REVIEW

Entrectinib and other ALK/TRK inhibitors for the treatment of neuroblastoma

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Abstract: RTK plays important roles in many cellular signaling processes involved in cancer growth and development. ALK, TRKA, TRKB, TRKC, and ROS1 are RTKs involved in several canonical pathways related to oncogenesis. These proteins can be genetically altered in malignancies, leading to receptor activation and constitutive signaling through their respective downstream pathways. Neuroblastoma (NB) is the most common extracranial solid tumor in childhood, and despite intensive therapy, there is a high mortality rate in cases with a high-risk disease. Alterations of ALK and differential expression of TRK proteins are reported in a proportion of NB. Several inhibitors of ALK or TRKA/B/C have been evaluated both preclinically and clinically in the treatment of NB. These agents have had variable success and are not routinely used in the treatment of NB. Entrectinib (RXDX-101) is a pan-ALK, TRKA, TRKB, TRKC, and ROS1 inhibitor with activity against tumors with *ALK*, *NTRK1*, *NTRK2*, *NTRK3*, and *ROS1* alterations in Phase I clinical trials in adults. Entrectinib's activity against both ALK and TRK proteins suggests a possible role in NB treatment, and it is currently under investigation in both pediatric and adult oncology patients.

Keywords: neuroblastoma, entrectinib, ALK, TRK, ROS1

Introduction

The human genome contains 58 known RTK genes that activate common intracellular signaling cascades and are frequently altered in cancer.[1] Different oncologic alterations in RTKs include activating mutations, gain of function mutations, fusion rearrangements, and overexpression. ALK, TRKA, TRKB, TRKC, and ROS1 are RTKs that may be altered or overexpressed in cancer, including neuroblastoma (NB).[2–4]

NB is the most common extracranial tumor of childhood that develops from progenitors arising from neural crest cells in the adrenal medulla or along the sympathetic chain.[5] It accounts for 10% of all pediatric cancers and results in ~15% of pediatric cancer mortality.[5,6] NB is known for its clinical heterogeneity, ranging from infants, where the disease spontaneously regresses or matures, to children with a highly aggressive metastatic disease.[5] The treatment is risk stratified based on clinical and genetic features associated with outcome. Genetic alterations associated with outcomes include *MYCN* amplification, DNA ploidy, gain of chromosome 17q, and deletions of chromosome arms 1p or 11q.[7–16] The current treatment for high-risk disease uses a multimodal approach incorporating chemotherapy, surgery, radiation therapy, autologous stem cell transplantation, and immunotherapy.[5] Despite intensified regimens, ~50% of patients with a high-risk NB relapse or are treatment refractory, demonstrating a critical need for novel therapies to improve cure rates and decrease toxicities.[17,18]

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The genetic landscape of NB has been widely studied, and several genetic aberrations have been identified. *MYCN* is a transcription factor located at 2p24 and is amplified in 20% of all patients at diagnosis.[19,20] *MYCN* amplification is associated with metastatic disease and a poor prognosis; however, therapeutic inhibition of *MYCN* has been difficult due to the ubiquitous presence of this transcription factor and the lack of available drug-binding sites.[19–21] Targetable genetic alterations such as *ALK* mutations/amplification are seen in 14% of NB cases.[22] Less common alterations are mutations in *ATRX*, *PTPN11*, and *NRAS* genes; each is reported in fewer than 10% of NB cases.[22–24] In addition to genetic alterations, there are genes that exhibit differential expression in NB, such as *NTRK1/2/3*.[25] The overall low frequency of mutations combined with difficulty in targeting the more frequently altered genes has resulted in a paucity of molecularly targeted therapeutic options for NB to date.[21,22,24,26] However, genes that are differentially expressed, such as *NTRK1/2/3*, and those that are genetically altered, such as *ALK*, are potential opportunities for molecularly driven therapy in NB.

ALK is an RTK in the insulin receptor superfamily and is located on chromosome 2. The ligands for ALK are pleiotrophin and midkine; their binding leads to receptor dimerization, autophosphorylation, adaptor protein recruitment, and downstream signal transduction through the RAS/MAPK, PI3K/AKT, and JAK/STAT pathways.[27,28] ALK is expressed in both the murine and human nervous systems and not in other tissues.[29–32] Studies in murine models identified high levels of ALK in the neonatal brain and low levels of ALK in adults, suggesting that this protein may be important in embryogenesis.[29] Constitutive *ALK* activation through translocation or mutation occurs in multiple malignancies, supporting its role in oncogenesis.[3] In fact, the *ALK* gene was initially discovered in the setting of anaplastic large cell lymphoma (ALCL) where most cases express a t(2;5) translocation, resulting in the fusion of *ALK* with *NPM*.[33] *ALK* translocations are present in 50% of inflammatory myofibroblastic tumor (IMT) and in 3%–7% of non-small-cell lung cancer (NSCLC).[34–37] *ALK*-activating mutations and amplification are also described in NB tumors and are more common in patients with a high-risk disease.[38]

TRK proteins, TRKA, TRKB, and TRKC, are another class of RTKs involved in oncogenesis. The proteins are encoded by *NTRK1*, *NTRK2*, and *NTRK3*, respectively. The ligand for TRKA is NGF; for TRKB is BDNF, NT3, and NT4/5; and for TRKC is NT3. Of note, some ligands like NT3 bind multiple TRK receptors.[39–43] Ligand binding results in receptor homodimerization and activation, which lead to signaling through various canonical pathways including RAS/MAPK, AKT, PLCγ1, and PKC.[44,45] TRK proteins are expressed in the human central and peripheral nervous system during embryogenesis.[46] Studies in animal models have identified that the TRK proteins have different roles and functions, depending on the timing and location of their expression during development. For example, TRKB is expressed in early sensory neuron development, while TRKA in the later stages.[47] Similarly, TRKC is expressed early in the development of sympathetic neurons of mouse embryos, while TRKA predominates later in development.[48]

Alterations in TRK proteins, including rearrangements and atypical expression, are described in a variety of cancers.[49–55] Rearrangements of *NTRK* result in novel fusion proteins, which cause constitutive activation of the kinase. Such fusions are found in a majority of infantile fibrosarcomas but are also described in lung cancer, papillary thyroid carcinoma, glioblastoma, and colorectal carcinomas.[49–53,55] Differential expression of TRK has also been reported in a variety of tumors including adrenal, pancreatic, ovarian, esophageal, bladder, pheochromocytoma, and NB.[54] TRK expression levels have prognostic significance in some tumors; high levels of TRKB are associated with increased mortality in Wilms tumor, while TRKC expression is associated with a favorable outcome in medulloblastoma.[56,57] Differential expression of TRK proteins in NB is also associated with disease severity and prognosis.[58]

ROS1 is a third RTK with an unknown ligand that thereby limits knowledge of its function.[2] This protein is expressed primarily in epithelial cells and is found in a variety of tissues including the kidney, cerebellum, stomach, and intestine.[2,59–61] *ROS1* translocations leading to increased ROS1 activation have been reported in malignancies and were originally described in glioblastoma where an intrachromosomal deletion leads to the formation of a ROS1–FIG fusion protein.[2,60–63] Other cancers where ROS1 translocations have been described include NSCLC, ovarian carcinoma, and cholangiocarcinoma.[62,64–66] Of note, *ROS1* translocations/alterations have not been reported in NB.[67]

To date, targeted inhibitors of ALK, TRKA/B/C, and/or ROS1 have shown effectiveness in the treatment of target-mutated malignancies in both preclinical and clinical settings.[68–77] Entrectinib (RXDX-101, NMS-E628, NMS-01191372; Ignyta, San Diego, CA, USA) is a newly developed pan-TRK, ALK, and ROS1 inhibitor that has demonstrated preclinical efficacy in tumors with N*TRK1/2/3*, *ALK*, and *ROS1* alterations, including NB (Figure 1).
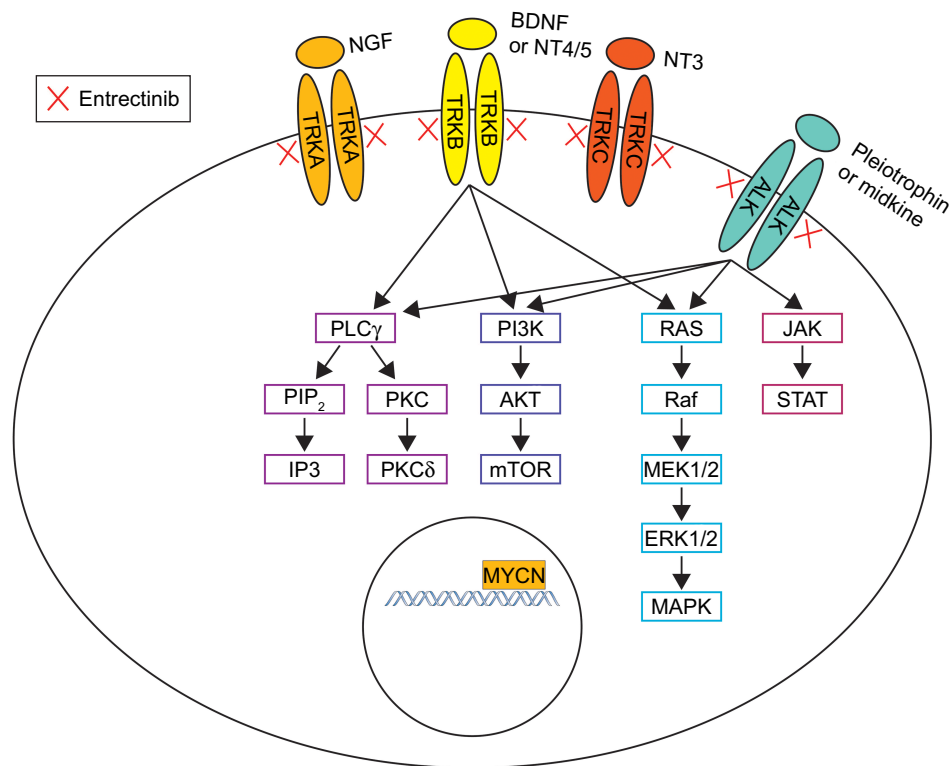
Figure 1 Mechanism of entrectinib in NB.
Abbreviation: NB, neuroblastoma.

Entrectinib was well tolerated in Phase I adult clinical trials and demonstrated activity against tumors with *NTRK1/2/3*, *ALK*, and *ROS1* translocations, providing the support for an ongoing Phase II study in adults.[73,78]

ALK expression and alterations in NB

ALK is recognized as an oncogenic driver of NB; and increased expression of ALK mRNA in NB is correlated with poor prognostic factors such as metastatic disease, *MYCN* amplification, and decreased survival.[79,80] *ALK* alterations present in NB include copy number gain, amplification, and mutations. *ALK* copy number gain is seen in 15%–25% of NB, and amplification is seen in 4% of high-risk NB; both are associated with advanced-stage disease and decreased survival.[81–85]

ALK mutations have been identified in both familial and sporadic NB. *ALK* germline mutations are reported in 50% of cases of hereditary NB.[85,86] These mutations are typically missense mutations within the kinase domain of *ALK* and lead to ALK hyperphosphorylation and constitutive activation of the kinase.[82,84–86] Three different germline mutations have been identified: R1192P, G1128A, and the most frequent R1275Q.[85,86] *ALK* mutations also occur in a small proportion (6%–10%) of somatic NB (Table 1).[81–83,85–88] In all, 12 somatic *ALK* mutations have been identified in NB, the majority of which are missense mutations within the kinase domain.[82,83,87–89] The three most common mutations are F1174L, R1275Q, and F1245C.[81–83,86–88] Cells transduced with either the F1174L or R1275Q *ALK* mutation lead to cytokine-independent growth of IL-3-dependent Ba/F3 cells, supporting the role of ALK mutations as oncogenic drivers in NB.[88] Of note, the F1174 mutation demonstrates increased oncogenic potential with faster transformation of Ba/F3 cells and stronger auto-phosphorylation compared to R1275Q.[83] Similarly, while all *ALK* mutations in NB are correlated with lower survival rates, those associated with F1174 mutations lead to even worse outcome than those with the R1275Q mutation.[81,83,87,90]

ALK alterations (amplification or mutations) are more common in cases with *MYCN* amplification. There is a strong correlation between the F1174L mutation and *MYCN* amplification in HR NB.[81,83–85,89] The association between *ALK* alterations and *MYCN* amplification is felt to be due in part to close localization of *ALK* and *MYCN* on chromosome 2. *MYCN* regulates *ALK* expression, and *ALK* is a transcriptional target of *MYCN*.[79] Additionally, ALK stimulates transcription of *MYCN* in NB cell lines, suggesting that the combination

Table I The frequency of ALK mutations in NB

Author, year	Number of NB samples	Frequency of ALK mutations	
Mossé et al, 2008	167 (high-risk NB samples)	Total	8.4% (14/167)
Janoueix-Lerosy et al, 2008	115	Total	6.1% (7/115)
		F1174	14.3% (1/7)
		R1275	71.4% (5/7)
		Other	14.3% (1/7)
Chen et al, 2008	215	Total	6.1% (13/215)
		F1174	50% (7/14)
		R1275	35.7% (5/14)
		Other	14.3% (2/14)
de Brouwer et al, 2010	254	Total	6.7% (17/254)
		F1174	29.4% (5/17)
		R1275	58.8% (10/17)
		Other	11.8% (2/17)
George et al, 2008	93	Total	7.5% (7/93)
		F1174	57.1% (4/7)
		R1275	14.3% (1/7)
		Other	28.6% (2/7)
Pugh et al, 2013	240	Total	9.2% (22/240)
Bellini et al, 2015	277	Total	9.7% (27/277)
		F1174	55.5% (15/27)
		R1275	44.4% (12/27)
Bresler et al, 2014	1,596	Total	8% (126/1,596)
		F1174	30% (38/126)
		R1275	43% (54/126)
		Others	27% (34/126)

Abbreviation: NB, neuroblastoma.

of *MYCN* amplification and *ALK* alterations may lead to increased oncogenic activity in NB.[91]

The presence of *ALK* alterations in NB and the association between both ALK overexpression and *ALK* alterations with decreased survival and more aggressive disease confirm that ALK is an important driver of NB and a potential therapeutic target.

TRK expression in NB

TRK proteins are differentially expressed in NB and have distinct roles in the pathogenesis of NB.[58] TRKA expression is associated with favorable prognostic factors such as localized disease (stages 1, 2, MS), younger age, absence of *MYCN* amplification, and improved survival.[92–94] Additionally, TRKA expression levels are decreased in patients with advanced disease and are inversely associated with *MYCN* amplification.[92–94] When NGF is applied to low-risk NB cells in vitro, which typically have high levels of TRKA, they undergo terminal differentiation, suggesting that TRKA may have a role in the regression or maturation of low-risk NB.[95] Similarly, high levels of TRKC in NB are associated with a low-risk disease and favorable prognosis and have a negative

correlation with *MYCN* amplification.[96,97] Low-risk NBs are more likely to express the full-length TRKC receptor, and high-risk cases more likely to have truncated TRKC or no TRKC expression at all.[96,97] Furthermore, tumors with TRKC also tend to express high levels of TRKA.[97]

In contrast, TRKB expression is associated with a poor prognosis in NB, present in >50% of high-risk cases and correlates with *MYCN* amplification.[45] TRKB activation leads to enhanced oncogenic potential in NB cells. When BDNF, the TRKB ligand, is applied to *MYCN*-amplified NB cells, there is improved cell survival and neurite growth.[45] TRKB is also associated with the angiogenic factors, VEGF and bFGF, suggesting that it may promote angiogenesis and metastatic ability.[25,98,99] Furthermore, TRKB expressing cell lines are less sensitive to doxorubicin, etoposide, and cisplatin, suggesting that TRKB may abrogate response to chemotherapy.[100] The association between TRKB/BDNF and cell survival, angiogenesis, metastasis, and drug resistance suggests that TRKB in NB may be a useful therapeutic target.[76,101]

ALK agents in oncologic development
Preclinical studies

ALK inhibition has been evaluated as a therapeutic option in NB with *ALK* amplification, mutations, and wild-type *ALK*. Knockdown of ALK expression in NB cells lines resulted in growth inhibition but was more effective in cells with *ALK* alterations than in those with wild-type *ALK*.[82,85,86] Although abrogation of ALK was less effective in the wild-type cells, several ALK inhibitors have been tested in models of NB with both wild-type and mutated *ALK*.

Crizotinib (Xalkori®; Pfizer, Inc., New York City, NY, USA) is a first-generation ALK inhibitor that competitively inhibits the binding of ATP to the active kinase site of ALK, MET, and ROS1.[102] Crizotinib was evaluated in both *ALK*-altered and wild-type NB cell lines and xenografts.[69] There was increased growth inhibition in vitro and decreased tumor growth in vivo in the cells with *ALK* alterations compared to wild-type *ALK* in response to crizotinib.[69] However, there was differential sensitivity to crizotinib based on the type of mutation. NBs with *ALK* R1275Q mutations were more sensitive to crizotinib, whereas those with *ALK* F1174L and F1245C exhibited a relative resistance.[69,103] The mechanism of relative resistance was due to the different ATP-binding affinities of the *ALK* mutations. For example, the F1174L *ALK* mutation demonstrates increased ATP binding compared to the R1275, thereby decreasing the ability

of crizotinib to bind leading to the resistant phenotype.[81] Crizotinib was also evaluated in combination with chemotherapy. In NB xenograft models with either *ALK* mutations (R1275Q, F1174L, F1245C) or amplification, crizotinib and cyclophosphamide/topotecan (C/T) in combination resulted in synergistic cytotoxicity and increased apoptosis compared to either agent alone.[104] This suggests that crizotinib in conjunction with chemotherapy may be more effective in *ALK*-altered NB including those with crizotinib-resistant *ALK* mutations.

Second- and third-generation ALK inhibitors have also been tested in NB. Alectinib (Alecensa®; Chugai Pharmaceutical Co., Tokyo, Japan) is a second-generation ALK/RET inhibitor that has improved affinity for the ATP-binding site and thereby increased potency against the ALK kinase compared to crizotinib.[105,106] Alectinib was evaluated in NB cell lines with both wild-type *ALK* and *ALK* F1174L mutations.[107] Alectinib treatment resulted in growth inhibition in all cell lines, including cells with the F1174L mutations. Additionally, the combination of alectinib and doxorubicin led to enhanced cell death compared to alectinib alone in both *ALK* wild-type and mutant cell lines.[107] While both crizotinib and alectinib demonstrate activity against *ALK* wild-type and mutant cell lines as a single agent and in combination with chemotherapy, alectinib has improved efficacy compared to crizotinib in the inhibition of the F1174L mutation.

Lorlatinib (PF-6463922; Pfizer, Inc.) is a third-generation ALK/ROS1 inhibitor designed to have improved inhibition of ALK compared to the previous agents.[108] Treatment with lorlatinib resulted in decreased growth of *ALK*-amplified NB cell lines and NB cell lines with the R1275Q, F1174L, and F1245C *ALK* mutations.[103] In xenograft models, complete and sustained tumor regression was seen in all animals with *ALK* mutations in response to lorlatinib therapy, whereas animals treated with crizotinib exhibited a more limited and transient delay in tumor growth.[103] These results suggest that lorlatinib is not only effective against *ALK* amplifications and mutations in NB in vitro and in vivo but is also more effective than crizotinib.

Preclinical studies suggest that ALK inhibition is effective in NB with *ALK* alterations and has some activity in NBs with wild-type *ALK*. Furthermore, there is differential sensitivity of *ALK* mutations in response to ALK inhibitors with relative resistance of the F1174L and F1245C mutations to crizotinib. The later generation ALK inhibitors are able to overcome this resistance, suggesting that these agents may be effective in individuals with tumors that contain crizotinib-resistant mutations.

Clinical trials

In clinical trials, ALK inhibitors have been widely studied in the treatment of NSCLC and several have been approved for clinical use. Crizotinib was the first of these agents to be approved for the treatment of *ALK*- and *ROS1*-rearranged NSCLC.[102] However, the clinical utility of crizotinib has been limited by the development of resistance and disease progression.[109] Patients with ALK-rearranged tumors who are treated with crizotinib can acquire *ALK* mutations, such as those of the F1174L, which leads to the development of drug resistance.[110] Furthermore, crizotinib does not cross the blood–brain barrier and brain metastases are a common location of disease progression in patients treated with crizotinib.[111]

The second- and third-generation ALK inhibitors (alectinib, ceritinib [Zykadia®; Novartis International AG, Basel, Switzerland], and lorlatinib) are able to overcome crizotinib resistance in clinical trials and have improved central nervous system (CNS) penetrance.[109] While lorlatinib is under investigation in Phase III clinical trial for patients with NSCLC, both ceritinib and alectinib are approved for the treatment of ALK-positive NSCLC.[112–114] However, the clinical utility of these agents is also limited by the eventual development of resistance. The mechanisms of acquired resistance include the addition of new mutations within the *ALK* or *ROS1* kinase domain, which prevent the drug from binding to the active site, amplification of *ALK* itself, and activation of bypass signaling pathways.[59,115–121] While the development of resistance remains a limitation of all ALK inhibitors, these agents are commonly used in the treatment of ALK-positive NSCLC and have been studied in pediatric malignancies.

Crizotinib was evaluated in a pediatric Phase I clinical trial that enrolled 79 patients with relapsed/refractory solid tumors, CNS tumors, or ALCL.[102] The main side effects were nausea and vomiting, seen in 65% and 57% of patients, respectively, and mild visual disturbances in 37% of patients. Nine patients had a complete response (CR) and five had a partial response (PR). Responses were more common in patients with known *ALK* aberrations, with eight of nine patients with *ALK*-translocated ALCL demonstrating an objective response (CR or PR). In all, 34 NB patients were enrolled in this Phase I single-agent trial, 11 with known *ALK* mutations; of them, one patient had a CR and three patients demonstrated stable disease (SD). The individual with a CR had a germline R1275Q mutation, and the three patients with SD had a germline R1275Q mutation, a somatic mutation at R1275L, and a somatic mutation at F1174L. The other seven patients with *ALK* mutations had progressive

disease, including three patients with F1174L mutations. Among the 23 patients with NB who had an unknown *ALK* mutation status, one patient had a CR and five had prolonged SD ranging from five to 39 cycles.[102] These results suggest activity of crizotinib in NB in a subset of individuals with *ALK* mutations or unknown *ALK* status. Additionally, despite prior preclinical and clinical evidence that the F1174 mutation is crizotinib-resistant, there was activity in a patient with a F1174L mutation, suggesting that the resistance is not absolute.

Although there was some efficacy in the Phase I pediatric study, the preclinical evidence suggests that crizotinib may be more effective in combination with chemotherapy. A pediatric Phase I trial evaluated crizotinib in combination with conventional chemotherapy for relapsed or refractory solid tumors or ALCL.[122] In this trial, crizotinib was combined with either C/T or vincristine/doxorubicin. Dose-limiting toxicities (DLTs) occurred in both groups and included nausea, diarrhea, dehydration, and prolonged QT in a total of four patients.[123] It was suspected that these gastrointestinal (GI) adverse effects may have been related to the poor palatability of the oral solution of crizotinib, and subsequent patients received a capsule formulation.[123] Neither the efficacy results nor the individual results of those receiving the capsule formulation are available. Crizotinib in combination with chemotherapy is also being evaluated in the current children's oncology group high-risk NB study, which includes a cohort for patients with *ALK*-mutated or -amplified tumors who will receive crizotinib in combination with standard high-risk multiagent chemotherapy.[124]

The second- and third-generation ALK inhibitors may be more effective than crizotinib and are being studied in pediatrics. There is an open Phase I pediatric trial evaluating the second-generation ALK inhibitor ceritinib in patients with tumors who have *ALK* alterations.[125] An interim report noted that 22 patients enrolled including seven patients with NB with *ALK* alterations. The adverse events were primarily GI related and included nausea, vomiting, diarrhea, transaminitis, abdominal pain, pyrexia, and fatigue.[126] There were two DLTs, which were grade 3 elevation in ALT and grade 2 persistent abdominal pain. Reported responses included two patients with ALCL and four patients with IMT. The results were less favorable in the seven patients with NB, where one patient with an F1174L *ALK* mutation experienced a mixed response with decrease in the size of a retroperitoneal mass but progression of intracranial disease.[126] It is difficult to draw conclusions about the efficacy of ceritinib in NB given the small number of patients. The third-generation ALK inhibitor lorlatinib may be more effective in NB due

to increased potency against the *ALK* kinase and improved CNS penetrance. There is an ongoing Phase I pediatric trial studying lorlatinib in NB, but preliminary results are not yet available.[127]

TRK agents in oncologic development
Preclinical studies

There are several inhibitors of TRKA/B/C in development for the treatment of TRK-altered malignancies. Inhibition of TRKB in NB is particularly intriguing due to the association between TRKB expression and high-risk disease. Several preclinical studies have evaluated the efficacy of TRK inhibitors in NB models that express TRKB. CEP-751 (KT-6587; Cephalon, Inc., Frazer, PA, USA) is an inhibitor of TRKA/B/C and has activity against PDGFR, EGFR, and PKC.[128] CEP-751 was evaluated in NB cells with varying levels of TRKB, both in vitro and in vivo, and was most effective in cells with high levels of TRKB.[74,75] AZ64 (AstraZeneca plc, London, UK) and GNF-4256 (Genomics Institute of the Novartis Research Foundation, San Diego, CA, USA) are selective and potent inhibitors of TRKA/B/C; and lestaurtinib (CEP-701; Teva Pharmaceutical, Peta Tikva, Israel) is a potent inhibitor of Flt3 that also has activity against JAK2 and TRKA/B/C.[71,76,129–131] Each of these agents was tested in a NB cell line that was transfected with TRKB. In these studies, drug treatment resulted in growth inhibition and decreased phosphorylation of TRKB, suggesting that the effect of the drugs was related to the inhibition of TRKB.[71,76,130] When these agents were tested in xenograft models, there was decreased tumor growth and improved survival, suggesting that TRKB inhibition in NB may be beneficial.[71,76,130]

While multiple TRK inhibitors have demonstrated effectiveness as single agents in preclinical NB, they were also tested in combination with chemotherapy. AZ64 and GNF-4256 were evaluated in combination with irinotecan/temozolomide (I/T) in xenograft models that express TRKB.[71,76] In both studies, the TRK inhibitor in combination with I/T led to decreased tumor growth and prolonged survival rates compared to single-agent TRK inhibition.[71,76] Similarly, lestaurtinib led to improved antitumor efficacy in xenografts when administered in combination with C/T or with I/T.[130] These studies suggest that inhibition of TRKB may be an important adjunct to conventional chemotherapy.

Clinical trials

While there are multiple preclinical reports of effective TRK inhibitors in NB, there are few agents in clinical trials. Neither GNF-4256 nor AZ64 was pursued for further

development.[67] Lestaurtinib was evaluated in a Phase I NB-specific clinical trial and enrolled 47 patients with refractory high-risk NB.[77] The most notable adverse effects included grade 1/2 transaminitis in 63% of patients with dose-limiting transaminitis in four patients and reversible pancreatitis in three patients with prolonged drug exposure. In all, 26 patients were treated at a dose level of at least 70 mg/m², two of whom experienced a PR and nine had an SD lasting a median of six cycles.[77] This study demonstrated a clinical benefit for some of the patients treated at a biologically effective dose. The lack of significant toxicities and the preclinical evidence that lestaurtinib is more effective with chemotherapy provided evidence for trials evaluating lestaurtinib in combination with chemotherapy. Unfortunately, further development of lestaurtinib has been halted.

Larotrectinib (LOXO-101, ARRY-470; Loxo Oncology, Stamford, CT, USA) is a highly selective inhibitor of TRKA/B/C, which was studied preclinically in tumors with *NTRK* fusions and remains in clinical development.[132] Larotrectinib was recently shown to be effective in early phase trials of patients (including children) with solid tumors who had *NTRK* fusions.[72,133] A total of 55 patients with *NTRK* fusions were enrolled in the combined adult and pediatric trial. The overall response rate was 75%, and seven patients had a CR and 34 had a PR, with 71% of the responses persisting beyond 1 year.[72] The pediatric specific Phase I study enrolled patients with and without *NTRK* fusions. There were 15 evaluable patients with infantile fibrosarcoma, papillary thyroid carcinoma, or other soft tissue sarcomas, which had documented *NTRK* fusions. Among the *NTRK* fusion-positive patients, the objective response rate (ORR) was 93% (four CR and 10 PR). The study enrolled seven patients without documented *NTRK* fusions including one patient with NB, and all developed progressive disease.[133] Although there were no responses in the patients without *NTRK* fusions, the number of fusion-negative patients on the trial was small. The most common drug-related adverse effects were transaminitis, hematologic toxicities, and vomiting.[72,133] In the pediatric-specific study, the only DLT was one grade 3 elevation in ALT.[133] Additionally, as is seen with other TKIs, 10 patients in the combined adult/pediatric study and one patient in the pediatric study developed acquired resistance through the development of new mutations within the *NTRK* kinase domain.[72,133] Larotrectinib was effective in treating tumors with *NTRK* fusions, which provided evidence for Phase II clinical trials studying larotrectinib in these patients.[134,135] However, its role in tumors without *NTRK* fusions, such as NB, is unclear due to the small number of patients enrolled.

Entrectinib: a TRKA/B/C, ALK, and ROS1 inhibitor in oncologic development
Preclinical studies

Entrectinib is an ATP-competitive TKI with activity against TRKA, TRKB, TRKC, ALK, and ROS1.[68] Entrectinib's activity is specific to the TRK, ALK, and ROS1 targets. In vitro proliferation profiling of >200 tumor cell lines revealed that entrectinib's antiproliferative effect was limited to cell lines dependent on entrectinib-specific RTK targets only.[68] Entrectinib has 10 to 100 times more potency in inhibiting ROS1 and is seven to eight times more potent against ALK compared to crizotinib, and it is also more potent than lestaurtinib.[67,68,136] Entrectinib's ability to inhibit both ALK and TRKA/B/C may provide a therapeutic advantage over previous agents with specificity to either ALK or TRKA/B/C.

Entrectinib has also been studied in preclinical models of NB. To evaluate its ability to inhibit *ALK*-driven NB, *ALK* wild-type, -amplified, or -mutated cell lines were treated with entrectinib.[137] Decreased cell proliferation and induction of apoptosis occurred in response to entrectinib as measured by Ki-67 and activation of caspase-3, respectively. The treated cell lines demonstrated decreases in ERK1/2 and STAT3 phosphorylation, supporting that entrectinib's antiproliferative effects are mediated through downstream inhibition of the ALK signaling pathway. *ALK*-amplified cells were the most sensitive to entrectinib. Cells with *ALK* mutations, especially F1174L, were less sensitive to entrectinib due to the induction of autophagy, as measured by microtubule-associated protein 1 LC3. This relative resistance was abrogated when entrectinib was studied in combination with chloroquine, an inhibitor of autophagy. NB cells with F1174L mutations treated with entrectinib and chloroquine had greater growth inhibition compared to either agent alone, suggesting that this combination may lead to improved efficacy of the drug in *ALK*-mutated tumors.[137]

NB cell lines with the *ALK* F1174L mutation, and thereby resistant to entrectinib, were transduced to express TRKB.[67] When entrectinib in increasing doses was applied to these transduced cells, there was decreased TRKB phosphorylation and cell viability suggesting that the effect was related to TRKB inhibition. Similarly, in xenograft models with the same *ALK* F1174 TRKB-transfected cell line, entrectinib therapy led to decreased tumor growth and improved survival compared to animals that were not treated. In the same in vitro model, combination treatment with chemotherapy resulted in increased growth inhibition compared to entrectinib or

I/T alone. Furthermore, entrectinib in combination with I/T resulted in decreased tumor growth and improved xenograft survival compared to either regimen alone.[67] These studies suggest that TRKB inhibition by entrectinib may provide a therapeutic benefit in NB, particularly when used in combination with chemotherapy.

Clinical trials

Entrectinib has been evaluated in two adult Phase I clinical trials, STARTRK-1 and ALKA-372-001, for patients with refractory or metastatic solid tumors and molecular alterations or rearrangements involving *NTRK1*, *NTRK2*, *NTRK3*, *ROS1*, or *ALK*. A total of 119 adult patients with advanced solid tumors were enrolled in these studies: 54 patients in ALKA-372-001 and 65 patients in STARTRK-1. The majority of patients had NSCLC (60%), and 15% had cancers of the GI tract. The drug was well tolerated, and the most common side effects were fatigue, dysgeusia, paresthesias, nausea, and myalgias. The majority of adverse events were grade 1 or 2, and all were reversible with dose modification. At the dose of 800 mg daily, there were three patients with DLTs: grade 3 cognitive disturbances, grade 3 fatigue, and grade 4 eosinophilic myocarditis. All the grade 3 and higher AEs resolved when the drug was held. The maximum tolerated dose was determined to be 600 mg daily and was the recommended Phase II dose (RP2D).[73]

A sub-analysis was performed on the patients with *NTRK1/2/3*, *ROS1*, or *ALK* fusions who were inhibitor naïve and received the RP2D. Of the 119 patients treated in the Phase I trials, 25 patients met these criteria and were evaluable for disease response. The ORR, those with PR or CR, was 79%. Responses were observed in patients with NSCLC, colorectal cancer, mammary analog secretory carcinoma, melanoma, and renal cell carcinoma.[73] When responses were analyzed by the type of fusion, the response rate in each group remained high. Three patients had *NTRK1/2/3* rearrangements. This cohort's ORR was 100%. The 14 patients with *ROS1* rearrangements had an ORR of 86% and included two patients with a CR. Patients with *ALK*-rearranged tumors had an ORR of 57% (n=7). The longest duration of response was 32 months in a patient with *ROS1*-rearranged lung cancer who remained on therapy at the time of study completion. Entrectinib also demonstrated antitumor activity within the CNS with five of eight (63%) patients with CNS disease having an objective response. This included one patient with a *NTRK1* rearrangement who had a CR within the brain and an ongoing response at 15 months.[73] These results demonstrate that entrectinib is well tolerated

and may be beneficial in inhibitor-naïve patients with ALK, TRKA/B/C, and ROS1 fusions and patients with CNS involvement. However, there are two reports of acquired resistance to entrectinib.[138,139] The first was a patient with mammary analog secretory carcinoma who had a *NTRK3* fusion and developed a secondary *NTRK3* mutation at G623R after treatment with entrectinib.[138] The second was a patient with colorectal cancer who had a *NTRK1* rearrangement and developed 2 *NTRK1* point mutations at G595R and G667C following progression on entrectinib.[139]

While the responses were favorable in individuals with *NTRK1/2/3*, *ROS1*, and *ALK* translocations who had not previously received therapies targeting those molecular alterations, there were no responses in 25 patients with *ROS1* or *ALK* translocations who had been previously treated with ROS1 or ALK inhibitors.[73] This suggests that entrectinib was unable to overcome acquired resistance to other inhibitors and that this agent may be most effective when used upfront. Additionally, there was only one response in the other 59 patients who did not have a fusion. Notably, this response was in a patient with NB with the *ALK* F1245V mutation. This individual had a PR that lasted 8.3 months and was continued on the drug for 3.5 years due to a clinical benefit. Although only one patient without a fusion protein who had a response, it suggests that entrectinib may have some clinical efficacy against certain *ALK* mutations in NB.[73]

As a result of the favorable responses in the Phase I studies, entrectinib was granted US Food and Drug Administration (FDA) orphan drug designation for the treatment of TRKA/B/C, ALK, or ROS1 positive colorectal cancer, NSCLC, and NB.[140] The Phase I adult trial also provided evidence for expanding further clinical studies evaluating entrectinib. STARTRK-2 is a global, multicenter, Phase II basket study for patients with *NTRK1/2/3*-, *ROS1*-, and *ALK*- rearranged cancers and is currently enrolling patients.[78] There is also an open pediatric Phase I (RXDX-101-03) trial for children with refractory solid tumors and CNS tumors.[141] The pediatric study includes both patients with and without *NTRK1/2/3*, *ROS1*, and *ALK* fusions or alterations. In an interim report from this study, there were 16 patients enrolled and 15 were evaluable, including 10 patients with NB, two with IMT, one with salivary gland adenocarcinoma, one with synovial sarcoma, and one with infantile fibrosarcoma.[142] There were three DLTs reported. One was a grade 2 increase in creatinine for >7 days in one of the six patients at the 550 mg/m² dose level. Two of the three patients at the 750 mg/m² dose level had DLTs, one had grade 2 dysgeusia/fatigue >7 days and the other had grade 3 pulmonary edema. As a result, the RP2D

was defined as 550 mg/m². There were three fusion-positive patients (two IMT and one infantile fibrosarcoma) enrolled to date, and all demonstrated an objective response and continue on protocol therapy. The genetic alterations in the two IMT patients were ALK (*DCTN1–ALK*) or ROS1 (*TFG–ROS1*) translocations, and the patient with infantile fibrosarcoma had a TRKC (*ETV6–NTRK3*) translocation. The responses of the other patients were not reported, although there is one patient with NB who continues on protocol therapy.[142] Although the outcome of the other nine NB patients has not been reported, the NB patient with an ongoing response suggests that there may be some efficacy of entrectinib in NB.

Conclusion

Both ALK and TRK play important roles in the pathogenesis of NB and are associated with aggressive disease and decreased survival. Inhibition of either ALK or TRK has been evaluated as a potential treatment for NB, and several agents demonstrated preclinical efficacy. However, relatively few NB patients have been treated with these agents in clinical trials and there has been limited efficacy. Furthermore, the ALK inhibitors tested in NB (crizotinib, ceritinib, alectinib, lorlatinib) do not inhibit TRKA/B/C, and similarly, the TRK inhibitors (CEP-751, AZ64, GNF-4256, lestaurtinib, larotrectinib) do not inhibit ALK (Table 2). Entrectinib is the first TKI that is highly selective for both ALK and TRK

A/B/C and has increased potency compared to other ALK and TRK inhibitors and has demonstrated some preclinical efficacy in NB models.

This ability to potently inhibit dual pathways that may be activated in NB suggests entrectinib may have improved efficacy compared to other targeted inhibitors previously evaluated in NB. Treatment with entrectinib in A*LK* wild-type and *ALK*-amplified NB cells in vitro resulted in growth inhibition although *ALK*-mutated cells were generally less sensitive and the F1174L-mutated cells were resistant. The ability of entrectinib to inhibit TRKB in NB was also evaluated. Interestingly, entrectinib was effective in a NB model with the F1174L *ALK* mutation that also expresses TRKB. This suggests that entrectinib's ability to inhibit TRKB may be sufficient to overcome resistance due to the F1174L ALK mutation. However, this requires further validation in preclinical studies. TRKB and TRKC expression are also important in the pathogenesis of NB and are seen in individuals with a low-risk disease, but entrectinib has not been studied in this setting.

Early preclinical data suggest that entrectinib may be most effective in combination with other therapies that may incorporate well into the current paradigm of multimodal therapy for high-risk NB. Further clinical trials evaluating entrectinib in combination with either chloroquine or with more standard cytotoxic chemotherapy are needed to confirm

Table 2 ALK and TRK inhibitors under investigation for NB

Drug name	Company	Targets	Status
Crizotinib (Xalkori®)	Pfizer, Inc.	ALK (first generation), MET, ROS1	Approved • ALK- and ROS1-rearranged NSCLC
Ceritinib (Zykadia®)	Novartis International AG	ALK (second generation), IGF-1R[143]	Approved • ALK-positive NSCLC
Alectinib (Alecensa®)	Chugai Pharmaceutical Co.	ALK (second generation), RET	Approved • ALK-positive NSCLC
Lorlatinib • **PF-6463922**	Pfizer, Inc.	ALK (third generation), ROS1	Clinical • Phase III
CEP-751 • KT-6587	Cephalon, Inc.	TRKA/B/C, PDGFR, EGFR, PKC	Preclinical • No longer under investigation
AZ64	AstraZeneca, Inc.	TRKA/B/C	Preclinical • No longer under investigation
GNF-4256	Genomics Institute of the Novartis Research Foundation	TRKA/B/C	Preclinical • No longer under investigation
Lestaurtinib • CEP-701	Teva Pharmaceutical Industries Ltd	TRKA/B/C, JAK2, Flt3	Clinical • No longer under investigation
Larotrectinib • **LOXO-101, ARRY-470**	Loxo Oncology, Inc.	TRKA/B/C	Clinical • Phase II
Entrectinib • **RXDX-101, NMS-E628, NMS-01191372**	Ignyta, Inc.	TRKA/B/C, ALK, ROS1	Clinical • Phase II

Note: Drugs approved or those that remain under investigation are highlighted in bold.
Abbreviation: NB, neuroblastoma.

the utility of this regimen. If combination therapy proves effective, this could be used to improve outcomes for the 50% of patients who currently do not respond or relapse. Moreover, if the combination of chloroquine and entrectinib is effective, this could be particularly appealing, as it might be able to decrease or limit the use of cytotoxic chemotherapy, which current therapy relies on heavily.

While there is intriguing preclinical evidence for the use of entrectinib in the treatment of NB, particularly in patients with *TRK*, *ALK* and *ROS1* alterations, the clinical efficacy in NB remains under investigation. As ALK expression/mutations and TRKB expression are associated with a high-risk disease and poor outcomes in NB, this agent is particularly exciting to consider as a potential treatment option. In the published Phase I clinical trials, there were relatively few patients with NB, and there is only one report of an individual with NB who had a PR to entrectinib. The ongoing pediatric phase I trial will provide necessary additional information regarding the efficacy as a single agent in this population. Additional clinical studies are needed, both as a single agent and in combination, to determine whether this is a beneficial and tolerable therapy for NB and which subset of patients is most likely to benefit.

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Disclosure

The authors report no conflicts of interest in this work.

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