Current Pharmacology Reports (2023) 9:43–58
https://doi.org/10.1007/s40495-022-00306-8

NANODRUGS (ATY LAU, SECTION EDITOR)

Promising Molecular Targets and Novel Therapeutic Approaches in Neuroblastoma

Xu Yang[1] · Jixia Li[2,3] · Jigang Yang[1]

Accepted: 31 August 2022 / Published online: 31 December 2022

Abstract

Purpose of Review This article provides a brief and up-to-date overview of promising molecular targets and novel therapeutic approaches in neuroblastoma (NB).

Recent Findings High-risk NB is hard to manage with existing treatment modalities, so more than half of those cases are unable to achieve long-term survival. With a deep understanding of molecular pathogenesis, numerous therapeutic targets have been discovered, offering a wide range of novel strategies to treat high-risk NB. Several molecular targets or pathways of NB are well studied, such as GD2, *MYCN*, ALK, p53/MDM2, PI3K/Akt/mTOR/, and RAS/MAPK signaling. Novel targeted drugs and combined therapies are being developed and investigated for treating high-risk NB in preclinical and clinical trials. Considering different NB patients respond to molecular-guided therapy and conventional therapy differently, how to design an effective personalized therapy remains a big challenge.

Summary Anti-GD2 monoclonal antibodies have been approved to treat high-risk NB. Inhibitors targeting *MYCN*, ALK, p53/MDM2, RAS/MAPK, and PI3K/Akt/mTOR are being tested in phase I/II clinical trials. However, most research on molecularly targeted therapy stays at the preclinical level. More valuable targets need to be identified, and more efficient therapies need to be developed. Further, exploration of new combinations using inhibitors targeting multiple targets and conventional therapy is still the most important research direction in future, which would advance treatment regimens, improve outcomes, and prolong survival in children with high-risk NB.

Keywords Neuroblastoma · GD2 · *MYCN* · ALK · Targeted therapy · Signaling pathway

Introduction

Neuroblastoma (NB) is an embryonal tumor arising from the adrenal medulla and the sympathetic nervous system. This disorder is the most common extracranial solid tumor of childhood, and 90% of the cases occur in young children before the age of 5 years, particularly in infants (under 1 year old) [1]. The incidence is 10.7 cases per million persons aged 0–14 years, with approximately 700–800 new patients reported annually in the USA [1, 2]. The median age at presentation is 22–23 months, and less than 10% of the cases are diagnosed after the age of 5 years [1, 2]. Indeed, it rarely presents in adolescence and adulthood, but this age population has a worse prognosis [2]. NB accounts for 8–10% of all pediatric malignancies and is responsible for 15% of all cancer-related mortality in children [3]. It is worth noting that NB is highly heterogeneous in terms of pathogenesis, clinical presentation, and prognosis. NB is considered as a spectrum of diseases other than a single pathological condition, as no solo molecular aberration contributes to all patients [4••]. Based on a number of prognostic factors, such as age at onset, histological category, grade of tumor differentiation, INRG (International Neuroblastoma Risk Group) stage, *MYCN* (MYCN proto-oncogene, bHLH

This article is part of the Topical Collection on *Nanodrugs*

✉ Jixia Li
j.li@auckland.ac.nz

✉ Jigang Yang
yangjigang@ccmu.edu.cn

[1] Department of Nuclear Medicine, Beijing Friendship Hospital, Capital Medical University, Beijing, China

[2] Department of Laboratory Medicine, School of Medicine, Foshan University, Foshan, China

[3] Blood and Cancer Biology Laboratory, Department of Molecular Medicine and Pathology, The University of Auckland, Auckland, New Zealand

(basic helix-loop-helix) transcription factor) amplification, 11q aberration, and DNA ploidy, this disease can be divided into four categories: very low-, low-, intermediate-, and high-risk [5]. Remarkably, high-risk patients, accounting for around 50% of all NB cases, have the worst prognosis, with the 5-year event-free survival (EFS) of 51% [6, 7•]. In contrast, the outcome of low-risk or intermediate-risk cases is much better with the 5-year EFS of larger than 85% [7•, 8]. Conventional treatments for NB include surgical tumor resection, systemic chemotherapy, radiation therapy, 13-cis retinoic acid (isotretinoin) differentiation induction therapy, autologous hematopoietic stem cell transplantation, and immunotherapy. However, even with intensive multimodal therapeutics, approximately 60% of high-risk patients eventually relapsed [9]. Up to 20% of children with high-risk NB have suboptimal responses to induction therapy because of drug resistance [10]. Furthermore, the 5-year overall survival (OS) of cases at INSS (International Neuroblastoma Staging System) stage 4 is only about 42% [8]. Thus, exploring potential targets and developing novel treatments are extremely important to improve clinical outcome for high-risk NB.

In recent years, with a deep understanding of molecular etiology of NB, therapeutic markers have been discovered, and targeted drugs have been being examined in clinical trials. Here, we focused on several well-studied molecular targets and relevant targeting therapeutics, ranging from disialoganglioside (GD2), *MYCN* and anaplastic lymphoma kinase (ALK) to p53/ mouse double minute 2 homolog (MDM2), phosphoinositide 3-kinases (PI3K)/protein kinase B (PKB, also known as Akt)/mammalian target of rapamycin (mTOR), and rat sarcoma virus (RAS)/mitogen-activated protein kinase (MAPK) (Table 1), providing a brief and up-to-date summary of such findings in this review.

Targeting GD2

GD2, a surface ganglioside, is one of the distinctive tumor-associated carbohydrate antigens [33••, 34]. GD2 overexpression is associated with poor prognosis and recurrence in NB, which controls survival, apoptosis, and microenvironment in cancer cells (Fig. 1) [33••, 34, 35••, 36•, 37]. It is well documented that GD2 can promote tumor cell survival through activation of multiple signaling pathways including *MYCN*/aurora kinase (AURKA), AKT/mTOR/eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1), 70-kDa ribosomal protein S6 (p70-S6) kinase, and AMP-activated protein kinase (AMPK) β subunits [35••]. GD2 is also able to modulate p53, pleckstrin homology–like domain family A member 1 (PHLDA1), and caspase 9, leading to apoptotic inhibition in NB [35••]. Further, GD2 reduces the gene expression of *PHLDA1*, supervillin (*SVIL*), ras-association domain family protein 6 (*RASSF6*), and *JUN* (Jun proto-oncogene, AP-1 transcription factor subunit), but upregulates the gene expression of inhibitor of DNA binding 1 (*ID1*), T cell leukemia homeobox 2 (*TLX2*), and cyclin-dependent kinase inhibitor 1A (*CDKN1A*), which may cause abnormal cell differentiation and RNA metabolic processes in tumor [36•]. It is known that GD2 can be released from tumor cells into the circulation or the microenvironment, thereby promoting T cell apoptosis and suppressing T cell proliferation, as well as blocking CD34 + cells maturation into dendritic cells [35••]. GD2 may also mediate metastases by enhancing focal adhesion kinase phosphorylation [37]. However, the detailed molecular mechanisms by which GD2 serves these biological functions have not been fully elucidated.

GD2 is an exciting, non-protein target for immunotherapy, as it is highly expressed on the surface of NB cells, with a relatively low expression level in normal tissues (e.g., neurons, skin melanocytes, and peripheral nerve fibers), and all NB cases express GD2 virtually despite grading and staging of tumor [4••, 38, 39]. Here, we mainly discuss anti-GD2 monoclonal antibody therapy and anti-GD2 CAR T cell therapy.

Anti-GD2 monoclonal antibody therapy is the most widely used immunotherapy for NB in recent years. Importantly, anti-GD2 monoclonal antibodies have different types including murine, chimeric, or humanized antibodies, which are extensively investigated in preclinical and clinical trials. Those novel drugs are currently used in the disease management, such as chemotherapy induction and retinoic acid maintenance. For example, dinutuximab (ch14.18) and dinutuximab beta (ch14.18/CHO), chimeric antibodies containing murine anti-GD2 variable regions of IgG3 and human constant regions of IgG1, have been approved to treat high-risk NB by the US Food and Drug Administration (FDA) in 2015 and by the European Medicines Agency (EMA) in 2017, respectively [11, 12]. In addition, a number of humanized monoclonal antibodies with low immunogenicity, including Hu14.18K322A and naxitamab, are being evaluated in clinical trials [14•, 40]. Notably, naxitamab has been approved to treat relapsed or refractory high-risk NB children with the age older than 1 year by the FDA in November 2020 [13•].

Given the biological roles of GD2 are poorly understood, the mechanism of anti-GD2 monoclonal antibodies against NB is still not clear. However, emerging evidence highlighted that anti-GD2 monoclonal antibodies may regulate immune system as well as survival, apoptosis, and invasion of tumor cells (Fig. 1) [41, 42•, 43, 44]. By way of illustration, the monoclonal antibodies binding to the highly expressed GD2 in cells can trigger dual immune responses that are antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity

Table 1 New molecular targeting therapeutics for NB in clinical trials

Therapies	Mechanism of action	Current drug developmental stage in NB	References
Antibodies			
Dinutuximab (ch14.18)	Human-murine chimeric mAb targeting GD2	FDA-approved for high-risk NB	[11, 12]
Dinutuximab beta	Human-murine chimeric mAb targeting GD2	EMA-approved for high-risk NB	[12]
Naxitamab (hu3F8, Danyelza, naxitamab-gqgk)	Humanized mAb targeting GD2	FDA-approved for r/r high-risk NB in the bone or bone marrow > 1 year of age	[13•]
Hu14.18K322A	Humanized mAb targeting GD2	Phase II trial for high-risk NB (NCT01857934)	[14•, 15]
Hu14.18-IL2	Humanized anti-GD2 mAb conjugated to IL-2	Completed phase II trial for r/r NB (NCT00082758) (NCT01334515)	[16]
Chimeric antigen receptor T cells (CAR-T)			
GD2-targeting CAR-T	T cells genetically modified to target GD2	Phase I/II trial for r/r NB (NCT03373097) (NCT02761915)	[17•, 18]
Inhibitors			
Alisertib (MLN8237)	Aurora A kinase inhibitor	Completed phase II trial for r/r NB (NCT01601535)	[19]
LY3295668 Erbumine	Aurora A kinase inhibitor	Phase I trial for r/r NB (NCT04106219)	[20••]
BMS-986158 BMS-986378	BET inhibitor	Phase I trial for NB (NCT03936465)	[21]
Difluoromethylornithine (DFMO, Eflornithine)	ODC1 inhibitor	Phase II trial for high-risk NB in remission or r/r NB (NCT02395666)	[22•]
Ribociclib	CDK4/6 inhibitor	Phase II trial for r/r NB (NCT05429502) (NCT01747876)	[23]
Crizotinib	First-generation ALK inhibitor	Completed phase II trial for r/r NB (NCT00939770)	[24•]
Ceritinib	Second-generation ALK inhibitor	Phase II trial for high-risk NB (NCT02559778) (NCT01742286)	[24•, 25]
Lorlatinib	Third-generation ALK inhibitor	Phase III trial for high-risk NB (NCT03126916)	[26, 27]
Idasanutlin	Small-molecule antagonist of MDM2	Phase I/II trial for r/r NB (NCT04029688)	[28]
Perifosine	AKT inhibitor	Completed phase I trial for r/r NB (NCT00776867)	[29•]
Temsirolimus	mTOR inhibitor	Phase II trial for r/r NB (NCT01767194)	[30]
Trametinib	MEK 1/2 inhibitor	Completed phase II trial for high-risk NB (NCT02124772)	[31•]
Regorafenib (BAY73-4506)	Multi-kinase inhibitor of the RAS-MAPK, PI3K/AKT/mTOR, and Fos/Jun pathways	Phase I trial for r/r NB (NCT02085148)	[32]

NB, neuroblastoma; *FDA*, Food and Drug Administration; *EMA*, European Medicines Agency; *mAb*, monoclonal antibody; r/r, relapsed/refractory; *BET*, bromodomain and extra-terminal domain; *ODC1*, ornithine decarboxylase 1; *CDK*, cyclin-dependent kinase; *ALK*, anaplastic lymphoma kinase; *MDM2*, murine double minute 2; *AKT*, protein kinase B; *mTOR*, mammalian target of rapamycin; *MEK*, mitogen-activated protein

(CDC) [41]. Subsequently, ADCC and CDC perform an anti-tumor function [41]. This type of antibodies can also restore immune activation through the block of immune checkpoints [44]. Intriguingly, the anti-GD2 monoclonal antibodies are able to directly regulate survival, invasion, and apoptosis by targeting multiple molecules or pathways, such as PI3K/Akt/mTOR and MYCN/AURKA, which are not associated with immune mechanisms [43].

To improve the efficacy of anti-GD2 monoclonal antibody therapy, the combination of antibodies and cytokines has been explored recently. Data suggested that granulocyte–macrophage colony–stimulating factor (GM-CSF) and interleukin-2 (IL-2) in cooperation with dinutuximab could enhance ADCC with IL-2 promoting NK cells and monocytes and GM-CSF stimulating neutrophils and macrophages [11]. Meanwhile, this regimen may also reduce

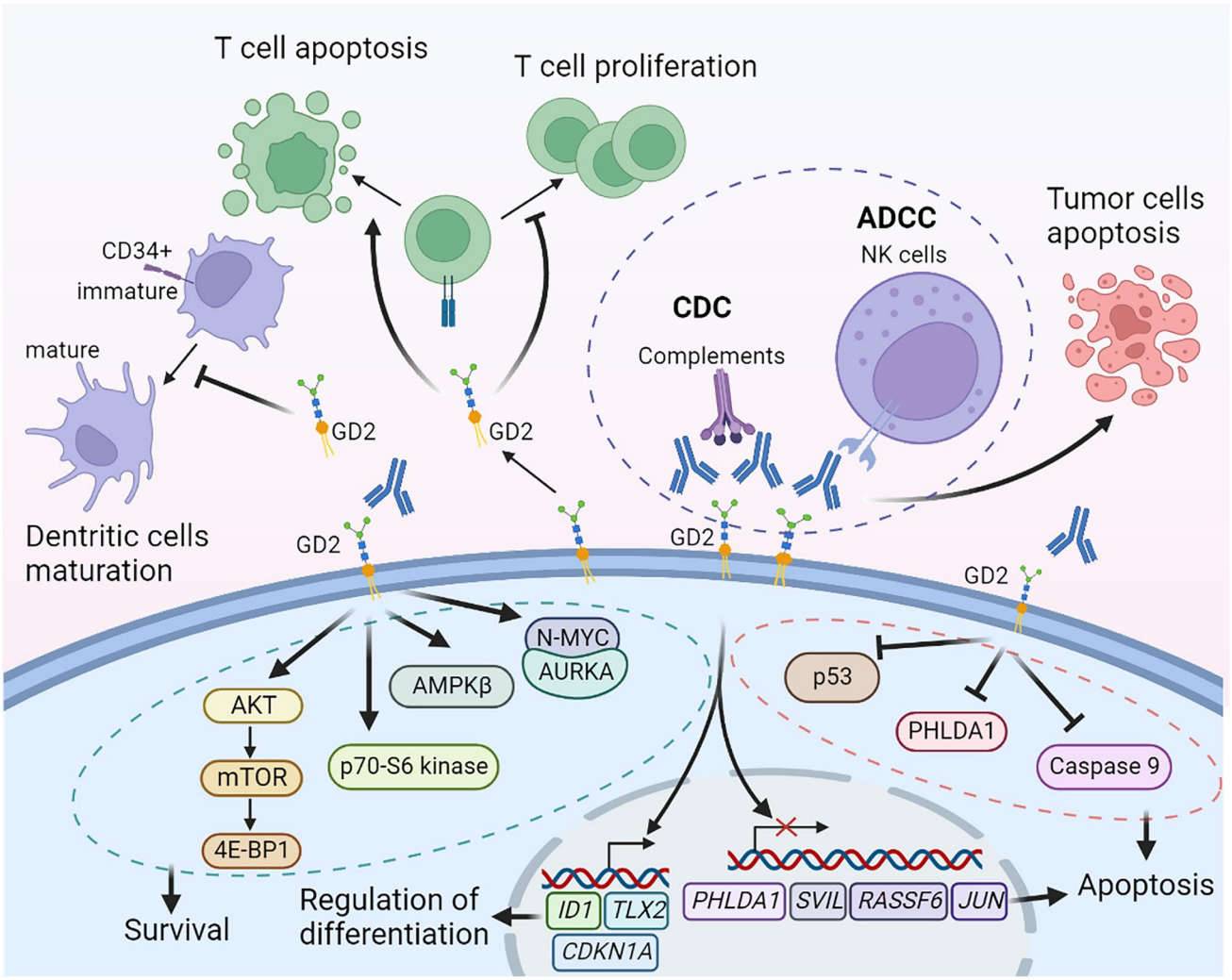

Fig. 1 The functions of GD2 in NB. GD2 overexpression increases the levels of N-MYC, AKT, mTOR, 4E-BP1, p70-S6 kinase, and AMPKβ subunits, consequently resulting in enhanced survival in NB. Meanwhile, high GD2 expression downregulates the levels of p53, PHLDA1, and caspase 9, leading to apoptotic inhibition. GD2 also modulates *PHLDA1*, *SVIL*, *RASSF6*, *JUN*, *ID1*, *TLX2*, and *CDKN1A* gene expression, which causes abnormal cell differentiation and RNA metabolic processes in tumor. In addition, GD2 can be released from tumor cells into the circulation or the microenvironment, which suppresses T cell proliferation, induces T cell apoptosis, and blocks CD34 + cells maturation into dendritic cells. Anti-GD2 monoclonal antibodies binding to GD2 triggers the activation of ADCC and CDC, subsequently resulting in apoptosis and cytolysis. NB, neuroblastoma; AKT, protein kinase B; mTOR, mammalian target of rapamycin; 4E-BP1, eukaryotic translation initiation factor 4E (eIF4E)-binding protein 1; AMPK, AMP-activated protein kinase; PHLDA1, pleckstrin homology-like domain family A member 1; ADCC, antibody-dependent cell-mediated cytotoxicity; CDC, complement-dependent cytotoxicity

the dose and the toxicity of monoclonal antibody against GD2 [11]. One randomized phase III trial (NCT00026312) demonstrated that the synergy of dinutuximab with IL-2/GM-CSF improves the survival of high-risk NB, compared to single drug treatments [45•]. Nonetheless, the multicenter phase III trial (NCT01704716) in 2018 showed that subcutaneous IL-2 does not increase the efficacy of dinutuximab beta for treating high-risk NB [46]. Another phase II trial (NCT01334515) uses the hu14.18-IL2 (the humanized 14.18 anti-GD2 monoclonal antibody linked to IL2) in combination with GM-CSF and isotretinoin to treat patients with refractory/relapsed NB, suggesting this combination strategy may be tolerable and suitable for cases with non-bulky disease [16].

Regarding the adverse side effect of anti-GD2 monoclonal antibodies, neuropathic pain is the most significant. This is mainly because the complement is activated when monoclonal antibodies bind to GD2-expressed peripheral nerve fibers. A few novel antibodies against GD2 have been designed to reduce neuropathic pain. For instance, Hu14.18K332A with FC mutations decreases complement fixation and lowers allodynia [47]. Another antibody drug (8B6 monoclonal

antibody) highly selectively binds to O-acetyl GD2, and does not cause neuropathic pain, as O-acetyl GD2, a derivative of GD2, is expressed together with GD2 but not expressed in peripheral nerves [48]. Although advances have been made, more studies are required to evaluate the efficacy, the toxicity, and the side effect of these newly developed drugs in NB.

Engineered chimeric antigen receptor (CAR) T cell therapy is another potent targeted GD2 therapy, which integrates the specificity of an antibody with the cytolytic capacity of T cells [49]. The anti-GD2 CAR T cells have advantages, such as increasing potency and crossing the blood–brain barrier, in comparison with monoclonal antibody therapy [50••]. However, anti-GD2 CAR T cell therapy has the same adverse side effect with antibodies, which is neurotoxicity [49]. Recently, the phase I clinical trial (NCT02761915) showed that anti-GD2 CAR T cell therapy is well tolerated without on-target off-tumor toxicity but does not achieve objective clinical response in children with relapsed/refractory NB [17•, 51••]. Therefore, more efforts should be made on the modification of GD2 CAR T cells to enhance antitumor activity and promote CAR T cell longevity.

Targeting *MYCN*

The *MYCN* gene belongs to the *MYC* proto-oncogene family, encoding a basic helix-loop-helix transcription factor N-MYC. N-MYC, a master regulator of cell fate, interacts with other transcription factors to regulate gene expression (Fig. 2). In normal condition, *MYCN* is predominantly

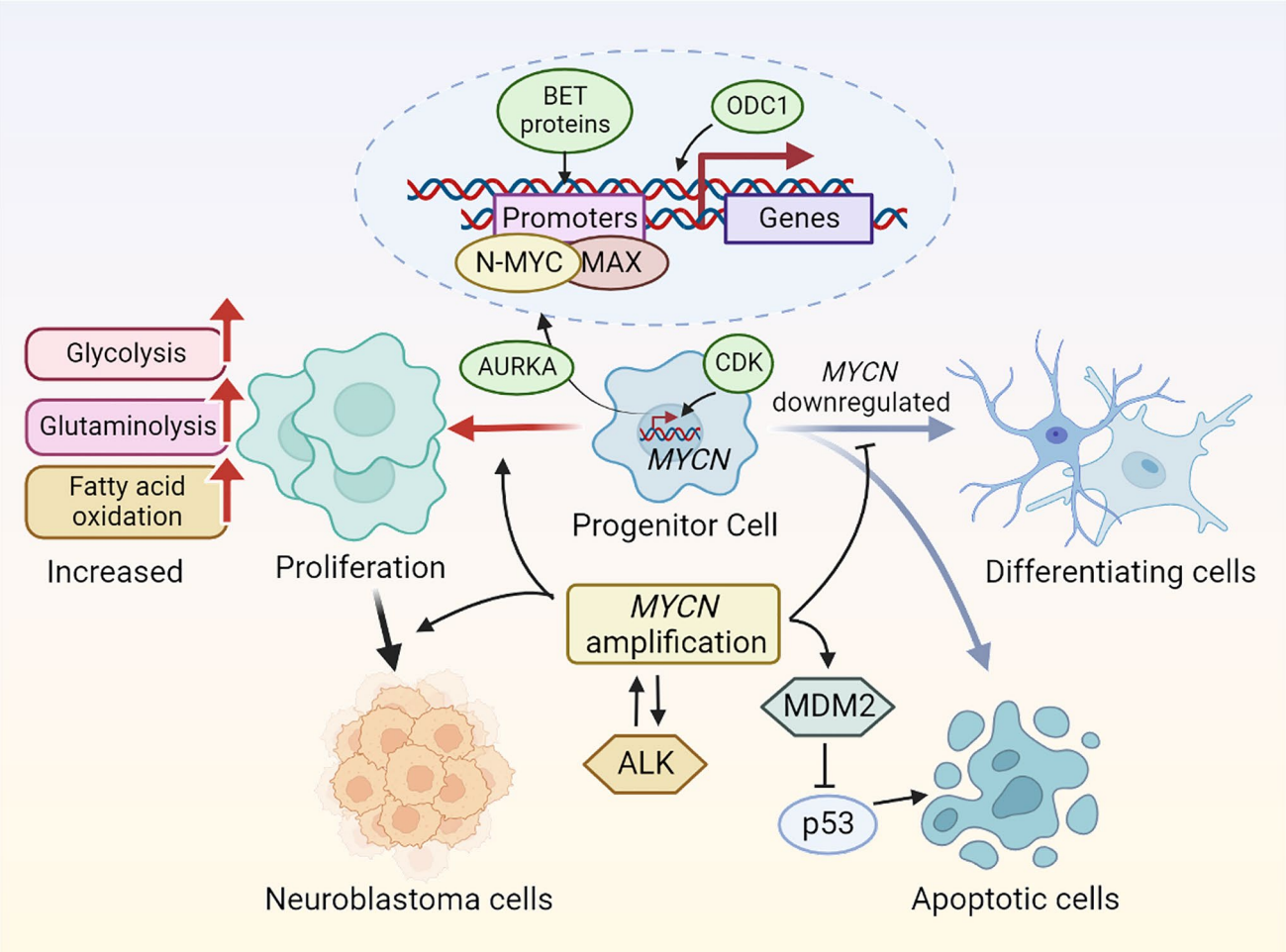

Fig. 2 The functions and regulators of *MYCN* (N-MYC) in NB. *MYCN* amplification strengthen proliferation and block differentiation, accompanied with an increase in glycolysis, fatty acid oxidation and glutaminolysis in NB. *MYCN* amplification also represses p53-induced apoptosis by increasing *MDM2* gene transcription. The crosstalk of *MYCN* with ALK is critical for disease initiation and progression. Key regulators of *MYCN* transcription, N-MYC degradation and function include CDKs, AURKA, BET, ODC1 and N-MYC/MAX interactions. Targeting those regulators is an important method to treat *MYCN*-amplificated cancer. MDM2, murine double minute 2; CDKs, Cyclin-dependent kinases; AURKA, Aurora A kinase; MAX, MYC-associated factor X; BET, Bromodomain and extra-terminal domain; ODC1, ornithine decarboxylase 1

expressed in hematopoietic stem cells and cells within the developing nervous system. By contrast, gene amplification of *MYCN* has been observed in 20–30% of NB patients with a dismal prognosis [9]. Regardless of age and INSS stage, *MYCN* amplification is thought to be an independent poor prognostic factor [52••]. The biological functions of N-MYC have been implicated in various cellular processes including proliferation, differentiation, apoptosis, metabolism, and maintenance of stem cell (Fig. 2) [53, 54, 55]. N-MYC overexpression results in uncontrolled cell proliferation, apoptosis inhibition, and differentiation arrest in NB. *MYCN* gene amplification can modulate tumor microenvironment and influence immune response by regulating cytokines [53, 56]. Additionally, *MYCN* amplification or N-MYC overexpression is one of the earliest molecular abnormalities identified in NB, which is closely associated with stage, aggressiveness, and prognosis of tumor [53]. Considering its important roles in NB pathogenesis, N-MYC has become an attractive target for curing this disorder.

Currently, there is no specific small molecular drug available for directly targeting N-MYC, since it acts as a general transcription factor in normal and tumor cells and its protein structure lacks enzymatic pocket for drug binding. Thus, approaches to target N-MYC mostly consist of two strategies: (1) downregulation of N-MYC expression by targeting its transcription, translation, and degradation; (2) impairment of N-MYC functions by targeting N-MYC/MYC-associated factor x (Max) interactions or other molecules. Here, a few simplified examples are presented for those strategies.

Exploring the inhibitors of bromodomain and extra-terminal domain (BET) and cyclin-dependent kinases (CDKs) to treat NB is an example of targeting *MYCN* transcription. The BET family recruits transcriptional regulatory complexes to acetylate chromatin, thereby regulating the transcription of *MYCN* [57]. BET inhibitors JQ1 and GSK1324726A (I-BET726) were firstly found to have the therapeutic effects on NB in xenograft mouse models [57, 58]. MZ1, a novel BET inhibitor, utilizes proteolytic-targeting chimera (PROTAC) technology to facilitate BET proteasomal degradation, resulting in a rapid and effective inhibition of *MYCN*-amplified NB in vitro and in vivo [59]. Another two BET inhibitors, BMS-986158 and BMS-986378, have entered a phase I clinical trial (NCT03936465) for treating NB with no data reported yet [21]. Moreover, CDKs are oncogenic drivers through regulating *MYCN* transcription [60•, 61]. The CDK2/9 inhibitor fadraciclib has been shown to repress growth and promote apoptosis in *MYCN*-amplified NB through *MYCN* inhibition in a preclinical study [61]. Importantly, the combination of CDK7 inhibitor YKL-5–124 and BET inhibitor JQ1 showed a synergistic effect in xenograft mice with NB [62]. Current studies about BET inhibitors and CDKs inhibitors for curing NB maintain in the preclinical stage, so further drug testing on animal models and clinical trials is required.

Targeting protein stability is another method to decrease N-MYC expression. In normal cells, N-MYC is precisely controlled by the ubiquitin–proteasome system (UPS) with a short half-life (less than 30 min) [63]. AURKA is considered as one of the negative regulators to control N-MYC degradation in NB, which means N-MYC degradation can be increased by AURKA inhibition [63]. A recent study demonstrated that one AURKA inhibitor MLN8237 (alisertib) induces cell senescence, blocks cell cycle arrest at G2/M and accelerates N-MYC degradation in NB cell line IMR32 [64]. Further, the tolerability and the anti-tumor activity of alisertib combined with irinotecan and temozolomide were evaluated by phases 1 and 2 clinical trials (NCT01601535), highlighting the potency of this regimen for managing NB [19]. Another AURKA inhibitor LY3295668 (erbumine) is being tested for treating relapsed/refractory NB, with the primary aim of assessing safety and tolerability and anti-tumor activity (phase I clinical trial NCT04106219) [20••]. Notably, PHA-680626, a novel small molecule inhibitor targeting interactions between AURKA and N-MYC, is designed to selectively decrease N-MYC protein level, and not to impair other functions of AURKA (e.g., cell cycle and mitosis). This inhibitor is currently being trialed at preclinical level [65••].

Targeting N-MYC/MYC associated factor X (Max) interplays is one of the first strategies to suppress NB development and progression. N-MYC forms heterodimers with MAX, which is essential for N-MYC to properly function as a transcription factor [66••]. For example, 10,058-F4 and 10,074-G5, small molecular drugs, are known to inhibit the N-MYC/MAX interaction, consequently inducing differentiation and apoptosis in *MYCN*-amplified NB cells [67]. Recently, the small molecule MYCMI-6 with a high affinity for MYC is reported to selectively inhibit the tumor growth in *MYCN*-amplified cell and animal models, and this compound has no cytotoxic effect on normal cells [68]. Remarkably, MYCI975 has multiple roles against NB rather than disrupting N-MYC heterodimerization with MAX, including enhancement of N-MYC phosphorylation, promotion of N-MYC degradation, and sensitization of anti-programmed cell death protein 1 (PD1) immunotherapy [69]. It is worth noting that omomyc, a dominant-negative form of MYC, binds to MAX to inhibit MYC-mediated gene transcription, because MYC/MAX heterodimers are replaced by omomyc/MAX heterodimers [70]. However, the anti-tumor activity of omomyc in NB has not been assessed [71].

Impairment of N-MYC function by targeting other molecules is also employed to treat NB. By way of explanation, the biological function of N-MYC depends on polyamine homeostasis [72]. Hence, targeting ornithine decarboxylase 1 (ODC1) is another method to disrupt N-MYC functions,

as this enzyme is a rate-limiting enzyme in polyamine synthesis. Difluoromethylornithine (DFMO), an inhibitor of ODC1, was initially reported to impede cell proliferation, block tumor initiation, and enhance chemotherapeutic efficacy in NB cells [73]. The data from a phase II clinical study (NCT02395666) showed DFMO maintenance after completion of standard therapy has a positive association with improved outcomes in high-risk NB, with 5-year EFS and 5-year OS being 85.2% and 95.1%, respectively [22•]. Remarkably, the integration of DFMO and AMXT 1501 (a polyamine transport inhibitor) significantly reduces tumor progression and prolongs survival in vitro and in vivo, suggesting incorporation of polyamine synthesis and uptake inhibitors with standard chemotherapy could be a new therapeutic approach [74].

Taken together, numerous drugs that impede *MYCN* directly and indirectly are being intensively investigated at preclinical and clinical levels, from monotherapy to combination therapy. These indicate that *MYCN*-targeted strategies are very promising to be incorporated into the clinical management of NB in future.

Targeting ALK

ALK, a tyrosine kinase receptor, has an important role in the initiation and progression of malignancies including NB. ALK is the second most common oncogenic driver with its mutations being correlated with familial NB, which accounts for 1–2% of all NB cases [75]. In sporadic NB, approximately 9–14% of patients carry *ALK* mutations or amplifications [76]. *ALK* mutations within the tyrosine kinase domain often occur in NB, among which the most common variants are Arg1275Gln, Phe1174Leu, and Phe1245Cys. The mutated ALK leads to ligand-independent autophosphorylation and activation of ALK, which consequently activates RAS/MAPK and PI3K/Akt/mTOR pathways (Fig. 3) [77••]. It is noted that hyperactivated ALK in NB contributes to proliferation, migration, and recurrence [78]. More importantly, *ALK* and *MYCN* have a close relationship (Figs. 2 and 3), since they are adjacently located on chromosome 2, at 2p23 and 2p24, respectively [79]. Further, *ALK* and *MYCN* are generally co-amplified [80], and *ALK* mutations are more frequently observed in *MYCN*-amplification positive NB [81]. Both ALK and *MYCN* have a correlation with a poor prognosis and interact with each other in a synergic manner. *MYCN* can induce ALK activation, which in turn enhances *MYCN* transcription and stability; however, the development of NB may require both activated proteins [82]. *MYCN* expression is also positively linked with ALK expression in *MYCN* non-amplified NB patients [83•]. Nevertheless, the elevated ALK expression may be found in cases without *ALK* mutations as well, which indicates a grim prognosis [84]. Briefly, ALK is a crucial therapeutic target, particularly for high-risk NB with *MYCN* amplification.

To date, there are two types of drugs for targeting ALK that are small molecular inhibitors and monoclonal antibodies. ALK small molecular inhibitors have been well developed to treat a range of cancers over the past years, while monoclonal antibodies against ALK are still largely unknown. The first-generation ALK inhibitor crizotinib competitively binds to the receptor tyrosine kinases. Treatment of ALK-positive cancers with crizotinib achieved a prolonged survival, in comparison with chemotherapy [85]. This agent has been approved to treat ALK or ROS1-positive non-small cell lung cancer (NSCLC), relapsed or refractory systemic anaplastic large cell lymphoma (ALCL), and ALK-positive inflammatory myofibroblastic by the FDA in 2016, 2021, and 2022, respectively [85, 86, 87]. In 2021, the third-generation ALK inhibitor lorlatinib has been approved to be the first-line therapy for metastatic ALK-positive NSCLC [85]. Regarding the efficiency of ALK inhibitors in NB, the response rate is low in clinical trials, although the inhibitors exert a good anti-tumor activity in tumor cell lines and xenograft mice [24•, 25, 88]. For example, a phase II clinical study (NCT00939770) found only three of twenty ALK-aberrant patients with relapsed/refractory NB effectively respond to crizotinib [24•]. Ceritinib is a second-generation ALK inhibitor and 20% of NB cases ($n = 30$) respond to this drug in a clinical study (NCT01742286) [25]. Lorlatinib (PF-06463922), a third-generation ALK inhibitor, has been demonstrated to be more efficient for treating ALK-driven NB than crizotinib [26, 89]. In a preclinical study, lorlatinib (ALK/ROS1 inhibitor) shows ALK inhibition and induces tumor regression in crizotinib-resistant and crizotinib-sensitive xenograft mice as well as in patient-derived xenografts carrying the crizotinib-resistant Phe1174Leu and Phe1245Cys mutations [89]. Another case study showed that one patient with relapsed refractory, ALK Phe1174Leu-mutated NB had a complete response to lorlatinib, but the disease recurred after 13 months of treatment [26]. Typically, ALK inhibitor efficacy partially depends on ALK-mutant sites, as evidenced by the fact that ALK Arg1275Gln mutation is susceptible to crizotinib in NB [24•]. Data about monoclonal antibodies against ALK are rather limited, with an ALK antibody identified to suppress the tumor cell growth and trigger ADCC in human NB–derived cell lines [90].

In summary, there is currently no ALK inhibitors or monoclonal antibodies approved for the treatment of NB by the FDA or the EMA. New findings pointed out that combining chemotherapy with ALK inhibitors could be a potential method to improve the efficacy and the sensitivity to ALK-targeted drugs. More studies should focus on how to improve ALK inhibitors efficacy and avoid resistance and adverse side effects. More anti-ALK monoclonal antibodies should

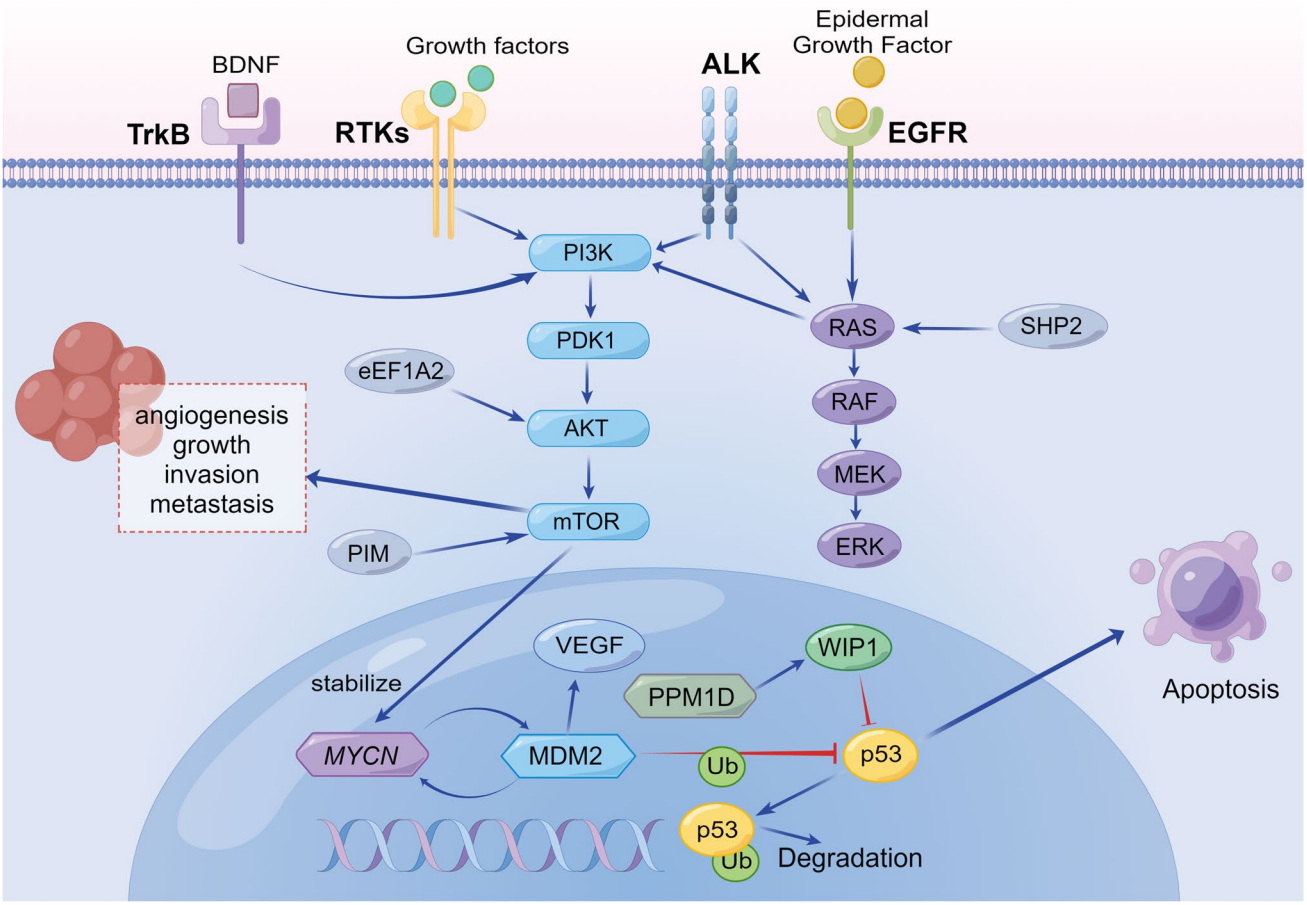

Fig. 3 The functions of ALK and PI3K/AKT/mTOR and RAS/MAPK pathways in NB. ALK activates PI3K/AKT/mTOR and RAS/RAF/MEK/ERK pathways, which regulates growth, proliferation, survival, apoptosis, invasion, metastasis, and angiogenesis in NB. PI3K signaling can also be triggered by TrkB and RTKs, while EGFR can stimulate RAS/MAPK pathway. Of note, a range of modulators can control different sites of these pathways, including eEF1A2, PIM and SHP2. N-MYC or *MYCN* that is regulated by PI3K/AKT/mTOR pathway, can interact with *MDM2*, thus causing aberrant VEGF-mediated angiogenesis and p53-induced apoptosis. (Fig. 3 was generated by Figdraw with permission ID being TYUIWc48a4). ALK, anaplastic lymphoma kinase; PI3K, phosphatidylinositol-3-kinase; AKT, protein kinase B; mTOR, mammalian target of rapamycin; RAS, rat sarcoma; MAPK, mitogen-activated protein kinase; RAF, serine/threonine kinase; MEK, mitogen-activated protein; ERK, extracellular signal-regulated kinase; NB, neuroblastoma; TrkB, tyrosine kinase receptor tropomyosin-related kinase B; RTK, receptor tyrosine kinase; EGFR, epidermal growth factor receptor; eEF1A2, eukaryotic translation elongation factor-1, alpha-2; PIM, proviral insertion site in Moloney murine leukemia virus; SHP2, Src homology-2 domain-containing protein tyrosine phosphatase-2; MDM2, murine double minute 2; VEGF, vascular endothelial growth factor; BDNF, brain-derived neurotrophic factor; Wip1, wild-type p53-induced phosphatase 1, Ub, ubiquitin; WIP1, wild-type p53-induced phosphatase 1

also be further designed, developed, and tested in cell and animal models and clinical trials.

Targeting Intracellular Signaling Transduction Pathways

Several intracellular signaling pathways are involved in the development and the progression of NB, including p53/MDM2, PI3K/Akt/mTOR, and RAS/MAPK (Fig. 3). Nowadays, inhibition of multiple signaling pathways is being extensively explored as a novel and promising therapeutic strategy.

Targeting p53/MDM2 signaling pathway has a great potentiality for curing NB, as this pathway is central for protecting cells against genome instability and malignant transformation. The p53 tumor suppressor, known as the guardian of the genome, is a critical multi-function player that regulates DNA damage and cell cycle, represses cell division, and promotes apoptosis [91••]. *TP53* mutations occur in fewer than 2% of the relapsed or refractory NB cases, which is usually associated with multi-drug resistance [91••, 92•]. Despite *TP53* mutations rarely seen in NB, p53 accumulation with increased stability and MDM2 overexpression induced p53 inhibition have been commonly identified [91••]. MDM2 is an E3 ubiquitin ligase involved

in p53 degradation, and high expression of MDM2 is linked to metastasis, high staging, chemotherapy resistance, and poor prognosis of NB [91••]. Increasing data proved that the involvement of MDM2 in tumor initiation and progression is partially because of MDM2-mediated-p53 reduction [91••]. MDM2 also performs p53-indenpent activities, such as elevation of gene stability and gene transcription [55]. Given the functional wild-type p53 and the crosstalk of MDM2-p53 with *MYCN* in NB, it is prudent to develop inhibitors for directly targeting MDM2-p53 interaction, thus enhancing p53 function [93]. For instance, nutlin-3a is the first-generation inhibitor to block the interaction between MDM2 and p53 and reactivate the p53 activity in tumor cells and xenograft mice [94]. Derived from the structure modification of nutlin-3, the new inhibitor RG7112 is developed with an increased affinity and a decreased toxicity, but it is still not being examined in clinical trials [95]. Another second-generation MDM2 antagonist idasanutlin (RG7388) shows elevated potency, selectivity and bioavailability in preclinical studies and is being investigated in a phase I/II clinical trial (NCT04029688) for the treatment of relapsed or refractory NB [28]. Beyond small molecular antagonists directly disrupting MDM2-p53 interactions, targeting protein modulators to inhibit MDM2 or restore p53 is gaining more and more attention. For example, the serine/threonine phosphatase WIP1 (wild-type p53-induced phosphatase 1), encoded by protein phosphatase, Mg2 + /Mn2 + dependent 1D (*PPM1D*) gene at 17q23, can reduce p53 activation as well as control cell cycle, apoptosis, and DNA repair in NB [96]. WIP1 overexpression links to poor prognosis in NB patients, while inhibition WIP1 decreases survival and growth of tumor cells [96]. Moreover, the WIP1 inhibitor SL-176 has been reported to have similar or stronger effects on tumor growth than nutlin-3 in NB xenograft models [97]. Although disruption of p53/MDM2 is an appealing strategy, treatment-related toxicity and drug resistance have been discovered. Thus, better inhibitors should be designed and developed.

Targeting PI3K/Akt/mTOR pathway has been identified as a promising target because this pathway has a significant role in many cellular processes [29•, 98]. PI3K/Akt/mTOR pathway, the key pro-survival signaling, is activated in most cases of NB, which results in deregulated growth, angiogenesis, invasion, and metastasis [51••, 99]. The aberrant activation of PI3K/AKT/mTOR pathway likely predicts poor prognosis and drug resistance in NB [100, 101]. Further, PI3K/Akt/mTOR pathway has been found to affect *MYCN* amplification and stabilization to enhance the NB phenotype. For example, high Akt phosphorylation correlates with *MYCN* amplification, and in turn, inhibition of Akt causes a decreased N-MYC protein level [100]. Hence, inhibitors directly or indirectly targeting PI3K, Akt or mTOR could be employed to manage NB. For instance, a phase I trial (NCT00776867) found that Akt inhibitor perifosine retards disease progression with no apparent toxicity in 33% of patients with refractory/relapsed NB ($n = 27$) [29•]. PP242, a mTOR inhibitor, reverses hypoxia-induced gene expression profiles that contribute to an unfavorable prognosis in NB [101]. Another mTOR inhibitor temsirolimus has been approved for the treatment of renal cell carcinoma in 2007 and is currently undergoing a phase II trial (NCT01767194) for patients with relapsed or refractory NB [30, 51••]. AZD8055, a dual mTORC1/mTORC2 inhibitor, suppresses growth and induces apoptosis in NB cells and mice [102]. VS-5584, a dual PI3K/mTOR inhibitor, exhibits anti-tumor effects on NB in vitro and in vivo [103]. Targeting modulators of this pathway is also a potential therapeutic approach. For instance, the eukaryotic translation elongation factor-1, alpha-2 (eEF1A2) is considered as an activator of PI3K/AKT/mTOR pathway, and its knockdown leads to an inhibition of Akt/mTOR phosphorylation in NB cells [104]. This finding may highlight a role of eEF1A2 as a new molecular target for NB therapy [104]. Interestingly, the serine/threonine proviral insertion site in murine leukemia virus (PIM) kinases has a correlation with MYC overexpression, PI3K inhibitor resistance, and an unfavorable outcome, and its inhibition significantly prolongs the survival of NB mice with wild-type neurofibromatosis type 1 (NF1) [105, 106]. It is of interest that the triple PIM/PI3K/mTOR inhibitor IBL-302 is more effective than the single target inhibition, which causes enhanced apoptosis and differentiation and decreased N-MYC in NB cells and patient-derived xenografts [106]. It appears that simultaneously combining standard therapy with multiple targeted therapy could be more powerful. How to design and develop inhibitors with multiple targets is extremely important.

RAS/MAPK pathway is not only involved in the early development, but also emphasized in the recurrence, with nearly 80% of relapsed NB patients harboring mutations in this pathway (e.g., *NF1*, *BRAF*, protein tyrosine phosphatase non-receptor type 11 (*PTPN11*), fibroblast growth factor receptor 1 (*FGFR1*), *KRAS*, *NRAS*, *HRAS*, and *ALK*) [107]. Current treatment options are less effective in relapsed NB, so targeting RAS/MAPK may offer an approach to treat disease recurrence. In NB, RAS/MAPK pathway is often activated by receptor tyrosine kinase, in particular ALK. Mutations in *ALK* or *RAS* gene family members usually cause the constant activated RAS/MAPK signaling in NB. By way of illustration, *RAS* mutations lock RAS into an active form and enhance GTP-binding affinity and effector-binding affinity, thus leading to persistent activation of RAS/MAPK [108•]. Sotorasib (AMG510), an inhibitor of KRAS-G12C mutant protein, has been successfully approved by the FDA for treating NSCLC in 2021 [108•]. Nevertheless, there is no report about the effects of this inhibitor on NB. Among RAS inhibitors, tipifarnib, a general inhibitor of RAS proteins,

is the only one being tested in early-stage clinical trials for NB. Tipifarnib prevents RAS from binding to the cellular membrane, thereby inhibiting signaling transduction [109, 110••]. Strikingly, tipifarnib, an FDA-approved inhibitor, sensitizes NB tumors to dinutuximab, providing a promising treatment option for high-risk NB [109]. MEK 1/2 inhibitors, such as binimetinib and trametinib, also show potent cytotoxicity against *RAS* mutated NB in vitro and in vivo [107, 110••, 111]. Evidence from several studies proved that NB cells or mice xenografts with *RAS/BRAF* mutations are sensitive to MEK inhibition [107, 110••, 111, 112, 113]. For instance, binimetinib and trametinib inhibit the growth in several NB cell lines and xenograft models harboring *RAS* mutations [107, 110••, 111, 112 113]. A phase I/IIa clinical trial (NCT02124772) is ongoing for the investigation of safety and activity of trametinib monotherapy in NB, and the combined therapy of dabrafenib with trametinib in cancers harboring *BRAF* V600E mutations [31•, 51••]. To overcome the acquired resistance to RAS/MAPK inhibitors, concurrent inhibition of this pathway at distinct sites has been suggested. *PTPN11*, one of the most important mutations in relapsed NB, encodes tyrosine phosphatase SHP2. SHP2, an activator of RAS, promoting its dephosphorylation to enhance RAS binding to RAF and consequently activates RAS/MAPK signaling [114]. *RAS* mutations (e.g., NRAS Q61K) in NB confer resistance to SHP2 inhibitors; however, the combination of SHP2 inhibitors and RAS downstream component inhibitors (RAF, MEK, or ERK) shows synergistic effects in *RAS* mutated NB cells [114]. In brief, targeting RAS/MAPK could be an effective strategy for relapsed NB; nonetheless, it should be aware that single inhibition of this way may be insufficient to manage NB. Combination therapies could be vital to achieve effective treatments and avoid resistance development.

Combination Therapies

It is well recognized that rational combination therapies will be essential to further progress therapies for high-risk NB, such as increasing the response to standard therapies and developing new therapies for refractory/relapsed disease. In general, there are two types of combined therapies: one is conventional therapies synergized with molecular targeted therapies; the other is combination targeted therapy using inhibitors that target many different molecules or pathways. For maximum therapeutic benefit, molecularly targeted therapies are often combined with chemotherapeutic agents. For example, dinutuximab in combination with irinotecan and temozolomide has shown a significant anti-tumor activity [115••]. Another study implied that integrating conventional chemotherapy with PIM/PI3K/mTOR inhibition could improve outcomes and avoid adverse effects in children with high-risk NB [106]. Regorafenib (BAY73-4506), an inhibitor of multiple kinases, has been approved by the FDA for the treatment of metastatic colorectal cancer, advanced gastrointestinal stromal tumors (GIST), and progressive hepatocellular carcinoma [116]. In NB, regorafenib simultaneously inhibits RAS/MAPK, PI3K/AKT/mTOR, and Fos/Jun pathways, resulting in growth suppression and apoptosis induction [32, 116]. This inhibitor combined with standard therapy is being used to treat refractory/relapsed NB in a phase I clinical trial (NCT02085148). More molecular-guided therapy in combination with standard therapy is being currently explored for high-risk NB managements in preclinical and clinical trials.

Combining multiple molecular targeted therapies concentrated on genetic alterations and deregulated pathways represents a novel approach for NB, which could improve efficacy, overcome resistance, avoid relapse, and reduce toxicity. For example, several inhibitors have been discovered to improve the efficacy and reduce the adverse side effects of GD2 monoclonal antibodies, such as PD-1 inhibitors, RAS inhibitors as well as ODC1 inhibitors [109, 117, 118, 119]. A combined treatment with a PD-1 inhibitor and dinutuximab beta showed a synergistic anti-NB immune response in the mouse model, and two patients with refractory NB have a good response to the combination of nivolumab (PD-1/PD-L1 monoclonal antibody) and dinutuximab beta [120]. A phase I trial (NCT02914405) is going to investigate safety and efficacy of nivolumab in combination with dinutuximab beta for relapsed/refractory NB with no results available. In addition to PD-1 inhibitors, the effectiveness of dinutuximab can be elevated by tipifarnib (RAS inhibitor) [109]. In contrast, the combination of DFMO (ODC1 inhibitor) and dinutuximab can relieve anti-GD2-induced allodynia in rat models, and the potency of this regimen is being evaluated in a phase II clinical trial (NCT03794349) [119]. Additionally, the benefit of DFMO in synergy with ceritinib (ALK inhibitor) or bortezomib (proteasome inhibitor) is being studied in phase II clinical trials (NCT02559778, NCT02139397). Trametinib (MEK inhibitor) combined with dabrafenib (BRAF inhibitor) has been reported to resolve vasoactive intestinal peptide-induced diarrhea in two NB cases with BRAF V600E mutation [31•]. The combination of MEK inhibitors and ribociclib (CDK4/6 inhibitor) has also shown a synergistic effect [111], and a phase I clinical trials (NCT02780128) for investigation of the synergy of trametinib and ribociclib is ongoing. Strikingly, preclinical studies found that the combination of trametinib and a YAP (yes-associated protein 1) inhibitor (CA3) is capable to overcome trametinib resistance [113], whilst trametinib combined with GD2 immunotherapy can increase the killing efficacy of GD2-CAR T cell therapy [121]. More drug combinations among distinct inhibitors with different

targets are being tested intensively in NB preclinical studies, such as ALK inhibitors with ATR inhibitors (BAY1895344) [122], MDM2 inhibitors with targeted radiation therapy (^{177}Lu-DOTATATE) [123], MDM2 inhibitors (CGM097) with BET inhibitors (OTX015) [124], CDK7 inhibitors (YKL-5–124) with BET inhibitors (JQ1) [62], mTOR inhibitors (temsirolimus) with BET inhibitors (JQ1 or OTX-015) [125], and FGFR1 inhibitors (AZD4547) with PI3K inhibitors (GDC0941) [126]. More combination options using inhibitors to target many various pathways would be further inspected at preclinical and clinical levels.

Conclusion

Despite significant discoveries in the field of NB molecularly targeted therapy, the translation of such findings to clinical disease managements remains low in past years. To date, only anti-GD2 monoclonal antibodies have been approved for the treatment of pediatrics with relapsed or refractory high-risk NB. There is a remarkable gap between preclinical and clinical trials. On one hand, the biological and the pathological roles of molecular targets are not fully elucidated in NB. On the other hand, new drug testing is currently conducted on the traditional NB cell and mouse models. New disease models should be evolved using advanced technology, such as 3D tissue-engineered system and patient-derived xenograft model system. Owning to the heterogeneity of NB, different patients respond differently to therapies. How should an efficient personalized therapy be designed using molecularly targeted drugs and standard therapy? How should an effective therapy be progressed to prolong survival for high-risk NB or for refractory/relapsed NB? Overall, new drugs with more efficacy and less toxicity will be eagerly expected; novel combination options, employing inhibitors targeting multiple molecular targets, and conventional therapy, will be desperately needed.

Acknowledgements The figures were created by the BioRender and Figdraw.

Author contribution XY drafted the manuscript. JL and JY provided supervision and guidance, and revised the manuscript. All authors approved the submitted version.

Funding Open Access funding enabled and organized by CAUL and its Member Institutions This research was funded by the Capital's Funds for Health Improvement and Research (No: 2020–2-2025), National Natural Science Foundation of China (No: 81971642, 82001861, 82102088), and National Key Research and Development Plan (No: 2020YFC0122000).

Declarations

Conflict of Interest The authors declare competing interests.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:
• Of importance
•• Of major importance

1. Takita J. Molecular basis and clinical features of neuroblastoma. Jma j. 2021;4(4):321–31. https://doi.org/10.31662/jmaj.2021-0077.
2. Colon NC, Chung DH. Neuroblastoma. Adv Pediatr. 2011;58(1):297–311. https://doi.org/10.1016/j.yapd.2011.03.011.
3. Park JR, Eggert A, Caron H. Neuroblastoma: biology, prognosis, and treatment. Hematol Oncol Clin North Am. 2010;24(1):65–86. https://doi.org/10.1016/j.hoc.2009.11.011.
4.•• Ciaccio R, De Rosa P, Aloisi S, Viggiano M, Cimadom L, Zadran SK, et al. Targeting oncogenic transcriptional networks in neuroblastoma: from N-Myc to epigenetic drugs. Int J Mol Sci. 2021;22(23). https://doi.org/10.3390/ijms222312883. **This article provides a comprehensive review of the deregulated gene expression and transcriptional networks in neuroblastoma, and briefly describes the related epigenetic therapies.**
5. Pinto NR, Applebaum MA, Volchenboum SL, Matthay KK, London WB, Ambros PF, et al. Advances in risk classification and treatment strategies for neuroblastoma. J Clin Oncol. 2015;33(27):3008–17. https://doi.org/10.1200/jco.2014.59.4648.
6. Sabbih GO, Danquah MK. Neuroblastoma GD2 expression and computational analysis of aptamer-based bioaffinity targeting. Int J Mol Sci. 2021;22(16). https://doi.org/10.3390/ijms22169101.
7.• Irwin MS, Naranjo A, Zhang FF, Cohn SL, London WB, Gastier-Foster JM, et al. Revised neuroblastoma risk classification system: a report from the children's oncology group. J Clin Oncol. 2021;39(29):3229–41. https://doi.org/10.1200/jco.21.00278. **This article reports the most recent findings on the risk classification system for neuroblastoma**.
8. Cohn SL, Pearson AD, London WB, Monclair T, Ambros PF, Brodeur GM, et al. The International Neuroblastoma Risk Group (INRG) classification system: an INRG Task Force report. J Clin Oncol. 2009;27(2):289–97. https://doi.org/10.1200/jco.2008.16.6785.

9. Maris JM. Recent advances in neuroblastoma. N Engl J Med. 2010;362(23):2202–11. https://doi.org/10.1056/NEJMra0804577.
10. Shohet J, Foster J. Neuroblastoma BMJ. 2017;357: j1863. https://doi.org/10.1136/bmj.j1863.
11. Dhillon S. Dinutuximab: first global approval. Drugs. 2015;75(8):923–7. https://doi.org/10.1007/s40265-015-0399-5.
12. Kholodenko IV, Kalinovsky DV, Doronin II, Deyev SM, Kholodenko RV. Neuroblastoma origin and therapeutic targets for immunotherapy. J Immunol Res. 2018;2018:7394268. https://doi.org/10.1155/2018/7394268.
13. Markham A. Naxitamab: first approval. Drugs. 2021;81(2):291–6. https://doi.org/10.1007/s40265-021-01467-4.
14• Furman WL, McCarville B, Shulkin BL, Davidoff A, Krasin M, Hsu CW, et al. Improved outcome in children with newly diagnosed high-risk neuroblastoma treated with chemoimmunotherapy: updated results of a phase II study using hu14.18K322A. J Clin Oncol. 2022;40(4):335–44. https://doi.org/10.1200/jco.21.01375. **This article reported the latest promising outcomes of humanized antidisialoganglioside monoclonal antibody (hu14.18K322A) for high-risk neuroblastoma**.
15. Furman WL, Federico SM, McCarville MB, Shulkin BL, Davidoff AM, Krasin MJ, et al. A phase II trial of Hu14.18K322A in combination with induction chemotherapy in children with newly diagnosed high-risk neuroblastoma. Clin Cancer Res. 2019;25(21):6320–8. https://doi.org/10.1158/1078-0432.Ccr-19-1452.
16. Shusterman S, Naranjo A, Van Ryn C, Hank JA, Parisi MT, Shulkin BL, et al. Antitumor activity and tolerability of hu14.18-IL2 with GMCSF and isotretinoin in recurrent or refractory neuroblastoma: a children's oncology group phase II study. Clin Cancer Res. 2019;25(20):6044–51. https://doi.org/10.1158/1078-0432.Ccr-19-0798.
17. Straathof K, Flutter B, Wallace R, Jain N, Loka T, Depani S, et al. Antitumor activity without on-target off-tumor toxicity of GD2-chimeric antigen receptor T cells in patients with neuroblastoma. Sci Transl Med. 2020;12(571). https://doi.org/10.1126/scitranslmed.abd6169.
18 Tumino N, Weber G, Besi F, Del Bufalo F, Bertaina V, Paci P, et al. Polymorphonuclear myeloid-derived suppressor cells impair the anti-tumor efficacy of GD2.CAR T-cells in patients with neuroblastoma. J Hematol Oncol. 2021;14(1):191. https://doi.org/10.1186/s13045-021-01193-0.
19. DuBois SG, Mosse YP, Fox E, Kudgus RA, Reid JM, McGovern R, et al. Phase II trial of alisertib in combination with irinotecan and temozolomide for patients with relapsed or refractory neuroblastoma. Clin Cancer Res. 2018;24(24):6142–9. https://doi.org/10.1158/1078-0432.Ccr-18-1381.
20. Chu QS, Bouganim N, Fortier C, Zaknoen S, Stille JR, Kremer JD, et al. Aurora kinase A inhibitor, LY3295668 erbumine: a phase 1 monotherapy safety study in patients with locally advanced or metastatic solid tumors. Invest New Drugs. 2021;39(4):1001–10. https://doi.org/10.1007/s10637-020-01049-3.
21. Sun Y, Han J, Wang Z, Li X, Sun Y, Hu Z. Safety and efficacy of bromodomain and extra-terminal inhibitors for the treatment of hematological malignancies and solid tumors: a systematic study of clinical trials. Front Pharmacol. 2020;11: 621093. https://doi.org/10.3389/fphar.2020.621093.
22.• Lewis EC, Kraveka JM, Ferguson W, Eslin D, Brown VI, Bergendahl G, et al. A subset analysis of a phase II trial evaluating the use of DFMO as maintenance therapy for high-risk neuroblastoma. Int J Cancer. 2020;147(11):3152–9. https://doi.org/10.1002/ijc.33044. **This article reported that DMFO significantly improves the outcome of high-risk neuroblastoma in a clinical phase II trial**.
23. Geoerger B, Bourdeaut F, DuBois SG, Fischer M, Geller JI, Gottardo NG, et al. A phase I study of the CDK4/6 inhibitor ribociclib (LEE011) in pediatric patients with malignant rhabdoid tumors, neuroblastoma, and other solid tumors. Clin Cancer Res. 2017;23(10):2433–41. https://doi.org/10.1158/1078-0432.Ccr-16-2898.
24• Foster JH, Voss SD, Hall DC, Minard CG, Balis FM, Wilner K, et al. Activity of crizotinib in patients with ALK-aberrant relapsed/refractory neuroblastoma: a children's oncology group study (ADVL0912). Clin Cancer Res. 2021;27(13):3543–8. https://doi.org/10.1158/1078-0432.Ccr-20-4224. **This article reports the efficacy of the first-generation ALK inhibitor crizotinib in the treatment of patients with refratory neuroblastoma**.
25. Fischer M, Moreno L, Ziegler DS, Marshall LV, Zwaan CM, Irwin MS, et al. Ceritinib in paediatric patients with anaplastic lymphoma kinase-positive malignancies: an open-label, multicentre, phase 1, dose-escalation and dose-expansion study. Lancet Oncol. 2021;22(12):1764–76. https://doi.org/10.1016/s1470-2045(21)00536-2.
26. Liu T, Merguerian MD, Rowe SP, Pratilas CA, Chen AR, Ladle BH. Exceptional response to the ALK and ROS1 inhibitor lorlatinib and subsequent mechanism of resistance in relapsed ALK F1174L-mutated neuroblastoma. Cold Spring Harb Mol Case Stud. 2021;7(4). https://doi.org/10.1101/mcs.a006064.
27. Weiss BD, Yanik G, Naranjo A, Zhang FF, Fitzgerald W, Shulkin BL, et al. A safety and feasibility trial of (131) I-MIBG in newly diagnosed high-risk neuroblastoma: a children's oncology group study. Pediatr Blood Cancer. 2021;68(10): e29117. https://doi.org/10.1002/pbc.29117.
28. Chen L, Pastorino F, Berry P, Bonner J, Kirk C, Wood KM, et al. Preclinical evaluation of the first intravenous small molecule MDM2 antagonist alone and in combination with temozolomide in neuroblastoma. Int J Cancer. 2019;144(12):3146–59. https://doi.org/10.1002/ijc.32058.
29. Kushner BH, Cheung NV, Modak S, Becher OJ, Basu EM, Roberts SS, et al. A phase I/Ib trial targeting the Pi3k/Akt pathway using perifosine: long-term progression-free survival of patients with resistant neuroblastoma. Int J Cancer. 2017;140(2):480–4. https://doi.org/10.1002/ijc.30440.
30. Mody R, Naranjo A, Van Ryn C, Yu AL, London WB, Shulkin BL, et al. Irinotecan-temozolomide with temsirolimus or dinutuximab in children with refractory or relapsed neuroblastoma (COG ANBL1221): an open-label, randomised, phase 2 trial. Lancet Oncol. 2017;18(7):946–57. https://doi.org/10.1016/s1470-2045(17)30355-8.
31. Shahid S, Kushner BH, Modak S, Basu EM, Rubin EM, Gundem G, et al. Association of BRAF V600E mutations with vasoactive intestinal peptide syndrome in MYCN-amplified neuroblastoma. Pediatr Blood Cancer. 2021;68(10): e29265. https://doi.org/10.1002/pbc.29265.
32. Geoerger B, Morland B, Jiménez I, Frappaz D, Pearson ADJ, Vassal G, et al. Phase 1 dose-escalation and pharmacokinetic study of regorafenib in paediatric patients with recurrent or refractory solid malignancies. Eur J Cancer. 2021;153:142–52. https://doi.org/10.1016/j.ejca.2021.05.023.
33.•• Nazha B, Inal C, Owonikoko TK. Disialoganglioside GD2 expression in solid tumors and role as a target for cancer therapy. Frontiers in Oncology. 2020;10. https://doi.org/10.3389/fonc.2020.01000. **This article provides a comprehensive overview of the biological characteristics of disialoganalioside GD2 and its value as a therapeutic target for tumors**.
34. Valentino L, Moss T, Olson E, Wang HJ, Elashoff R, Ladisch S. Shed tumor gangliosides and progression of human neuroblastoma. Blood. 1990;75(7):1564–7.

35•• Yu J, Hung JT, Wang SH, Cheng JY, Yu AL. Targeting glycosphingolipids for cancer immunotherapy. FEBS Lett. 2020;594(22):3602–18. https://doi.org/10.1002/1873-3468.13917. **This article reviews the progression of glycosphingolipids as therapeutic targets for cancer**.

36. Horwacik I, Durbas M, Boratyn E, Sawicka A, Węgrzyn P, Krzanik S, et al. Analysis of genes involved in response to doxorubicin and a GD2 ganglioside-specific 14G2a monoclonal antibody in IMR-32 human neuroblastoma cells. Acta Biochim Pol. 2015;62(3):423–33. https://doi.org/10.18388/abp.2015_1035.

37. Chen YX, Chen XW, Li CG, Yue LJ, Mai HR, Wen FQ. Effect of tumor gangliosides on tyrosine phosphorylation of p125FAK in platelet adhesion to collagen. Oncol Rep. 2013;29(1):343–8. https://doi.org/10.3892/or.2012.2092.

38. Schulz G, Cheresh DA, Varki NM, Yu A, Staffileno LK, Reisfeld RA. Detection of ganglioside GD2 in tumor tissues and sera of neuroblastoma patients. Cancer Res. 1984;44(12 Pt 1):5914–20.

39. Mastrangelo S, Rivetti S, Triarico S, Romano A, Attinà G, Maurizi P, et al. Mechanisms, characteristics, and treatment of neuropathic pain and peripheral neuropathy associated with dinutuximab in neuroblastoma patients. Int J Mol Sci. 2021;22(23). https://doi.org/10.3390/ijms222312648.

40. I VK, D VK, E VS, I ID, M VK, A VK, et al. Multimerization through pegylation improves pharmacokinetic properties of scFv fragments of GD2-specific antibodies. Molecules. 2019;24(21). https://doi.org/10.3390/molecules24213835.

41. Sait S, Modak S. Anti-GD2 immunotherapy for neuroblastoma. Expert Rev Anticancer Ther. 2017;17(10):889–904. https://doi.org/10.1080/14737140.2017.1364995.

42. Durbas M, Horwacik I, Boratyn E, Kamycka E, Rokita H. GD2 ganglioside specific antibody treatment downregulates PI3K/Akt/mTOR signaling network in human neuroblastoma cell lines. Int J Oncol. 2015;47(3):1143–59. https://doi.org/10.3892/ijo.2015.3070.

43. Horwacik I, Durbas M, Boratyn E, Węgrzyn P, Rokita H. Targeting GD2 ganglioside and aurora A kinase as a dual strategy leading to cell death in cultures of human neuroblastoma cells. Cancer Lett. 2013;341(2):248–64. https://doi.org/10.1016/j.canlet.2013.08.018.

44. Shurin GV, Shurin MR, Bykovskaia S, Shogan J, Lotze MT, Barksdale EM Jr. Neuroblastoma-derived gangliosides inhibit dendritic cell generation and function. Cancer Res. 2001;61(1):363–9.

45.• Yu AL, Gilman AL, Ozkaynak MF, Naranjo A, Diccianni MB, Gan J, et al. Long-term follow-up of a phase III study of ch14.18 (dinutuximab) + cytokine immunotherapy in children with high-risk neuroblastoma: COG study ANBL0032. Clin Cancer Res. 2021;27(8):2179–89. https://doi.org/10.1158/1078-0432.Ccr-20-3909. **This article reported long-term follow-up of dinutuximab with cytokines in children with high-risk neuroblastoma**.

46 Ladenstein R, Pötschger U, Valteau-Couanet D, Luksch R, Castel V, Yaniv I, et al. Interleukin 2 with anti-GD2 antibody ch14.18/CHO (dinutuximab beta) in patients with high-risk neuroblastoma (HR-NBL1/SIOPEN): a multicentre, randomised, phase 3 trial. Lancet Oncol. 2018;19(12):1617–29. https://doi.org/10.1016/s1470-2045(18)30578-3.

47. Sorkin LS, Otto M, Baldwin WM 3rd, Vail E, Gillies SD, Handgretinger R, et al. Anti-GD(2) with an FC point mutation reduces complement fixation and decreases antibody-induced allodynia. Pain. 2010;149(1):135–42. https://doi.org/10.1016/j.pain.2010.01.024.

48. Bahri M, Kailayangiri S, Vermeulen S, Galopin N, Rossig C, Paris F, et al. SIRPα-specific monoclonal antibody enables antibody-dependent phagocytosis of neuroblastoma cells. Cancer Immunol Immunother. 2022;71(1):71–83. https://doi.org/10.1007/s00262-021-02968-7.

49. Richards RM, Sotillo E, Majzner RG. CAR T cell therapy for neuroblastoma. Front Immunol. 2018;9:2380. https://doi.org/10.3389/fimmu.2018.02380.

50 Navid F, Sondel PM, Barfield R, Shulkin BL, Kaufman RA, Allay JA, et al. Phase I trial of a novel anti-GD2 monoclonal antibody, Hu14.18K322A, designed to decrease toxicity in children with refractory or recurrent neuroblastoma. J Clin Oncol. 2014;32(14):1445–52. https://doi.org/10.1200/jco.2013.50.4423.

51.•• Zafar A, Wang W, Liu G, Wang X, Xian W, McKeon F, et al. Molecular targeting therapies for neuroblastoma: progress and challenges. Med Res Rev. 2021;41(2):961–1021. https://doi.org/10.1002/med.21750. **This article provides a comprehensive review of molecular targeting therapies for neuroblastoma**.

52•• Campbell K, Naranjo A, Hibbitts E, Gastier-Foster JM, Bagatell R, Irwin MS, et al. Association of heterogeneous MYCN amplification with clinical features, biological characteristics and outcomes in neuroblastoma: a report from the Children's Oncology Group. Eur J Cancer. 2020;133:112–9. https://doi.org/10.1016/j.ejca.2020.04.007. **This article reported the significant impact of MYCN on the clinical features, biological characteristics, and prognosis of neuroblastoma**.

53.•• Otte J, Dyberg C, Pepich A, Johnsen JI. MYCN function in neuroblastoma development. Frontiers in Oncology. 2021;10. https://doi.org/10.3389/fonc.2020.624079. **This article provides an overview of the progress of MYCN function in neruoblastoma.**

54. Durbin AD, Zimmerman MW, Dharia NV, Abraham BJ, Iniguez AB, Weichert-Leahey N, et al. Selective gene dependencies in MYCN-amplified neuroblastoma include the core transcriptional regulatory circuitry. Nat Genet. 2018;50(9):1240–6. https://doi.org/10.1038/s41588-018-0191-z.

55. Gu L, Zhang H, He J, Li J, Huang M, Zhou M. MDM2 regulates MYCN mRNA stabilization and translation in human neuroblastoma cells. Oncogene. 2012;31(11):1342–53. https://doi.org/10.1038/onc.2011.343.

56. Casey SC, Baylot V, Felsher DW. MYC: master regulator of immune privilege. Trends Immunol. 2017;38(4):298–305. https://doi.org/10.1016/j.it.2017.01.002.

57. Puissant A, Frumm SM, Alexe G, Bassil CF, Qi J, Chanthery YH, et al. Targeting MYCN in neuroblastoma by BET bromodomain inhibition. Cancer Discov. 2013;3(3):308–23. https://doi.org/10.1158/2159-8290.Cd-12-0418.

58. Wyce A, Ganji G, Smitheman KN, Chung CW, Korenchuk S, Bai Y, et al. BET inhibition silences expression of MYCN and BCL2 and induces cytotoxicity in neuroblastoma tumor models. PLoS ONE. 2013;8(8): e72967. https://doi.org/10.1371/journal.pone.0072967.

59. Zhang X, Guo X, Zhuo R, Tao Y, Liang W, Yang R, et al. BRD4 inhibitor MZ1 exerts anti-cancer effects by targeting MYCN and MAPK signaling in neuroblastoma. Biochem Biophys Res Commun. 2022;604:63–9. https://doi.org/10.1016/j.bbrc.2022.03.039.

60. Wells CI, Vasta JD, Corona CR, Wilkinson J, Zimprich CA, Ingold MR, et al. Quantifying CDK inhibitor selectivity in live cells. Nat Commun. 2020;11(1):2743. https://doi.org/10.1038/s41467-020-16559-0.

61. Poon E, Liang T, Jamin Y, Walz S, Kwok C, Hakkert A, et al. Orally bioavailable CDK9/2 inhibitor shows mechanism-based therapeutic potential in MYCN-driven neuroblastoma. J Clin Invest. 2020;130(11):5875–92. https://doi.org/10.1172/jci134132.

62. Gao Y, Volegova M, Nasholm N, Das S, Kwiatkowski N, Abraham BJ, et al. Synergistic anti-tumor effect of combining selective CDK7 and BRD4 inhibition in neuroblastoma. Front Oncol. 2021;11: 773186. https://doi.org/10.3389/fonc.2021.773186.

63. Greengard EG. Molecularly targeted therapy for neuroblastoma. Children (Basel). 2018;5(10). https://doi.org/10.3390/children5100142.
64. Yang Y, Ding L, Zhou Q, Fen L, Cao Y, Sun J, et al. Silencing of AURKA augments the antitumor efficacy of the AURKA inhibitor MLN8237 on neuroblastoma cells. Cancer Cell Int. 2020;20:9. https://doi.org/10.1186/s12935-019-1072-y.
65. Boi D, Souvalidou F, Capelli D, Polverino F, Marini G, Montanari R, et al. PHA-680626 Is an Effective Inhibitor of the Interaction between Aurora-A and N-Myc. Int J Mol Sci. 2021;22(23). https://doi.org/10.3390/ijms222313122.
66. Smith AG, Popov N, Imreh M, Axelson H, Henriksson M. Expression and DNA-binding activity of MYCN/Max and Mnt/Max during induced differentiation of human neuroblastoma cells. J Cell Biochem. 2004;92(6):1282–95. https://doi.org/10.1002/jcb.20121.
67. Müller I, Larsson K, Frenzel A, Oliynyk G, Zirath H, Prochownik EV, et al. Targeting of the MYCN protein with small molecule c-MYC inhibitors. PLoS One. 2014;9(5): e97285. https://doi.org/10.1371/journal.pone.0097285.
68. Castell A, Yan Q, Fawkner K, Hydbring P, Zhang F, Verschut V, et al. A selective high affinity MYC-binding compound inhibits MYC:MAX interaction and MYC-dependent tumor cell proliferation. Sci Rep. 2018;8(1):10064. https://doi.org/10.1038/s41598-018-28107-4.
69. Han H, Jain AD, Truica MI, Izquierdo-Ferrer J, Anker JF, Lysy B, et al. Small-molecule MYC inhibitors suppress tumor growth and enhance immunotherapy. Cancer Cell. 2019;36(5):483-97.e15. https://doi.org/10.1016/j.ccell.2019.10.001.
70. Madden SK, de Araujo AD, Gerhardt M, Fairlie DP, Mason JM. Taking the Myc out of cancer: toward therapeutic strategies to directly inhibit c-Myc. Mol Cancer. 2021;20(1):3. https://doi.org/10.1186/s12943-020-01291-6.
71. Sorolla A, Wang E, Golden E, Duffy C, Henriques ST, Redfern AD, et al. Precision medicine by designer interference peptides: applications in oncology and molecular therapeutics. Oncogene. 2020;39(6):1167–84. https://doi.org/10.1038/s41388-019-1056-3.
72. Evageliou NF, Hogarty MD. Disrupting polyamine homeostasis as a therapeutic strategy for neuroblastoma. Clin Cancer Res. 2009;15(19):5956–61. https://doi.org/10.1158/1078-0432.Ccr-08-3213.
73. Evageliou NF, Haber M, Vu A, Laetsch TW, Murray J, Gamble LD, et al. Polyamine antagonist therapies inhibit neuroblastoma initiation and progression. Clin Cancer Res. 2016;22(17):4391–404. https://doi.org/10.1158/1078-0432.Ccr-15-2539.
74. Gamble LD, Purgato S, Murray J, Xiao L, Yu DMT, Hanssen KM, et al. Inhibition of polyamine synthesis and uptake reduces tumor progression and prolongs survival in mouse models of neuroblastoma. Sci Transl Med. 2019;11(477). https://doi.org/10.1126/scitranslmed.aau1099.
75. Mossé YP, Laudenslager M, Longo L, Cole KA, Wood A, Attiyeh EF, et al. Identification of ALK as a major familial neuroblastoma predisposition gene. Nature. 2008;455(7215):930–5. https://doi.org/10.1038/nature07261.
76. Cheung NK, Dyer MA. Neuroblastoma: developmental biology, cancer genomics and immunotherapy. Nat Rev Cancer. 2013;13(6):397–411. https://doi.org/10.1038/nrc3526.
77.•• Wulf AM, Moreno MM, Paka C, Rampasekova A, Liu KJ. Defining pathological activities of ALK in neuroblastoma, a neural crest-derived cancer. Int J Mol Sci. 2021;22(21). https://doi.org/10.3390/ijms222111718. **This article provides a comprehensive review of the pathological role of ALK in the development of neuroblastoma and the ALK-associated treatments for neuroblastoma**.
78. Berry T, Luther W, Bhatnagar N, Jamin Y, Poon E, Sanda T, et al. The ALK(F1174L) mutation potentiates the oncogenic activity of MYCN in neuroblastoma. Cancer Cell. 2012;22(1):117–30. https://doi.org/10.1016/j.ccr.2012.06.001.
79. Bagci O, Tumer S, Olgun N, Altungoz O. Copy number status and mutation analyses of anaplastic lymphoma kinase (ALK) gene in 90 sporadic neuroblastoma tumors. Cancer Lett. 2012;317(1):72–7. https://doi.org/10.1016/j.canlet.2011.11.013.
80. Javanmardi N, Fransson S, Djos A, Umapathy G, Östensson M, Milosevic J, et al. Analysis of ALK, MYCN, and the ALK ligand ALKAL2 (FAM150B/AUGα) in neuroblastoma patient samples with chromosome arm 2p rearrangements. Genes Chromosomes Cancer. 2019. https://doi.org/10.1002/gcc.22790.
81. Kramer M, Ribeiro D, Arsenian-Henriksson M, Deller T, Rohrer H. Proliferation and survival of embryonic sympathetic neuroblasts by MYCN and activated ALK signaling. J Neurosci. 2016;36(40):10425–39. https://doi.org/10.1523/jneurosci.0183-16.2016.
82. Ono S, Saito T, Terui K, Yoshida H, Enomoto H. Generation of conditional ALK F1174L mutant mouse models for the study of neuroblastoma pathogenesis. Genesis. 2019;57(10): e23323. https://doi.org/10.1002/dvg.23323.
83• Somasundaram DB, Aravindan S, Gupta N, Yu Z, Baker A, Aravindan N. ALK expression, prognostic significance, and its association with MYCN expression in MYCN non-amplified neuroblastoma. World J Pediatr. 2022;18(4):285–93. https://doi.org/10.1007/s12519-022-00517-5. **This article describes the association between ALK and MYCN in neuroblastoma**.
84. Duijkers FAM, Gaal J, Meijerink JPP, Admiraal P, Pieters R, de Krijger RR, et al. High anaplastic lymphoma kinase immunohistochemical staining in neuroblastoma and ganglioneuroblastoma is an independent predictor of poor outcome. Am J Pathol. 2012;180(3):1223–31. https://doi.org/10.1016/j.ajpath.2011.12.003.
85. Peng L, Zhu L, Sun Y, Stebbing J, Selvaggi G, Zhang Y, et al. Targeting ALK rearrangements in NSCLC: current state of the art. Front Oncol. 2022;12: 863461. https://doi.org/10.3389/fonc.2022.863461.
86. Merino M, Kasamon Y, Li H, Ma L, Leong R, Zhou J, et al. FDA approval summary: crizotinib for pediatric and young adult patients with relapsed or refractory systemic anaplastic large cell lymphoma. Pediatr Blood Cancer. 2022;69(8): e29602. https://doi.org/10.1002/pbc.29602.
87. Baranov E, Winsnes K, O'Brien M, Voss SD, Church AJ, Janeway KA, et al. Histologic characterization of paediatric mesenchymal neoplasms treated with kinase-targeted therapy. Histopathology. 2022;81(2):215–27. https://doi.org/10.1111/his.14680.
88. Bresler SC, Weiser DA, Huwe PJ, Park JH, Krytska K, Ryles H, et al. ALK mutations confer differential oncogenic activation and sensitivity to ALK inhibition therapy in neuroblastoma. Cancer Cell. 2014;26(5):682–94. https://doi.org/10.1016/j.ccell.2014.09.019.
89. Infarinato NR, Park JH, Krytska K, Ryles HT, Sano R, Szigety KM, et al. The ALK/ROS1 inhibitor PF-06463922 overcomes primary resistance to crizotinib in ALK-Driven Neuroblastoma. Cancer Discov. 2016;6(1):96–107. https://doi.org/10.1158/2159-8290.Cd-15-1056.
90. Carpenter EL, Haglund EA, Mace EM, Deng D, Martinez D, Wood AC, et al. Antibody targeting of anaplastic lymphoma kinase induces cytotoxicity of human neuroblastoma. Oncogene. 2012;31(46):4859–67. https://doi.org/10.1038/onc.2011.647.
91•• Zafar A, Wang W, Liu G, Xian W, McKeon F, Zhou J, et al. Targeting the p53-MDM2 pathway for neuroblastoma therapy: Rays of hope. Cancer Lett. 2021;496:16–29. https://doi.org/10.1016/j.canlet.2020.09.023. **This article summarized the oncogenic**

functions of the p53-MDM2 pathway in neuroblastoma and the progress of targeting p53-MDM2 therapy.

92. Veneziani I, Infante P, Ferretti E, Melaiu O, Battistelli C, Lucarini V, et al. Nutlin-3a enhances natural killer cell-mediated killing of neuroblastoma by restoring p53-dependent expression of ligands for NKG2D and DNAM-1 receptors. Cancer Immunol Res. 2021;9(2):170–83. https://doi.org/10.1158/2326-6066.Cir-20-0313.
93. Carr J, Bell E, Pearson AD, Kees UR, Beris H, Lunec J, et al. Increased frequency of aberrations in the p53/MDM2/p14(ARF) pathway in neuroblastoma cell lines established at relapse. Cancer Res. 2006;66(4):2138–45. https://doi.org/10.1158/0008-5472.Can-05-2623.
94. Van Maerken T, Ferdinande L, Taildeman J, Lambertz I, Yigit N, Vercruysse L, et al. Antitumor activity of the selective MDM2 antagonist nutlin-3 against chemoresistant neuroblastoma with wild-type p53. J Natl Cancer Inst. 2009;101(22):1562–74. https://doi.org/10.1093/jnci/djp355.
95. Al-Ghabkari A, Narendran A. In vitro characterization of a potent p53-MDM2 inhibitor, RG7112 in neuroblastoma cancer cell lines. Cancer Biother Radiopharm. 2019;34(4):252–7. https://doi.org/10.1089/cbr.2018.2732.
96. Van Maerken T, Vandesompele J, Rihani A, De Paepe A, Speleman F. Escape from p53-mediated tumor surveillance in neuroblastoma: switching off the p14(ARF)-MDM2-p53 axis. Cell Death Differ. 2009;16(12):1563–72. https://doi.org/10.1038/cdd.2009.138.
97. Milosevic J, Treis D, Fransson S, Gallo-Oller G, Sveinbjörnsson B, Eissler N, et al. PPM1D is a therapeutic target in childhood neural tumors. Cancers (Basel). 2021;13(23). https://doi.org/10.3390/cancers13236042.
98. King D, Yeomanson D, Bryant HE. PI3King the lock: targeting the PI3K/Akt/mTOR pathway as a novel therapeutic strategy in neuroblastoma. J Pediatr Hematol Oncol. 2015;37(4):245–51. https://doi.org/10.1097/mph.0000000000000329.
99. Yang Q, Jiang W, Hou P. Emerging role of PI3K/AKT in tumor-related epigenetic regulation. Semin Cancer Biol. 2019;59:112–24. https://doi.org/10.1016/j.semcancer.2019.04.001.
100. Chilamakuri R, Rouse DC, Yu Y, Kabir AS, Muth A, Yang J, et al. BX-795 inhibits neuroblastoma growth and enhances sensitivity towards chemotherapy. Transl Oncol. 2022;15(1): 101272. https://doi.org/10.1016/j.tranon.2021.101272.
101. Uva P, Bosco MC, Eva A, Conte M, Garaventa A, Amoroso L, et al. Connectivity map analysis indicates PI3K/Akt/mTOR inhibitors as potential anti-hypoxia drugs in neuroblastoma. Cancers (Basel). 2021;13(11). https://doi.org/10.3390/cancers13112809.
102. Xu DQ, Toyoda H, Yuan XJ, Qi L, Chelakkot VS, Morimoto M, et al. Anti-tumor effect of AZD8055 against neuroblastoma cells in vitro and in vivo. Exp Cell Res. 2018;365(2):177–84. https://doi.org/10.1016/j.yexcr.2018.02.032.
103. Chen Y, Tsai HW, Tsai YH, Tseng SH. VS-5584, a PI3K/mTOR dual inhibitor, exerts antitumor effects on neuroblastomas in vitro and in vivo. J Pediatr Surg. 2021;56(8):1441–8. https://doi.org/10.1016/j.jpedsurg.2020.10.033.
104. Khwanraj K, Dharmasaroja P. Neuroblastoma cell death induced by eEF1A2 knockdown is possibly mediated by the inhibition of Akt and mTOR phosphorylation. Int J Hematol Oncol Stem Cell Res. 2021;15(4):221–9. https://doi.org/10.18502/ijhoscr.v15i4.7477.
105. Brunen D, de Vries RC, Lieftink C, Beijersbergen RL, Bernards R. PIM kinases are a potential prognostic biomarker and therapeutic target in neuroblastoma. Mol Cancer Ther. 2018;17(4):849–57. https://doi.org/10.1158/1535-7163.Mct-17-0868.
106. Mohlin S, Hansson K, Radke K, Martinez S, Blanco-Aparicio C, Garcia-Ruiz C, et al. Anti-tumor effects of PIM/PI3K/mTOR triple kinase inhibitor IBL-302 in neuroblastoma. EMBO Mol Med. 2020;12(1): e11749. https://doi.org/10.15252/emmm.201911749.
107. Eleveld TF, Oldridge DA, Bernard V, Koster J, Colmet Daage L, Diskin SJ, et al. Relapsed neuroblastomas show frequent RAS-MAPK pathway mutations. Nat Genet. 2015;47(8):864–71. https://doi.org/10.1038/ng.3333.
108.• Lin L, Miao L, Lin H, Cheng J, Li M, Zhuo Z, et al. Targeting RAS in neuroblastoma: is it possible? Pharmacol Ther. 2021;236:108054. https://doi.org/10.1016/j.pharmthera.2021.108054. **This article describes the progress of RAS being a promising therapeutic target for neuroblastoma**.
109. Liu X, Wills CA, Chen L, Zhang J, Zhao Y, Zhou M, et al. Small extracellular vesicles induce resistance to anti-GD2 immunotherapy unveiling tipifarnib as an adjunct to neuroblastoma immunotherapy. J Immunother Cancer. 2022;10(4). https://doi.org/10.1136/jitc-2021-004399.
110.•• Mlakar V, Morel E, Mlakar SJ, Ansari M, Gumy-Pause F. A review of the biological and clinical implications of RAS-MAPK pathway alterations in neuroblastoma. J Exp Clin Cancer Res. 2021;40(1):189. https://doi.org/10.1186/s13046-021-01967-x. **This article provided a review of the RAS-MAPK pathway in the treatment of neuroblastoma**.
111. Hart LS, Rader J, Raman P, Batra V, Russell MR, Tsang M, et al. Preclinical therapeutic synergy of MEK1/2 and CDK4/6 inhibition in neuroblastoma. Clin Cancer Res. 2017;23(7):1785–96. https://doi.org/10.1158/1078-0432.Ccr-16-1131.
112. Togashi Y, Tanaka T, Takemoto M, Takeuchi Y, Higashi M, Fumino S, et al. Anti-relapse effect of trametinib on a local minimal residual disease neuroblastoma mouse model. J Pediatr Surg. 2021;56(7):1233–9. https://doi.org/10.1016/j.jpedsurg.2021.03.031.
113. Takemoto M, Tanaka T, Tsuji R, Togashi Y, Higashi M, Fumino S, et al. The synergistic antitumor effect of combination therapy with a MEK inhibitor and YAP inhibitor on pERK-positive neuroblastoma. Biochem Biophys Res Commun. 2021;570:41–6. https://doi.org/10.1016/j.bbrc.2021.07.028.
114. Valencia-Sama I, Ladumor Y, Kee L, Adderley T, Christopher G, Robinson CM, et al. NRAS status determines sensitivity to SHP2 inhibitor combination therapies targeting the RAS-MAPK pathway in neuroblastoma. Cancer Res. 2020;80(16):3413–23. https://doi.org/10.1158/0008-5472.Can-19-3822.
115. Mody R, Yu AL, Naranjo A, Zhang FF, London WB, Shulkin BL, et al. Irinotecan, temozolomide, and dinutuximab with GM-CSF in children with refractory or relapsed neuroblastoma: a report from the children's oncology group. J Clin Oncol. 2020;38(19):2160–9. https://doi.org/10.1200/jco.20.00203.
116. Subramonian D, Phanhthilath N, Rinehardt H, Flynn S, Huo Y, Zhang J, et al. Regorafenib is effective against neuroblastoma in vitro and in vivo and inhibits the RAS/MAPK, PI3K/Akt/mTOR and Fos/Jun pathways. Br J Cancer. 2020;123(4):568–79. https://doi.org/10.1038/s41416-020-0905-8.
117. Heczey A, Louis CU, Savoldo B, Dakhova O, Durett A, Grilley B, et al. CAR T cells administered in combination with lymphodepletion and PD-1 inhibition to patients with neuroblastoma. Mol Ther. 2017;25(9):2214–24. https://doi.org/10.1016/j.ymthe.2017.05.012.
118 Siebert N, Zumpe M, Jüttner M, Troschke-Meurer S, Lode HN. PD-1 blockade augments anti-neuroblastoma immune response induced by anti-GD(2) antibody ch14.18/CHO. Oncoimmunology. 2017;6(10):e1343775. https://doi.org/10.1080/2162402x.2017.1343775.

119. Diccianni MB, Kempińska K, Gangoti JA, Yu AL, Sorkin LS. Anti-GD2 induced allodynia in rats can be reduced by pretreatment with DFMO. PLoS ONE. 2020;15(7): e0236115. https://doi.org/10.1371/journal.pone.0236115.
120. Ehlert K, Hansjuergens I, Zinke A, Otto S, Siebert N, Henze G, et al. Nivolumab and dinutuximab beta in two patients with refractory neuroblastoma. J Immunother Cancer. 2020;8(1). https://doi.org/10.1136/jitc-2020-000540.
121. Tomida A, Yagyu S, Nakamura K, Kubo H, Yamashima K, Nakazawa Y, et al. Inhibition of MEK pathway enhances the antitumor efficacy of chimeric antigen receptor T cells against neuroblastoma. Cancer Sci. 2021;112(10):4026–36. https://doi.org/10.1111/cas.15074.
122. Szydzik J, Lind DE, Arefin B, Kurhe Y, Umapathy G, Siaw JT, et al. ATR inhibition enables complete tumour regression in ALK-driven NB mouse models. Nat Commun. 2021;12(1):6813. https://doi.org/10.1038/s41467-021-27057-2.
123. Lundsten S, Berglund H, Jha P, Krona C, Hariri M, Nelander S, et al. p53-Mediated radiosensitization of (177)Lu-DOTATATE in neuroblastoma tumor spheroids. Biomolecules. 2021;11(11). https://doi.org/10.3390/biom11111695.
124. Maser T, Zagorski J, Kelly S, Ostrander A, Goodyke A, Nagulapally A, et al. The MDM2 inhibitor CGM097 combined with the BET inhibitor OTX015 induces cell death and inhibits tumor growth in models of neuroblastoma. Cancer Med. 2020;9(21):8144–58. https://doi.org/10.1002/cam4.3407.
125. Kling MJ, Griggs CN, McIntyre EM, Alexander G, Ray S, Challagundla KB, et al. Synergistic efficacy of inhibiting MYCN and mTOR signaling against neuroblastoma. BMC Cancer. 2021;21(1):1061. https://doi.org/10.1186/s12885-021-08782-9.
126. Cimmino F, Montella A, Tirelli M, Avitabile M, Lasorsa VA, Visconte F, et al. FGFR1 is a potential therapeutic target in neuroblastoma. Cancer Cell Int. 2022;22(1):174. https://doi.org/10.1186/s12935-022-02587-x.