain cases based on

inherent genetic and molecular properties of the tumor as they may not provide significant benefit. [124] From a molecular point of view, NF- κ B is a heterodimer containing a p50 and a p65 subunit that is kept from translocating to the nucleus by binding to its inhibitor I κ B α . Any of a number of diverse stimuli can activate the pathway to result in phosphorylation of I κ B α , ubiquitination of I κ B α and then degradation of I κ B α in 26S chromosome, leading to NF- κ B translocation to the nucleus. In the nucleus, NF- κ B activates the transcription of many genes involved in tumorigenesis, apoptosis, and inflammation. [125-127].

The elucidation of this theory has led to immense amounts of research. In one such study, curcumin, an agent documented to have anti-tumor effects (suppression of cellular transformation, proliferation, invasion, angiogenesis and metastasis) against many types of cancer was tested on human neuroblastoma cells and was found to induce apoptosis of 3 neuroblastoma lines (Lan-5, SK-N-SH and Kelly) through an increased induction of apoptosis by inhibition of NF- κ B. [24] Furthermore, Hewson and colleagues conducted a study that suggested that fenretinide-induced reactive oxygen species may be responsible for the induction of apoptosis in SH-SY5Y cells via inhibition of the NF- κ B pathway. This finding elucidates information that will help focus future development of drugs for neuroblastoma therapy. [8]

5.0 CONCLUSION

Neuroblastoma is a leading cause of cancer-related mortality in very young children due to difficulty in treating it effectively with current standard treatment modalities. Initial presentation often at an advanced stage and marked variability in tumor characteristics make neuroblastoma particularly difficult to treat. As a result, we have identified a dire need to further develop and investigate promising new therapies to treat neuroblastoma. Particularly of interest are the concepts of immunotherapy and anti-adhesion. Anti-adhesion data suggests that attenuation of NF- κ B activity may decrease development of tumor resistance to chemotherapy and radiation. Therefore, a significant unmet medical need for novel modulators of NF- κ B activity as potential direct or assisted therapeutic agents for tumors such as neuroblastoma exists. For this reason, it is imperative that anti-adhesion drugs be a strong focus in drug development for neuroblastoma.

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