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# Current management of neuroblastoma and future direction

Elizabeth R. Pastor, Shaker A. Mousa*

*The Pharmaceutical Research Institute, Albany College of Pharmacy and Health Sciences, Rensselaer, NY, USA*

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ABSTRACT

Neuroblastoma is the most common solid extracranial tumor in pediatrics and can regress spontaneously or grow and metastasize with resistance to multiple therapeutic approaches. The prognosis and approach to treatment depends on the tumor presentation and whether it expresses certain drivers such as *MYCN*, ALK, and TrkB. Expression or mutation of these genes and kinases correlates with high-risk and poor prognosis. Multiple therapeutic approaches are being used to target *MYCN*, ALK, and TrkB, as well as GD2, a surface antigen present on the surface of neuroblastoma tumor cells. This review discusses the nature of these targets and several current therapies for neuroblastoma. A focus is placed on recent therapeutic developments including targeted delivery of chemotherapy, novel radiation therapy, and immunotherapy.

## 1. Introduction

Neuroblastoma is a solid cancer that arises from neural crest cells (Maris et al., 2007), which mature and develop into other cell types such as melanocytes, cranial neurons and glia, cartilage, bone, connective tissue as well as peripheral sympathetic neurons and Schwann cells (Kholodenko et al., 2018). It is a cancer of the peripheral sympathetic nervous system that is typically found within the adrenal medulla. It can also be found in the sympathetic nerve ganglia, which are located along the sympathetic nervous system in the neck, chest, abdomen, and pelvis (Maris et al., 2007). Neuroblastoma primarily affects children and is the most common extracranial solid tumor in this population (Kholodenko et al., 2018). About 1.1 in 100,000 children aged 0–14 in the United States are diagnosed annually with neuroblastoma (American Cancer Society, 2018). Additionally, neuroblastoma represents approximately 6% of all childhood cancers in the United States (Siegel et al., 2018).

Neuroblastoma is marked by its heterogeneity (Kholodenko et al., 2018). Tumors may spontaneously regress without treatment, or they can develop and metastasize, with resistance to therapies (Kholodenko et al., 2018). Patients present with high genetic variability in neuroblastoma tumors, which contributes to conflict in developing novel therapies. Treatment of neuroblastoma is typically a patient-specific or tailored approach. Consequently, there is a large unmet medical need for these patients.

## 2. Staging and prognosis

Staging for neuroblastoma provides the basis for determining in which risk group a patient identifies and what type of therapy they should receive (Monclair et al., 2009; Cohn et al., 2009). Patients in lower risk groups tend to have a better prognosis and require less intensive treatments. Conversely, patients identifying with high-risk neuroblastoma (HR-NB) have poor prognosis, and therapies for these patients are often highly aggressive and provide minimal benefit in terms of survival rates (Kholodenko et al., 2018; Davidoff, 2012). The International Neuroblastoma Risk Group (INRG) developed the following staging system (INRGSS), located in Table 1, to assess risk in patients prior to treatment initiation (Monclair et al., 2009).

Pretreatment risk stratification, used to assess 5-year event-free survival rates (EFS), is determined based on a patient's INRG stage, age in months, tumor histology, grade of tumor differentiation, as well as whether they present with *MYCN* amplification, 11q aberration, and their DNA ploidy (Cohn et al., 2009). Patients presenting with very low risk are estimated to have a 5-year EFS of greater than 85%, low-risk $> 75\%$ to $\leq 85\%$, intermediate risk $\geq 50\%$ to $\leq 75\%$, and high-risk $< 50\%$. Ultra-high risk (UHR) neuroblastoma, a new term, still lacks a universal definition. Some authors use it to refer to refractory disease (less than complete or very good partial response after induction chemotherapy), others use "death from disease within 18 months of diagnosis," or patients with 5-year EFS of 10%–15% (Morgenstern et al., 2019).

Many genomic studies have demonstrated that single-nucleotide

* Corresponding author at: The Pharmaceutical Research Institute, Albany College of Pharmacy and Health Sciences, 1 Discovery Drive, Rensselaer, NY 12144, USA.

*E-mail address:* Shaker.mousa@acphs.edu (S.A. Mousa).

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**Table 1**
International Neuroblastoma Risk Group (INRG) staging.

| Stage | Description |
|---|---|
| L1 | Tumors localized to one region of the body with absence of image-defined risk factors (IDRF) |
| L2 | Tumors localized to one region of the body with presence of at least one of 20 IDRFs |
| M | Tumors with metastases, with the exception of stage MS |
| MS | Tumors in children under 18 months with metastases limited to skin, liver, and/or bone marrow |

polymorphisms (SNPs) in DUSP12 and HSD17B12 locus at chromosome 5q11.2 are associated with low-risk neuroblastoma, whereas SNPs within or upstream of CASC15 and CASC14 on chromosome 6p22, BARD1, LMO1, HACE1, and LIN28B as well as a common copy-number variation at 1q21 within NBPF23 have been associated with high-risk disease (Shang et al., 2009; Maris, 2010; Faisal et al., 2011; Capasso et al., 2013; Pinto et al., 2015). Furthermore, African genomic ancestry has also been significantly associated with high-risk neuroblastoma, supporting a genetic etiology for the racial disparities in survival observed in neuroblastoma (Pinto et al., 2015). Additional sequencing studies are needed to discover the new risk variants and develop a deeper understanding of the genetic etiology of neuroblastoma.

Therefore, especially in the high-risk patient population, it is necessary to detect and examine the genetic components of tumors to determine treatment strategy. This review will focus on current diagnostic and treatment strategies as well as recent therapeutic developments for patients with HR-NB.

## 3. Detection and therapeutic targets

Neuroblastoma can occur anywhere in the sympathetic nervous system, and therefore signs and symptoms upon presentation are variable (Maris et al., 2007). Patients with localized tumors tend to have few signs or symptoms. Conversely, patients presenting with metastatic disease may appear more ill and display distinctive signs of periorbital ecchymosis or “racoon eyes” with or without proptosis (Maris et al., 2007). Screening spot urine samples of infants can detect tumor-derived catecholamine, but this approach is not associated with reduced mortality and therefore is not widely used (Maris et al., 2007).

### 3.1. Detection and imaging

#### 3.1.1. Norepinephrine receptors

The primary tumor is imaged by computed tomography (CT) and/or magnetic resonance imaging (MRI) to determine image defined risk factors (IDRFs) and aid in staging (Monclair et al., 2009). Metastatic sites are also imaged as a marker of response to therapy. Scintigraphy with Iodine-123 metaiodobenzylguanidine (MIBG) also aids in diagnosis and imaging of metastases that may otherwise be unidentifiable on imaging scans alone. MIBG is transported from the extracellular milieu into the cytosol via the norepinephrine receptors, which are expressed on 90% of neuroblastoma tumor cells (Yanik et al., 2015; Matthay et al., 2012). In addition to imaging, MIBG may be used to treat neuroblastoma by targeting tumors that express the norepinephrine receptor.

#### 3.1.2. Somatostatin receptors

Somatostatin receptors are often expressed on neuroendocrine tumors. In addition to MIBG, tumors and metastases may be imaged by targeting somatostatin receptors with $^{68}$Ga-DOTATATE (Zhang et al., 2017), which targets these receptors and can be useful for imaging tumors that do not express norepinephrine receptors (Zhang et al., 2017; Georgantzi et al., 2011). Patients who present with high uptake of $^{68}$Ga-DOTATATE on positron emission tomography (PET) or CT scans may be candidates for treatment targeting these receptors. Additionally, somatostatin receptor-targeted therapy may be useful in patients who are refractory to treatment with MIBG.

### 3.2. Therapeutic targets

#### 3.2.1. GD2

Disialoganglioside (GD2) is a surface antigen found on cell membranes (Sait and Modak, 2017). Gangliosides are primarily involved in cellular adhesion and communication as well as in differentiation (Kholodenko et al., 2018). Their expression varies greatly when cells undergo malignant transformation, indicating that gangliosides may be a marker of tumorigenicity. Shedding and circulation of GD2 is also associated with poor survival rates in patients with neuroblastoma (Ladisch et al., 1987). In infants, GD2 is expressed in the central nervous system, peripheral neurons, and melanocytes (Kholodenko et al., 2018). Additionally, neuroblastoma cells of all stages express high levels of GD2 (Sait and Modak, 2017). Hence, GD2 is an attractive target for immunotherapeutic approaches to neuroblastoma.

#### 3.2.2. MYCN

*MYCN* is an oncogene whose amplification places patients in higher risk categories and translates to poor prognosis. Amplification of *MYCN* occurs in approximately 20% of primary neuroblastoma tumors (Schnepp and Maris, 2013). Because *MYCN* is only expressed in maturing neural crest cells, it has been the subject of many attempts at targeted therapy (Barone et al., 2013). Though there is a known connection between *MYCN* amplification and the severity of neuroblastoma, there are complications in directly targeting *MYCN*, namely the absence of binding pockets in MYC proteins where small molecule inhibitors would attach (Johnsen et al., 2018; Prochownik and Vogt, 2010). There is, however, potential for downstream targeting of *MYCN* through bromodomain and extraterminal (BET) inhibition, ornithine decarboxylase inhibition, and Aurora A kinase inhibition (Johnsen et al., 2018).

BET is a family of proteins consisting of BRD2, BRD3, BRD4, and BRDT (Barone et al., 2013). They are responsible for binding to acetylated histones wherein they recruit the transcription of many genes such as *MYCN* (Johnsen et al., 2018). Inhibition of *MYCN* through BET inhibition via small molecule inhibitors has been successful in prior studies (Henssen et al., 2016; Mertz et al., 2011; Puissant et al., 2013; Lee et al., 2015). Specific outcomes of these studies are discussed later in this article.

MYC proteins require polyamines for functional support, and it is proposed that targeting Ornithine decarboxylase (ODC) can produce a downstream inhibitory effect on *MYCN* (Bassiri et al., 2015). ODC is the rate-limiting enzyme involved in the synthesis of polyamines (Pinto et al., 2015; Johnsen et al., 2018). Additionally, ODC activity is increased in cells that are actively dividing, as in solid tumors, supporting the inhibition of ODC as a potential treatment strategy (LoGiudice et al., 2018).

Aurora A Kinase (AURKA) is a serine/threonine kinase that is essential for stabilization of *MYCN* and protection from degradation by proteasomes (Pinto et al., 2015; Johnsen et al., 2018). Neuroblastoma cells that express high levels of *MYCN* also tend to express increased AURKA (Brockmann et al., 2013). Additionally, AURKA is overexpressed in a number of other cancers and demonstrates oncogenic behavior (Shang et al., 2009). Small molecule inhibitors have been used

to target AURKA in an attempt of indirect targeting of *MYCN* (Faisal et al., 2011; Johnsen et al., 2018).

#### 3.2.3. ALK

Anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase that serves as an oncogene in many different cancers. Its presence was initially noted in anaplastic large cell lymphoma (Mosse, 2016). It is also found in a small percentage of non-small cell lung cancer and other childhood cancers including neuroblastoma (Matthay et al., 2012; Mosse, 2016). Within all stages of neuroblastoma, ALK mutations were found in 8% of patients, with mutations and amplifications found in 14% of patients with HR-NB (Bresler et al., 2014). Mutations and amplifications of ALK in these patients represents poor prognosis. ALK is only expressed in developing tissue of the nervous system, allowing it to be a target for small molecule inhibitors or immunotherapy (Mosse, 2016). There is, however, potential for resistance to targeted ALK therapy via mutations and alternative signaling pathways (Matthay et al., 2012).

#### 3.2.4. Trk

Tropomyosin receptor kinase (Trk) is a family of neurotrophin receptors that are essential in peripheral nervous system development (Brodeur et al., 2009). TrkA and its ligand nerve growth factor (NGF) are associated with more favorable outcomes such as spontaneous tumor regression or differentiation (Iyer et al., 2016). TrkB and its ligand brain-derived neurotrophic factor (BDNF) are associated with high-risk tumors and progression with unfavorable outcomes (Iyer et al., 2016; Croucher et al., 2015). When bound to BDNF, TrkB tumors have increased survival, drug resistance, and angiogenesis (Brodeur et al., 2009). Approximately 36% of patients with neuroblastoma present with overexpression of TrkB and BDNF (Brodeur et al., 2014). Therefore, targeting TrkB with small molecule inhibitors remains a plausible approach to treatment.

## 4. Current treatment options

Depending on a patient's risk category and prognosis, surgery may be performed to remove some or all of the primary tumor (Children's Oncology Group, 2019). Typically, in low-risk patients, tumors may be monitored for spontaneous differentiation or regression and chemotherapy or radiation may not be needed in these patients. In patients with intermediate or high risk, however, chemotherapy may be given to shrink the tumor prior to surgery. High-risk patients, in addition to chemotherapy and radiation therapy, may undergo stem cell transplant and immunotherapy. Further treatment of high-risk patients with the differentiating agent 13-cis-retinoic acid (isotretinoin) may be given to improve EFS (Matthay et al., 1999).

### 4.1. Radiation therapy

An Iodine-MIBG compound, Iodine-131 MIBG (Azedra; Progenics Pharmaceuticals) can be used in the treatment of neuroblastoma. As previously mentioned, MIBG targets norepinephrine receptors, which are expressed on 90% of neuroblastoma tumors (Matthay et al., 2012). Additionally, MIBG can target tumors regardless of *MYCN* or ALK amplification, or tumor histology (Yanik et al., 2015). Iodine-131 MIBG is typically used in addition to chemotherapy in patients with HR-NB. There is potential for resistance to $^{131}$I-MIBG and a need to target both resistant tumors and those that do not express the norepinephrine transporter.

Targeted radiotherapy of neuroblastoma using radiolabeled DOTATATE has great potential in somatostatin receptor (SSTR)-positive neuroblastoma that does not express the norepinephrine transporter (NET) or develop resistance to MIBG. However, resistance to targeted radiotherapy arises from the heterogeneous expression of SSTR and NET. Therefore, it has been hypothesized that a cocktail of MIBG and DOTATATE could improve treatment efficacy compared to single radiopharmaceutical treatment (Tesson et al., 2017). Lutathera ($^{177}$Lu-DOTATATE; Advanced Accelerator Applications) was approved by the FDA in January 2018. This radiopharmaceutical targets somatostatin receptors that are highly expressed in neuroblastoma (Georgantzi et al., 2011). In a phase 3 trial, $^{177}$Lu-DOTATATE showed marked improvements in EFS in patients with midgut neuroendocrine tumors when compared with a long-acting repeatable octreotide (Strosberg et al., 2017). For patients enrolled in this study, an estimated EFS rate at 20 months post-therapy in the treatment group was 65.2% compared with 10.8% in the control group (Strosberg et al., 2017). Effectiveness of $^{177}$Lu-DOTATATE was also found to be increased when used with radiosensitizing agents such as nutlin-3 and topotecan (Tesson et al., 2018). Patients who may be eligible for $^{177}$Lu-DOTATATE therapy are selected based on positive $^{68}$Ga-DOTATATE PET or CT scans, which identify tumors expressing somatostatin receptors (Gains et al., 2011).

### 4.2. Immunotherapy

Dinutuximab (Unituxin, ch14.18) is a chimeric monoclonal antibody composed of murine variable regions of IgG3 and human constant regions of IgG1 (Matthay et al., 2012; Sait and Modak, 2017). Dinutuximab was approved by the FDA in 2015 as anti-GD2 immunotherapy used to treat patients with HR-NB (Kholodenko et al., 2018). In these patients, a 20% increase in 2-year EFS was noted when dinutuximab was used in combination therapy (Yu et al., 2010). Combination therapy consists of dinutuximab, granulocyte-macrophage colony stimulating factor (GM-CSF), interleukin-2 (IL-2), and isotretinoin (Sait and Modak, 2017; Yu et al., 2010). This therapy is approved for use in patients who have undergone and responded to induction therapy and autologous stem cell transplant (ASCT). Prior to clinical uses of immunotherapy, standard of care treatment included myeloablative therapy with autologous stem cell transplant and isotretinoin, after which a majority of patients relapsed (Yu et al., 2010). Adding anti-GD2 with GM-CSF and IL-2 provides an effective treatment for residual disease (Yu et al., 2010).

Side effects of dinutuximab therapy include neuropathic pain, fever, hypotension, allergic reactions, and capillary leak syndrome that may be attributable to IL-2 (Ozkaynak et al., 2018). There is some suggestion that neuropathic pain may be related to activation of complement (Sait and Modak, 2017; Navid et al., 2014). Anti-GD2 monoclonal antibodies utilize both antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) to exert their effects (Sait and Modak, 2017).

## 5. Recent treatment developments

### 5.1. Etoposide drug delivery system

Drug delivery systems have long been pursued as a way to deliver cytotoxic chemotherapy directly to a tumor or other site of action, while potentially decreasing systemic side effects (Wolinsky et al., 2012). Yavuz et al. used silk wafers loaded with etoposide to target neuroblastoma tumors (Yavuz et al., 2018). Coburn et al. also used silk fibers and gels loaded with vincristine and/or doxorubicin to target these tumors (Coburn et al., 2017). Both studies were able to achieve higher drug concentrations in the tumors and limit tumor growth (Yavuz et al., 2018; Coburn et al., 2017).

### 5.2. SAHA (Vorinostat)/MG132

Wu et al. found that co-administration of a histone deacetylase inhibitor, suberoylanilide hydroxamic acid (SAHA or Vorinostat) with the proteasome inhibitor MG132 produced synergistically suppressive effects on the human neuroblastoma cell line SH-SY5Y (Wu et al., 2018).

The combination induced apoptosis through up-regulation of inhibitor of growth 5 (ING5). This may provide a new chemotherapeutic regimen that can potentially act on neuroblastoma cells. Vorinostat is currently FDA approved for treatment of cutaneous T-cell lymphoma. It is also undergoing two phase 2 trials to assess its use in combination with 131I-MIBG (NCT02035137) and for use with standard immunotherapy with or without difluoromethylornithine (DFMO) (NCT02559778) specifically in patients with neuroblastoma.

### 5.3. Gene/Kinase targeting

#### 5.3.1. MYCN targeting through BET inhibition

JQ1 is a small molecule competitive inhibitor that targets BRD4 (Puissant et al., 2013). It acts by interrupting transcription of *MYCN*, leading to its antitumoral effects. Lee et al. demonstrated in vitro that treatment with JQ1 suppressed N-Myc protein levels and decreased proliferation in *MYCN* amplified neuroblastoma cell lines (Lee et al., 2015). Additionally, JQ1 treatment assisted in differentiation of neuroblastoma cells both in vitro and in vivo in mouse xenograft models.

OTX015 (Birabresib) is a synthetic small molecule inhibitor that prevents binding of BRD2, BRD3, and BRD4 to acetylated histones (Henssen et al., 2016). Similar to JQ1, the therapeutic efficacy of OTX015 is related to its targeting of BRD4 (Henssen et al., 2016). Hessen et al. demonstrated that neuroblastoma cell lines that exhibited *MYCN* amplification were more sensitive to treatment with OTX015 (Henssen et al., 2016). In these cell lines, treatment with OTX015 showed significant reductions in cell viability. Additionally, in mouse xenograft models OTX015 inhibited tumor growth and significantly prolong survival (Henssen et al., 2016). The results of these preclinical studies suggest that exploration of BET inhibition could be pursued in clinical trials.

#### 5.3.2. MYCN targeting through ornithine decarboxylase inhibition

Difluoromethylornithine (DFMO, eflornithine) works by inhibiting ODC irreversibly. Eflornithine is currently approved for treatment of African sleeping sickness (trypanosomiasis), as well as hirsutism (Bassiri et al., 2015; LoGiudice et al., 2018). When targeting ODC in TH-MYCN mice, Hogarty et al. found that tumor progression could be slowed or prevented, and synergistic effects were observed with cytotoxic chemotherapy agents (Hogarty et al., 2008). It was demonstrated that ODC also had high correlation with *MYCN* amplification. When inhibiting ODC in both *MYCN* amplified and non-amplified cells, tumor growth was inhibited (Hogarty et al., 2008). DFMO was evaluated in a Phase I trial in patients with relapsed or refractory neuroblastoma and was deemed to be safe at doses up to 1500 mg/m2 twice daily (Saulnier Sholler et al., 2015). This same trial described a lack of dose-limiting toxicities related to DFMO (Saulnier Sholler et al., 2015). However, more studies are needed to determine efficacy of DFMO in patients with neuroblastoma.

#### 5.3.3. MYCN targeting through aurora A kinase inhibition

Alisertib (MLN8237) is a small molecule inhibitor of Aurora A kinase. It works competitively with ATP binding to AURKA (Mosse et al., 2012). Alisertib was shown to have antitumor effects in preclinical trials, however it has not shown much objective efficacy (zero of 11 patients) when used as a monotherapy agent in Phase I clinical trials (Mosse et al., 2012). It did show a better objective antitumor response (31.8%) when used in combination therapy with irinotecan and temozolomide in a Phase I trial by (DuBois et al. (2016)).

Another approach to target Aurora A kinase is by using alisertib combined with a BET (BRD4) inhibitor, I-BET151 (Felgenhauer et al., 2018). Felgenhauer et al. demonstrated synergy of these compounds in vitro. Additionally, in mouse xenograft models, the combination extended survival and induced regression in *MYCN*-amplified tumors (Felgenhauer et al., 2018). Using combination therapies as downstream inhibitors of *MYCN* may provide a novel strategy for treating *MYCN*-amplified neuroblastoma.

#### 5.3.4. ALK inhibitors

Crizotinib is a small molecule inhibitor of ALK / MET (mesenchymal-epithelial transition) / ROS1 (a receptor tyrosine kinase encoded by the *ROS1* gene). It was approved by the FDA in 2011 for ALK-positive non-small cell lung cancer (NSCLC) (Mosse, 2016). While crizotinib has demonstrated efficacy in ALK-positive NSCLC and some neuroblastomas, certain mutations to ALK in neuroblastoma, specifically F1174L-mutated ALK, contribute to resistance (Brodeur et al., 2014). Resistance to crizotinib and other ALK inhibitors will likely develop, either through mutations within the ALK kinase domain or by up-regulation of an alternative signaling pathway. This intrinsic resistance has been the target of several newer ALK inhibitors.

Lorlatinib is a small molecule ALK/ROS1 inhibitor. In preclinical studies, lorlatinib demonstrated potency surpassing that of crizotinib (Infarinato et al., 2016). Additionally, in mouse xenograft models containing F1174 L, R1275Q, and F1245C mutations, known to convey resistance to crizotinib, lorlatinib demonstrated strong antitumor effects (Infarinato et al., 2016). The results of preclinical studies of lorlatinib suggest that it has potential in crizotinib-resistant ALK-driven neuroblastoma and may be effective as a single agent treatment (Infarinato et al., 2016; Guan et al., 2016). The results of clinical trials of lorlatinib in NSCLC have been promising; however, there is a lack of clinical testing of lorlatinib in patients with neuroblastoma. A summary of other ALK inhibitors is provided in Table 2.

#### 5.3.5. Trk inhibitors

Entrectinib is a selective inhibitor of Trk, ALK, and ROS1. Iyer et al. examined the potential efficacy of entrectinib as a TrkB inhibitor (Iyer et al., 2016). Entrectinib was compared with vehicle in vitro and entrectinib was compared with irinotecan, temozolomide, and a combination of all three agents in vivo using mouse xenograft models (Iyer et al., 2016). Entrectinib inhibited autophosphorylation of TrkB and cell growth in vitro. Additionally, entrectinib inhibited tumor growth significantly in xenograft models, thereby improving EFS. This inhibition was further enhanced when used with irinotecan and temozolomide in combination therapy (Iyer et al., 2016). Entrectinib displayed

**Table 2**
Summary of current anaplastic lymphoma kinase (ALK) inhibitors.

| Drug Name | Code Name | Target | Status (Company) |
|---|---|---|---|
| Crizotinib | PF-02341066 | ALK/MET/ROS1 | Marketed (Pfizer) |
| Ceritinib | LDK378 | ALK/IGF-1 | Marketed (Novartis) |
| Lorlatinib | PF-06463922 | ALK/ROS1 | Pending Approval (Pfizer) |
| Brigatinib | AP26113 | ALK | Marketed (ARIAD) |
| Alectinib | CH5424802 | ALK | Marketed (Chugai) |
| Ensartinib | X-396 | ALK | Phase I/II (NCT02898116) (Betta Pharmaceuticals/Xcovery) |
| – | AZD3463 | ALK/IGF1R | Preclinical Wang et al. (2016) (AstraZeneca) |
| – | ASP3026 | ALK | Phase I (NCT01284192) (Astellas Pharma) |

MET, mesenchymal-epithelial transition; ROS1, receptor tyrosine kinase encoded by the *ROS1* gene; IGF-1, insulin-like growth factor 1; IGF1R, IGF-1 receptor.

**Table 3**
Summary of current clinical trials using CAR T cells in neuroblastoma.

| ClinicalTrials.gov Identifier | Summary | Phase | Status |
|---|---|---|---|
| NCT02919046 | Safety/efficacy of CAR T cells in relapsed/refractory neuroblastoma | – | Recruiting |
| NCT03373097 | Safety/efficacy of GD2-CART01 in high-risk neuroblastoma and/or relapsed/refractory neuroblastoma | I/II | Recruiting |
| NCT03635632 | Safety of GD2-C7R T cells and their effect on relapsed/refractory neuroblastoma | I | Not yet recruiting |
| NCT02761915 | Safety of 1RG-CART in relapsed/refractory neuroblastoma | I | Recruiting |
| NCT02765243 | Efficacy of Anti-GD2 4th generation CAR T cells in relapsed/refractory neuroblastoma | II | Recruiting |
| NCT02311621 | Maximum tolerated dose of CD171 CAR T cells in relapsed/refractory neuroblastoma | I | Recruiting |
| NCT02107963 | Safety of anti-GD2-CAR T cells in patients with solid tumors | I | Completed |
| NCT01822652 | Safety of iC9-GD2 T cells in relapsed/refractory neuroblastoma | I | Active, not recruiting |

promising results in several phase I clinical trials (Drilon et al., 2017) and is currently undergoing more phase I and II clinical trials specifically targeting solid tumors including neuroblastoma and neuroendocrine tumors (NCT02568267, NCT02650401).

### 5.4. Immunotherapy

Hu14.18K322A is a humanized version of ch14.18 (dinutuximab) with a point mutation at lysine 322 to alanine, which decreases its ability to bind complement proteins, thereby decreasing CDC (Matthay et al., 2012). Hu14.18K322A was developed to decrease complement activation, which may be the cause of neuropathic pain associated with dinutuximab (Matthay et al., 2012; Federico et al., 2017). Additionally, by using a humanized monoclonal antibody, hypersensitivity reactions during infusions can be potentially limited (Navid et al., 2014). A phase 2 clinical trial, sponsored by St. Jude Children's Research Hospital, is currently underway to investigate the use of this antibody when given with induction chemotherapy (NCT01857934). This trial currently has an estimated enrollment of 210 participants up to 19 years of age and is estimated to be completed by July 2021. Patients are eligible for inclusion if they present with newly diagnosed, advanced-stage HR-NB, have histologic proof of neuroblastoma or test positive for bone marrow tumor cells with increased urine catecholamine, have adequate renal and hepatic function, have not had prior treatment, and have provided written, informed consent.

In addition to monoclonal antibodies, there are ongoing studies exploring the efficacy of chimeric antigen receptor (CAR) T cells in preventing relapse of neuroblastoma. CAR T cells have been developed to target GD2, and they have been safely implemented in patients and can possibly contribute to prolonged survival (Louis et al., 2011). There are several clinical trials underway for the use of CAR T cells in neuroblastoma; these are summarized in Table 3.

## 6. Conclusion

Neuroblastoma, the most common extracranial solid cancer in pediatrics, is a highly heterogeneous disease. Children who present with very low risk neuroblastoma have favorable outcomes and typically do not require intensive treatment. Conversely, patients with HR-NB express tumors that progress despite multiple different therapeutic approaches. There have been several recent developments in targeting of specific oncogenic drivers of neuroblastoma, including *MYCN*, ALK, and TrkB that are associated with HR-NB. Additional recent developments in chemotherapy, immunotherapy, and radiation therapies have improved current treatment strategies. While these developments and improvements are moving towards extending survival and preventing relapse, there is still work to be done. Many recent therapeutic developments are in preclinical and phase I/II trials. The focus of future developments should be in moving these products through trials, to approval, and developing novel treatments that can target *MYCN*, ALK and TrkB in different ways than current therapies. Researchers are aiming to achieve this through targeted drug delivery, using small molecule inhibitors and silk wafers. Additionally, attempts are being made at using CAR T cells to promote a host attack against tumor cells. Continuation of innovative thinking will be essential in improving survival rates of children diagnosed with neuroblastoma.

## Conflict of interest statement

There are no conflicts to declare.

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