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Emerging and investigational therapies for neuroblastoma

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Abstract

Introduction—Treatment for children with clinically aggressive, high-risk neuroblastoma remains challenging. Less than 50% of patients with high-risk neuroblastoma will survive long-term with current therapies, and survivors are at risk for serious treatment-related late toxicities. Here, we review new and evolving treatments that may ultimately improve outcome for children with high-risk neuroblastoma with decreased potential for late adverse events.

Areas covered—New strategies for treating high-risk neuroblastoma are reviewed including: radiotherapy, targeted cytotoxics, biologics, immunotherapy, and molecularly targeted agents. Recently completed and ongoing neuroblastoma clinical trials testing these novel treatments are highlighted. In addition, we discuss ongoing clinical trials designed to evaluate precision medicine approaches that target actionable somatic mutations and oncogenic cellular pathways.

Expert opinion—Advances in genomic medicine and molecular biology have led to the development of early phase studies testing biologically rational therapies targeting aberrantly activated cellular pathways. Because many of these drugs have a wider therapeutic index than standard chemotherapeutic agents, these treatments may be more effective and less toxic than current strategies. However, to effectively integrate these targeted strategies, robust predictive biomarkers must be developed that will identify patients who will benefit from these approaches and rapidly match treatments to patients at diagnosis.

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Keywords

Neuroblastoma; immunotherapy; MIBG; molecularly targeted drugs; genomic trials

1. Introduction

Neuroblastoma, a common pediatric neoplasm of the sympathetic nervous system, is remarkable for its heterogeneous clinical behavior and varying response to treatment [1]. Subsets of patients are highly curable with surgery alone or with adjuvant chemotherapy [2, 3], while infants with localized tumors commonly achieve long-term survival without any treatment, including surgery [4]. Approximately 45% of patients have clinically aggressive, high-risk tumors that are relatively resistant to chemotherapy and radiation. Although treatment for this cohort remains challenging, increasingly intensive multi-modality treatments developed throughout the world that include myeloablative therapy and stem cell transplant have led to improved survival [5]. In addition, a seminal randomized Children's Oncology Group (COG) study demonstrated the efficacy of post-consolidation immunotherapy [6], leading to FDA approval of dinutuximab for high-risk patients. Most recently, 3-year overall survival rates of more than 70% were reported in a randomized COG trial testing further intensification of consolidation therapy with tandem cycles of high-dose therapy and autologous stem cell transplant [7]. While these results are promising, further follow-up will be needed to determine if this “more-is-better” approach will ultimately lead to improved long-term survival. Unfortunately, this aggressive treatment strategy confers an increased risk for organ dysfunction and other treatment-related late adverse events including second cancers [8]. Thus, it is clear that more effective strategies with a higher therapeutic index are desperately needed. This review will provide an overview of emerging and investigational therapies, many of which are based on a better understanding of the oncogenic drivers of this aggressive tumor. We will primarily focus on therapies that are currently being tested in early phase studies.

2. ^{131}I -meta-iodobenzylguanidine (MIBG)

Although the anti-tumor activity of the radiopharmaceutical ^{131}I -MIBG in refractory and relapsed neuroblastoma was first reported in the 1980's [9], it remains unclear if the addition of ^{131}I -MIBG in treatment strategies for newly diagnosed neuroblastoma patients will lead to improved survival. Response rates in early phase studies of patients with relapsed and refractory disease have been promising, and in a large Phase II study of 164 patients, 37% achieved a partial or complete response with ^{131}I -MIBG at a dose of 18 mCi/kg [10]. Efforts to further improve the rate of response have included rapid tandem infusion ^{131}I -MIBG with autologous stem cell rescue and combination therapy with radiosensitizers or chemotherapy [11]. To directly test if response to ^{131}I -MIBG is improved with radiosensitizers, The New Approaches to Neuroblastoma Therapy (NANT) consortium is currently conducting a randomized Phase II comparing single-agent ^{131}I -MIBG to combination regimens with ^{131}I -MIBG plus vincristine and irinotecan or vorinostat (NCT02035137, Table 1). The feasibility of incorporating ^{131}I -MIBG into consolidation regimens with myeloablative chemotherapy and autologous stem cell transplant has also been demonstrated [12].

A small number of studies have tested ^{131}I -MIBG during the induction or consolidation phase of treatment in newly diagnosed patients. Schmidt and colleagues retrospectively analyzed data from a German neuroblastoma trial in which 40 of 111 patients with residual disease following induction therapy were treated with ^{131}I -MIBG [13]. Although significantly improved 3-year event-free survival and overall survival was observed in the cohort treated with ^{131}I -MIBG, no difference in outcome was seen in a subgroup analysis of 66 patients who underwent high-dose chemotherapy with autologous stem cell transplantation. In a study conducted in the Netherlands, a response rate of 66% was observed following two cycles of ^{131}I -MIBG administered to 41 newly diagnosed children prior to induction chemotherapy [14]. Recently, COG conducted a pilot study (NCT01175356, Table 1) testing the feasibility and tolerability of 12 mCi/kg, 15 mCi/kg, or 18 mCi/kg ^{131}I -MIBG administered prior to myeloablative therapy with busulfan and melphalan in high-risk patients regardless of response to induction therapy. Significant toxicities were observed with the 18 mCi/Kg dose, although the stopping rules outlined in the protocol were not met. Based on these results, COG is now developing the first randomized Phase III study for newly diagnosed high-risk patients that will directly test if the addition of ^{131}I -MIBG at a dose of 15 mCi/kg following cycle 3 of induction chemotherapy improves survival.

3. Targeted cytotoxic therapy

3.1. Nanoparticle Tumor Targeting Strategies

Protein nanoparticles were originally designed to address the toxicity of solvent-based formulations of hydrophobic anti-cancer agents. Additionally, traditional cytotoxic agents are being linked to antibodies which direct these agents specifically to tumor cells while sparing normal tissue. To reduce Cremophor® EL (CrEL) associated, dose-limiting hypersensitivity to paclitaxel, a 130-nanometer albumin-bound paclitaxel (*nab*TM-paclitaxel) was first introduced and approved by the FDA in January 2005 for the treatment of relapsed metastatic breast cancer. The *nab*TM technology takes advantage of endogenous human albumin receptor mediated transcytosis and albumin binding proteins, such as secreted protein acidic and rich in cysteine (SPARC), to deliver higher doses of paclitaxel across the vascular endothelium and into tumors [15]. In preclinical studies, neuroblastoma xenografts were responsive to *nab*TM-paclitaxel in a dose dependent fashion and *nab*TM-paclitaxel produced significantly more favorable tumor/plasma ratios versus solvent-based drug, with enhanced delivery to tumor tissue, but lower systemic exposure [16]. These properties potentially allow for intratumoral dose intensification with decreased toxicity. A phase 2 trial of *nab*TM-paclitaxel in neuroblastoma, rhabdomyosarcoma, and Ewing sarcoma is ongoing (NCT01962103, Table 1). Several other nanoparticle tumor targeting strategies are in development, including liposomal irinotecan (MM-398; Onivyde®; NCT02013336, Table 1) and a poly(lactide)-poly(ethylene glycol) (PLA-PEG)-based polymeric nanoparticle containing SN-38, the active metabolite of irinotecan [17].

3.2. Antibody-drug conjugates (ADC)

ADC is another unique avenue to direct cytotoxic agents to tumor. For neuroblastoma, lorvotuzumab mertansine (IMGN901) is the agent in class furthest along in the development

pipeline. IMG901 is a high-affinity humanized antibody recognizing CD56 (NCAM, neural cell adhesion molecule) conjugated to a derivative of maytansine, a highly potent microtubule inhibitor (DM1; mertansine) [18]. NCAM is almost ubiquitously expressed by neuroblastic tumors, although the particular isoform may differ depending on maturational status [19]. When IMG901 binds CD56, it is internalized. The disulfide linker is cleaved within the cell to allow local concentrated release of mertansine and mitotic arrest, theoretically augmenting tumor kill while diminishing systemic side effects. A phase 2 study of IMG901 in neuroblastoma and other CD56 expressing tumors through COG (NCT02452554, Table 1) was recently suspended due to drug availability. Recently, IMG901 failed to show an improvement in survival in small-cell lung cancer patients and was attributed with increased infectious deaths [20]. Another potential ADC target being explored preclinically for neuroblastoma is the *ALK* receptor tyrosine kinase [21].

4. Immunotherapy

4.1. First Generation Anti-Neuroblastoma Antibodies

One of the most significant recent advances in the treatment of patients with high-risk neuroblastoma has been the development of therapies targeted to the cell surface GD2 disialoganglioside. Preclinical and early phase trials demonstrated that the chimeric antibody ch14.18 (dinutuximab) efficiently binds GD2 and culminated in the seminal COG ANBL0032 study which demonstrated that dinutuximab plus cytokines (GMS-CSF and IL-2) significantly improves EFS and OS [6]. In an effort to mitigate the pain and other toxicities that are seen when the antibody is infused over 8 to 20 hours, SIOPEN conducted a Phase II clinical trial testing a 10-day continuous infusion of Ch14.18 manufactured in Chinese hamster ovary (CHO) cells (ch14.18.CHO) in combination with sub-cutaneous (sc) IL-2 [22, 23]. Results of this Phase II study demonstrated clinically activity of the long-term delivery method of ch14.18/CHO and an improved toxicity profile. SIOPEN also recently conducted a randomized Phase III immunotherapy study to investigate the role of scIL-2 in the immune response, and no survival benefit of co-treatment with ch14.18/CHO and scIL-2 was observed [24] (NCT01704716, Table 2).

A randomized COG Phase II study has recently demonstrated the efficacy of combining chemotherapy (irinotecan and temozolomide) with dinutuximab for neuroblastoma patients in first relapse [25] (NCT01767194, Table 2). Specifically, nine of seventeen patients (52.9%) treated with dinutuximab and chemotherapy had a clinical response, and five (19.4%) achieved a complete remission. These encouraging results led an expanded enrollment on the antibody arm of this study in order to collect more data on the efficacy of this combination. In addition, a pilot study for newly diagnosed patient with high-risk neuroblastoma testing dinutuximab combined with induction chemotherapy is under development within the COG.

4.2. Second-generation GD2-targeted immunotherapy

A humanized version of the murine 3F8 (hu3F8) has been developed [26] and is being tested in multiple trials combined or not with sc GM-CSF in the relapsed setting (NCT01757626, NCT01419834, Table 2) or with sc GM-CSF and isotretinoin as consolidation therapy for

patients in first remission (NCT03033303, Table 2). COG tested a fusion protein of humanized antibody (hu14.18) and IL-2 (hu14.18-IL2) (APN301) in a Phase II clinical trial and demonstrated a 21.7% response rate in relapsed patients without bulky disease [27]. A modified humanized antibody (hu14.18K322A) with a single amino acid substitution that blunts the complement activation thought to be responsible for neuropathic pain has shown a 19% response rate in patients with only MIBG detectable relapsed disease [28]. This antibody is now being evaluated as part of first line therapy (NCT01857934, Table 2) during induction.

4.3. Chimeric Antigen Receptor (CAR) T-cells

Based on the remarkable responses seen with CD19-CAR T cells in patients with acute lymphoblastic leukemia studies [29], CAR T-cells engineered to target neuroblastoma have been developed. Early trials have shown that it is feasible to engineer cells to target the neuroblastoma cell surface markers L1-CAM or GD2 [30]. Though these efforts were limited by the transiency of the engineered T-cells, they did demonstrate a tolerable safety profile [31]. Newer generations of CARs are thus being designed to improve efficacy while minimizing T-cell exhaustion.

4.4. Natural Killer (NK) cell therapy

Because NB cells do not express HLA class I, they can be a target of NK cell killing. Thus, administering NK cells in conjunction with anti-GD2 antibody or other immunostimulatory approaches may improve ADCC [32]. Multiple clinical trials are ongoing to test this hypothesis in relapsed neuroblastoma patients (NCT02573896, NCT01576692, NCT02650648, NCT02100891, Table 2).

4.5. Checkpoint inhibitors

In order to activate T-cells against tumor cells, both major histocompatibility complexes (MHC) and other co-stimulatory signals are required. Several signaling molecules, known as immune checkpoints, are capable of repressing T-cell activation by preventing co-stimulation. Thus, a patient's immune system could be induced to have antitumor activity by inhibiting these checkpoints. Agents targeting Cytotoxic T-lymphocyte antigen 4 (CTLA-4) became the first to demonstrate the potential of this approach [33]. Ipilimumab was the initial agent to target CTLA-4 and received FDA approval for metastatic melanoma in 2011 following successful phase 3 trials [34]. Subsequently, agents were developed against two other checkpoints, program death (PD-1) and its ligand (PD-L1). Phase 3 trials in metastatic melanoma led to approval of the PD-1 inhibitors nivolumab and pembrolizumab for this indication [35, 36] and subsequent large trials in other tumor types have also been successful. The broad efficacy of these agents, which do not rely on disease specific targets, has led COG ADVL1412 is testing nivolumab with or without ipilimumab and (NCT02304458, Table 2) and ADVL1621 evaluating single agent pembrolizumab (NCT02332668, Table 2).

4.6. Tumor vaccines

Despite the intensive therapy given to patients with high-risk neuroblastoma, many patients in clinical remission clearly have minimal residual disease leading to late relapse [1], making vaccine approaches attractive in the post-consolidation setting. Multiple antigens have been explored as targets for these treatments including NY-ESO1 [37], GD2 [38], survivin [39], and MYCN [40]. However, efforts to use these approaches have had modest success, though new vaccines hold the potential for improved the immune response against neuroblastoma (NCT00911560, NCT02745756, Table 2).

5. Biologic agents

5.1. Fenretinide

Several agents are being explored for their immune-modulatory properties. The synthetic retinoid N-(4-hydroxyphenyl) retinamide (fenretinide) has been shown to have clinically relevant activity in neuroblastoma trials [41]. Unlike isotretinoin, fenretinide does not induce differentiation, but rather is selectively cytotoxic for cancer cells. The mechanism of action is complex, retinoic acid receptor and p53 independent, and includes the induction of de novo ceramide synthesis, generation of reactive oxygen species, and inhibition of angiogenesis [42]. Fenretinide also causes large, dose-dependent increases of intracellular dihydroceramides, which are precursors of GD2. Recently it has been shown that fenretinide can induce GD2 target antigen expression, resulting in a significantly enhanced GD2-specific ADCC, as well as augmented complement-mediated, and NK-mediated killing suggesting that suggest that clinical activity of Dinutuximab would be boosted by fenretinide [43].

5.2. Lenalidomide

Lenalidomide is FDA approved in combination with dexamethasone for the treatment of multiple myeloma, is also being studies as an immune adjuvant. Although the mechanisms of lenalidomide are not completely understood, the overall effect in adult hematologic malignancy has been to increase anti-inflammatory cytokines, decrease pro-inflammatory cytokines, and induce proliferation, activation and survival of NK and cytotoxic T-lymphocytes, effectively enhancing both NK cell-mediated cytotoxicity and ADCC [44]. In children with refractory solid tumors and myelodysplastic syndrome, lenalidomide induces increases in plasma IL-2, IL-15 and GM-CSF, to augment NK and T-cell mediated cytotoxicity, and to reduce the fraction of CD4+CD25+ regulatory T-cells thought to foster immune privilege [45]. In preclinical models, lenalidomide reversed the immunosuppressive effects of monocyte derived IL-6 and monocyte and neuroblastoma derived TGFβ1 on NK cell activity *in vitro*, and augmented the efficacy of ch14.18 immunotherapy *in vivo* [46]. A NANT phase I trial of lenalidomide in combination with dinutuximab and isotretinoin to evaluate the safety, preliminary activity and immunologic activity of this cassette in patients with recurrent/ refractory neuroblastoma (NCT01711554, Table 3) is nearing completion. Results of this study will provide clinically relevant information regarding the potential of replacing pharmacologic exogenous cytokines, particularly IL-2, during anti-GD2 immunotherapy which is perceived to be the most toxic component of the regimen, with lenalidomide.

5.3. Vascular Endothelial Growth Factor (VEGF) Inhibitors

Intriguingly, biologic agents can have multiple mechanisms by which they affect the tumor microenvironment. VEGF and multi-tyrosine kinases inhibitors (MTKI) were primarily designed to curtail endothelial survival, inhibit new vessel growth, and normalize tumor vasculature. In doing so, they can also modulate the immune response and the effects of pro-inflammatory cytokines. VEGF is known to inhibit immune infiltration of tumors, decrease the proliferation and activity of dendritic cells and alter the activity of T regulatory cells. Multiple studies are exploiting this connection by combining the VEGF inhibitor bevacizumab or MTKI in combination with immunotherapies [47]. The best studied MTKI in neuroblastoma is sorafenib which inhibits VEGF, platelet-derived growth factor β (PDGFB), c-Kit, and RET receptor signaling, as well as B-Raf and C-Raf activation [48]. In subcutaneous and orthotopic xenografts, sorafenib inhibits tumor progression, reduces blood vessel density, decreases neuroblastoma proliferation, induces cell cycle arrest and diminishes signaling through the MAPK/ERK pathway (Figure 1) [49]. Furthermore, sorafenib has also been shown to inhibit the JAK2/ STAT3 pathway as well as activation of STAT3 by the pleiotropic, stromal derived cytokine IL-6, which is known to promote neuroblastoma proliferation, survival and drug resistance [50]. In pediatric embryonal tumors, the clinical activity of single agent sorafenib, as well as other VEGF blocking agents, has been disappointing [51, 52]. The BEACON trial studying temozolomide and irinotecan with and without bevacizumab (NCT02308527, Table 3) and NANT13-02 looking at the combination of cyclophosphamide, topotecan and sorafenib in refractory neuroblastoma (NCT02298348, Table 3) may provide further insight into the role of these agents which primarily target the tumor microenvironment.

6. Molecularly targeted agents

6.1. ALK Inhibitors

Anaplastic lymphoma kinase (*ALK*), encoded by the *ALK* gene on chromosome 2p23.1, is a receptor tyrosine kinase belonging to the insulin receptor superfamily. It is normally expressed in the developing nervous system and is thought to play a role in neuronal differentiation [53]. Oncogenic *ALK* translocations have been described in multiple malignancies of adults. In neuroblastoma, *ALK* is also a tractable molecular target, although activating point mutations are more commonly detected than *ALK* translocations [54, 55].

6.1.1. Crizotinib—Crizotinib, a small molecule competitive inhibitor of *ALK* and *MET* kinase activity, has shown significant response rates in non-small cell lung cancer with *ALK* translocations, for which it is FDA approved [56]. A phase 1/2 trial of crizotinib (NCT00939770) was conducted in children, adolescents, and young adults with relapsed or refractory solid tumors or anaplastic large cell lymphoma, with a dedicated phase 2 cohort for individuals with neuroblastoma with *ALK*+ disease. In the phase 1 portion of the study, the recommended phase 2 dose (RP2D) was declared as 280 mg/m² BID, almost twice the RP2D determined in adults. Therapy was overall well tolerated, and the most common toxicities during nausea and vomiting. Of the 11 patients with neuroblastoma and known *ALK* mutations, 1 patient had a CR and 2 had SD. Individuals with neuroblastoma who had a CR or SD received anywhere from 4-39 cycles of therapy, with some continuing on

treatment at the time of publication of the phase 1 results [57]. The phase 2 portion of this study investigating response in those with neuroblastoma with *ALK* mutations or amplification has met accrual and may provide additional information on which patients may benefit from *ALK* inhibition. A COG phase 1 study of crizotinib with combination chemotherapy (cyclophosphamide/ topotecan or vincristine/ dexrazoxane/ doxorubicin) in children with relapsed or refractory solid tumors or anaplastic large cell lymphoma (NCT01606878, Table 4) is ongoing. Development plans for the next COG phase 3 study (ANBL1531) include integrating crizotinib in the upfront setting for individuals with *ALK*+ neuroblastoma.

6.1.2. Lorlatinib—Pediatric phase 1 results of crizotinib showed modest responses in neuroblastoma with *ALK* mutations compared to *ALK*-rearranged malignancies, indicating differences in therapeutic targeting of the various *ALK* aberrations [58]. While crizotinib has shown antitumor activity in preclinical neuroblastoma models, cell lines and xenografts harboring F1174L-mutated *ALK* have shown reduced sensitivity. Crizotinib resistance to F1174L-mutated *ALK* is thought to be, in part, secondary to increased ATP-binding affinity, which may be overcome by higher doses of crizotinib or higher-affinity inhibitors [59]. This has prompted investigation of the *ALK*/ROS1 inhibitor lorlatinib (PF-6463922), which has increased *ALK*-binding affinity to all variants and may, therefore, overcome the effects of primary resistance mutations. Indeed, lorlatinib was a more potent *ALK*-inhibitor than crizotinib across neuroblastoma mutations in vitro. Lorlatinib demonstrated significant antitumor activity in mouse xenograft models of neuroblastoma with and without primary crizotinib resistance and crizotinib naïve patient derived xenografts with F1174L and F1245C mutations. A NANT study with this agent is planned.

6.1.3. Ceritinib—Ceritinib is a second-generation small molecule ATP-competitive tyrosine inhibitor of *ALK* and IGF-1 [60]. Preclinical studies have shown significant antitumor activity against both crizotinib-sensitive and most crizotinib-resistant *ALK*-rearranged non-small cell lung cancer [61]. Enzymatic assay studies have shown that ceritinib is 20 times more potent against *ALK* than crizotinib. A phase 1 trial (+ expansion) of ceritinib in *ALK*-rearranged non-small cell lung cancer revealed high clinical activity, with an overall response rate of 56% (95% CI 45-67) was seen among patients who had received crizotinib previously [60]. As a result, ceritinib was FDA approved for the treatment of patients with *ALK* positive, metastatic non-small cell lung cancer (NSCLC) who have progressed on or are intolerant to crizotinib [62]. A pediatric phase 1 study of ceritinib in malignancies with *ALK* aberrations is ongoing (NCT01742286, Table 4).

6.2. TRK inhibitors

The TRK neurotrophin receptors play critical roles in development and maintenance of the central and peripheral nervous system. Activation of TRK family receptors (TrkA/NTRK1, TrkB/NTRK2, TrkC/NTRK3) is implicated in both pediatric and adult tumors. TrkB is co-expressed with its ligand, BDNF, and this autocrine pathway leads to invasion, metastasis, angiogenesis and drug resistance [63, 64]. Blocking TrkB with inhibitors leads to apoptosis and increased sensitivity to chemotherapy [64, 65]. Many pediatric and adult cancers have rearrangements or aberrant expression of TRK genes. However, mutations in or

translocations involving TRK genes have not been found in neuroblastomas. Rather, autocrine activation of TrkB/BDNF pathway occurs in 50-60% of high-risk neuroblastoma, particularly in *MYCN*-amplified tumors [66]. Due to its frequency of activation, TRK has been proposed as a promising target for neuroblastoma.

6.2.1. Entrectinib—Entrectinib (RXDX-101) is a potent, small molecule inhibitor of the receptor tyrosine kinases TrkA/B/C, ROS1, and ALK. Entrectinib crosses the blood brain barrier, potentially enabling targeting of central nervous system (CNS) metastases and primary CNS tumors [67]. *In vivo* (SY5Y-TrkB xenografts), entrectinib demonstrates significant tumor growth inhibition and prolonged event free survival (EFS) when compared to vehicle alone. The addition of entrectinib to irinotecan and temozolomide enhanced tumor growth inhibition both *in vitro* and *in vivo* [68].

Phase 1 studies of entrectinib in adults with advanced or metastatic solid tumors harboring NTRK1/2/3, ROS1, or ALK molecular alterations have been completed and demonstrated a tolerable safety profile [69]. Phase 2 studies of entrectinib in adults are ongoing (NCT02097810, NCT02568267, NCT02587650). A phase 1/1b study of entrectinib is currently open in children with recurrent or refractory solid tumors and primary CNS tumors (NCT02650401, Table 4). Upon identification of the RP2D, one of the expansion cohorts in the phase 1b portion will open specifically for individuals with relapsed or refractory neuroblastoma to assess response.

6.2.2. Larotrectinib (LOXO-101)—Larotrectinib (LOXO-101), a selective small molecule inhibitor of TrkA/B/C is under clinical development for adult (NCT02122913, NCT02576431) and pediatric advanced solid tumors and brain tumors (NCT02122913). Once a RP2D is determined, expansion cohorts will open for individuals with alterations in one of the three NTRK genes or TRK proteins.

6.3. RAS-MAPK and MEK inhibitors

The RAS-MAPK pathway plays a role in cellular growth, survival, and differentiation and has been implicated in variety of malignancies (Figure 1) [70]. RAS-pathway related mutations are infrequent at diagnosis in neuroblastoma [71]. However, a recent report by Eleveld, et al. [72] showed an increased frequency of activating mutations not present at diagnosis in the RAS-MAPK pathway in relapsed neuroblastoma tumors. Specifically, mutations were identified in *ALK*, *NFI*, *BRAF*, *PTPN11*, *FGFR1*, *KRAS*, *HRAS*, and *NRAS*. Thus, inhibition of the RAS-MAPK pathway may serve as therapeutic target in the relapsed/refractory neuroblastoma population.

MAPK/ERK kinase (MEK) inhibitors have demonstrated anti-tumor effects in neuroblastoma *in vitro* [73]; binimetinib (MEK162) has shown tumor growth inhibition and increased survival *in vivo* [72]. Trametinib (MEK 1/2 inhibitor) was FDA approved in 2013 as an oral monotherapy for the treatment of adult patients with unresectable or metastatic melanoma containing *BRAF*V600E or V600K mutations [74], and in 2014 trametinib in combination with dabrafenib was FDA approved for the same indication [75]. A Phase I/IIa trial of trametinib alone or with dabrafenib for V600 mutations (NCT02124772, Table 4) is

ongoing for pediatrics, and the study includes a dedicated expansion cohort (n=10) for individuals with relapsed or refractory neuroblastoma to begin to assess response.

6.4. PI3K/mTOR inhibitors

Aberrant activation of the PI3K/Akt/mTOR cell survival pathway has been demonstrated in neuroblastoma and is a focus of targeted therapy development (Figure 1). Chesler, et al. [76] showed that PI3K inhibition as a therapeutic strategy in neuroblastoma is, in part, based on its relationship with MYCN. The PI3K pathway appears to play a role in MYCN stabilization, and PI3K inhibition in *MYCN*-driven murine neuroblastoma leads to decreased levels of MYCN protein and tumor mass. *MYCN* mRNA levels are unchanged, suggesting that PI3K inhibition blocks MYCN at a post-transcriptional level. Thus, PI3K inhibition is of great interest, given the difficulty of directly blocking MYCN and other transcriptional factors [76, 77].

Chanthery, et al. [78] further showed that dual PI3K/mTOR inhibition with dactolisib (NVP-BEZ235) decreased levels of MYCN protein in tumor cells, with secondary paracrine blockade of angiogenesis in *MYCN*-driven neuroblastoma models. Elimination of MYCN protein by PI3K/mTOR inhibition, with compounds such as dactolisib, is closely associated with effective targeting of both mTORC1 and mTORC2 and suggests that mTOR kinase plays a significant role in the maintenance of MYCN stability [80]. The degree of *MYCN*-amplification and protein expression correlates with sensitivity to mTOR inhibition [78]. In addition, direct inhibition of AKT has been proposed as a therapeutic strategy in neuroblastoma. The AKT inhibitor perifosine (D21266) induced caspase-dependent apoptosis and decreased AKT phosphorylation in neuroblastoma cell lines and demonstrated tumor growth inhibition and/or tumor regression and longer survival in mouse xenograft models of neuroblastoma [79].

6.4.1. PI3K/mTOR inhibition—A phase 1 trial of SF1126, a pan-PI3K/mTOR inhibitor, is being conducted in children, adolescents, and young adults through the NANT consortium (NCT02337309, Table 4) using a 3+3 dose escalation design. SF1126 is a novel RGDS-conjugated LY294002 prodrug, which shows increased solubility and binds to integrins in the tumors compartment, improving delivery of drug to the tumor vasculature and tumor [80]. Upon determination of a RP2D, an expansion cohort of 10 patients with tumors demonstrating *MYCN*-amplification, *MYCN* over-expression, or *MYC* over-expression will be treated. COG is also planning a phase 1 trial using the PI3K/mTOR inhibitor PQR309 for patients with relapsed and refractory solid tumors.

6.4.2 Perifosine (AKT inhibitor)—A phase 1/1b study of the PI3K/AKT pathway inhibitor perifosine (NCT01049841) has recently been completed in children, adolescents, and young adults with advanced solid tumors, with a 1b expansion cohort for individuals with relapsed/ refractory neuroblastoma. Twenty-seven patients with neuroblastoma received perifosine. One of 24 patients had tumor with *MYCN*-amplification and none of the 21 tumor samples tested had *ALK* mutations. Therapy was overall well tolerated, without any dose limiting toxicities. Nine patients have had a progression free survival for a median of 54 months from study entry (range: 43-74 months) [81].

6.5. Epigenetic inhibitors

Targeting epigenetic modifications, or changes in the regulators of patterns of gene expression rather than the DNA sequence itself, continues to be a growing area of anti-cancer therapy. Epigenetic pathways involving DNA methylation, histone modification, nucleosome remodeling, and non-coding RNAs are linked to oncogenesis, and several epigenetic inhibitors are under clinical development [82].

6.5.1. BET inhibitors—The bromodomain and extraterminal domain (BET) family (BRD2, BRD3, BRD4, and BRDT) plays a critical role in the activation of transcription. Bromodomains recognize and bind acetylated lysine residues on histone tails and regulate chromatin structure; thus, BET proteins are known as epigenetic “readers”. BET inhibitors have been found to block transcription of *MYCN*. BET inhibition with JQ1, I-BET726 (GSK1324726A), and OTX015 (now MK-8628) induced cell cycle arrest, apoptosis and down-regulation of *MYCN* expression and *MYCN* target genes in *MYCN*-amplified neuroblastoma cell lines [83, 84, 85]. *MYCN*-amplification was a strong predictive marker of sensitivity to JQ1 [83]. I-BET726 also induced growth inhibition and cytotoxicity in cell lines regardless of *MYCN* copy number or expression level, as well as direct suppression of *BCL2* [84]. In various *in vivo* neuroblastoma models, treatment with JQ1, I-BET726, and OTX015 decreased tumor volume and prolonged overall survival [83, 84, 85]. Various BET inhibitors are under clinical investigation for adults. A phase 1 study of I-BET762 (GSK525762) is currently ongoing for individuals with NUT midline carcinoma and other cancers, including neuroblastoma and *MYCN*-driven solid tumors, and includes individuals 16 years of age and older (NCT01587703, Table 4).

6.6. Modulation of MYCN

Polyamines are essential polycations that enhance transcription, translation and replication, as well as support *MYCN* functions. The ornithine decarboxylase gene *ODC1*, a direct target of *MYCN*, encodes the rate-limiting enzyme in polyamine synthesis, and thus inhibition of ODC1 mediates *MYCN* effects and serves as a therapeutic target in neuroblastoma. Alpha-difluoromethylornithine (DFMO), an irreversible ODC1 inhibitor, is FDA approved for the treatment of *Trypanosomiasis* and has been investigated in neuroblastoma for its role in polyamine depletion [86, 87]. *In vivo*, DFMO delays tumor initiation and enhances the antitumor activity of conventional cytotoxic chemotherapy [86]. DFMO in combination with celecoxib (an inducer of polyamine catabolism) alone or with conventional cytotoxic chemotherapy leads to tumor regression *in vivo* [87], possibly by altering the tumor microenvironment to support anti-tumor immunity. In a phase 1 trial of DFMO alone and in combination with oral etoposide conducted in patients with relapsed/refractory neuroblastoma, individuals tolerated 500-1500 mg/m² of DFMO twice daily, without dose limiting toxicity [88]. A phase 1 study of DFMO and celecoxib with cyclophosphamide/topotecan is ongoing (NCT02030964, Table 4).

In addition to *ODC1*, Aurora A kinase (*AURKA*) plays an important role in stabilizing the *MYCN* protein. In preclinical testing, the *AURKA* inhibitor alisertib (MLN8237) led to a complete response in three out of seven neuroblastoma xenografts [89]. A recent NANT Phase 1/2 study demonstrated that alisertib is well tolerated and an objective response was

seen in 6 of 32 (18.8%) patients taking either the tablet or solution formulation [90]. Somewhat paradoxically, patients with *MYCN*-amplification had a trend towards lower response rates, necessitating improved biomarkers of activity in future trials of this agent.

6.7. Cell Cycle Checkpoint Kinase inhibitors

Loss of cell cycle regulatory control is a key step in tumorigenesis and is thus another promising therapeutic target. Numerous studies have shown *in vitro* and *in vivo* activity of agents targeting checkpoint kinases (CHK) 1/2/4/6, WEE1, and polo-like kinase 1 (PLK1) [91, 92]. Phase 1 testing in adults with refractory tumors has demonstrated tolerability and efficacy of the CHK1 inhibitor prexasertib (LY2606368), the WEE1 inhibitor AZD1775 and the PLK1 inhibitor volasertib (BI-6727) [93, 94, 95]. Additionally, the CDK4/6 inhibitor ribociclib recently received FDA approval after demonstrated improved survival in women with breast cancer. In pediatrics, phase 1 trials are ongoing for prexasertib (NCT02808650, Table 4), AZD1775 (NCT02095132, Table 4), ribociclib (NCT01747876, Table 4), and volasertib (NCT01971476, Table 4) for relapsed and refractory solid tumors.

6.8. Histone Deacetylase (HDAC) inhibitors

Decreased histone acetylation has long been a hallmark of cancer, altering chromatin structure and modifying transcriptional pathways [96]. HDAC inhibition leads to increased acetylation and a less malignant transcriptional profile. In neuroblastoma, vorinostat has shown the most potential in this category with a R2PD of 230mg/m² [97]. Additionally, vorinostat is a sensitizer to ¹³¹I-MIBG and is tolerated at 180mg/m² in combination with 18 mCi/kg ¹³¹I-MIBG [12]. This combination is currently being compared to ¹³¹I-MIBG alone or in combination with vincristine and irinotecan (NCT02035137, Table 1).

7. Genomic trials

In order to tackle the challenge of personalizing the use of the targeted agents described, several groups have evaluated genomic biomarkers to identify appropriate agents for patients with relapsed neuroblastoma [98, 99, 100, 101]. The goal of these trials was to determine the feasibility of using somatic and germline sequencing to recommend targeted treatment options. While feasibility was demonstrated with 31-50% of patients having a potentially targetable mutation, it is notable that only 3-19% received a drug specific to somatic aberrations with even fewer patients deriving clinical benefit from this approach. The NANT consortium recently activated a feasibility trial to sequence tumors from patients with relapsed neuroblastoma and provide informed treatment options (NCT02868268, Table 5). The important next question being addressed by these consortia is whether genomic approaches for drug selection provides a survival advantage compared to standard treatment options for relapsed and refractory patients. The Genomic Assessment Improves Novel Therapy (GAIN) consortium is currently accruing a total of 875 patients in an effort to demonstrate that genomic approaches will improve the survival of patients with relapsed solid tumors by recommending options to the family and provider through a molecular tumor board (NCT02520713, Table 5).

One of the biggest barriers to precision medicine that emerged in feasibility studies was that many children did not receive a drug matched to their genomic profile because the patient could not obtain the recommended medication. To address this, groups have partnered with industry to design trials that have cassettes of readily available molecularly targeted agents and aim to identify patients for whom one of these drugs may be beneficial. The NCI-MATCH trial is accruing adult oncology patients regardless of tumor histology into one of 24 different treatment arms depending on identified somatic mutations (NCT02465060, Table 5). Plans are underway to allow children to enroll on this study through the NCI Pediatric MATCH which is likely to open for enrollment in 2017. The American Society of Clinical Oncology is also enrolling adults into the Targeted Agent and Profiling Utilization Registry (TAPUR) Study (NCT02693535, Table 5) which is determining the efficacy of using commercially available agents based on mutations detected in tumor profiling and will open the trial to children 12 years and older. Additionally, the NEPENTHE trial is accruing relapsed neuroblastoma patients into one of three treatment arms defined by *ALK* mutations, activations of the RAS-MAPK pathway, and those with wild-type p53. Patients in these three arms will be treated with ceritinib and ribociclib, trametinib, or HDM201 (an inhibitor of the HDM2 protein which inactivates p53), respectively (NCT02780128, Table 5). Thus, over the next several years, we will know if it is possible to identify a patient's individual tumor profile, treat them with a drug specific for those aberrations, and hopefully improve their prognosis.

8. Conclusion

The recent success of intensive induction chemotherapy and surgery, followed by tandem high-dose chemotherapy with autologous stem cell transplant and radiation and post-consolidation therapy with dinutuximab has improved outcomes for children with high-risk neuroblastoma. However, further gains in survival and a reduction in both short- and long-term side effects are needed. Advances in ¹³¹I-MIBG, immunotherapy, biologics, and molecularly targeted agents hold the promise of achieving these goals, but much work remains. Improved biomarkers will help guide new approaches for risk stratification and drug selection for patients with high-risk neuroblastoma.

9. Expert opinion

Advances in genomic medicine and molecular biology have led to a better understanding of the oncogenic drivers in neuroblastoma, and promising results have been observed in early phase studies testing therapies that target these aberrantly active cellular pathways. By implementing multiple therapeutic approaches including improved cytotoxic agents, immunotherapy, biologics, and molecularly targeted agents, advances are being made that have improved the outcomes for children diagnosed with high-risk neuroblastoma. Because many of these drugs have a wider therapeutic index and are more “precise” than standard chemotherapeutic agents, these treatments have the potential to be more effective and less toxic, leading to further improvement in survival for patients with newly diagnosed or relapsed neuroblastoma.

Currently, induction and consolidation therapy for patients with newly diagnosed, high-risk neuroblastoma consists primarily of large doses of traditional cytotoxic agents and radiation followed by immunotherapy. Patients are assigned to this treatment based on clinical and biologic variables identified decades ago. Unfortunately, up to 20% will not respond or will progress through induction therapy and more than 30% will relapse at later time points. To ultimately improve the survival of children with high-risk neuroblastoma, it will be critical to develop systems that will enable the rapid matching of effective treatments to patients in the diagnostic setting, thus decreasing the frequency of primary refractive disease. This will require the development of robust biomarkers that will distinguish patients who will respond to traditional treatment strategies from those who will not. For patients in the latter cohort, additional biomarkers will be needed to identify alternative treatment approaches that are most likely to be effective. Further, novel trial designs that will enable accurate assessment of the activity of these alternative regimens in small numbers of patients will be required.

DNA mutations serve as the most commonly used biomarkers for targeted agents at the present time, but are difficult to implement in neuroblastoma due to the paucity of these changes in this disease. However, DNA copy number changes and alterations in the transcriptome, epigenome, and proteome are all well described in neuroblastoma. Thus, a combination of “omic” measures are likely to be needed to develop robust predictive biomarkers for new agents, whether they be small molecules, biologics, or immunotherapeutics. For example, if an expression signature or genomic/epigenomic profile is prospectively validated to be predictive of response and outcome, it would open the way for the development of a molecular test that, ideally, would be rapidly implemented in the clinic and have clear therapeutic implications. A similar approach is currently planned for children with high-risk acute lymphoblastic leukemia wherein a low-density array expression panel will be used in the next phase 3 COG trial to identify those who would likely benefit from specific kinase inhibitors based on Philadelphia-like profiles.

Ultimately, emerging therapies for neuroblastoma are likely to continue to improve the survival rates for high-risk patients. With a deeper understanding of the underlying biology driving high-risk disease, we will continue to be able to develop additional precision medicine strategies that will hopefully be more effective and associated with less acute and long-term adverse effects. Intensification of therapy has improved outcomes for children with high-risk neuroblastoma thus far, but new, biomarker driven therapies will determine our future success.

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Article highlights

- Long-term survival rates for patients with high-risk neuroblastoma remain at less than 50% despite intensive, multimodal therapy.
- The Children's Oncology Group is developing a Phase 3 clinical trial for patients with newly diagnosed high-risk neuroblastoma to evaluate the efficacy of the targeted radiopharmaceutical ^{131}I -MIBG for patients with *ALK*- tumors and crizotinib for patients with *ALK*+ neuroblastoma.
- Multiple agents including biologics, immunotherapies, and molecularly targeted compounds show promise in early phase trials.
- Genomic driven trials have been developed to match patients with actionable tumor mutations with specific targeted agents.
- Robust predictive biomarkers are needed to identify patients that will benefit most from novel therapies.

Figure 1. PI3K and RAS are activated by multiple growth factors in neuroblastoma tumors. This activation leads to cellular proliferation and has been associated with relapsed disease and poor outcomes. Multiple drugs (purple) are in clinical development which inhibit one or more of the proteins in these pathways and may help improve outcomes for patients with high-risk neuroblastoma.

Table 1
Description of clinical trials currently available using ¹³¹I-MIBG and targeted cytotoxic therapy for patients with high-risk neuroblastoma

Trial Identifier	Study question	Sponsor	Study agent(s)	Phase	Disease eligibility	Age Range
NCT02035137	Efficacy of single-agent ¹³¹ I-MIBG compared to combination regimens with ¹³¹ I-MIBG plus vincristine and irinotecan or vorinostat	NANT	¹³¹ I-MIBG, vincristine, irinotecan, vorinostat	2	Recurrent/ refractory neuroblastoma	2 – 30 yrs.
NCT01175356	Feasibility and tolerability of 12 mCi/kg, 15 mCi/kg, or 18 mCi/kg ¹³¹ I-MIBG administered prior to myeloablative therapy with busulfan and melphalan	COG	¹³¹ I-MIBG	Pilot	New diagnosis neuroblastoma	1 – 30 yrs.
NCT01962103	Dose finding and efficacy of <i>naβ</i> TM -paclitaxel in recurrent/ refractory solid tumors	Celgene	<i>naβ</i> TM -paclitaxel	1/2	Recurrent/ refractory solid tumors	6 mo. – 24 yrs.
NCT02013336	Dose finding and tolerability of irinotecan sucrosfate liposome injection (MM-398) with cyclophosphamide in recurrent/ refractory solid tumors	Merrimack Pharmaceuticals/ SPOC	irinotecan sucrosfate (MM-398)	1	Recurrent/ refractory solid tumors	1 – 20 yrs.
NCT02452554	Efficacy of lorvotuzumab mertansine in recurrent/ refractory solid tumors	COG	lorvotuzumab mertansine (IMGN901)	2	Recurrent/ refractory solid tumors	1 – 30 yrs.

Abbreviations: COG; Children's Oncology Group, SPOC; South Plains Oncology Consortium, NANT; New Advances in Neuroblastoma

Description of clinical trials currently available using immunotherapy for patients with high-risk neuroblastoma

Table 2

Trial Identifier	Study question	Sponsor	Study agent(s)	Phase	Disease eligibility	Age Range
NCT01704716	Compare efficacy of ch14.18/CHO with or without IL-2 in consolidation	SIOPEX	ch14.18/CHO, IL-2	3	New diagnosis neuroblastoma	1 mo. – 21 yrs.
NCT01767194	Efficacy of dinutuximab plus irinotecan and temozolomide in first relapse	COG	dinutuximab, irinotecan, temozolomide	2	First recurrence/ progression neuroblastoma	All ages
NCT01757626	Dose finding and efficacy of hu3F8 plus GM-CSF	Memorial Sloan Kettering Cancer Center	hu3F8, GM-CSF	1/2	Recurrent/ refractory neuroblastoma	1 yr.
NCT01419834	Dose finding and tolerability of hu3F8 alone	Memorial Sloan Kettering Cancer Center	hu3F8	1	Recurrent/ refractory neuroblastoma	1 yr.
NCT03033303	Efficacy of hu3F8, GM-CSF, isotretinoin for consolidation of high-risk neuroblastoma in first remission	Memorial Sloan Kettering Cancer Center	hu3F8, GM-CSF, isotretinoin	2	Neuroblastoma in first remission	All ages
NCT01857934	Efficacy of two courses of cyclophosphamide and topotecan combined with hu14.18K322A in induction chemotherapy	St. Jude	hu14.18K322A	2	New diagnosis neuroblastoma	18 yrs.
NCT02573896	Dose finding and tolerability of autologous NK cells, lenalidomide, and dinutuximab in recurrent/ refractory neuroblastoma	NANT	NK cells, lenalidomide, and dinutuximab	1	Recurrent/ refractory neuroblastoma	1 mo. – 30 yrs.
NCT01576692	Feasibility and tolerability of hu14.18K322A with allogeneic NK cells in recurrent/ refractory neuroblastoma	St. Jude	NK cells and hu14.18K322A	1	Recurrent/ refractory neuroblastoma	21 yrs.
NCT02650648	Feasibility and tolerability of hu3F8, cyclophosphamide, and allogeneic NK cells in recurrent/ refractory neuroblastoma	Memorial Sloan Kettering Cancer Center	NK cells, hu3F8, and cyclophosphamide,	1	Recurrent/ refractory neuroblastoma	All ages
NCT02100891	Efficacy of allogeneic NK cells and haploidentical hematopoietic cell transplantation in recurrent/ refractory solid tumors	Medical College of Wisconsin	NK cells, stem-cell transplant	2	Recurrent/ refractory solid tumors	All ages
NCT02304458	Dose finding and efficacy of nivolumab with or without ipilimumab in recurrent/ refractory solid tumors	COG	nivolumab, ipilimumab	1/2	Recurrent/ refractory solid tumors	1 – 30 yrs.
NCT02332668	Dose finding and efficacy of pembrolizumab in recurrent/ refractory solid tumors	Merck	pembrolizumab	1/2	Recurrent/ refractory solid tumors	6 mo. – 17 yrs.

Trial Identifier	Study question	Sponsor	Study agent(s)	Phase	Disease eligibility	Age Range
NCT00911560	Dose finding and efficacy of a bivalent vaccine, OPT-821, and β -glucan for patients in neuroblastoma patients in remission > 6 months from end of treatment	Memorial Sloan Kettering Cancer Center	OPT-821, immunological adjuvant GD2L and GD3L, β -glucan	1/2	Neuroblastoma in remission	21 yrs.
NCT02745756	Feasibility and tolerability of a tumor lysate dendritic cell vaccine	H. Lee Moffitt Cancer Center and Research Institute	Dendritic cell vaccine	Pilot	All neuroblastoma patients undergoing autologous stem cell transplant and surgery	21 yrs.

Abbreviations: COG; Children's Oncology Group, SIOPEN; International Society of Pediatric Oncology Europe Neuroblastoma, NANT; New Advances in Neuroblastoma, IL-2; Interleukin-2, GM-CSF; Granulocyte macrophage colony-stimulating factor, NK; Natural killer

Table 3
Description of clinical trials currently available using biologic agents for patients with high-risk neuroblastoma

Trial Identifier	Study question	Sponsor	Study agent(s)	Phase	Disease eligibility	Age Range
NCT01711554	Dose finding and tolerability of lenalidomide, dinutuximab, with or without isotretinoin in recurrent/refractory neuroblastoma	NANT	lenalidomide, dinutuximab, isotretinoin	1	Recurrent/ refractory neuroblastoma	21 yrs.
NCT02308527	Efficacy of bevacizumab and chemotherapy in recurrent/ refractory neuroblastoma	University of Birmingham	bevacizumab, temozolomide, irinotecan, and topotecan	2	Recurrent/ refractory neuroblastoma	1 – 21 yrs.
NCT02298348	Dose finding and tolerability of sorafenib, topotecan, and cyclophosphamide in recurrent/refractory neuroblastoma	NANT	sorafenib, cyclophosphamide, and topotecan	1	Recurrent/ refractory neuroblastoma	30 yrs.

Abbreviations: NANT; New Advances in Neuroblastoma

Table 4
Description of clinical trials currently available using molecularly targeted agents for patients with high-risk neuroblastoma

Trial Identifier	Study question	Sponsor	Study agent(s)	Phase	Disease eligibility	Age Range
NCT01606878	Dose finding and tolerability of crizotinib and chemotherapy in recurrent/ refractory solid tumors	COG	crizotinib	1	Recurrent/ refractory solid tumors	1 – 21 yrs.
NCT01742286	Dose finding and tolerability of ceritinib in recurrent/ refractory solid tumors	Novartis	ceritinib	1	Recurrent/ refractory solid tumors	1 – 17 yrs.
NCT02650401	Dose finding and tolerability of entrectinib in recurrent/ refractory solid tumors	Ignity Inc.	entrectinib (RXDX-101)	1/1b	Recurrent/ refractory solid tumors	2 – 22 yrs.
NCT02124772	Dose finding and efficacy of trametinib in recurrent/ refractory solid tumors	GlaxoSmithKline	trametinib	1/2	Recurrent/ refractory solid tumors	1 mo. – 17 yrs.
NCT02337309	Dose finding and tolerability of SF1126 in recurrent/ refractory solid tumors	NANT	SF1126	1	Recurrent/ refractory neuroblastoma	1 – 21 yrs.
NCT01587703	Dose finding and efficacy of I-BET762 in NUT midline carcinoma or recurrent/ refractory solid tumors with <i>MYCN</i> amplification	GlaxoSmithKline	I-BET762 (GSK525762)	1/2	NUT midline carcinoma or recurrent/ refractory <i>MYCN</i> -amplified solid tumors	16 yrs.
NCT02030964	Dose finding and tolerability of DFMO in combination with celecoxib, cyclophosphamide, and topotecan in recurrent/ refractory neuroblastoma	NANT	DFMO, celecoxib, cyclophosphamide, topotecan	1	Recurrent/ refractory neuroblastoma	2 – 30 yrs.
NCT02808650	Dose finding and tolerability of prexasertib in recurrent/ refractory solid tumors	COG	prexasertib (LY2606368)	1	Recurrent/ refractory solid tumors	1 – 21 yrs.
NCT02095132	Dose finding and efficacy of AZD1775 in recurrent/ refractory solid tumors	COG	AZD1775	1/2	Recurrent/ refractory solid tumors	1 – 21 yrs.
NCT01747876	Dose finding and tolerability of ribociclib in recurrent/ refractory malignant rhabdoid tumors and neuroblastoma	Novartis	ribociclib	1	Recurrent/ refractory malignant rhabdoid tumors and neuroblastoma	1 – 21 yrs.
NCT01971476	Dose finding and tolerability of volasertib in recurrent/ refractory solid tumors	Boehringer-Ingelheim	volasertib (BI-6727)	1	Recurrent/ refractory solid tumors	2 – 17 yrs.

Abbreviations: COG; Children's Oncology Group, NANT; New Advances in Neuroblastoma

Table 5
Description of clinical trials currently available using genomic guided therapy for patients with high-risk neuroblastoma

Trial Identifier	Study question	Sponsor	Study agent(s)	Phase	Disease eligibility	Age Range
NCT02868268	Identify targetable genomic alterations in ALK, MAPK pathway, metabolism, and immunologic mechanisms in recurrent/refractory neuroblastoma	NANT	Recommendations made from genomic results	2	Recurrent/ refractory neuroblastoma	1 – 30 yrs.
NCT02520713	Identify targetable alterations using genome wide sequencing approaches in recurrent/refractory solid tumors	Dana-Farber Cancer Institute	Recommendations made from genomic results	2	Recurrent/ refractory solid tumors	30 yrs.
NCT02465060	Identify targetable alterations using genomic approaches in recurrent/ refractory tumors	NCI	24 unique treatment arms	2	Recurrent/ refractory solid tumors	18 yrs.
NCT02693535	Identify targetable alterations using genomic approaches in recurrent/ refractory tumors	ASCO	15 unique treatment arms	2	Recurrent/ refractory solid tumors	18 yrs.
NCT02780128	Identify targetable genomic alterations in ALK, MAPK pathway, and p53 related mechanisms in recurrent/ refractory neuroblastoma	Children's Hospital of Philadelphia	3 unique treatment arms	2	Recurrent/ refractory neuroblastoma	1 – 21 yrs.

Abbreviations: NANT; New Advances in Neuroblastoma, NCI; National Cancer Institute, ASCO; American Society of Clinical Oncology