# Vandetanib inhibits cisplatin-resistant neuroblastoma tumor growth and invasion

CHANGCHUN LI[1-3], CHAO YANG[1-3] and GUANGHUI WEI[2-4]

[1]Department of Pediatric Surgical Oncology, Children's Hospital of Chongqing Medical University, Ministry of Education Key Laboratory of Child Development and Disorders;
[2]China International Science and Technology Cooperation Base of Child Development and Critical Disorders;
[3]Chongqing Key Laboratory of Pediatrics, Children's Hospital of Chongqing Medical University;
[4]Department of Urology, Children's Hospital of Chongqing Medical University, Ministry of Education Key Laboratory of Child Development and Disorders, Chongqing 400014, P.R. China

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**Abstract.** Resistance is the major cause of cisplatin treatment failure in neuroblastoma (NB). Vandetanib is widely used in the treatment of several cancers. In the present study, we aimed to determine the potential of vandetanib in cisplatin-resistant NB therapy. Immunohistochemistry (IHC) staining was employed to detect p-RET and CXCR4 expression in cisplatin-resistant or -sensitive NB tissues from patients. Vandetanib was added to treat selected cisplatin-resistant SH-SY5Y cells (SH-SY5Y-R); this was followed by CCK8 assay, colony formation assay, and invasion assay. Furthermore, the effect of vandetanib on subcutaneous tumor growth was investigated in mice. Our results demonstrated greater expression of p-RET and CXCR4 in cisplatin-resistant neuroblastomas (NBs). Vandetanib significantly inhibited SH-SY5Y-R cell proliferation, colony formation, and invasion, while downregulating p-RET and CXCR4 expression. Furthermore, vandetanib was as effective as high-dose cisplatin in impairing cisplatin-resistant NB subcutaneous tumor growth. Notably, vandetanib caused less severe liver toxicity in mice compared with high-dose cisplatin. In summary, this study identified Vandetanib as a potential drug for cisplatin-resistant NB treatment.

## Introduction

Neuroblastomas (NBs) are known for their unpredictable behavior; some spontaneously regress, some mature, whereas others develop into aggressive forms (1). Moreover, around 7% of all tumors observed in children are NBs, next only to leukemia and brain/central nervous system tumors (2). Notably, NB accounts for approximately 15% of childhood cancer-related mortality (2,3). Though aggressive treatment strategies such as surgery, radiation, and/or chemotherapy have improved in recent decades, the prognosis for patients with disseminated NB is grim, with a 5-year survival rate of ~30% (4,5).

Multi-agent chemotherapy, including cisplatin, rapamycin, 13-*cis*-retinoic acid (CRA), and vincristine, is the conventional therapy for patients with advanced stages of NB (6-8). However, drug resistance arises in the majority of stage IV and relapsed NB, often leading to treatment failure (9,10). Furthermore, aggressive therapy also causes severe, long-term side effects in patients, including deafness, cardiac failure, and secondary malignancies (3,11). Cisplatin is one of the frontline chemotherapeutic drugs for NB and widely used in clinical therapy (12). Unfortunately, due to acquired cisplatin resistance of NBs, the prognosis of advanced NB patients after cisplatin treatment is still poor (13-15). Thus, the development of novel antitumor strategies is essential to overcome cisplatin resistance and to prevent tumor progression.

Rearranged during transfection (RET) is a receptor tyrosine kinase that is expressed in various neurons including NB. Activation of RET is correlated with poor progression of NB and associated with promoting cell proliferation and metastasis (16,17). RET is triggered by anaplastic lymphoma kinase (ALK) in NB and inhibition of RET impaired tumor growth *in vivo* in ALK mutated NB (16). A previous study using cell lines and primary cancer samples has also demonstrated a correlation between high C-X-C chemokine receptor type 4 (CXCR4) expression levels in NB cells and increased occurrence of bone marrow metastases (18). CXCR4 was also demonstrated to support the development of NB primary tumors (19). Thus, RET and CXCR4 are the potential therapeutic targets of NB.

Vandetanib (Caprelsa, AstraZeneca Pharmaceuticals) is a small-molecule receptor tyrosine kinase inhibitor of VEGF receptor 2 (VEGFR2), EGF receptor (EGFR), and RET tyrosine kinase activity as well as mutated RET (20,21). Vandetanib is widely used as a chemotherapeutic agent in

*Correspondence to:* Dr Guanghui Wei, China International Science and Technology Cooperation Base of Child Development and Critical Disorders, 136 Zhongshan 2nd Road, Yuzhong, Chongqing 400014, P.R. China
E-mail: ghwei@cqmu.edu.cn

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thyroid carcinoma (22-24), glioblastoma (25), non-small cell lung cancer (26), and pulmonary adenocarcinoma (27). Vandetanib has been demonstrated to inhibit NB migration and invasion by reducing CXCR4 expression (28). The combination of vandetanib with CRA was more effective in reducing tumor growth than either treatment alone in NB (29). However, whether vandetanib exhibits antitumor activity in cisplatin-resistant NB is still unclear. In the present study, we aimed to determine the potential of vandetanib in cisplatin-resistant NB therapy. The NB cell line SH-SY5Y, with a strong ability for proliferation and invasion, and which is established from a metastatic bone tumor, was used in our study.

## Materials and methods

*Primary NB tumors*. In total, 30 diagnostic primary NB tumor samples were obtained from the Department of Pediatric Surgical Oncology, Children's Hospital of Chongqing Medical University. Research was approved by the Research Ethics Committees of Chongqing Medical University. Written informed consent was signed by the parents or guardians of the pediatric patients. The patients were classified as cisplatin-resistant and -sensitive according to the prognosis of patients with cisplatin treatment and the expression of ERCC1 gene, a marker of cisplatin sensitivity. Before surgery, four cisplatin treatments, combined with vincristine, cyclophosphamide and etoposide, were performed. During the treatments, the tumor volume was assessed by B ultrasound examination once a month. Following surgery, the tumor tissues were collected for ERCC1 mRNA detection. The patients with reduction of tumor volume and ERCC1 negative expression were classified as cisplatin-sensitive. The patients without reduction of tumor volume and ERCC1 positive expression were classified as cisplatin-resistant.

*Cell culture and treatment*. The NB cell line SH-SY5Y was purchased from ATCC (Manassas, VA, USA). The cells were grown at 37˚C in 5% $CO_2$ in DMEM (Invitrogen, Carlsbad, CA, USA) supplemented with 10% heat-inactivated fetal bovine serum (EMD Millipore, Billerica, MA, USA), L-glutamine, sodium pyruvate, nonessential amino acids, and penicillin/streptomycin (Sigma-Aldrich, St. Louis, MO, USA). The SH-SY5Y cells were maintained at the initial cisplatin concentration of 10 $\mu$M ($IC_{50}$). The dose of cisplatin was titrated gradually to a final concentration of 80 $\mu$M after 6 weeks. The selected cisplatin-resistant SH-SY5Y cells were named SH-SY5Y-R cells, and the cisplatin-sensitive SH-SY5Y cells were named SH-SY5Y-S cells. SH-SY5Y-R cells were established and then were maintained in DMEM medium with 10% FBS containing 80 $\mu$M cisplatin.

*Cell viability assay*. Cells (1,000) were plated in each well of a 96-well plate. Then cisplatin at different concentrations and vandetanib (0, 2.5, 5 and 10 $\mu$M) were added into the cell and incubated for 24-72 h. Cell viability was evaluated by Cell Counting Kit-8 (CCK8) assay (Dako; Agilent Technologies, Inc., Dallas, TX, USA). The relative cell viability was calculated as the OD 450 nm of the treated group/the OD 450 nm of the blank group. The $IC_{50}$ was calculated using SPSS (version 21.0; IBM SPSS, Armonk, NY, USA) according to the guidelines published by Sebaugh (30).

*Colony formation assay*. Cells (1,000) were plated in each well of a 6-well plate. Then vandetanib (5 $\mu$M) was added into the cells and incubated for 7-10 days. The plate was fixed with 4% paraformaldehyde for 15 min and stained with crystal violet (Beyotime, Beijing, China) for 10 min at room temperatures. The number of colonies formed were counted and analyzed.

*Invasion assay*. Following dilution with DMEM medium (1:5), Matrigel was added into an 8.0-$\mu$m Transwell (BD, Franklin Lakes, NJ, USA). Then, 30 min later, $2x10^4$ SH-SY5Y-S or SH-SY5Y-R cells were added into the upper well containing serum-free medium, with or without vandetanib (5 $\mu$M) treatment. The lower well was fixed with DMEM medium containing 10% FBS. Subsequently, 24 h later, the Transwell was fixed with 4% paraformaldehyde and stained with crystal violet (Beyotime, Beijing, China). The invaded cells were counted and analyzed.

*Western blotting*. Western blotting analysis was performed as previously described (31). Briefly, SH-SY5Y-R cells with or without vandetanib treatment were lysed in RIPA lysis buffer (Beyotime, Beijing, China) containing 1% protease inhibitor cocktail (EMD Millipore). Following concentration determination by BCA assay (Beyotime), 10 $\mu$g of total protein was added and separated by 10-12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The protein was transferred to a polyvinylidene fluoride (PVDF) membrane (EMD Millipore) and blocked with 5% non-fat milk in TBS/T buffer. The following antibodies were used: p-RET (rabbit monoclonal antibody; cat no. 3221; 1:800; Cell Signaling Technology, Inc., Danvers, MA, USA), CXCR4 (rabbit monoclonal antibody; cat no. ab124824; 1:1,000; Abcam, Cambridge, UK), and GAPDH (mouse monoclonal antibody; cat no. sc-293335; 1:5,000; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) was used as a loading control. The density of each band was assessed with ImageJ software (NIH, Bethesda, MA, USA).

*Animal study*. BALB/c-nu mice (5-6 weeks old, 18-20 g) were purchased from the Model Animal Center of the Nanjing University and housed in barrier facilities on a 12-h light/dark cycle. All experimental procedures were approved by the Institutional Animal Care and Use Committee of Chongqing Medical University. All mouse care and experiments were carried out in accordance with institutional guidelines concerning animal use and care of Chongqing Medical University. One week after receiving the mice, $5x10^6$ SH-SY5Y-R cells were subcutaneously injected into the left dorsal flank. When the tumor reached ~100 $mm^3$, the mice were randomly assigned to four groups (n=4/group). The mice were injected intratumorally with 100 $\mu$l PBS, 20 nmol cisplatin diluted in 100 $\mu$l PBS, 100 nmol cisplatin diluted in 100 $\mu$l PBS and 0.6 mg vandetanib diluted in 100 $\mu$l PBS every day. The tumor size was assessed every five days with calipers by the same investigators and the tumor volume was calculated using the equation (length x $width^2$ x 0.52). On day 35

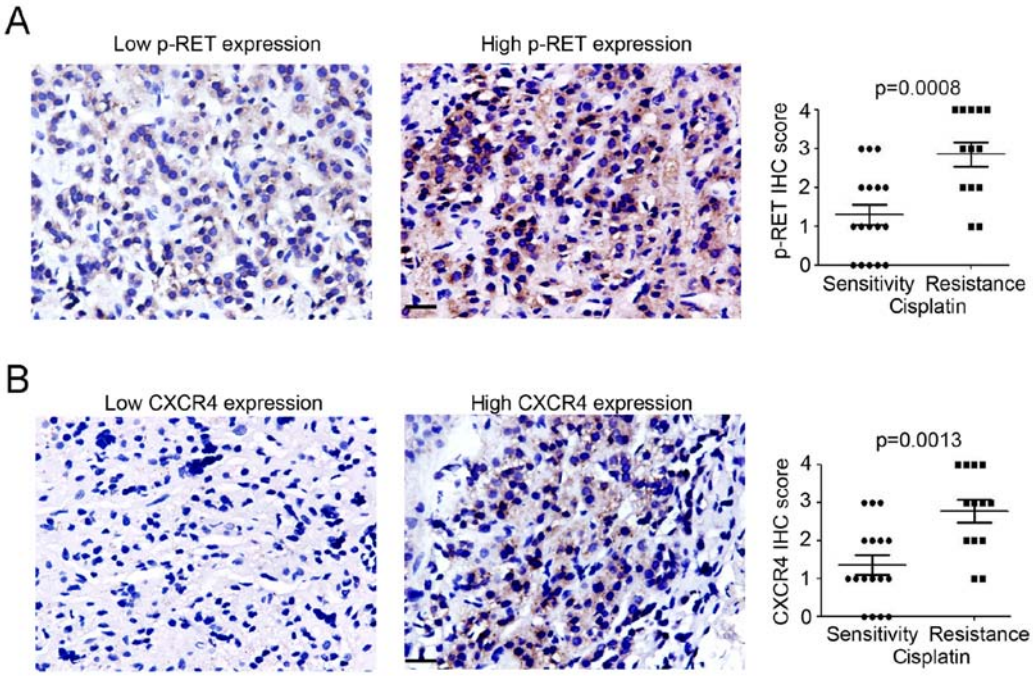


Figure 1. High expression of p-RET and CXCR4 in cisplatin-resistant NB tissues. (A) Staining of p-RET in cisplatin-sensitive and -resistant malignant NB tissues. Scale bar, 50 $\mu$m. The IHC score of p-RET expression in cisplatin-sensitive and -resistant malignant NB tissues (n=30; $^{**}$P<0.01). (B) Staining of CXCR4 in cisplatin-sensitive and -resistant malignant NB tissues. Scale bar, 50 $\mu$m. The IHC score of CXCR4 expression in cisplatin-sensitive and -resistant malignant NB tissues (n=30; $^{**}$P<0.01).

post-tumor cell injection, the animals were euthanized, and the tumors were excised, weighed, and paraffin-embedded.

*Immunostaining and TUNEL assay*. Immunostaining was performed as previously described (32). The following antibodies were used: Anti-PCNA antibody (mouse monoclonal antibody; cat no. sc25280; 1:100; Santa Cruz Biotechnology, Inc.), anti-p-RET antibody (rabbit monoclonal antibody; cat no. 3221; 1:200; Cell Signaling Technology, Inc.), anti-CXCR4 antibody (rabbit monoclonal antibody; cat no. ab124824; 1:200; Abcam), anti-CD31 antibody (mouse monoclonal antibody; cat no. 555444; 1:100; BD Biosciences). All specimens were evaluated using Olympus BX600 microscope and Spot Flex camera (Olympus, Tokyo, Japan). The positive and total cells in 3-5 random fields were counted and analyzed.

Apoptotic DNA fragmentation was examined using an *in situ* DeadEnd™ Fluorometric TUNEL System assay kit (Promega, Madison, WI, USA) according to the manufacturer's protocol. The localized green fluorescence of apoptotic cells from the fuorescein-12-dUTP was detected by fluorescence microscopy. The cell nuclei were stained with DAPI (Beyotime). The apoptotic cells in 5 random fields were counted and analyzed.

*Statistical analysis*. All statistical analyses were carried out using SPSS 19.0 statistical software (SPSS Inc., Chicago, IL, USA). The 2-tailed Student's t-test was used to evaluate the significance of differences between two groups of data and one-way ANOVA was used for statistics in multiple groups in all pertinent experiments. All experiments were performed 3-5 times. P<0.05 was considered to indicate a statistically significant result.

## Results

*High expression of p-RET and CXCR4 in cisplatin-resistant NB tissues*. To determine the potential utility of vandetanib in cisplatin-resistant NB patients, IHC staining was employed to analyze p-RET and CXCR4 expression in 30 NB tissue samples, which were classified as originating from either cisplatin-sensitive or -resistant patients. As shown in Fig. 1, increased p-RET- and CXCR4-positive cells were found in the cisplatin-resistant NB tissues. This suggested that p-RET and CXCR4 may play a crucial role in maintaining the cisplatin resistance of NB tissues.

*High expression of p-RET and CXCR4 in cisplatin-resistant NB cells*. To further investigate the expression of p-RET and CXCR4 in cisplatin-sensitive and -resistant NB cells, cisplatin was used to treat SH-SY5Y cells, and the cisplatin-resistant cells were selected and named SH-SY5Y-R. As shown in Fig. 2A and B, the $IC_{50}$ values of cisplatin for SH-SY5Y-S (cisplatin-sensitive SH-SY5Y cells) and SH-SY5Y-R were approximately 10 and 130 $\mu$M, respectively. These results were identical to a previous study conducted by our laboratory (33). A CCK8 assay demonstrated higher viability of proliferation in SH-SY5Y-R cells compared with SH-SY5Y-S cells (Fig. 2C). Furthermore, increased colony formation (Fig. 2D and E) and invading cells (Fig. 2F and G) were observed in the SH-SY5Y-R cells as determined by colony formation assay and Matrigel invasion assay *in vitro*, respectively. Western blotting demonstrated that the expression of p-RET and CXCR4 was significantly increased in SH-SY5Y-R cells (Fig. 2H and I). Collectively, these results indicated that cisplatin-resistant NB cells exhibited increased malignancy and invasive properties, combined with upregulation of p-RET and CXCR4 expression.

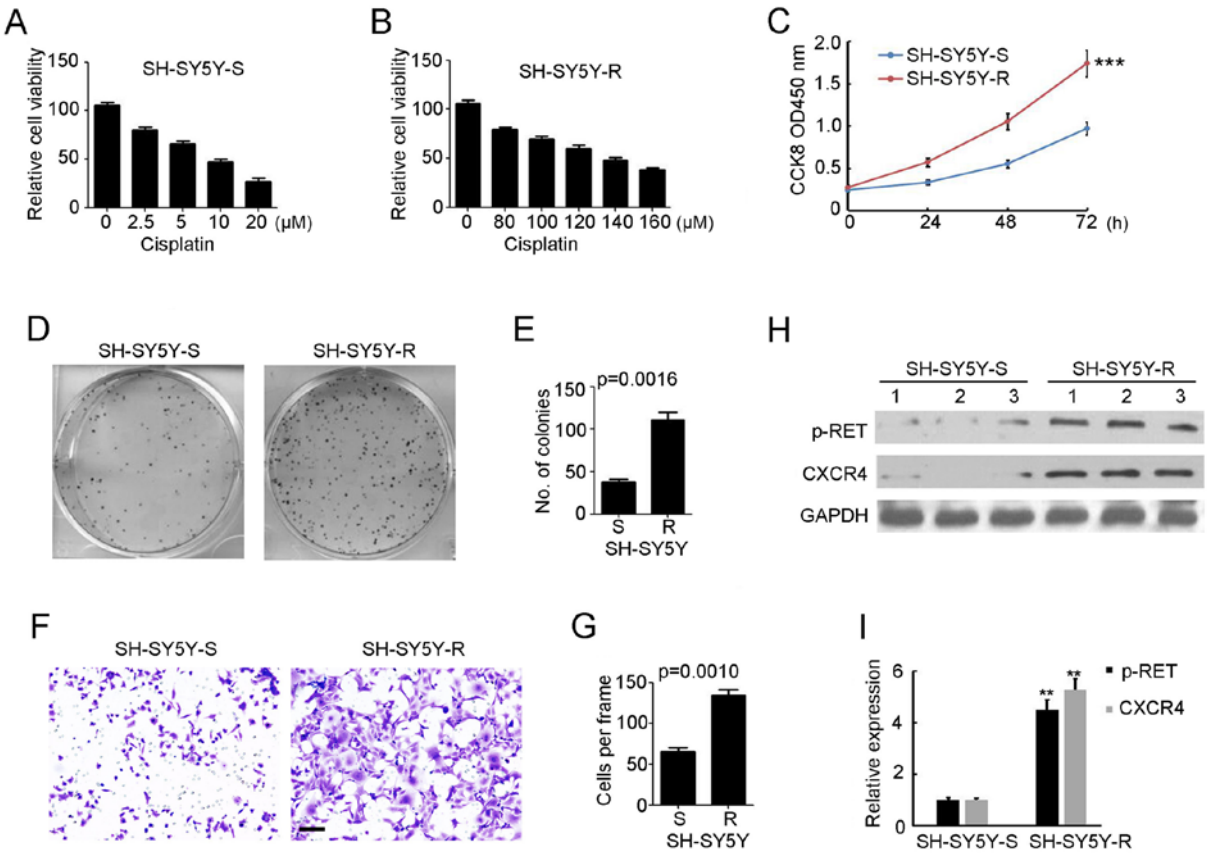


Figure 2. High expression of p-RET and CXCR4 in cisplatin-resistant NB cells. (A and B) A Cell Counting Kit-8 assay was performed to determine cell proliferation of selected cisplatin-sensitive SH-SY5Y cells (SH-SY5Y-S) and cisplatin-resistant SH-SY5Y cells (SH-SY5Y-R) that were established in our laboratory and used in a previous study (33) under different concentrations of cisplatin treatment. Data represent the means ± SD from three independent experiments. (C) A Cell Counting Kit-8 assay was performed to determine cell proliferation of SH-SY5Y-S and SH-SY5Y-R cells. Data represent the means ± SD from three independent experiments (***P<0.001). (D) A colony formation assay was performed to determine tumorgenesis of SH-SY5Y-S and SH-SY5Y-R cells. (E) The number of colonies was counted and analyzed. Data represent the means ± SD from three independent experiments (**P<0.01). (F) A Matrigel-mediated invasion assay was performed to determine invasion of SH-SY5Y-S and SH-SY5Y-R cells. Scale bar, 50 $\mu$m. (G) The number of invaded cells in 4 random selected frames was counted and analyzed. Data represent the means ± SD from three independent experiments (**P<0.01). (H) Total cellular extracts from SH-SY5Y-S and SH-SY5Y-R cells were prepared and subjected to western blotting using antibodies against p-RET and CXCR4. GAPDH was used as a loading control. (I) The relative expression of p-RET and CXCR4 was analyzed. Data represent the means ± SD from three independent experiments (**P<0.01).

*Vandetanib inhibits cisplatin-resistant NB tumorigenesis and invasion in vitro*. We employed vandetanib to treat cisplatin-resistant NB cells. As shown in Fig. 3A, vandetanib efficiently reduced p-RET and CXCR4 expression in SH-SY5Y-R cells. A cell viability assay demonstrated that SH-SY5Y-R cell proliferation was significantly inhibited by vandetanib in a concentration-dependent manner (Fig. 3B). In addition, we demonstrated that the $IC_{50}$ of vandetanib for SH-SY5Y-R cells was ~5 $\mu$M (Fig. 3B). Thus, 5 $\mu$M vandetanib was used in the following experiments. A colony formation assay, revealed that fewer colonies were formed by the vandetanib-treated SH-SY5Y-R (Fig. 3C and D) cells. Then, a Transwell invasion assay was performed to determine the effects of vandetanib on NB. We determined that vandetanib markedly prevented SH-SY5Y-R cell invasion (Fig. 3E and F). In summary, vandetanib may be an effective agent for cisplatin-resistant NB therapy.

*Vandetanib inhibits cisplatin-resistant NB tumor growth in vivo*. To further investigate whether vandetanib inhibited cisplatin-resistant NB tumor growth and enhanced sensitivity of NB to cisplatin *in vivo*, SH-SY5Y-R cells were injected into the flank of female wild-type (WT) BALB/c nude mice to establish a subcutaneous tumor model. When the tumor volume reached ~100 $mm^3$, vandetanib and cisplatin were administered to the mice. As shown in Fig. 4A, treatment of mice with a high dose of cisplatin (100 nmol/day) markedly reduced tumor volume (Fig. 4B) and weight (Fig. 4C) by 65.7 and 65.4%, respectively. However, a low-dose of cisplatin had no observed inhibitory effect on tumor volume (Fig. 4B) or weight (Fig. 4C). In contrast, injection of vandetanib alone significantly reduced tumor volume (Fig. 4B) and weight (Fig. 4C) by 70.8 and 71.8%, respectively. IHC staining demonstrated a reduction in p-RET and CXCR4 expression in vandetanib-treated NB tumors (Fig. 4D and E). The aforementioned data demonstrated that vandetanib may be an effective agent for cisplatin-resistant NB therapy.

*Vandetanib exerts low toxicity in cisplatin-resistant NB treatment*. PCNA staining and TUNEL assay were performed to detect the proliferative and apoptotic cells in NB tissues. Less proliferative cells were revealed in both high-dose

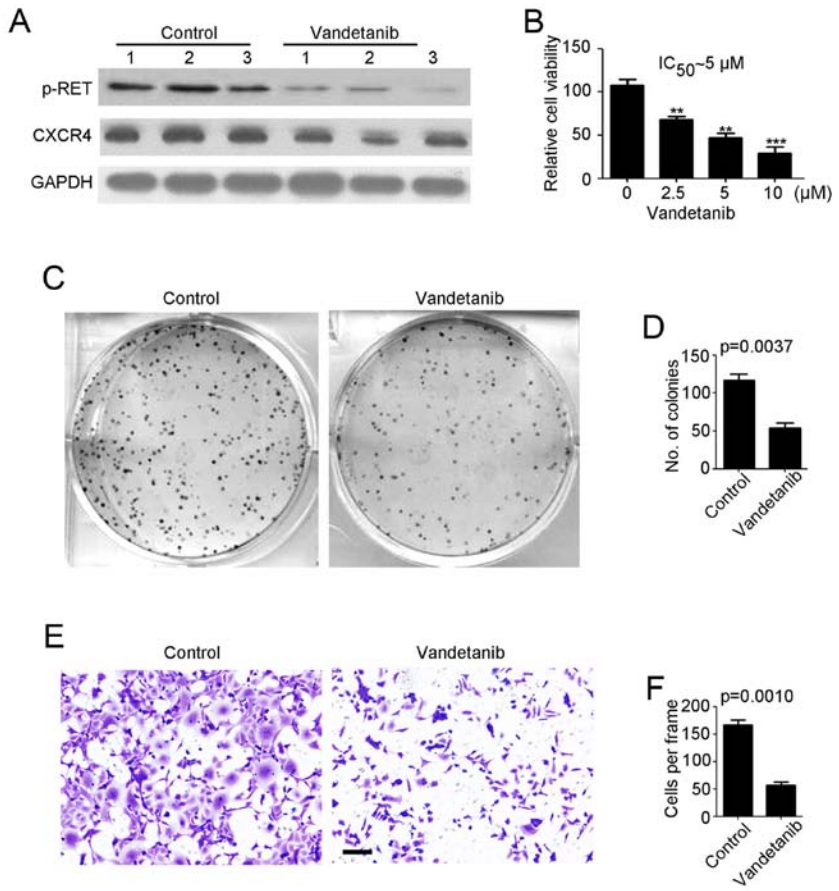


Figure 3. Vandetanib inhibits cisplatin-resistant NB tumorgenesis and invasion *in vitro*. (A) Total cellular extracts from SH-SY5Y-R cells after vandetanib treatment were prepared and subjected to western blotting using antibodies against p-RET and CXCR4. GAPDH was used as a loading control. (B) A Cell Counting Kit-8 assay was performed to determine cell viability of SH-SY5Y-R cells after vandetanib (0, 2.5, 5 and 10 $\mu$M) treatment. The relative cell viability was calculated. Data represent the means ± SD from three independent experiments ($^{**}$P<0.01; $^{***}$P<0.001). (C) A colony formation assay was performed to determine tumorgenesis of SH-SY5Y-R cells after vandetanib treatment. (D) The number of colonies was counted and analyzed. Data represent the means ± SD from three independent experiments ($^{**}$P<0.01). (E) A Transwell-mediated invasion assay was performed to determine invasion of SH-SY5Y-R cells after vandetanib treatment. Scale bar, 50 $\mu$m. (F) The number of invaded cells in 4 random selected frames were counted and analyzed. Data represent the means ± SD from three independent experiments ($^{**}$P<0.01).

cisplatin-treated and vandetanib-treated tumors (Fig. 5A). Additionally, more apoptotic cells were revealed in the aforementioned tumors (Fig. 5B). Furthermore, decreased angiogenesis was observed in the vandetanib-treated tumors than in those exposed to cisplatin alone (Fig. 5C). Notably, severe liver toxicity occurred in the high-dose cisplatin treatment group, in contrast to the low dose cisplatin and control groups (Fig. 5D). No liver toxicity was observed in the vandetanib treatment group (Fig. 5D). The aforementioned data demonstrated that vandetanib exhibited low toxicity in cisplatin-resistant NB treatment.

## Discussion

New treatment strategies are clearly needed for children with recurrent or refractory NBs. In the present study we demonstrated greater expression of p-RET and CXCR4 in cisplatin-resistant NB tumors compared with cisplatin-sensitive tumors. Vandetanib rapidly inhibited cisplatin-resistant NB cell proliferation, tumorigenesis, and invasion. Vandetanib alone induced a significant reduction in cisplatin-resistant NB tumor growth *in vivo* in a xenograft mouse model. While high-dose cisplatin treatment yielded similar results, it caused severe liver toxicity in mice.

Cisplatin is one of the frontline chemotherapeutic drugs for NB and widely used in clinical therapy (12), however the use of cisplatin is limited due to the therapy resistance (13-15). In our study, we determined that cisplatin-resistant cells possessed more aggressive characteristics. Furthermore, we determined that p-RET and CXCR4 expression was significantly upregulated in cisplatin-resistant NB cells and tumor tissues of patients. This indicated that p-RET and CXCR4 upregulation may be an adaptation to cisplatin treatment and could play a crucial role in NB cisplatin resistance. Furthermore, treatment of chemosensitive NB cells with cisplatin reversibly increased EGFR expression, whereas cisplatin-resistant cells revealed enhanced EGFR expression independent of the presence of cisplatin (34). Inhibition of EGFR, using gefitinib, revealed minor chemosensitizing effects in NB (35), whereas EGFR-targeted antibodies and growth factor toxins scFv(14E1)-Pseudomonas exotoxin A (ETA) and TGF-α-ETA exerted anticancer effects in NB cell lines (34). In the present study we revealed that EGFR expression was upregulated in cisplatin-resistant SH-SY5Y cells (data not shown), but its

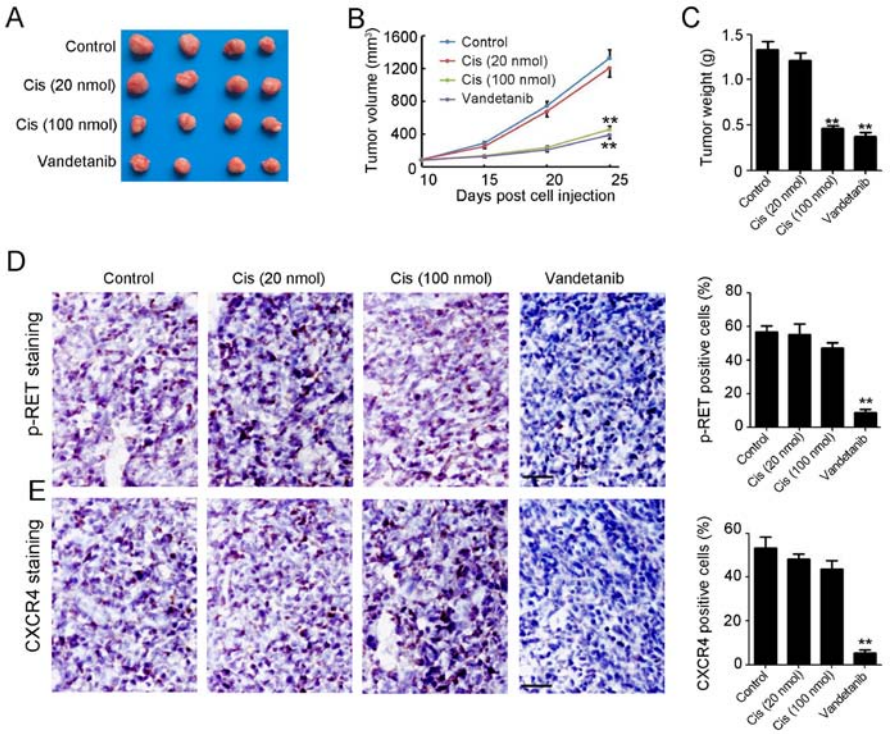


Figure 4. Vandetanib inhibits cisplatin-resistant NB tumor growth *in vivo*. (A) Representative macroscopic findings of NB tumors. (B and C) The tumor volumes (n=4; $^{**}$P<0.01), and end-stage tumor weights (n=4; $^{**}$P<0.01) after treatment of SH-SY5Y-R tumors with vandetanib or cisplatin. (D and E) IHC staining of p-RET and CXCR4 expression in SH-SY5Y-R tumors. Scale bar, 50 $\mu$m. The number of p-RET- and CXCR4-positive cells and total cells were counted in 5 random fields and analyzed ($^{**}$P<0.01).

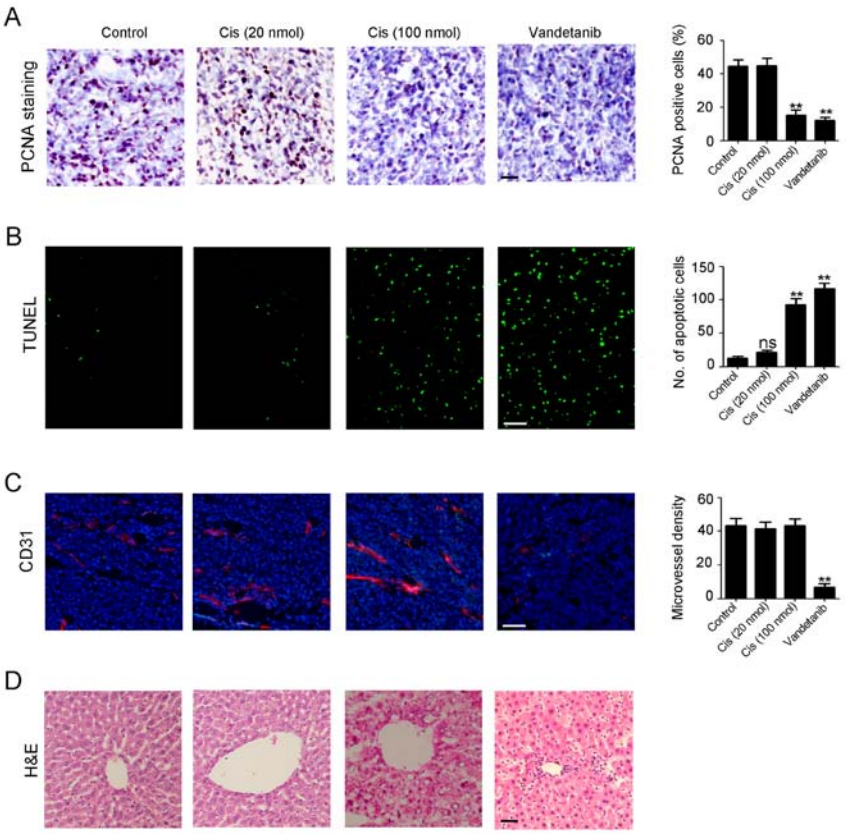


Figure 5. Vandetanib exerts low toxicity in cisplatin-resistant NB treatment. (A) IHC staining of PCNA expression in SH-SY5Y-R tumors. Scale bar, 50 $\mu$m. The number of PCNA-positive cells and total cells were counted in 5 random fields and analyzed ($^{**}$P<0.01). (B) A TUNEL assay was performed to detect apoptotic cells in SH-SY5Y-R tumors. Scale bar, 100 $\mu$m. The number of apoptotic cells was counted in 5 random fields and analyzed ($^{**}$P<0.01). (C) CD31 staining in SH-SY5Y-R tumors. Scale bar, 100 $\mu$m. The number of microvessel densities was counted in 5 random fields and analyzed ($^{**}$P<0.01). (D) H&E staining of liver in nude mice after treatment. Scale bar, 50 $\mu$m.

expression was not inhibited by vandetanib at the concentration of 5 $\mu$M (data not shown). Therefore, EGFR may be another adaptation to cisplatin treatment in NB, but it was not the effector of vandetanib in the inhibition of cisplatin-resistant NB tumor progression at low concentrations.

Increasing the concentration of cisplatin is the most common strategy to offset cisplatin resistance. However, high-dose cisplatin may cause severe liver toxicity, which is the main side-effect of cisplatin therapy (36,37). In the present study we demonstrated that vandetanib was as effective as high-dose cisplatin in impairing cisplatin-resistant NB subcutaneous tumor growth *in vivo*. Notably, less liver toxicity was observed in the vandetanib treatment group than in the high-dose cisplatin treatment group. These results provide solid evidence, demonstrating the advantages of vandetanib in the treatment of cisplatin-resistant NB. Whether combination of vandetanib with cisplatin produces a better therapeutic effect in NB will be investigated in a future study. Different concentrations of vandetanib will be used to treat cisplatin resistance in NB after combination with different concentrations of cisplatin. The potential synergy will be analyzed according to previous models (38,39).

Previously, RET rearrangements have been reported in NB (17). Activated ALK triggered RET upregulation in mouse sympathetic ganglia at birth, as well as in murine and human NB (16). RET inhibition strongly impaired tumor growth *in vivo* in both MYCN/KI AlkR1279Q and MYCN/KI AlkF1178L mice (16). Inhibition of RET phosphorylation by vandetanib treatment resulted in the induction of apoptosis in the majority of NB cell lines *in vitro*, and inhibited tumor growth in a mouse xenograft model, via both reduction in tumor vascularity and induction of apoptosis (16). Notably, in the present study we first demonstrated that inhibition of RET phosphorylation resulted in the inhibition of proliferation, invasion, and induction of apoptosis in cisplatin-resistant NB cells. Vandetanib treatment was an efficient therapy for cisplatin-resistant NB tumor growth, inducing apoptosis and inhibiting proliferation and angiogenesis.

CXCR4 has been demonstrated to be one of the most frequently expressed chemokines, affecting tumor cell proliferation, survival, and metastasis in various cancers (40,41). In NB, CXCR4 has been proposed to be involved in the mechanisms by which cells metastasize to specific sites from the primary site (18,42). Greater expression of CXCR4 in NBs was correlated with high-stage disease and worse clinical outcome than lower expression of CXCR4 (43,44). Functional studies have demonstrated that inhibition of CXCR4 was an efficient strategy to inhibit NB cell proliferation and metastasis (45-47). A previous study by Ding *et al* demonstrated the inhibitory role of vandetanib on NB cell migration and invasion through downregulation of CXCR4 and MMP-14 expression (28). The present study for the first time also indicated that vandetanib treatment caused a significant decrease in CXCR4 expression and cisplatin-resistant NB cell invasion. Ding *et al* demonstrated that the $IC_{50}$ of vandetanib for SH-SY5Y cells was ~10 $\mu$M. However we demonstrated that the $IC_{50}$ of vandetanib for SH-SY5Y-R cells was ~5 $\mu$M. This could be attributed to the higher expression of CXCR4 in cisplatin-resistant SH-SY5Y cells, which enhances the sensitivity of vandetanib.

In conclusion, the present study indicated that vandetanib is an efficient therapeutic agent for cisplatin-resistant NBs, inhibiting p-RET and CXCR4 expression. It identified vandetanib as a potential therapy for cisplatin-resistant NBs. In particular, the combination of vandetanib with cisplatin may represent a novel therapeutic strategy in NB patients.

## Competing interests

The authors declare that they have no competing interests.

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