Review

Cell Proliferation in Neuroblastoma

Laura L. Stafman and Elizabeth A. Beierle *

Received: 20 November 2015; Accepted: 8 January 2016; Published: 12 January 2016

Academic Editor: Vita Golubovskaya

Department of Surgery, Division of Pediatric Surgery, University of Alabama, Birmingham, AL 35233, USA; lstafman@uabmc.edu

* Correspondence: elizabeth.beierle@childrensal.org; Tel.: +1-205-638-9688; Fax: +1-205-975-4972

Abstract: Neuroblastoma, the most common extracranial solid tumor of childhood, continues to carry a dismal prognosis for children diagnosed with advanced stage or relapsed disease. This review focuses upon factors responsible for cell proliferation in neuroblastoma including transcription factors, kinases, and regulators of the cell cycle. Novel therapeutic strategies directed toward these targets in neuroblastoma are discussed.

Keywords: neuroblastoma; MYCN; kinases; cell cycle check point inhibitors

1. Neuroblastoma

Neuroblastoma, a tumor of neural crest origin, is the most common extracranial solid tumor of childhood, accounting for 7% of childhood malignancies and 15% of childhood cancer mortality [1,2]. More than 50% of these tumors occur in children less than 2 years of age. The incidence of neuroblastoma has increased in recent years and it continues to carry a poor prognosis in children over two years of age with a survival of only 38% [3,4]. Overall 5-year survival was 74% from 1999–2005 [5]. Neuroblastoma exhibits a wide array of biological characteristics and behaviors, which are important in predicting outcomes.

2. Cell Proliferation

Cell proliferation refers to an increase in the number of cells due to cell growth and division and is necessary not only for growth and normal tissue function but also tumorigenesis. From embryological development through senescence, cell proliferation is maintained by a strict coordination of cellular signals and deregulation of cell proliferation is the defining feature of all tumors. The mechanisms of deregulation vary but are inevitably via perturbations in signal transduction within the cell. Many of these perturbations are in signaling pathways important for proliferation—those regulating cellular growth, differentiation, and developmental signals—and are the result of mutations, amplifications, gene overexpression, or chromosomal deletions [6]. Therefore, manipulation of cell proliferation pathways may decrease the malignant potential of tumors, including neuroblastoma, making these pathways an attractive target for novel therapeutics. Manipulation of the pathways involved in cell differentiation can also decrease the malignant potential of neuroblastoma. Differentiation and proliferation are separate but concurrent processes that are regulated independently by signaling pathways, although some of the pathways overlap and crosstalk [7]. This review will discuss some of the common cell proliferation proteins and pathways involved in neuroblastoma, including transcription factors, kinases, and regulators of the cell cycle.

3. Neuroblastoma and Transcription Factors

Multiple transcription factors, which initiate and regulate gene transcription, have been implicated in neuroblastoma pathogenesis via an increase in cell proliferation. These include N-MYC, nuclear...
factor kappa-light-chain-enhancer of activated B cells (NF-κB), paired-like homeobox 2b (PHOX2B), and P53.

MYCN oncogene encodes N-MYC, a phosphoprotein in the MYC family of transcription factors. N-MYC binds to the E-box sequences CACGTG and CATGTG predominantly in the MYCN-amplified state [8,9]. When bound to these promoter sequences, N-MYC acts as a classical transcription factor, increasing expression of individual genes that increase cell proliferation, and changes global chromatin structure by DNA hypermethylation, which also increases cell proliferation. N-MYC is expressed early in development up through a few weeks following birth [10]. After that, it is only found in adult B cells. MYCN amplification is detected in approximately 20% of neuroblastoma tumors [11,12], and amplification of greater than ten copies of MYCN is the strongest adverse prognostic indicator for this disease [13]. The significance of MYCN amplification in neuroblastomas was first noted in the 1980s [13,14]. More recently, Molenaar et al. found that the majority of high-risk neuroblastoma tumors had genomic amplifications of LIN28B, which suppresses LET-7 and resulted in increased N-MYC protein expression [15].

Using in vitro studies, it has been demonstrated that the level of N-MYC expression correlates with cell proliferation in neuroblastoma [16,17]. N-MYC has been shown to increase cell proliferation in neuroblastoma via multiple mechanisms. Transient receptor potential cation channels M6 and M7 (TRPM6 and TRPM7) are transcriptional targets of N-MYC. These proteins promote cellular calcium and magnesium uptake and enhance cell proliferation in neuroblastoma [18]. N-MYC upregulates high mobility group protein protein of the A type 1 (HMGA1), an important regulator of cell growth which also leads to increased cell proliferation [19]. E2F2 is transcriptionally activated by N-MYC and induces transcription activation and progression through the cell cycle, with the ultimate effect of increasing cell proliferation [20,21]. N-MYC also upregulates serine hydroxymethyltransferase 2 (SHMT2), which breaks down serine into glycine and a one-carbon moiety. The glycine produced is an important source of methyl groups for biosynthesis, and as such, SHMT2 leads to increased cell proliferation. Using a panel of diverse human cancer cell lines, it was demonstrated that glycine consumption correlated with rapid cell proliferation in cancer cells [22]. SHMT2 has been found to be highly expressed in aggressive MYCN-amplified neuroblastomas [23]. MYC also regulates transcription of PHGDH, an enzyme upstream in the SHMT2 pathway that converts 3-phosphoglycerate to serine. Six of the eleven genes in this pathway have been identified as targets of MYC [24]. The protein sulfatase 2 (SULF-2) is overexpressed in MYCN-amplified neuroblastoma cells but expressed at a much lower level in MYCN-unamplified cells [25]. Suppression of SULF-2 using small interference RNA (siRNA) decreased cell proliferation in MYCN-amplified neuroblastoma cells, indicating that SULF-2 is a potential drug target. A SULF-2 small molecule inhibitor, OKN-007, has been developed, but has yet to be examined in neuroblastoma.

Many researchers have targeted MYCN transcription in the development of anti-tumor therapies. Using antisense oligonucleotides directed against human MYCN in MYCN-amplified human neuroblastoma cells yielded approximately half as much N-MYC protein expression compared to control and in vivo led to decreased tumor growth in transgenic MYCN mice [26]. Antisense oligonucleotides are quickly degraded by nucleases, limiting their clinical potential, but more recently, morpholino antisense oligomers have been developed. These are more stable and function to knock down genes by modifying the splicing of pre-mRNA. However, to our knowledge, these have not yet been utilized to target MYCN in neuroblastoma. Kang et al. used siRNA directed against MYCN and found a downregulation in N-MYC protein expression in MYCN-amplified neuroblastoma cells but not in MYCN unamplified cells [27]. Additionally, proliferation in MYCN-amplified neuroblastoma cells was decreased with siMYCN treatment. The delivery method to the target cells and off-target effects in the clinical setting remain challenges for the clinical application of siRNA technology. An anti-gene peptide nucleic acid (PNA) conjugated with a nuclear localization signal peptide targeted against a sequence of MYCN DNA was determined to decrease N-MYC expression. Additionally, in MYCN-amplified and MYCN-unamplified/low-expressed human neuroblastoma cell lines cell proliferation decreased significantly, but in MYCN-unamplified/unexpressed cells there was no
decrease in cell proliferation [28]. PNA therapy is promising given the strong binding between PNA and DNA, but PNAs are hydrophobic and difficult to deliver to target cells. MicroRNAs (miRNAs) occur naturally in humans and regulate expression of genes by binding to mRNAs and inhibiting protein production. Using miRNAs targeted against MYCN mRNA, Buechner and colleagues noted reduced endogenous N-MYC expression and significantly impaired proliferation in MYCN-amplified neuroblastoma cell lines [29]. In contrast to siRNAs, miRNAs’ complementarity to the target mRNA is not exact, so each miRNA may target many different mRNAs and concerns remain regarding off-target effects. Recently, Puissant showed that bromodomain inhibition suppressed MYCN transcription in neuroblastoma [30]. Using a novel ligand, JQ1, which competitively displaced bromodomain and extra-terminal domain (BET) bromodomains from chromatin, MYCN transcription was decreased in vitro. Additionally, in a MYCN-amplified neuroblastoma murine model, JQ1 treatment significantly decreased tumor volume and increased survival compared to controls. Other BET bromodomain inhibitors are in preclinical trials including I-BET762 [31]. OTX015 is a BET bromodomain inhibitor which decreased cell proliferation in MYCN-amplified neuroblastoma cells in preclinical trials and is currently in Phase I clinical trials for other solid tumors [32].

Other researchers have targeted the N-MYC protein. In order for N-MYC to act as a transcription factor, it must localize to the nucleus and dimerize with MAX protein. This dimer then binds to a specific E-box sequence in DNA to transcribe genes important for cell proliferation. However, when cells have higher levels of MAX, there is not enough N-MYC available to form heterodimers, so MAX homodimerizes. Instead of stimulating proliferation, MAX homodimers stimulate transcription of genes important for cell differentiation [33]. An inhibitor of N-MYC/MAX dimerization, 10058-F4, caused cell cycle arrest, apoptosis, and differentiation in vitro, and increased survival in MYCN transgenic mice [34]. MAD is another protein that forms heterodimers with MAX. However, as opposed to N-MYC, which is expressed in proliferating tissues, MAD is a protein expressed in differentiated tissue. When MAD forms a heterodimer with MAX, it binds to the same E-box sequence, but this dimer represses transcription, thereby inhibiting cell proliferation [35,36].

NF-κB is another important transcription factor that may be activated in neuroblastoma [37]. In cells other than B cells and macrophages, NF-κB is sequestered in the cytoplasm through an interaction with inhibitor of κB (IκB) proteins [38]. It is not until IκB is phosphorylated that NF-κB is free to translocate to the nucleus where it binds to promoter sequences to induce transcription [39]. When phosphorylated, IκB is ubiquitinated and then degraded by proteasomes. Bortezomib is a reversible inhibitor of the 26S proteasome [40]. By inhibiting the proteasomal degradation of a host of proteins including IκB, bortezomib decreased cell proliferation in neuroblastoma cells and decreased growth and the number of mitotic cells in murine neuroblastoma models [41–43]. A phase I clinical trial found that bortezomib was well-tolerated in children with recurrent or refractory solid tumors, two of whom had neuroblastoma [37,44]. In some forms of neuroblastoma, NF-κB has been shown to lead to increased migration and invasion but in others it induced apoptosis [45,46]. Curcumin suppresses NF-κB activity, and was shown to inhibit proliferation in neuroblastoma cell lines and decrease tumor growth and cell proliferation in a murine neuroblastoma model [47]. Gao et al. used oleanolic acid derivatives, CDDO-Im and CDDO-Me, for NF-κB inhibition and saw decreased neuroblastoma cell proliferation [48]. CDDO-Me has also been demonstrated to delay tumor growth in neuroblastoma xenografts [49].

A third transcription factor, PHOX2B, is expressed exclusively in the nervous system. Germline mutations of PHOX2B predispose to hereditary neuroblastoma and have also been observed in sporadic cases [50–53], but remain a rare cause of neuroblastoma [54]. Sympathetic neural cells derived from chick and mouse embryos transfected with gain-of-function PHOX2B variants seen in neuroblastoma demonstrated significantly more cell proliferation than wild-type PHOX2B, indicating that wild-type PHOX2B may act a tumor suppressor [55]. PHOX2B has not yet been targeted for development of therapeutics given the low frequency of involvement in neuroblastoma.

Mutations in the tumor suppressor, P53, are the most frequent genetic alterations found in human cancer [56]. Mutations in P53 are infrequent in neuroblastoma and are mostly limited to
relapsed tumors [57–60], but wild-type P53 is often destabilized and not functional. In undifferentiated neuroblastomas, wild-type P53 was sequestered in the cytoplasm preventing its function as a transcription factor [61]. N-MYC has been found to upregulate P53 expression in neuroblastoma [62], but the protooncogene MDM2 which negatively regulates P53 is a direct transcriptional target of N-MYC, leading to increased MDM2 and subsequent decreased P53 function in MYCN-amplified neuroblastoma cells [63]. Nutlin-3, an MDM2 antagonist, releases P53 from negative control by MDM2 and thus stabilizes and activates P53. Using an in vitro model of acquired drug resistance in neuroblastoma, van Maerken treated cells with nutlin-3 and saw a significant decrease in cell proliferation in cell lines with wild-type P53 but not in those with mutant P53 [64]. They also noted increased expression of P53 and its target genes, indicating that P53 activation in neuroblastoma by nutlin-3 may yield a new therapy for drug-resistant neuroblastoma with wild-type P53. Additionally, MYCN-amplified neuroblastoma cells treated with MDM2 antagonists, nutlin-3 and MI-63, had decreased cell proliferation when compared to MYCN-unamplified cells, further illuminating the importance of the relationship between MYCN and P53 in neuroblastoma [65]. A summary of the drugs discussed in this section is provided in Table 1.

Table 1. Proteins that affect proliferation in neuroblastoma through transcription and their targeted drugs. Mechanism of action and stage of development in neuroblastoma is listed along with references.

| Target Protein | Drug               | Mechanism of Action                                      | Stage of Development | References |
|----------------|--------------------|-----------------------------------------------------------|----------------------|------------|
| SULF-2         | siSULF-2           | Double-stranded RNA that cleaves SULF-2 mRNA              | In vitro             | [25]       |
| N-MYC          | siMYCN             | Double-stranded RNA that cleaves MYCN mRNA                | In vitro             | [27]       |
| PNA            | Synthetic polymer  | Binds MYCN DNA to inhibit N-MYC expression                | In vitro             | [28]       |
| miRNAs         | Single-stranded RNA| Inhibits translation of MYCN mRNA                         | In vitro             | [29]       |
| JQ1            | Inhibits BET bromodomains |                                                  | In vivo             | [30]       |
| I-BET762       | Inhibits BET bromodomains |                                                | In vivo             | [31]       |
| OTX015         | Inhibits BET bromodomains |                                                | Clinical trial     | [32]       |
| 10058-F4       | Inhibits N-MYC/Max dimerization |                                              | In vivo             | [34]       |
| NFkB           | Bortezomib         | Inhibits proteasomal degradation of IxB, thus deactivating NFkB | Clinical trial     | [40–44]    |
| Curcumin       | Inhibits activation of NFkB |                                                | In vivo             | [47]       |
| Oleanolic acid derivatives (CDDO-Im and CDDO-Me) | Inhibits TNFα-induced targeting of NFkB to the nucleus | In vivo             | [48,49]    |
| MDM2           | Nutlin-3           | Releases P53 from negative control by MDM2                | In vivo             | [64]       |
| MI-63          | Releases P53 from negative control by MDM2                | In vivo             | [65]       |

4. Neuroblastoma and Kinases

Kinases are proteins defined by their ability to phosphorylate other proteins in cell signaling pathways. Phosphorylation either activates or inactivates the downstream protein, which in turns affects the signaling cascade. In neuroblastoma, many kinases have been found to play a role in cell proliferation.

One of the well-described signaling pathways is the RAS/RAF/MEK/ERK cascade. RAS is a small GTPase that is activated when a mitogen binds to a membrane receptor tyrosine kinase. A serine/threonine kinase, RAF, is then targeted to the cell membrane, interacts with RAS, dimerizes, and undergoes phosphorylation for activation. RAF phosphorylates MEK and MEK in turn phosphorylates ERK. Once activated by phosphorylation, ERK phosphorylates a number of proteins involved in proliferation. Abnormal activation of this pathway has been seen
Cancers 2016, 8, 13 of 21

in neuroblastoma. A recent study found that the majority (78%) of relapsed neuroblastomas had mutations predicted to activate the RAS/RAF/MEK/ERK pathway, but very few primary tumors had these mutations [66]. In preclinical studies, treatment with the MEK inhibitor U0126 decreased proliferation in neuroblastoma cell lines with high constitutive total ERK, but not in those with less total ERK, indicated that this pathway plays a role in cell proliferation in some forms of neuroblastoma [67]. Treatment of RAS/RAF-mutated neuroblastoma cell lines with the MEK inhibitors trametinib, cobimetinib, and binimetinib also decreased cell growth [66]. MEK inhibitors warrant further study as targeted therapies for relapsed neuroblastoma.

The phosphoinositide 3-kinase (PI3K)/Protein kinase B (AKT) cascade has been implicated in neuroblastoma [68–70]. When activated, PI3K phosphorylates phosphatidylinositol to produce a secondary messenger that binds downstream targets, including AKT, recruiting them to the cell membrane. The serine/threonine kinase, AKT, then acts on downstream targets to promote cell survival and proliferation. Via a series of intermediaries, the serine/threonine kinase, mechanistic target of rapamycin (mTOR), is activated [71]. Neuroblastoma tissue was found to have significant levels of activated AKT and mTOR compared to the normal adrenal medulla [68,72]. Multiple human neuroblastoma cell lines including SK-N-SH, SK-N-BE, and IMR-32 have been found to have activated AKT. Activated AKT correlated with advanced stage, unfavorable histology, poor outcome, and MYCN amplification in human tumor specimens [70]. Inhibition of mTOR with rapamycin and an analog, CCI-779, decreased neuroblastoma cell proliferation, indicating a role for the PI3K/AKT/mTOR pathway in tumorigenesis [68]. There are conflicting data regarding the relationship between MYCN amplification and the PI3K/AKT/mTOR pathway. Johnsen et al. found that the decrease in cell proliferation in MYCN-amplified tumors was greater than that observed in MYCN non-amplified tumors [68], but Misawa et al. found that maintenance of cell proliferation in neuroblastoma required mTOR function, but it appeared to be independent of MYCN induction [69].

There are a number of drugs targeting the PI3K/AKT/mTOR cascade currently in development. mTOR inhibitors rapamycin (also termed sirolimus) and its analogues, including temsirolimus, everolimus, and ridaforolimus, function by binding to the cytosolic protein FK-binding protein 12 (FKBP12). Once bound to FKBP12, the complex binds directly to mTOR, inhibiting its action [73]. Rapamycin is currently in clinical trials and has been demonstrated to decrease cell proliferation in neuroblastoma [68,69,74]. However, in neuroblastoma cells, rapamycin introduced potential for resistance by way of inducing expression of survivin, a protein that protects cells from apoptosis [74]. A phase II study of temsirolimus in children with relapsed or refractory high-grade neuroblastoma was halted as there were no objective responses. In that study it was noted that 32% of neuroblastoma subjects did experience disease stabilization, so temsirolimus may be used in the future in combination with other therapies [75]. A phase I study of everolimus in neuroblastoma has been completed and the drug is well-tolerated in children, but no objective responses were reported [76]. Similar findings were observed in a phase I study of ridaforolimus in children with refractory solid tumors [77]. It is clear that rapamycin and its analogues have only modest activity against neuroblastoma, but their effectiveness as combination therapies remains to be elucidated.

mTOR inhibitors that act as ATP-competitive inhibitors are also being evaluated. These include INK128/MLN0128, AZD2014, and OSI027. In both MYCN-amplified and non-amplified neuroblastoma cell lines, treatment with INK128/MLN0128 suppressed cell proliferation. Using xenograft models of neuroblastoma, INK128/MLN0128 led to significant tumor growth inhibition in one study, but failed to prevent progressive disease in another, although some tumors did exhibit significantly less growth [78,79]. AZD2014 has shown promise in breast cancer [80] and OSI027 had anti-proliferative activity in several human cancer cell lines, but they have not yet been examined in neuroblastoma [81]. Similar to rapamycin, the ATP-competitive mTOR inhibitors appear to have limited activity against neuroblastoma cell proliferation.

There is a feedback loop that causes reactivation of PI3K and the RAS/RAF/MEK/ERK cascade when mTOR activity is decreased [82]. Given the modest activity of both groups of mTOR inhibitors
against neuroblastoma and this feedback loop, researchers have turned to dual inhibitors targeting both PI3K and mTOR. NVP-BEZ235 is one such compound that has been used in vitro and found to have an anti-proliferative effect in neuroblastoma cell lines [83]. In an in vitro neuroblastoma model with TH-MYCN mice, NVP-BEZ235 suppressed proliferation of tumor cells and angiogenesis [84]. SF1126, another dual PI3K/mTOR inhibitor, has been demonstrated to inhibit neuroblastoma cell proliferation [85]. NVP-BKM120, developed with a different profile of targets within the PI3K/mTOR family, also inhibited cell proliferation in a variety of tumor cells, but preferentially inhibited tumor cells with PIK3CA oncogenic mutations [86]. This mutation is rare in neuroblastoma; Dam et al. found PIK3CA mutations in only 2.9% of 69 human neuroblastoma samples [87], limiting its utility for neuroblastoma. Finally, GDC-0980, has been shown to decrease cell proliferation in a variety of adult cancer cell lines, but has not been studied in neuroblastoma [88]. All of these dual PI3K/mTOR inhibitors are in various stages of clinical trials.

Another mechanism to circumvent the feedback loops that occur with mTOR inhibition is targeting the AKT protein upstream from mTOR. MK-2206 is an AKT inhibitor that has been shown to inhibit cell proliferation in neuroblastoma cells and inhibit tumor growth and increase survival in mice bearing xenograft neuroblastoma tumors [89]. A phase I trial of MK-2206 that included 3 children with neuroblastoma found that the drug was well-tolerated, but there were no objective responses or prolonged stabilization of disease [90]. Perifosine, an AKT inhibitor that also inhibits the ERK and JNK pathways [91,92], decreased neuroblastoma cell proliferation, decreased tumor growth, and increased survival in mice bearing neuroblastoma xenografts [93]. Perifosine is currently in phase I trials in pediatric solid tumors.

RET is a receptor tyrosine kinase required for normal development of the nervous system that has been found to be expressed in most neuroblastomas and overexpressed in some [94]. Upon addition of glial cell line-derived neurotrophic factor (GDNF), a growth factor and ligand for RET that neuroblastoma cells secrete, cell proliferation was stimulated in non-adherent neuroblastoma cells but not in adherent cells. These findings indicated that RET/GDNF played a role in cell proliferation in neuroblastoma [95]. Additionally, a RET-overexpressing transgenic mouse spontaneously developed neuroblastoma [96]. Vandetanib/ZD6474 inhibits activation of RET and was found to decrease neuroblastoma cell viability and proliferation and inhibit growth in neuroblastoma xenografts [97,98].

Another receptor tyrosine kinase implicated in neuroblastoma is CD117, also known as C-KIT and mast/stem cell growth factor receptor. CD117 has been correlated with both favorable and unfavorable tumors [99,100]. Vitali et al. found that 13% of neuroblastomas expressed CD117 and 23% expressed its ligand, stem cell factor (SCF), and MYCN-amplified tumors were more likely to express CD117 and SCF than non-amplified tumors. CD117 stimulation actively promoted cell proliferation in neuroblastoma [100]. Additionally, in a murine xenograft model, animals treated with imatinib developed smaller tumors than controls [102]. The concentration of imatinib required to achieve growth inhibition in neuroblastoma was higher than that required to inhibit CD117 activation indicating that there was an additional mechanism of action. In neuroblastoma cells, imatinib was also found to inhibit C-ABL, a tyrosine kinase implicated in multiple cancers due to its role in cell differentiation, proliferation, and adhesion. Imatinib-induced C-ABL inhibition yielded a dose-dependent decrease in neuroblastoma cell proliferation [103]. A phase II clinical trial studying imatinib in refractory and relapsing metastatic neuroblastoma found that it was well-tolerated and effective with 21% showing a complete response and 8% a partial response [104].

Neuroblastoma cells have been shown to have significantly higher activity of the kinase C-SRC than normal human fibroblasts or glioblastoma cells [105]. Expression of C-SRC is correlated with a favorable prognosis and is inversely correlated with MYCN amplification [106]. Investigators have targeted C-SRC in an attempt to treat neuroblastoma. The C-SRC inhibitor PP2 and the dual C-SRC/ABL inhibitor dasatinib decreased cell proliferation in neuroblastoma cells [107,108].
As selective C-SRC inhibitors continue to be developed, their anti-proliferative activity improves, making them a potential treatment for neuroblastoma [109].

Focal adhesion kinase (FAK) is a non-receptor tyrosine kinase that is regulated by N-MYC and is more frequently expressed in aggressive forms of neuroblastoma [110]. When phosphorylated, FAK binds to SRC and activates downstream signaling pathways that control cell proliferation, viability, motility, and survival [111–114]. Inhibition of FAK has been achieved using a variety of methods. By using adenoviral gene transduction of the carboxyl-terminal domain of FAK (AdFAK-CD), FAK phosphorylation was decreased [115] and AdFAK-CD decreased neuroblastoma cell proliferation, with a larger proportional decrease in proliferation of MYCN-amplified cells than MCYN non-amplified cells [116]. siRNA has also been used to decrease expression of FAK. In neuroblastoma cells, treatment with siFAK led to decreased cell survival, with a larger effect in MYCN-amplified cell lines [117]. Small molecule inhibitors of FAK include NVP-TAE226, 1,2,4,5-benzenetetramine tetrahydrochloride (Y15), and PF-04554878. Treatment of neuroblastoma cells with NVP-TAE226 resulted in a decrease in cell viability [118]. Y15 has been demonstrated to decrease viability in neuroblastoma cells and to inhibit neuroblastoma tumor growth in mouse xenograft models [119]. A phase I clinical trial studying the small molecular FAK inhibitor, PF-04554878, in adults with advanced solid tumors has been completed and others are currently in progress. Another approach to inhibiting FAK is by interrupting its interaction with vascular endothelial growth factor receptor 3 (VEGFR-3) using a small molecular inhibitor, chloropyramine hydrochloride (C4). Treatment of neuroblastoma cells with C4 decreased cell viability and treatment of mouse xenografts with C4 decreased neuroblastoma tumor growth [120].

Aurora kinase A is a serine/threonine kinase that has been associated with poor prognosis in neuroblastoma [121]. Aurora kinase A stabilizes N-MYC, thereby increasing neuroblastoma cell proliferation [122,123]. Additionally, Aurora kinase A activity increased VEGF secretion and angiogenesis. Inhibition of Aurora kinase A with the small molecule alisertib/MLN8237 decreased cell proliferation and inhibited anchorage-independent growth in neuroblastoma cells [124]. A structurally similar inhibitor, MLN8054, was shown to inhibit proliferation in neuroblastoma cells and both MLN8054 and alisertib/MLN8237 led to complete response rates of about 50% in a transgenic TH-MYCN mouse model of neuroblastoma [123]. In a phase I/II clinical trial for MLN8237 in children with refractory/recurrent solid tumors 2 of 11 children with neuroblastoma attained prolonged stable disease [125]. Aurora kinase A inhibitors offer a promising new direction for treatment of neuroblastoma, particularly in MYCN-amplified neuroblastomas given the relationship between Aurora kinase A and N-MYC.

Aurora kinase B is a related serine/threonine kinase that plays a role in the attachment of the mitotic spindle to the centromere during mitosis. Patients with high Aurora kinase B expression have a worse prognosis than those with low/normal expression [126]. Aurora kinase B does not stabilize N-MYC and does not affect N-MYC levels [123]. Conversely, it is transcriptionally regulated by N-MYC. Barasertib is a specific Aurora kinase B inhibitor which has been shown to decrease cell growth in neuroblastoma cell lines which are MYCN-amplified and have wild-type P53 [126]. However, MYCN-unamplified and mutant P53 cell lines were less sensitive to the drug. Using the IMR5 neuroblastoma cell line, which is MYCN-amplified and has wild-type P53, barasertib induced cell cycle arrest at G2/M. In a xenograft model with the same cell line, barasertib decreased tumor growth. Barasertib has not yet been examined in pediatric clinical trials. CCT137690, an inhibitor of both Aurora kinase A and B, inhibited cell proliferation in MYCN-amplified neuroblastoma cells and tumor growth in MYCN-driven transgenic tumors in vivo [127].

Anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase expressed in the developing nervous system [128]. Oncogenic mutations in the ALK gene play a role in neuroblastoma pathogenesis and are highly correlated with MYCN amplification [129]. Mutant ALK in neuroblastoma has increased kinase activity compared to wild-type ALK. Using RNA interference (RNAi) to knock down ALK in neuroblastoma cells harboring mutant ALK resulted in decreased cell proliferation. Crizotinib/PF-02341066 is a small molecule inhibitor targeting ALK and other receptor tyrosine
kinases, MET and ROS1, that decreased proliferation in neuroblastoma cell lines with abnormal ALK, particularly those with the R1275Q mutation [130]. In mice with neuroblastoma xenografts containing R1275Q-ALK, crizotinib caused tumor regression within three weeks and complete regression over the fourth week. Crizotinib is FDA-approved for treatment of non-small cell lung cancer. It has also been examined in a phase I trial in pediatric patients with refractory solid tumors including 34 patients with neuroblastoma. Crizotinib was well-tolerated with two of the 34 patients with neuroblastoma experiencing complete response and eight experiencing stable disease [131]. Multiple clinical trials are underway examining crizotinib in neuroblastoma.

Sorafenib is a multi-kinase inhibitor targeting VEGFR, PDGFR, CD117, RAF, and RET [132]. It is currently approved for treatment of renal cell carcinoma, hepatocellular carcinoma, and thyroid cancer in adults. Sorafenib has been studied in neuroblastoma and was shown to decrease cell proliferation both in vivo and in vitro with cell cycle arrest at the G1 checkpoint [133]. In the Pediatric Preclinical Test Program in vivo and in vitro panels, which included 4 neuroblastoma cell lines and 5 neuroblastoma xenografts, sorafenib was shown to decrease growth in the majority of neuroblastoma samples [134]. A phase I pediatric study revealed similar toxicities to those seen in adults, but no further clinical studies have been completed [135]. A summary of targets and drugs discussed in this section is available in Table 2.

Table 2. Kinases affecting proliferation in neuroblastoma and their targeted drugs. Mechanism of action for each drug and stage of development in neuroblastoma is listed along with references.

| Target Protein | Drug | Mechanism of Action | Stage of Development | References |
|----------------|------|---------------------|----------------------|------------|
| MEK            | U0126| Binds/inhibits MEK  | In vitro             | [67]       |
|                | Trametinib | Binds/inhibits MEK  | In vitro             | [66]       |
|                | Cobimetinib | Binds/inhibits MEK  | In vitro             | [66]       |
|                | Binimetinib | Binds/inhibits MEK  | In vitro             | [66]       |
| mTOR           | Rapamycin | Binds FKBP12 inhibits mTOR | Clinical trial | [68,69,73,74] |
|                | CCI-779/Temsirolimus | Binds FKBP12 inhibits mTOR | Clinical trial | [68,73,75] |
|                | Everolimus | Binds FKBP12 inhibits mTOR | Clinical trial | [73,76]   |
|                | Ridaforolimus | Binds FKBP12 inhibits mTOR | Clinical trial | [73,77]   |
|                | INK128/MLN0128 | ATP-competitive mTOR inhibitor | In vivo | [78,79]  |
| PI3K/mTOR      | NVP-BEZ235 | Inhibits PI3K and mTOR | In vivo             | [83,84]   |
|                | SF1126 | Reversibly inhibits PI3K and mTOR | Clinical trial | [85,136] |
|                | NVP-BKM120 | Inhibits PI3K and mTOR in ATP-competitive manner | In vitro | [86]      |
| AKT            | MK-2206 | Inhibits AKT via allosteric binding | Clinical trial | [89,90] |
|                | Perifosine | Binds AKT, inhibiting translocation to the plasma membrane | Clinical trial | [93,137] |
| RET            | Vandetanib/ZD6474 | Inhibits activation of RET | In vivo | [97,98] |
| CD117/ABL/PDGFR | Imatinib | Binds active site and inhibits kinases | Clinical trial | [100,102–104] |
| C-SRC          | PP2 | Binds and acts as a mixed competitive inhibitor for C-SRC | In vitro | [107]     |
| C-SRC/ABL      | Dasatinib | Binds adenosine pocket inhibits C-SRC and ABL | Clinical trial | [108,138] |
| FAK            | AdFAK-CD | Adenoviral gene transduction of the carboxyl-terminal domain of FAK | In vitro | [115,116] |
|                | siFAK | Double-stranded RNA cleaves FAK mRNA | In vitro | [117]     |
Table 2. Cont.

| Target Protein | Drug | Mechanism of Action | Stage of Development | References |
|----------------|------|---------------------|----------------------|------------|
| NVP-TAE226     | Inhibits phosphorylation of FAK | In vivo | [118,139] |
| Y15            | Inhibits phosphorylation of FAK | In vivo | [119] |
| Chloropyramine hydrochloride/C4 | Inhibits interaction between FAK and VEGFR-3 | In vivo | [120] |
| Aurora kinase A | Alisertib/MLN8237 | Binds/inhibits Aurora kinase A | Clinical trial | [123,125] |
| MLN8054        | Binds/inhibits Aurora kinase A | In vivo | [123] |
| Aurora kinase B | Barasertib | Binds ATP pocket/inhibits Aurora kinase B | In vivo | [126] |
| Aurora kinase A/B | CCT137690 | Binds/inhibits Aurora kinase A and B | In vivo | [127] |
| ALK            | siALK | Double-stranded RNA cleaves ALK mRNA | In vitro | [129] |
| ALK/MET/ROS1   | Crizotinib/PF-02341066 | Binds/inhibits ALK, MET, and ROS1 | Clinical trial | [130,131] |
| VEGFR/PDGFR/CD117/RAF/RET | Sorafenib | Binds/inhibits VEGFR, PDGFR, CD117, RAF, and RET | Clinical trial | [132–135] |

5. Neuroblastoma and Cell Cycle Checkpoints

In normal cells, the cell cycle is highly regulated at checkpoints by multiple protein families—cyclins, cyclin dependent kinases (CDKs), CDK inhibitors (CKIs), and tumor suppressors including P53 and RB [140]. These proteins function as accelerators or brakes to advance the cell cycle to the next phase or halt the cycle at the checkpoint.

P53 regulates the G1/S and G2/M checkpoints in the cell cycle. It has been demonstrated that tumor cells lacking normal P53 function continue through the G1/S checkpoint. P53 is a transcription factor for WAF1/CIP1/P21, a CKI [141]. By upregulating P21 expression, CDKs are inhibited, leading to arrest of the cell cycle and therefore a decrease in cell proliferation. In some neuroblastoma cells treated with nutlin-3, a P53 activator, induction of P21 and arrest of the cell cycle arrest at the G1/S checkpoint was observed [65], but this effect was cell line dependent.

Another tumor suppressor, RB, has been demonstrated to play a role in neuroblastoma. It acts as a regulator of the G1/S checkpoint, restricting cell cycle progression and thus cell proliferation [142]. Unphosphorylated RB remains bound to the transcription factor E2F. RB phosphorylation leads to the release of E2F, which activates a multitude of proto-oncogenes and DNA polymerase to start the replication process [143,144]. In a study examining the bone marrow of 4 children with neuroblastoma, only one child had a mutation in the RB protein and that child was the only one with bone marrow infiltration who died, correlating with advanced disease [145]. RB has been indirectly targeted in neuroblastoma cells using difluoromethylornithine (DFMO). DFMO decreased RB phosphorylation in MYCN-amplified neuroblastoma cells and significantly decreased cell growth, likely mediated by the protein P27 [146]. To our knowledge, no drugs have been developed specifically targeting RB in neuroblastoma.

RB phosphorylation is regulated by CDK4 and CDK6. CDK4/6 mRNA expression in human neuroblastoma tissue was determined to be high compared to normal tissue [147]. CDK4 expression correlated with a poor prognosis and CDK6 expression correlated with undifferentiated histology, also correlating with poor prognosis. The specific CDK4/6 inhibitor, ribociclib/LEE011, reduced proliferation in MYCN-amplified neuroblastoma in vitro and decreased growth of murine neuroblastoma xenografts [148]. A phase I clinical trial examining LEE011 in children with malignant rhabdoid tumors and neuroblastoma is underway. Palbociclib/PD-0332991 is a CKI targeting CDK4/6 that is FDA-approved for breast cancer and has been demonstrated to effectively decrease
phosphorylation of RB, causing a potent anti-proliferative effect in a variety of adult tumor cells [149]. Palbociclib has recently been found to reduce the growth of neuroblastoma cells in vitro, but has not yet been studied in neuroblastoma in vivo or in clinical trials [150].

While DFMO indirectly targets RB, it directly inhibits ornithine decarboxylase (ODC), the rate-limiting enzyme in polyamine biosynthesis [146,151,152]. Polyamines are critical for cell survival; ODC along with an increase in polyamines has been seen in a variety of cancers [153,154]. A decrease in polyamines, such as that caused by DFMO, initiates cell cycle arrest or apoptosis whereas an increase in polyamines allows the cell to continue through the cell cycle [146]. The gene expressing ODC, ODC1, is activated by N-MYC and is upregulated in MYCN-amplified human neuroblastomas [155,156]. Even some tumors without MYCN amplification had elevated ODC mRNA levels. High ODC expression, regardless of MYCN status, correlates with poor survival [156,157]. The use of DFMO in MYCN-amplified neuroblastoma cells has been demonstrated to deplete polyamines, impair proliferation, and induce G1 cell cycle arrest without apoptosis [146,158]. The cell cycle arrest was associated with an increase in the P27Kip1 cyclin-dependent kinase inhibitor.

Aside from CDK4 and 6, other CDKs have been examined as potential targets for neuroblastoma treatment given their importance in cell proliferation through regulation of the cell cycle. SNS-032, designed as a CDK2 inhibitor, also inhibits CDK7 and 9 and causes G2 cell cycle arrest [160,161]. CDK2 is necessary for progression through the G1/S checkpoint through its interaction with cyclin E and is necessary for progression through the S phase through its interaction with cyclin A [162,163]. CDK7 and 9 play critical roles in transcription initiation and elongation and expression of both was decreased with treatment of UKF-NB-3 and IMR-32 neuroblastoma cells with SNS-032 [164]. However, most of the drug’s effects were due to cytotoxicity as opposed to a decrease in proliferation. CDK2 has also been targeted using miRNA and siRNA. miR-885-5p is a miRNA that downregulates CDK2. Both miR-885-5p and siCDK2 inhibited neuroblastoma proliferation in a number of cell lines [165]. A specific inhibitor targeting CDK7, THZ1, has also been examined in neuroblastoma. In MYCN-driven neuroblastoma cells, THZ1 led to cell cycle arrest. Additionally, in MYCN-amplified neuroblastoma xenograft tumors in mice, cell cycle arrest and a decrease in markers associated with transcription were observed [166].

The genes encoding multiple proteins involved at cell cycle checkpoints are targets for a group of enzymes that remove acetylate groups from histones, termed histone deacetylases (HDACs). The balance between histone acetylation and deacetylation determines chromatin configuration, which determines the amount of gene transcription that can occur. Histone acetylation causes the chromatin to have an open configuration, allowing for transcription, whereas histone deacetylation compacts chromatin and blocks transcription. Genes that are repressed by HDACs are often tumor suppressors, cell-cycle inhibitors, and inducers of apoptosis. A variety of HDAC inhibitors have been developed as potential treatments for cancer. In the Pediatric Preclinical Testing Program, vorinostat, a nonselective HDAC inhibitor, yielded significant growth inhibition in four neuroblastoma cell lines [167]. However, in neuroblastoma xenografts, vorinostat treatment did not lead to any objective responses. Other nonselective HDAC inhibitors—sodium butyrate, suberoylanilide hydroxamic acid, and trichostatin A—induced cell cycle arrest in the G2/M phase in neuroblastoma cells [168]. BL1521 is another HDAC inhibitor that caused G1 arrest along with an increase in the CK1 P21, decreased CDK4, and RB hypophosphorylation [169,170]. The expression of HDAC8, a specific protein within the family, correlates with advanced disease and poor outcome in neuroblastoma [171]. By knocking down HDAC8 using siRNA, neuroblastoma cell proliferation decreased. The small molecule inhibitor of HDAC8, PCI-48012, decreased cell proliferation in neuroblastoma both in vitro and in vivo [172]. HDAC5 also promotes neuroblastoma cell proliferation and has been targeting using siRNA, yielding
a decrease in proliferation [173]. However, no small molecule inhibitor specific for HDAC5 has been used in neuroblastoma. Other specific HDAC inhibitors also have efficacy in neuroblastoma, but through mechanisms other than a change in cell proliferation—HDAC1 and HDAC2 inhibitors induce differentiation, HDAC6 inhibitors decrease migration and invasion, and HDAC10 inhibitors decrease cell survival by autophagy [174–176]. Vorinostat has been examined in a phase I clinical trial in children with recurrent solid tumors, including two with neuroblastoma, and found to be well-tolerated [177]. None of the patients had objective responses to vorinostat alone, but one of the patients with neuroblastoma had a complete response to a combination of vorinostat and 13-cis retinoic acid. A number of other HDAC inhibitors are currently in pediatric clinical trials.

A kinome screen with RNAi found that loss of checkpoint kinase 1 (CHK1) was cytotoxic with ≥50% growth inhibition in neuroblastoma cells but no inhibition in normal somatic cells [178]. Given that it only decreases growth in the tumor cells, this is a promising therapeutic target. CHK1 regulates the G1/S and G2/M checkpoints and is expressed at a significantly higher level in MYCN-amplified compared to MYCN-unamplified neuroblastoma [178]. The small molecule checkpoint kinase inhibitors SB218078 and TCS2312 decreased proliferation of neuroblastoma cells. PF-00477736 is another CHK1 inhibitor that has been demonstrated to decrease tumor growth in mouse neuroblastoma xenografts. A phase I clinical trial was begun for PF-00477736, but was terminated for business reasons. Multiple clinical trials examining the CHK1 inhibitor LY2606368 in adults are in progress, but this drug has not yet been examined in neuroblastoma. A summary of drugs discussed in this section is available in Table 3.

Table 3. Regulators of the cell cycle affecting proliferation in neuroblastoma and the drugs that target them. Mechanism of action for each drug and stage of development in neuroblastoma is listed along with references.

| Target Protein | Drug | Mechanism of Action | Stage of Development | References |
|----------------|------|---------------------|----------------------|------------|
| ODC            | DFMO | Binds/inhibits ODC irreversibly | Clinical trial       | [146,151,152,155–159] |
| CDK4/6         | Ribociclib/LEE011 | Inhibits CDK4 and 6 | Clinical trial | [148] |
|                | Palbociclib/PD-0332991 | Inhibits CDK4 and 6 | In vitro          | [149,150] |
| CDK2/7/9       | SNS-032 | Inhibits CDK2, 7 and 9 | In vitro          | [160,161,164] |
| CDK2           | siCDK2 | Double-stranded RNA cleaves CDK2 mRNA | In vitro          | [165] |
|                | miR-885-5p | Single-stranded RNA inhibits translation of CDK2 mRNA | In vitro          | [165] |
| CDK7           | THZ1  | Inhibits CDK7        | In vivo            | [166] |
| Nonselective HDACs | Vorinostat | Nonselectively inhibits HDACs | Clinical trial | [167,177] |
|                | Sodium butyrate | Nonselectively inhibits HDACs | In vivo          | [168] |
|                | Suberoylanilide hydroxamic acid | Nonselectively inhibits HDACs | In vivo          | [168] |
|                | Trichostatin A | Nonselectively inhibits HDACs | In vivo          | [168,179] |
|                | BL1521 | Nonselectively inhibits HDACs | In vivo          | [169,170] |
| HDAC8          | siHDAC8 | Double-stranded RNA cleaves HDAC8 mRNA | In vivo          | [171] |
|                | PCI-48012 | Binds metal binding site/inhibits HDAC8 | In vivo          | [172] |
| HDAC5          | siHDAC5 | Double-stranded RNA cleaves HDAC5 mRNA | In vivo          | [173] |
| CHK1           | SB218078 | Binds ATP pocket/inhibits CHK1 competitively | In vitro          | [178] |
|                | TCS2312 | Binds ATP pocket/inhibits CHK1 competitively | In vitro          | [178] |
|                | PF-00477736 | Binds ATP pocket/inhibits CHK1 competitively | In vivo          | [178] |
6. Conclusions

In conclusion, transcription factors, kinases, and regulators of cell cycle checkpoints play a significant role in cell proliferation in neuroblastoma. Despite the poor prognosis associated with neuroblastoma, there is vast potential for therapeutic targets, many of which are still in the early stages of examination and development, and many more of which have yet to be discovered. These novel therapies have the potential to transform the treatment of neuroblastoma in the future.

Acknowledgments: This work was supported in part by a grant from the National Cancer Institute T32CA091078 (L.L.S.). The content of this manuscript was solely the responsibility of the authors and does not necessarily represent the official views of the National Cancer Institute.

Author Contributions: L.L.S.—manuscript preparation. E.A.B.—manuscript revision. All authors reviewed the manuscript.

Conflicts of Interest: The authors declare no conflicts of interest.

References
1. Lanzkowsky, P. Manual of Pediatric Hematology and Oncology; Elsevier: Amsterdam, The Netherlands, 2011; pp. 671–694.
2. Maris, J.M.; Hogarty, M.D.; Bagatell, R.; Cohn, S.L. Neuroblastoma. *Lancet* 2007, 369, 2106–2120. [CrossRef]
3. Cotterill, S.J.; Parker, L.; More, L.; Craft, A.W. Neuroblastoma: Changing incidence and survival in young people aged 0–24 years. A report from the north of England young persons’ malignant disease registry. *Med. Pediatr. Oncol.* 2001, 36, 231–234. [CrossRef]
4. Spix, C.; Pastore, G.; Sankila, R.; Stiller, C.A.; Steliarova-Foucher, E. Neuroblastoma incidence and survival in European children (1978–1997): Report from the automated childhood cancer information system project. *Eur. J. Cancer* 2006, 42, 2081–2091. [CrossRef] [PubMed]
5. Maris, J.M. Recent advances in neuroblastoma. *N. Engl. J. Med.* 2010, 362, 2202–2211. [CrossRef] [PubMed]
6. Blume-Jensen, P.; Hunter, T. Oncogenic kinase signalling. *Nature* 2001, 411, 355–365. [CrossRef] [PubMed]
7. Brown, G.; Hughes, P.J.; Michell, R.H. Cell differentiation and proliferation—Simultaneous but independent? *Exp. Cell Res.* 2003, 291, 282–288. [CrossRef]
8. Alex, R.; Sözeri, O.; Meyer, S.; Dildrop, R. Determination of the DNA sequence recognized by the BHLH-zip domain of the N-Myc protein. *Nucleic Acids Res.* 1992, 20, 2257–2263. [CrossRef] [PubMed]
9. Murphy, D.M.; Buckley, P.G.; Bryan, K.; Das, S.; Alcock, L.; Foley, N.H.; Prenter, S.; Bray, I.; Watters, K.M.; Higgins, D.; et al. Global MYCN transcription factor binding analysis in neuroblastoma reveals association with distinct e-box motifs and regions of DNA hypermethylation. *PLoS ONE* 2009, 4, e8154. [CrossRef] [PubMed]
10. Zimmerman, K.A.; Yancopoulos, G.D.; Collum, R.G.; Smith, R.K.; Kohl, N.E.; Denis, K.A.; Nau, M.M.; Witte, O.N.; Toran-Allerand, D.; Gee, C.E. Differential expression of myc family genes during murine development. *Nature* 1986, 319, 780–783. [CrossRef] [PubMed]
11. Deyell, R.J.; Attiyeh, E.F. Advances in the understanding of constitutional and somatic genomic alterations in neuroblastoma. *Cancer Genet.* 2011, 204, 113–121. [CrossRef] [PubMed]
12. Wang, L.L.; Teshiba, R.; Ikegaki, N.; Tang, X.X.; Naranjo, A.; London, W.B.; Hogarty, M.D.; Gastier-Foster, J.M.; Look, A.T.; Park, J.R.; et al. Augmented expression of MYC and/or MYCN protein defines highly aggressive MYC-driven neuroblastoma: A children’s oncology group study. *Br. J. Cancer* 2015, 113, 57–63. [CrossRef] [PubMed]
13. Brodeur, G.M.; Seeger, R.C.; Schwab, M.; Varmus, H.E.; Bishop, J.M. Amplification of N-Myc in untreated human neuroblastomas correlates with advanced disease stage. *Science* 1984, 224, 1121–1124. [CrossRef] [PubMed]
14. Schwab, M.; Alitalo, K.; Klemmpnauer, K.H.; Varmus, H.E.; Bishop, J.M.; Gilbert, F.; Brodeur, G.; Goldstein, M.; Trent, J. Amplified DNA with limited homology to MYC cellular oncogene is shared by human neuroblastoma cell lines and a neuroblastoma tumour. *Nature* 1983, 305, 245–248. [CrossRef] [PubMed]
15. Molenaar, J.J.; Domingo-Fernández, R.; Ebus, M.E.; Lindner, S.; Koster, J.; Drabek, K.; Mestdagh, P.; van Sluis, P.; Valentijn, L.J.; van Nes, J.; et al. Lin28b induces neuroblastoma and enhances MYCN levels via let-7 suppression. *Nat. Genet.* 2012, 44, 1199–1206. [CrossRef] [PubMed]
16. Schweigerer, L.; Breit, S.; Wenzel, A.; Tsunamoto, K.; Ludwig, R.; Schwab, M. Augmented MYCN expression advances the malignant phenotype of human neuroblastoma cells: Evidence for induction of autocrine growth factor activity. *Cancer Res.* 1990, 50, 4411–4416. [PubMed]

17. Negroni, A.; Scarpa, S.; Romeo, A.; Ferrari, S.; Modesti, A.; Raschella, G. Decrease of proliferation rate and induction of differentiation by a MYCN antisense DNA oligomer in a human neuroblastoma cell line. *Cell Growth Differ.* 1991, 2, 511–518. [PubMed]

18. Zhang, Z.; Fauzi, M.; Huang, J.; Geerts, D.; Yu, H.; Fleig, A.; Penner, R. N-Myc-induced up-regulation of TRPM6/TRPM7 channels promotes neuroblastoma cell proliferation. *Oncotarget* 2014, 5, 7625–7634. [CrossRef] [PubMed]

19. Giannini, G.; Cerignoli, F.; Mellone, M.; Massimi, I.; Ambrosi, C.; Rinaldi, C.; Dominici, C.; Frati, L.; Screpani, I.; Gulino, A. High mobility group A1 is a molecular target for MYCN in human neuroblastoma. *Cancer Res.* 2005, 65, 8308–8316. [CrossRef] [PubMed]

20. Sears, R.; Ohtani, K.; Nevins, J.R. Identification of positively and negatively acting elements regulating expression of the E2F2 gene in response to cell growth signals. *Mol. Cell. Biol.* 1997, 17, 5227–5235. [CrossRef] [PubMed]

21. DeGregori, J.; Leone, G.; Miron, A.; Jakoi, L.; Nevins, J.R. Distinct roles for E2F proteins in cell growth control and apoptosis. *Proc. Natl. Acad. Sci. USA* 1997, 94, 7245–7250. [CrossRef] [PubMed]

22. Jain, M.; Nilsson, R.; Sharma, S.; Madhusudhan, N.; Kitami, T.; Souza, A.L.; Kafri, R.; Kirschner, M.W.; Chish, C.B.; Mootha, V.K. Metabolite profiling identifies a key role for glycine in rapid cancer cell proliferation. *Science* 2012, 336, 1040–1044. [CrossRef] [PubMed]

23. Ye, J.; Fan, J.; Venneti, S.; Wan, Y.W.; Pawel, B.R.; Zhang, J.; Finley, L.W.; Lu, C.; Lindsten, T.; Cross, J.R.; et al. Serine catabolism regulates mitochondrial redox control during hypoxia. *Cancer Discov.* 2014, 4, 1406–1417. [CrossRef] [PubMed]

24. Vazquez, A.; Markert, E.K.; Oltvai, Z.N. Serine biosynthesis with one carbon catabolism and the glycine cleavage system represents a novel pathway for ATP generation. *PLoS ONE* 2011, 6, e25881. [CrossRef] [PubMed]

25. Solari, V.; Borriello, L.; Turcatel, G.; Shimada, H.; Sposto, R.; Fernandez, G.E.; Asgharzadeh, S.; Yates, E.A.; Turnbull, J.E.; DeClerck, Y.A. MYCN-dependent expression of sulfatase-2 regulates neuroblastoma cell survival. *Cancer Res.* 2014, 74, 5999–6009. [CrossRef] [PubMed]

26. Burkhart, C.A.; Cheng, A.J.; Madafiglio, J.; Kavallaris, M.; Marshall, G.M.; Weiss, W.A.; Khachigian, L.M.; Norris, M.D.; Haber, M. Effects of MYCN antisense oligonucleotide administration on tumorigenesis in a murine model of neuroblastoma. *J. Natl. Cancer Inst.* 2005, 97, 1394–1403. [CrossRef] [PubMed]

27. Kang, J.H.; Rychahou, P.G.; Ishola, T.A.; Qiao, J.; Evers, B.M.; Chung, D.H. MYCN silencing induces differentiation and apoptosis in human neuroblastoma cells. *Biochem. Biophys. Res. Commun.* 2006, 351, 192–197. [CrossRef] [PubMed]

28. Tonelli, R.; Purgato, S.; Camerin, C.; Franzoni, R.; Alboresi, S.; Franzoni, M.; Corradini, R.; Faccini, A.; et al. Anti-gene peptide nucleic acid specifically inhibits MYCN expression in human neuroblastoma cells leading to cell growth inhibition and apoptosis. *Cancer Discov.* 2013, 3, 308–323. [CrossRef] [PubMed]

29. Buechner, J.; Tamte, E.; Haug, B.H.; Henriksen, J.R.; Løkke, C.; Flægstad, T.; Einvik, C. Tumour-suppressor microRNAs let-7 and mir-101 target the proto-oncogene MYCN and inhibit cell proliferation in MYCN-amplified neuroblastoma. *Br. J. Cancer* 2011, 105, 296–303. [CrossRef] [PubMed]

30. Puissant, A.; Frumm, S.M.; Alexe, G.; Qi, J.; Chanthery, Y.H.; Zeid, R.; Gustafson, W.C.; Greninger, P.; et al. Targeting MYCN in neuroblastoma by bet bromodomain inhibition. *Cancer Discov.* 2013, 3, 308–323. [CrossRef] [PubMed]

31. Wyce, A.; Ganji, G.; Smitheman, K.N.; Chung, C.W.; Korenchuk, S.; Bai, Y.; Barbash, O.; Le, B.; Craggs, P.D.; McCabe, M.T.; et al. Bet inhibition silences expression of MYCN and BCL2 and induces cytotoxicity in neuroblastoma tumor models. *PLoS ONE* 2013, 8, e72967. [CrossRef] [PubMed]

32. Schulte, J.H.; Althoff, K.; Bell, E.; Odersky, A.; Beckers, A.; Spelman, F.; Schafers, S.; Schramm, A.; Eggert, A.; Westermann, F.; et al. Abstract 3967: Bet protein inhibitor OTX015 has selective anti-tumoral activity in preclinical models of MYCN-amplified neuroblastoma. In Proceedings of the AACR Annual Meeting 2014, San Diego, CA, USA, 5–9 April 2014.
33. Albanus, R.D.; Juliani Siqueira Dalmolin, R.; Alves Castro, M.A.; Augusto de Bittencourt Pasquali, M.; de Miranda Ramos, V.; Pens Gelain, D.; Fonseca Moreira, J.C. Reverse engineering the neuroblastoma regulatory network uncovers max as one of the master regulators of tumor progression. *PloS ONE* 2013, 8, e82457. [CrossRef] [PubMed]

34. Zirath, H.; Frenzel, A.; Oliynyk, G.; Segerström, L.; Westermark, U.K.; Larsson, K.; Munksgaard Persson, M.; Hultenby, K.; Lehtio, J.; Einvik, C.; et al. MYC inhibition induces metabolic changes leading to accumulation of lipid droplets in tumor cells. *Proc. Natl. Acad. Sci. USA* 2013, 110, 10258–10263. [CrossRef] [PubMed]

35. Ayer, D.E.; Kretzner, L.; Eisenman, R.N. Mad: A heterodimeric partner for max that antagonizes MYC transcriptional activity. *Cell* 1993, 72, 211–222. [CrossRef]

36. Grandori, C.; Cowley, S.M.; James, L.P.; Eisenman, R.N. The MYC/MAX/MAD network and the regulatory network uncovers max as one of the master regulators of tumor progression. *Cancers* 2016, 8, 13, 14 of 21.

37. Körner, M.; Tarantino, N.; Pleskoff, O.; Lee, L.M.; Debré, P. Activation of nuclear factor κB in human neuroblastoma cell lines. *J. Neurochem.* 1994, 62, 1716–1726. [CrossRef] [PubMed]

38. Baeuerle, P.A.; Baltimore, D. I κB: A specific inhibitor of the NF-κB transcription factor. *Science* 1988, 242, 540–546. [CrossRef] [PubMed]

39. DiDonato, J.A.; Hayakawa, M.; Rothwarf, D.M.; Zandi, E.; Karin, M. A cytokine-responsive IκB kinase that activates the transcription factor NF-κB. *Nature* 1997, 388, 548–554. [PubMed]

40. Chen, D.; Frezza, M.; Schmitt, S.; Kanwar, J.; Dou, Q.P. Bortezomib as the first proteasome inhibitor anticancer drug: Current status and future perspectives. *Curr. Cancer Drug Targets* 2011, 11, 239–253. [CrossRef] [PubMed]

41. Brignole, C.; Marimpietri, D.; Pastorino, F.; Nico, B.; di Paolo, D.; Cioni, M.; Piccardi, F.; Cilli, M.; Pezzolo, A.; Corrias, M.V.; et al. Effect of bortezomib on human neuroblastoma cell growth, apoptosis, and angiogenesis. *J. Natl. Cancer Inst.* 2006, 98, 1142–1157. [CrossRef] [PubMed]

42. Valentiner, U.; Haane, C.; Nehmann, N.; Schumacher, U. Effects of bortezomib on human neuroblastoma cells in vitro and in a metastatic xenograft model. *Anticancer Res.* 2009, 29, 1219–1225. [CrossRef] [PubMed]

43. Hamner, J.B.; Dickson, P.V.; Sims, T.L.; Zhou, J.; Spence, Y.; Ng, C.Y.; Davidoff, A.M. Bortezomib inhibits angiogenesis and reduces tumor burden in a murine model of neuroblastoma. *Surgery* 2007, 142, 185–191. [CrossRef] [PubMed]

44. Bortezomib inhibits angiogenesis and reduces tumor burden in a murine model of neuroblastoma. *Surgery* 2007, 142, 185–191. [CrossRef] [PubMed]

45. Blaney, S.M.; Bernstein, M.; Neville, K.; Ginsberg, J.; Kitchen, B.; Berg, S.L.; Krailo, M.; Adamson, P.C. Phase I study of the proteasome inhibitor bortezomib in pediatric patients with refractory solid tumors: A children’s oncology group study (advl0015). *J. Clin. Oncol.* 2004, 22, 4804–4809. [CrossRef] [PubMed]

46. Chen, D.; Frezza, M.; Schmitt, S.; Kanwar, J.; Dou, Q.P. Bortezomib as the first proteasome inhibitor anticancer drug: Current status and future perspectives. *Curr. Cancer Drug Targets* 2011, 11, 239–253. [CrossRef] [PubMed]

47. Orr, W.S.; Denbo, J.W.; Saab, K.R.; Myers, A.L.; Ng, C.Y.; Zhou, J.; Morton, C.L.; Pfeffer, L.M.; Davidoff, A.M. NF-κB signaling pathway confers neuroblastoma cells migration and invasion ability via the regulation of c-cxcr4. *Med. Sci. Monit.* 2014, 20, 2746–2752. [PubMed]

48. Bian, X.; McAllister-Lucas, L.M.; Shao, F.; Schumacher, K.R.; Feng, Z.; Porter, A.G.; Castle, V.P.; Opipari, A.W. NF-κB activation mediates doxorubicin-induced cell death in N-type neuroblastoma cells. *J. Biol. Chem.* 2001, 276, 48921–48929. [CrossRef] [PubMed]

49. Orr, W.S.; Denbo, J.W.; Saab, K.R.; Myers, A.L.; Ng, C.Y.; Zhou, J.; Morton, C.L.; Pfeffer, L.M.; Davidoff, A.M. NF-κB signaling pathway confers neuroblastoma cells migration and invasion ability via the regulation of c-cxcr4. *Med. Sci. Monit.* 2014, 20, 2746–2752. [PubMed]

50. Blaney, S.M.; Bernstein, M.; Neville, K.; Ginsberg, J.; Kitchen, B.; Berg, S.L.; Krailo, M.; Adamson, P.C. Phase I study of the proteasome inhibitor bortezomib in pediatric patients with refractory solid tumors: A children’s oncology group study (advl0015). *J. Clin. Oncol.* 2004, 22, 4804–4809. [CrossRef] [PubMed]

51. Zhi, Y.; Duan, Y.; Zhou, X.; Yin, X.; Guan, G.; Zhang, H.; Dong, Q.; Yang, K. NF-κB signaling pathway confers neuroblastoma cells migration and invasion ability via the regulation of c-cxcr4. *Med. Sci. Monit.* 2014, 20, 2746–2752. [PubMed]
Cancers 2016, 8, 13

52. Van Limpt, V.; Schramm, A.; van Lakeman, A.; Sluis, P.; Chan, A.; van Noesel, M.; Baas, F.; Caron, H.; Eggert, A.; Versteeg, R. The PHOX2B homeobox gene is mutated in sporadic neuroblastomas. Oncogene 2004, 23, 9280–9288. [CrossRef] [PubMed]

53. McConvile, C.; Reid, S.; Baskcomb, L.; Douglas, J.; Rahman, N. PHOX2B analysis in non-syndromatic neuroblastoma cases shows novel mutations and genotype-phenotype associations. Am. J. Med. Genet. A 2006, 140, 1297–1301. [CrossRef] [PubMed]

54. Raabe, E.H.; Laudenslager, M.; Winter, C.; Wasserman, N.; Cole, K.; LaQuaglia, M.; Maris, D.J.; Mosse, Y.P.; Maris, J.M. Prevalence and functional consequence of PHOX2B mutations in neuroblastoma. Oncogene 2008, 27, 469–476. [CrossRef] [PubMed]

55. Reiff, T.; Tsarovina, K.; Majdazari, A.; Schmidt, M.; del Pino, I.; Rohrer, H. Neuroblastoma PHOX2B variants stimulate proliferation and dedifferentiation of immature sympathetic neurons. J. Neurosci. 2010, 30, 905–915. [CrossRef] [PubMed]

56. Hamrroun, D.; Kato, S.; Ishioka, C.; Claustres, M.; Béroud, C.; Soussi, T. The UMD TP53 database and website: Update and revisions. Hum. Mutat. 2006, 27, 14–20. [CrossRef] [PubMed]

57. Vogan, K.; Bernstein, M.; Leclerc, J.M.; Brisson, L.; Brossard, J.; Brodeur, G.M.; Pelletier, J.; Gros, P. Absence of p53 gene mutations in primary neuroblastomas. Cancer Res. 1993, 53, 5269–5273. [PubMed]

58. Nicolai, S.; Pieraccioli, M.; Peschiaroli, A.; Melino, G.; Raschellà, G. Neuroblastoma: Oncogenic mechanisms and therapeutic exploitation of necroptosis. Cell Death Dis. 2015, 6, e2010. [CrossRef] [PubMed]

59. Tweddle, D.A.; Pearson, A.D.; Haber, M.; Norris, M.D.; Xue, C.; Flemming, C.; Lunec, J. The p53 pathway and its inactivation in neuroblastoma. Cancer Lett. 2003, 197, 93–98. [CrossRef]

60. Diskin, S.J.; Capasso, M.; Diamond, M.; Oldridge, D.A.; Conkrite, K.; Bosse, K.R.; Russell, M.R.; Iolascon, A.; Hakonarson, H.; Devoto, M.; et al. Rare variants in tp53 and susceptibility to neuroblastoma. J. Natl. Cancer Inst. 2014, 106, dju047. [CrossRef] [PubMed]

61. Moll, U.M.; LaQuaglia, M.; Bénard, J.; Riou, G. Wild-type p53 protein undergoes cytoplasmic sequestration in undifferentiated neuroblastomas but not in differentiated tumors. Proc. Natl. Acad. Sci. USA 1995, 92, 4407–4411. [CrossRef] [PubMed]

62. Chen, L.; Iraci, N.; Gherardi, S.; Gamble, L.D.; Wood, K.M.; Perini, G.; Lunec, J.; Tweddle, D.A. P53 is a direct transcriptional target of MYCN in neuroblastoma. Cancer Res. 2010, 70, 1377–1388. [CrossRef] [PubMed]

63. Slack, A.; Chen, Z.; Tonelli, R.; Pule, M.; Hunt, L.; Pession, A.; Shohet, J.M. The p53 regulatory gene MDM2 is a direct transcriptional target of MYCN in neuroblastoma. Proc. Natl. Acad. Sci. USA 2005, 102, 731–736. [CrossRef] [PubMed]

64. Van Maerken, T.; Ferdinande, L.; Taildeman, J.; Lambertz, I.; Yigit, N.; Vercruysse, L.; Rihani, A.; Michaelis, M.; Slack, A.; Chen, Z.; et al. Antitumor activity of the selective MDM2 antagonist nutlin-3 against chemoresistant neuroblastoma with wild-type p53. J. Natl. Cancer Inst. 2009, 101, 1562–1574. [CrossRef] [PubMed]

65. Gamble, L.D.; Kees, U.R.; Tweddle, D.A.; Lunec, J. MYCN sensitizes neuroblastoma to the MDM2-p53 antagonists nutlin-3 and mi-63. Oncogene 2012, 31, 752–763. [CrossRef] [PubMed]

66. Eleveld, T.F.; Oldridge, D.A.; Bernard, V.; Koster, J.; Daage, L.C.; Diskin, S.J.; Schild, L.; Bentahar, N.B.; Bellini, A.; Chicard, M.; et al. Relapsed neuroblastomas show frequent RAS-MAPK pathway mutations. Nat. Genet. 2015, 47, 864–871. [CrossRef] [PubMed]

67. Eppstein, A.C.; Sandoval, J.A.; Klein, P.J.; Woodruff, H.A.; Grosfeld, J.L.; Hickey, R.J.; Malkas, L.H.; Schmidt, C.M. Differential sensitivity of chemoresistant neuroblastoma subtypes to mapk-targeted treatment correlates with erk, p53 expression, and signaling response to u0126. J. Pediatr. Surg. 2006, 41, 252–259. [CrossRef] [PubMed]

68. Johnsen, J.I.; Segerström, L.; Orrego, A.; Elfman, L.; Henriksson, M.; Kågedal, B.; Eksborg, S.; Sveinbjörnsson, B.; Kogner, P. Inhibitors of mammalian target of rapamycin downregulate MYCN protein expression and inhibit neuroblastoma growth in vitro and in vivo. Oncogene 2008, 27, 2910–2922. [CrossRef] [PubMed]

69. Misawa, A.; Hosoi, H.; Tsuchiya, K.; Sugimoto, T. Rapamycin inhibits proliferation of human neuroblastoma cells without suppression of MYCN. Int. J. Cancer 2003, 104, 233–237. [CrossRef] [PubMed]

70. Opel, D.; Poremba, C.; Simon, T.; Debatin, K.M.; Fulda, S. Activation of Akt predicts poor outcome in neuroblastoma. Cancer Res. 2007, 67, 735–745. [CrossRef] [PubMed]
71. Hay, N.; Sonenberg, N. Upstream and downstream of mtor. *Genes Dev.* 2004, 18, 1926–1945. [CrossRef] [PubMed]

72. Iżycka-Świeszewska, E.; Drożyńska, E.; Rzepko, R.; Kobierska-Gulida, G.; Grajkowska, W.; Perc, D.; Baklerska, A. Analysis of PI3K/Akt/mTOR signalling pathway in high risk neuroblastic tumours. *Pol. J. Pathol.* 2010, 61, 192–198. [PubMed]

73. Dumont, F.J.; Su, Q. Mechanism of action of the immunosuppressant rapamycin. *Life Sci.* 1996, 58, 373–395. [CrossRef]

74. Samkari, A.; Cooper, Z.A.; Holloway, M.P.; Liu, J.; Altura, R.A. Rapamycin induces the anti-apoptotic protein survivin in neuroblastoma. *Int. J. Biochem. Mol. Biol.* 2012, 3, 28–35. [PubMed]

75. Geoerger, B.; Kieran, M.W.; Grupp, S.; Perc, D.; Clancy, J.; Krygowski, M.; Ananthakrishnan, R.; Boni, J.P.; Berkenblit, A.; Spunt, S.L. Phase II trial of temsirolimus in children with high-grade glioma, neuroblastoma and rhabdomyosarcoma. *Eur J. Cancer* 2012, 48, 253–262. [CrossRef] [PubMed]

76. Fouladi, M.; Laningham, F.; Wu, J.; O’Shaughnessy, M.A.; Molina, K.; Broniscer, A.; Spunt, S.L.; Luckett, I.; Stewart, C.F.; Houghton, P.J.; et al. Phase I study of everolimus in pediatric patients with refractory solid tumors. *J. Clin. Oncol.* 2007, 25, 4806–4812. [CrossRef] [PubMed]

77. Gore, L.; Trippett, T.M.; Katzenstein, H.M.; Boklan, J.; Narendran, A.; Smith, A.; Macy, M.E.; Rolla, K.; Narashimhan, N.; Squillace, R.M.; et al. A multicenter, first-in-pediatrics, phase 1, pharmacokinetic and pharmacodynamic study of ridaforolimus in patients with refractory solid tumors. *Clin. Cancer Res.* 2013, 19, 3649–3658. [CrossRef] [PubMed]

78. Zhang, H.; Dou, J.; Yu, Y.; Zhao, Y.; Fan, Y.; Cheng, J.; Xu, X.; Liu, W.; Guan, S.; Chen, Z.; et al. mTOR ATP-competitive inhibitor INK128 inhibits neuroblastoma growth via blocking mTORC signaling. *Apoptosis* 2015, 20, 50–62. [CrossRef] [PubMed]

79. Kang, M.H.; Reynolds, C.P.; Maris, J.M.; Gorlick, R.; Kolb, E.A.; Lock, R.; Carol, H.; Keir, S.T.; Wu, J.; Lyalin, D.; et al. Initial testing (stage 1) of the investigational mTOR kinase inhibitor MLN0128 by the pediatric preclinical testing program. *Pediatr. Blood Cancer* 2014, 61, 1486–1489. [CrossRef] [PubMed]

80. Guichard, S.; Howard, Z.; Heathcote, D.; Roth, M.; Hughes, G.; Curwen, J.; Yates, J.; Logie, A.; Holt, S.; Chresta, C.; et al. AZD2014, a dual mTORC1 and mTORC2 inhibitor is differentiated from allosteric inhibitors of mtorc1 in ER+ breast cancer. In Proceedings of the AACR 103rd Annual Meeting, Chicago, IL, USA, 31 March–4 April 2012.

81. Bhagwat, S.; Crew, A.; Gokhale, P.; Yao, Y.; Kahler, J.; Epstein, D.; Wild, R.; Pachter, J. Osi-027, a potent and PI3-kinase inhibitor. *Mol. Cancer Ther.* 2008, 7, 118–127. [PubMed]

82. Carracedo, A.; Ma, L.; Teruya-Feldstein, J.; Rojo, F.; Salmena, L.; Alimonti, A.; Egia, A.; Sasaki, A.T.; Bhagwat, S.; Crew, A.; Gokhale, P.; Yao, Y.; Kahler, J.; Epstein, D.; Wild, R.; Pachter, J. Osi-027, a novel class i PI3K/mTOR kinase inhibitor with robust activity in cancer models driven by the PI3K pathway. *Mol. Cancer Ther.* 2011, 10, 2426–2436. [CrossRef] [PubMed]

83. Peirce, S.K.; Findley, H.W.; Prince, C.; Dasgupta, A.; Cooper, T.; Durden, D.L. The PI-3 kinase-Akt-MDM2-survivin signaling axis in high-risk neuroblastoma: A target for PI-3 kinase inhibitor intervention. *Cancer Chemother. Pharmacol.* 2011, 68, 325–335. [CrossRef] [PubMed]

84. Maira, S.M.; Pecchi, S.; Huang, A.; Burger, M.; Knapp, M.; Sterker, D.; Schnell, C.; Guthy, D.; Nagel, T.; Wiesmann, M.; et al. Identification and characterization of NVP-BKM120, an orally available pan-class i PI3 kinase inhibitor. *Mol. Cancer Ther.* 2012, 11, 317–328. [CrossRef] [PubMed]

85. Dam, V.; Morgan, B.T.; Mazanek, P.; Hogarty, M.D. Mutations in PIK3CA are infrequent in neuroblastoma. *BMC Cancer* 2006, 6, 177. [CrossRef] [PubMed]

86. Wallin, J.J.; Edgar, K.A.; Guan, J.; Berry, M.; Prior, W.W.; Lee, L.; Lesnick, J.D.; Lewis, C.; Nonomiya, J.; Pang, J.; et al. GDC-0980 is a novel class i PI3K/mTOR kinase inhibitor with robust activity in cancer models driven by the PI3K pathway. *Mol. Cancer Ther.* 2011, 10, 2426–2436. [CrossRef] [PubMed]
89. Li, Z.; Yan, S.; Attayyan, N.; Ramalingam, S.; Thiele, C.J. Combination of an allosteric akt inhibitor MK-2206 with etoposide or rapamycin enhances the antitumor growth effect in neuroblastoma. *Clin. Cancer Res.* **2012**, *18*, 3603–3615. [CrossRef] [PubMed]

90. Fouladi, M.; Perentesis, J.P.; Phillips, C.L.; Leary, S.; Reid, J.M.; McGovern, R.M.; Ingle, A.M.; Ahern, C.H.; Ames, M.M.; Houghton, P.; et al. A phase I trial of MK-2206 in children with refractory malignancies: A children's oncology group study. *Pediatr. Blood Cancer* **2014**, *61*, 1246–1251. [CrossRef] [PubMed]

91. Kondapaka, S.B.; Singh, S.S.; Dasmahapatra, G.P.; Sausville, E.A.; Roy, K.K. Perifosine, a novel alkylphospholipid, inhibits protein kinase B activation. *Mol. Cancer Ther.* **2003**, *2*, 1093–1103. [PubMed]

92. Chiarini, F.; Del Sole, M.; Mongiorgi, S.; Gaboardi, G.C.; Cappellini, A.; Mantovani, I.; Mantovani, I.; Follo, M.Y.; McCubrey, J.A.; Martelli, A.M. The novel Akt inhibitor, perifosine, induces caspase-dependent apoptosis and downregulates P-glycoprotein expression in multidrug-resistant human T-acute leukemia cells by a JNK-dependent mechanism. *Leukemia* **2008**, *22*, 1106–1116. [CrossRef] [PubMed]

93. Li, Z.; Tan, F.; Liewehr, D.J.; Steinberg, S.M.; Thiele, C.J. *In vitro and in vivo* inhibition of neuroblastoma tumor cell growth by Akt inhibitor perifosine. *J. Natl. Cancer Inst.* **2010**, *102*, 758–770. [CrossRef] [PubMed]

94. Nagao, M.; Ishizaka, Y.; Nakagawara, A.; Kohno, K.; Kawanaka, M.; Tahira, T.; Itoh, F.; Ikeda, I.; Sugimura, T. Expression of retroproto-oncogene in human neuroblastomas. *Jpn. J. Cancer Res.* **1990**, *81*, 309–312. [CrossRef] [PubMed]

95. Futami, H.; Sakai, R. RET protein promotes non-adherent growth of NB-39-nu neuroblastoma cell line. *Cancer Sci.* **2009**, *100*, 1034–1039. [CrossRef] [PubMed]

96. Iwamoto, T.; Taniguchi, M.; Wajjwalku, W.; Nakashima, I.; Takahashi, M. Neuroblastoma in a transgenic mouse carrying a metallothionein/ret fusion gene. *Br. J. Cancer* **1993**, *67*, 504–507. [CrossRef] [PubMed]

97. Beaudry, P.; Nilsson, M.; Ritho, M.; Prox, D.; Poon, D.; Xu, L.; Zweidler-McKay, P.; Ryan, A.; Folkman, J.; Ryeom, S.; et al. Potent antitumor effects of ZD6474 on neuroblastoma via dual targeting of tumor cells and tumor endothelium. *Mol. Cancer Ther.* **2008**, *7*, 418–424. [CrossRef] [PubMed]

98. Zage, P.E.; Zeng, L.; Palla, S.; Fang, W.; Nilsson, M.B.; Heymach, J.V.; Zweidler-McKay, P.A. A novel therapeutic combination for neuroblastoma: The vascular endothelial growth factor receptor/epidermal growth factor receptor/rarregulated during transfection inhibitor vandetanib with 13-cis-retinoic acid. *Cancer* **2010**, *116*, 2465–2475. [CrossRef] [PubMed]

99. Krams, M.; Parwaresch, R.; Sipos, B.; Heidorn, K.; Harms, D.; Rudolph, P. Expression of the c-kit receptor characterizes a subset of neuroblastomas with favorable prognosis. *Oncogene* **2004**, *23*, 588–595. [CrossRef] [PubMed]

100. Vitali, R.; Cesi, V.; Nicotra, M.R.; McDowell, H.P.; Donfrancesco, A.; Mannarino, O.; Natali, P.G.; Raschella, G.; Dominici, C. C-kit is preferentially expressed in MYCN-amplified neuroblastoma and its effect on cell proliferation is inhibited in *vivo* by sti-571. *Int. J. Cancer* **2003**, *106*, 147–152. [CrossRef] [PubMed]

101. Cohen, P.S.; Chan, J.P.; Lipkunskaya, M.; Biedler, J.L.; Seeger, R.C. Expression of stem cell factor and c-kit in associated with amino-terminal tyrosine phosphorylation of the src gene product. *Proc. Natl. Acad. Sci. USA* **1990**, *81*, 793–798. [CrossRef] [PubMed]

102. Beppu, K.; Jaboine, J.; Merchant, M.S.; Mackall, C.L.; Thiele, C.J. Expression of neuronal src mRNA as a favorable marker and inverse correlation to N-MYC gene amplification in human neuroblastomas. *Int. J. Cancer* **1994**, *58*, 793–798. [CrossRef] [PubMed]
107. Hishiki, T.; Saito, T.; Sato, Y.; Mitsunaga, T.; Terui, E.; Matsuura, G.; Saito, E.; Shibata, R.; Mise, N.; Yokoyama, Y.; et al. Src kinase family inhibitor pp2 induces aggregation and detachment of neuroblastoma cells and inhibits cell growth in a PI3 kinase/Akt pathway-independent manner. *Pediatr. Surg. Int.* 2011, 27, 225–230. [CrossRef] [PubMed]

108. Vitali, R.; Mancini, C.; Cesi, V.; Tanno, B.; Piscitelli, M.; Mancuso, M.; Sesti, F.; Pasquali, E.; Calabretta, B.; Dominici, C.; et al. Activity of tyrosine kinase inhibitor dasatinib in neuroblastoma cells *in vitro* and in orthotopic mouse model. *Int. J. Cancer* 2009, 125, 2547–2555. [CrossRef] [PubMed]

109. Radi, M.; Brullo, C.; Crespan, E.; Tintori, C.; Musumeci, F.; Biava, M.; Schenone, S.; Dreassi, E.; Zamperini, C.; Maga, G.; et al. Identification of potent c-Src inhibitors strongly affecting the proliferation of human neuroblastoma cells. *Bioorg. Med. Chem. Lett.* 2011, 21, 5928–5933. [CrossRef] [PubMed]

110. Schaller, M.D.; Hildebrand, J.D.; Shannon, J.D.; Fox, J.W.; Vines, R.R.; Parsons, J.T. Autophosphorylation of

111. Hanks, S.K.; Polte, T.R. Signaling through focal adhesion kinase. *Cancer Res.* 2007, 67, 299–305. [CrossRef] [PubMed]

112. Gabarra-Niecko, V.; Schaller, M.D.; Dunty, J.M. Fak regulates biological processes important for the pathogenesis of cancer. *Cancer Metastasis Rev.* 2003, 22, 359–374. [CrossRef] [PubMed]

113. Romain, C.; Paul, P.; Kim, K.W.; Lee, S.; Qiao, J.; Chung, D.H. Targeting Aurora kinase-A downregulates cell proliferation and angiogenesis in neuroblastoma. *J. Biol. Chem.* 2015, 290, 275–286. [CrossRef] [PubMed]

114. Beijersbergen, R.; Christiansen, H.; et al. Identification of potent c-Src inhibitors strongly affecting the proliferation of human neuroblastoma cells. *Bioorg. Med. Chem. Lett.* 2011, 21, 5928–5933. [CrossRef] [PubMed]

115. Xu, L.H.; Yang, X.; Bradham, C.A.; Brenner, D.A.; Baldwin, A.S.; Craven, R.J.; Cance, W.G. The focal adhesion kinase decreases tumorigenicity in neuroblastoma. *Cancer Metastasis Rev.* 2003, 22, 359–374. [CrossRef] [PubMed]

116. Beierle, E.A.; Ma, X.; Nabers, H.; Cance, W.G.; Kurenova, E.V.; Beijersbergen, R.; Christiansen, H.; et al. Small Molecule Inhibitors of Aurora-A Induce Proteasomal Degradation of N-MYC in Childhood Neuroblastoma. *Cancer Cell* 2013, 24, 75–89. [CrossRef] [PubMed]

117. Megison, M.L.; Gillory, L.A.; Stewart, J.E.; Nabers, H.C.; Mrozcek-Musulman, E.; Beierle, E.A. FAK inhibition abrogates the malignant phenotype in aggressive pediatric renal tumors. *Cancer Res.* 2014, 74, 514–526. [CrossRef] [PubMed]

118. Beierle, E.A.; Ma, X.; Trujillo, A.; Kurenova, E.V.; Cance, W.G.; Golubovskaya, V.M. Inhibition of focal adhesion kinase and src increases detachment and apoptosis in human neuroblastoma cell lines. *Cancer Metastasis Rev.* 2010, 49, 224–234. [CrossRef] [PubMed]

119. Henk, S.K.; Ryzhova, L.; Shin, N.Y.; Brábek, J. Focal adhesion kinase signaling activities and their implications in the control of cell survival and motility. *Front. Biosci.* 2003, 8, d982–d996. [CrossRef] [PubMed]

120. Beierle, E.A.; Ma, X.; Trujillo, A.; Kurenova, E.V.; Cance, W.G.; Golubovskaya, V.M. Inhibition of focal adhesion kinase and src increases detachment and apoptosis in human neuroblastoma cell lines. *Cancer Metastasis Rev.* 2010, 49, 224–234. [CrossRef] [PubMed]

121. Megison, M.L.; Gillory, L.A.; Stewart, J.E.; Nabers, H.C.; Mrozcek-Musulman, E.; Beierle, E.A. FAK inhibition abrogates the malignant phenotype in aggressive pediatric renal tumors. *Cancer Res.* 2014, 74, 514–526. [CrossRef] [PubMed]

122. Otto, T.; Horn, S.; Brockmann, M.; Eilers, U.; Schüttrumpf, L.; Popov, N.; Kenney, A.M.; Schulte, J.H.; Beijersbergen, R.; Christiansen, H.; et al. Stabilization of N-MYC is a critical function of Aurora A in human neuroblastoma. *Cancer Cell* 2009, 15, 67–78. [CrossRef] [PubMed]

123. Brockmann, M.; Poon, E.; Berry, T.; Carstensen, A.; Deubzer, H.E.; Rycak, L.; Jamin, Y.; Thway, K.; Robinson, S.P.; Roels, F.; et al. Small Molecule Inhibitors of Aurora-A Induce Proteasomal Degradation of N-MYC in Childhood Neuroblastoma. *Cancer Cell* 2013, 24, 75–89. [CrossRef] [PubMed]

124. Romain, C.; Paul, P.; Kim, K.W.; Lee, S.; Qiao, J.; Chung, D.H. Targeting Aurora kinase-A downregulates cell proliferation and angiogenesis in neuroblastoma. *J. Pediatr. Surg.* 2014, 49, 159–165. [CrossRef] [PubMed]

125. Mossé, Y.P.; Lipsitz, E.; Fox, E.; Teachey, D.T.; Maris, J.M.; Weigel, B.; Adamson, P.C.; Ingle, M.A.; Ahern, C.H.; Blaney, S.M. Pediatric phase I trial and pharmacokinetic study of MLN8237, an investigational oral selective small-molecule inhibitor of Aurora kinase A: A children’s oncology group phase I consortium study. *Clin. Cancer Res.* 2012, 18, 6058–6064. [CrossRef] [PubMed]
126. Bogen, D.; Wei, J.S.; Azorsa, D.O.; Ormanoglu, P.; Buehler, E.; Guha, R.; Keller, J.M.; Mathews Griner, L.A.; Ferrer, M.; Song, Y.K.; et al. Aurora B kinase is a potent and selective target in MYCN-driven neuroblastoma. Oncotarget 2015, 6, 35247–35262. [PubMed]

127. Faisal, A.; Vaughan, L.; Bavetsias, V.; Sun, C.; Atrash, B.; Avery, S.; Jamin, Y.; Robinson, S.P.; Workman, P.; Blagg, J.; et al. The Aurora kinase inhibitor CCT137690 downregulates MYCN and sensitizes MYCN-amplified neuroblastoma in vivo. Mol. Cancer Ther. 2011, 10, 2115–2123. [CrossRef] [PubMed]

128. Iwahara, T.; Fujimoto, J.; Wen, D.; Cupples, R.; Bucay, N.; Arakawa, T.; Mori, S.; Ratzkin, B.; Yamamoto, T. Molecular characterization of ALK, a receptor tyrosine kinase expressed specifically in the nervous system. Oncogene 1997, 14, 439–449. [CrossRef] [PubMed]

129. Chen, Y.; Takita, J.; Choi, Y.L.; Kato, M.; Ohira, M.; Sanada, M.; Wang, L.; Soda, M.; Kikuchi, A.; Igarashi, T.; et al. Oncogenic mutations of ALK kinase in neuroblastoma. Nature 2008, 455, 971–974. [CrossRef] [PubMed]

130. Bresler, S.C.; Wood, A.C.; Haglund, E.A.; Courtright, J.; Belcastro, L.T.; Plegaria, J.S.; Cole, K.; Megison, M.L.; Stewart, J.E.; Nabers, H.C.; Gillory, L.A.; Beierle, E.A.; Golias, C.H.; Charalabopoulos, A.; Charalabopoulos, K. Cell proliferation and cell cycle control: A mini review. Int. J. Clin. Pract. 2004, 58, 1134–1141. [CrossRef] [PubMed]

131. Wilhelm, S.; Carter, C.; Lynch, M.; Lowinger, T.; Dumas, J.; Schwartz, B.; Simantov, R.; Kelley, S. Discovery and development of sorafenib: A multikinase inhibitor for treating cancer. Nat. Rev. Drug Discov. 2006, 5, 835–844. [CrossRef] [PubMed]

132. Bogen, D.; Wei, J.S.; Azorsa, D.O.; Ormanoglu, P.; Buehler, E.; Guha, R.; Keller, J.M.; Mathews Griner, L.A.; Ferrer, M.; Song, Y.K.; et al. Aurora B kinase is a potent and selective target in MYCN-driven neuroblastoma. Oncotarget 2015, 6, 35247–35262. [PubMed]

133. Kakodkar, N.C.; Peddinti, R.R.; Tian, Y.; Guerrero, L.J.; Chlenski, A.; Yang, Q.; Salwen, H.R.; Maitland, M.L.; Cohn, S.L. Sorafenib inhibits neuroblastoma cell proliferation and signaling, blocks angiogenesis, and impairs tumor growth. Pediatr. Blood Cancer 2012, 59, 642–647. [CrossRef] [PubMed]

134. Kohn, S.T.; Mas, M.J.; Lock, R.; Kolb, E.A.; Gorlick, R.; Carol, H.; Morton, C.L.; Reynolds, C.P.; Kang, M.H.; Watkins, A.; et al. Initial testing (stage 1) of the multi-targeted kinase inhibitor sorafenib by the pediatric preclinical testing program. Pediatr. Blood Cancer 2010, 55, 1126–1133. [CrossRef] [PubMed]

135. Widemann, B.C.; Kim, A.; Fox, E.; Baruchel, S.; Adamson, P.C.; Ingle, A.M.; Glade Bender, J.; Burke, M.; Weigel, B.; Stempak, D.; et al. Aurora B kinase is a potent and selective target in MYCN-driven neuroblastoma. Mol. Cancer Ther. 2013, 12, 472–480. [CrossRef]

136. Wilhelm, S.; Carter, C.; Lynch, M.; Lowinger, T.; Dumas, J.; Smith, R.A.; Schwartz, B.; Simantov, R.; Kelley, S. Discovery and development of sorafenib: A multikinase inhibitor for treating cancer. Nat. Rev. Drug Discov. 2006, 5, 835–844. [CrossRef] [PubMed]
145. Markaki, E.A.; Tsopanomichalou, M.; Dimitriou, H.; Stiakaki, E.; Perdikoyanni, C.; Spandidios, D.; Kalmanti, M. Mutations of retinoblastoma gene (Rb-1) as a prognostic factor in children with acute leukemia and neuroblastoma. *Pediatr. Hematol. Oncol.* 2001, 18, 101–110. [CrossRef] [PubMed]

146. Wallick, C.J.; Gamper, I.; Thorne, M.; Feith, D.J.; Takasaki, K.Y.; Wilson, S.M.; Seki, J.A.; Pegg, A.E.; Byus, C.V.; Bachmann, A.S. Key role for p27Kip1, retinoblastoma protein Rb, and MYCN in polyamine inhibitor-induced G1 cell cycle arrest in MYCN-amplified human neuroblastoma cells. *Oncogene* 2005, 24, 5606–5618. [CrossRef] [PubMed]

147. Molenaar, J.J.; Koster, J.; Ebus, M.E.; van Sluis, P.; Westerhout, E.M.; de Preter, K.; Gisselsson, D.; Øra, L.; Speleman, F.; Caron, H.N.; et al. Copy number defects of G1-cell cycle genes in neuroblastoma are frequent and correlate with high expression of E2F target genes and a poor prognosis. *Genes Chromosomes Cancer* 2012, 51, 10–19. [CrossRef] [PubMed]

148. Rader, J.; Russell, M.R.; Hart, L.S.; Nakazawa, M.S.; Belcastro, L.T.; Martinez, D.; Li, Y.; Carpenter, E.L.; Attiyeh, E.F.; Diskin, S.J.; et al. Dual CDK4/CDK6 inhibition induces cell-cycle arrest and senescence in neuroblastoma. *Clin. Cancer Res.* 2013, 19, 6173–6182. [CrossRef] [PubMed]

149. Fry, D.W.; Harvey, P.J.; Keller, P.R.; Elliott, W.L.; Meade, M.; Trachet, E.; Albassam, M.; Zheng, X.; Leopold, W.R.; Pryer, N.K.; et al. Specific inhibition of cyclin-dependent kinase 4/6 by pd 0332991 and associated antitumor activity in human tumor xenografts. *Mol. Cancer Ther.* 2004, 3, 1427–1438. [PubMed]

150. Rihani, A.; Vandesompele, J.; Speleman, F.; van Maerken, T. Inhibition of CDK4/6 as a novel therapeutic option for neuroblastoma. *Cancer Cell Int.* 2015, 15, 76. [CrossRef] [PubMed]

151. Mamont, P.S.; Danzin, C.; Wagner, J.; Siat, M.; Joder-Ohlenbusch, A.M.; Claverie, N. Accumulation of decarboxylated s-adenosyl-l-methionine in mammalian cells as a consequence of the inhibition of putrescine biosynthesis. *Eur. J. Biochem.* 1982, 123, 499–504. [CrossRef] [PubMed]

152. Tabor, C.W.; Tabor, H. Polyamines. *Annu. Rev. Biochem.* 1984, 53, 749–790. [PubMed]

153. Balasundaram, D.; Tyagi, A.K. Polyamine—DNA nexus: Structural ramifications and biological implications. *Mol. Cell. Biochem.* 1991, 100, 129–140. [CrossRef] [PubMed]

154. Gerner, E.W.; Meyskens, F.L. Polyamines and cancer: Old molecules, new understanding. *Nat. Rev. Cancer* 2004, 4, 781–792. [CrossRef] [PubMed]

155. Lutz, W.; Stöhr, M.; Schürmann, J.; Wenzel, A.; Löhr, A.; Schwab, M. Conditional expression of N-MYC in human neuroblastoma cells increases expression of α-prothymosin and ornithine decarboxylase and accelerates progression into s-phase early after mitogenic stimulation of quiescent cells. *Oncogene* 1996, 13, 803–812. [PubMed]

156. Hogarty, M.D.; Norris, M.D.; Davis, K.; Liu, X.; Evageliou, N.F.; Hayes, C.S.; Pawel, B.; Guo, R.; Zhao, H.; Sekyere, E.; et al. ODC1 is a critical determinant of MYCN oncogenesis and a therapeutic target in neuroblastoma. *Cancer Res.* 2008, 68, 9735–9745. [CrossRef] [PubMed]

157. Hop, M.W.; de la Haba, R. Formation and activation of a cyclin E-cdk2 complex during the G1 phase of the human cell cycle. *Science* 2003, 299, 1131–1134. [CrossRef] [PubMed]

158. Rounbehler, R.J.; Li, W.; Hall, M.A.; Yang, C.; Fallahi, M.; Cleveland, J.L. Targeting ornithine decarboxylase impairs development of MYCN-amplified neuroblastoma. *Cancer Res.* 2009, 69, 547–553. [CrossRef] [PubMed]

159. Saulnier Sholler, G.L.; Gerner, E.W.; Bergendahl, G.; MacArthur, R.B.; VanderWerff, A.; Ashikaga, T.; Bond, J.P.; Ferguson, W.; Roberts, W.; Wada, R.K.; et al. A phase I trial of DFMO targeting polyamine addiction in patients with relapsed/refractory neuroblastoma. *PloS ONE* 2015, 10, e0127246. [CrossRef] [PubMed]

160. Nuwayhid, S.; Stockett, D.; Hyde, J.; Aleshin, A.; Walker, D.; Arkin, M. SNS-032 is a potent and selective inhibitor of CDK2, 7 and 9 and induces cell death by inhibiting cell cycle progression and the expression of antiapoptotic proteins. *Proc. Am. Assoc. Cancer Res.* 2006, 47, 491.

161. Chen, R.; Wierda, W.G.; Chubb, S.; Hawtin, R.E.; Fox, J.A.; Keating, M.J.; Gandhi, V.; Plunkett, W. Mechanism of action of SNS-032, a novel cyclin-dependent kinase inhibitor, in chronic lymphocytic leukemia. *Blood* 2009, 113, 4637–4645. [CrossRef] [PubMed]

162. Woo, R.A.; Poon, R.Y. Cyclin-dependent kinases and s phase control in mammalian cells. *Cell Cycle* 2003, 2, 316–324. [CrossRef] [PubMed]

163. Koff, A.; Giordano, A.; Desai, D.; Yamashita, K.; Harper, J.W.; Elledge, S.; Nishimoto, T.; Morgan, D.O.; Franca, B.R.; Roberts, J.M. Formation and activation of a cyclin E-cdk2 complex during the G1 phase of the human cell cycle. *Science* 1992, 257, 1689–1694. [CrossRef] [PubMed]
164. Löschmann, N.; Michaelis, M.; Rothweiler, F.; Zehner, R.; Cinatl, J.; Voges, Y.; Sharifi, M.; Riecken, K.; Meyer, J.; von Deimling, A.; et al. Testing of SNS-032 in a panel of human neuroblastoma cell lines with acquired resistance to a broad range of drugs. Transl. Oncol. 2013, 6, 685–696. [CrossRef] [PubMed]

165. Afanasyeva, E.A.; Mestdagh, P.; Kumps, C.; Vandesompele, J.; Ehemann, V.; Theissen, J.; Fischer, M.; Zapatka, M.; Brors, B.; Savelyeva, L.; et al. MicroRNA miR-885-5p targets CDK2 and MCM5, activates p53 and inhibits proliferation and survival. Cell Death Differ. 2011, 18, 974–984. [CrossRef] [PubMed]

166. Chipumuro, E.; Marco, E.; Christensen, C.L.; Kwiatkowski, N.; Zhang, T.; Hatheway, C.M.; Abraham, B.J.; Sharma, B.; Yeung, C.; Altabet, A.; et al. CDK7 inhibition suppresses super-enhancer-linked oncogenic transcription in MYCN-driven cancer. Cell 2014, 159, 1126–1139. [CrossRef] [PubMed]

167. Keshelava, N.; Houghton, P.J.; Morton, C.L.; Lock, R.B.; Carol, H.; Keir, S.T.; Maris, J.M.; Reynolds, C.P.; Gorlick, R.; Koll, E.A.; et al. Initial testing (stage 1) of vorinostat (SAHA) by the pediatric preclinical testing program. Pediatr. Blood Cancer 2009, 53, 505–508. [CrossRef] [PubMed]

168. Mühlethalera-Mottet, A.; Meier, M.; Flahaut, M.; Bourloud, K.B.; Nardou, K.; Joseph, J.M.; Gross, N. Complex molecular mechanisms cooperate to mediate histone deacetylase inhibitors anti-tumour activity in neuroblastoma cells. Mol. Cancer 2008, 7, 55. [CrossRef] [PubMed]

169. Ouwehand, K.; de Ruiter, A.J.; van Bree, C.; Caron, H.N.; van Kuilenburg, A.B. Histone deacetylase inhibitor BL1521 induces a G1-phase arrest in neuroblastoma cells through altered expression of cell cycle proteins. FEBS Lett. 2005, 579, 1523–1528. [CrossRef] [PubMed]

170. De Ruiter, A.J.; Kemp, S.; Kramer, G.; Meinsma, R.J.; Kaufmann, J.O.; Caron, H.N.; van Kuilenburg, A.B. The novel histone deacetylase inhibitor BL1521 inhibits proliferation and induces apoptosis in neuroblastoma cells. Biochem. Pharmacol. 2004, 68, 1279–1288. [CrossRef] [PubMed]

171. Oehme, I.; Deubzer, H.E.; Wegener, D.; Pickert, D.; Linke, J.P.; Hero, B.; Kopp-Schneider, A.; Westermann, F.; Ulrich, S.M.; von Deimling, A.; et al. Histone deacetylase 8 in neuroblastoma tumorigenesis. Clin. Cancer Res. 2009, 15, 91–99. [CrossRef] [PubMed]

172. Rettig, I.; Koenneke, E.; Trippel, F.; Mueller, W.C.; Burhenne, J.; Kopp-Schneider, A.; Fabian, J.; Schober, A.; Fernekorn, U.; von Deimling, A.; et al. Selective inhibition of HDAC8 decreases neuroblastoma growth in vitro and in vivo and enhances retinoic acid-mediated differentiation. Cell Death Dis. 2015, 6, e1657. [CrossRef] [PubMed]

173. Sun, Y.; Liu, F.Y.; Scarlett, C.J.; Malyukova, A.; Liu, B.; Marshall, G.M.; MacKenzie, K.L.; Biankin, A.V.; Liu, T. Histone deacetylase 5 blocks neuroblastoma cell differentiation by interacting with N-MYC. Oncogene 2014, 33, 2987–2994. [CrossRef] [PubMed]

174. Frumm, S.M.; Fan, Z.P.; Ross, K.N.; Duvall, J.R.; Gupta, S.; VerPlank, L.; Suh, B.C.; Holson, E.; Wagner, F.F.; Smith, W.B.; et al. Selective HDAC1/HDAC2 inhibitors induce neuroblastoma differentiation. Chem. Biol. 2013, 20, 713–725. [CrossRef] [PubMed]

175. Zhang, L.; Liu, N.; Xie, S.; He, X.; Zhou, J.; Liu, M.; Li, D. HDAC6 regulates neuroblastoma cell migration and may play a role in the invasion process. Cancer Biol. Ther. 2014, 15, 1561–1570. [CrossRef] [PubMed]

176. Oehme, I.; Linke, J.P.; Böck, B.C.; Mildt, T.; Lodrini, M.; Hartenstein, B.; Wiegand, I.; Eckert, C.; Roth, W.; Kool, M.; et al. Histone deacetylase 10 promotes autophagy-mediated cell survival. Proc. Natl. Acad. Sci. USA 2011, 108, E2592–E2601. [CrossRef] [PubMed]

177. Fouladi, M.; Park, J.R.; Stewart, C.F.; Gilbertson, R.J.; Schaiquevich, P.; Sun, J.; Reid, J.M.; Ames, M.M.; Speights, R.; Ingle, A.M.; et al. Pediatric phase I trial and pharmacokinetic study of vorinostat: A children’s oncology group phase I consortium report. J. Clin. Oncol. 2010, 28, 3623–3629. [CrossRef] [PubMed]

178. Cole, K.A.; Huggins, J.; Laquaaglia, M.; Hulderman, C.E.; Russell, M.R.; Bosse, K.; Diskin, S.J.; Attiyeh, E.F.; Sennett, R.; Norris, G.; et al. Rnai screen of the protein kinome identifies checkpoint kinase 1 (CHK1) as a therapeutic target in neuroblastoma. Proc. Natl. Acad. Sci. USA 2011, 108, 3336–3341. [CrossRef] [PubMed]

179. Hamner, J.B.; Sims, T.L.; Cutshaw, A.; Dickson, P.V.; Rosati, S.; McGee, M.; Ng, C.Y.; Davidoff, A.M. The efficacy of combination therapy using adeno-associated virus—Interferon β and trichostatin a in vitro and in a murine model of neuroblastoma. J. Pediatr. Surg. 2008, 43, 177–183. [CrossRef] [PubMed]