Acta Pharmacologica Sinica

www.nature.com/aps

ARTICLE

NGF monoclonal antibody DS002 alleviates chemotherapy-induced peripheral neuropathy in rats

Zhi-juan Liang[1,2], Jie Tan[3,4], Lei Tang[3], Zuo-bin Xie[3], Gan-jun Chen[3,4], Guo-jian Liu[3], Lin Yuan[3], Kai-xin Wang[3], Hua-ping Ding[3], Hong Qiu[1], Qi Wang[1,2], Gui-feng Wang[1,2], Yi-li Chen[3,4] and Chun-he Wang[1,2,3]

Chemotherapy-induced peripheral neuropathy (CIPN) is one of the pervasive side effects of chemotherapy, leading to poor quality of life in cancer patients. Discovery of powerful analgesics for CIPN is an urgent and substantial clinical need. Nerve growth factor (NGF), a classic neurotrophic factor, has been identified as a potential therapeutic target for pain. In this study, we generated a humanized NGF monoclonal antibody (DS002) that most effectively blocked the interaction between NGF and tropomyosin receptor kinase A (TrkA). We showed that DS002 blocked NGF binding to TrkA in a dose-dependent manner with an IC_{50} value of 6.6 nM; DS002 dose-dependently inhibited the proliferation of TF-1 cells by blocking the TrkA-mediated downstream signaling pathway. Furthermore, DS002 did not display noticeable species differences in its binding and blocking abilities. In three chemotherapy-induced rat models of CIPN, subcutaneous injection of DS002 produced a significant prophylactic effect against paclitaxel-, cisplatin- and vincristine-induced peripheral neuropathy. In conclusion, we demonstrate for the first time that an NGF inhibitor effectively alleviates pain in animal models of CIPN. DS002 has the potential to treat CIPN pain in the clinic.

Keywords: nerve growth factor; neuropathic pain; chemotherapy; monoclonal antibody

Acta Pharmacologica Sinica (2022) 43:2841–2847; https://doi.org/10.1038/s41401-022-00904-8

INTRODUCTION

Chemotherapy-induced peripheral neuropathy (CIPN), commonly characterized by sensory symptoms, including mechanical and cold allodynia, numbness, and weakness [1, 2], leads to dose-limiting toxicities and even cessation of chemotherapies. Prolonged application of widely used chemotherapeutic agents, such as paclitaxel, cisplatin, and vincristine [1, 3], can induce acute and chronic CIPN, dramatically affecting the survival and quality of life of cancer patients [2, 4].

There are two main strategies for treating CIPN: targeting the underlying pathologic mechanism responsible for CIPN or addressing the symptoms [5]. To date, the mechanism of CIPN remains unclear [6], and no agents can effectively prevent or control the neuropathic pain caused by CIPN without affecting the antitumor activities of chemotherapeutics [5, 7]. Duloxetine is the only agent that has been recommended by the American Society of Clinical Oncology to treat CIPN, but its effectiveness is limited [7–9]. Other medications commonly used to treat neuropathic pain, such as amitriptyline, gabapentin, and pregabalin are not effective [8]. Thus, there is an urgent need to identify novel therapeutic agents for CIPN pain.

Studies conducted by different laboratories have demonstrated that NGF, a member of the neurotrophin superfamily [10, 11], is a peripheral pain mediator involved in a wide range of inflammatory and neuropathic pain conditions [12, 13], in addition to its role in the nervous system regulating the growth and development of peripheral and central neurons and maintaining neuronal cell survival in embryos and juvenile animals [14]. Through interaction with tropomyosin receptor kinase A (TrkA) expressed on the surface of many nociceptors [15, 16], NGF lowers the threshold of the neuronal action potential and then improves neuronal sensitivity to potential pain stimulation via ERK, PLC/PKC, and other signaling pathways [13]. Monoclonal antibodies (mAbs) antagonizing NGF were highly effective in several human clinical trials [17–22], as well as in animal models of many acute and chronic pain states, and they did not result in severe adverse effects [23, 24]. Moreover, NGF levels are elevated in several pain conditions in humans [23]. Although previous studies showed that CIPN patients, in general, had lower serum NGF levels [25, 26], CIPN patients with pain had elevated levels of NGF in serum, indicating that NGF may be a viable therapeutic target for neuropathic pain in CIPN [27]. To date, however, the efficacies of NGF antagonists in treating CIPN pain have not yet been tested in any clinical trials or animal models.

Here, we reported that a novel NGF mAb, DS002, successfully alleviated neuropathic pain in paclitaxel-, cisplatin- and vincristine-induced rat models of CIPN. We believe that this study has provided the necessary preclinical evidence to support clinical testing of DS002 in CIPN patients with neuropathic pain.

MATERIALS AND METHODS

Reagents

DS002, tanezumab, human TrkA-Fc, and antigens from human and rat NGF were synthesized by Dartsbio Pharmaceuticals, Ltd. (Zhongshan, China). The isotype control antibody was prepared

[1]Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China; [2]University of Chinese Academy of Sciences, Beijing 100049, China; [3]Dartsbio Pharmaceuticals, Ltd., Zhongshan 528400, China and [4]Shanghai Mabstone Biotechnologies, Ltd., Shanghai 201203, China
Correspondence: Chun-he Wang (wangc@simm.ac.cn)
These authors contributed equally: Zhi-juan Liang, Jie Tan, Lei Tang.

Received: 17 January 2022 Accepted: 27 March 2022
Published online: 25 April 2022

by Shanghai Institute of Materia Medica (Shanghai, China). Human NGF-Fc was purchased from Peprotech (Rocky Hill, NJ, USA). Mouse NGF (mNGF) was purchased from Sino Biological (Beijing, China). Cell Counting Kit-8 (CCK8) was purchased from Dojindo (Kumamoto, Kyushu, Japan).

Experimental animals

Male Sprague-Dawley (SD) rats (200–220 g starting weight) were purchased from Vital River Laboratory (Zhejiang, China) and were raised in an SPF environment (temperature 20–26 ℃, relative humidity 40%–70%, 12-h light/dark cycle) with food and water available ad libitum. Animals were adapted for 1 week before treatment and then randomly divided into groups according to weight.

Antibody discovery

Female BALB/c mice were immunized with recombinant human NGF-Fc fusion protein emulsified in complete Freund's adjuvant and then boosted with the same protein in incomplete Freund's adjuvant every 3 weeks. Three days after the last boost, mouse spleen cells were harvested and fused with the myeloma SP2/0 cell line to construct hybridoma cells. Hybridoma clones secreting NGF antibodies that blocked the interaction between NGF and TrkA were screened by high-throughput ELISAs. The murine antibody ANGF56 was identified as the most effective blocker of the hNGF and TrkA interaction. The variable regions of the heavy chain (VH) and light chain (VL) were amplified by PCR and then humanized by "CDR grafting". First, a human-mouse chimeric antibody 5C1 was constructed by grafting the heavy chain variable (VH) region of ANGF56 into the heavy chain constant region of human IgG1 and the light chain variable (VL) region of ANGF56 into the light chain constant region of human kappa. Then, computer simulation technology was used to analyze the amino acid sequences of the CDR regions and their surrounding frameworks. The framework amino acid sequences of ANGF56 were compared to those from human germlines to obtain sequences with high homology. By calculating the electrostatic force, van der Waals forces, hydrophobicity, and entropy in a 3D model constructed by using antibodies with the highest overall homology from the PDB database, we identified the key amino acids interacting with NGF and maintaining the spatial frameworks in the amino acid sequence of ANGF56 and then mutated them back from the human to murine version. Among them, humanized antibodies 5C2 and 5C3 were generated by constructing ANGF56 with the human IGHV4–38–2 * 02 heavy chain variable region and human IGKV1–33 * 01 light chain variable region as template sequences. We found that 5C3 maintained both the binding and blocking abilities of ANGF56, while 5C2 lost its blocking ability. Therefore, 5C3 was selected as a candidate molecule and designated DS002 (Supplementary Fig. S1a, b). The antibodies with an hIgG1 backbone were obtained after transient expression in HEK293F cells and purification by a protein A column (Fig. 1a). Protein quality was examined by reduced and nonreduced sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE, Fig. 1b). ELISA analysis indicated that the EC_{50} value of DS002 with hNGF-his was 0.031 nM (Fig. 1d, Table 1), and its K_D value with hNGF-his was less than 1×10^{-12} M, as determined by BLI (Fig. 1c).

SDS-PAGE

Samples were boiled in 1× loading buffer for 5 min, and samples containing 5 μg of DS002 were separated by SDS-PAGE (10% Bio-Rad Criterion Tris-HCl gels), stained for 10 min with Coomassie blue, and imaged on a Bio-Rad ChemiDoc MP system.

SEC-HPLC

SEC-HPLC was carried out to monitor the homogeneity of DS002. Briefly, the antibody solution was analyzed by HPLC (Agilent, Santa Clara, CA) on an SEC column (Thermo MAbPac SEC-1) with a mobile phase of phosphate-buffered saline (pH 6.8). The column was maintained at 26 ℃ during separation. The detector was set at 280 nm. The flow rate was 0.2 mL/min. The sample injection quantity was 20 μg.

Functional enzyme-linked immunosorbent assay

For the binding assay, 2 μg/mL human NGF (hNGF)-His, mouse NGF (mNGF), or rat NGF (rNGF)-His was immobilized on a 96-well ELISA plate and incubated at 4 ℃ for 16–20 h. ELISA plates were blocked with 1% skim milk powder-PBST (PBS containing 0.05% Tween 20, pH 7.4) at room temperature for 1 h. Then, dilutions of DS002 were added to the wells and incubated at room temperature for 1 h. The binding was subsequently detected with goat anti-human IgG-HRP followed by TMB substrate.

For the competition assay, a 96-well ELISA plate was coated with 2 μg/mL hTrkA-Fc at 4 ℃ overnight. ELISA plates were sealed with 1% skim milk powder-PBST for 1 h. Then, 0.25 μg/mL biotinylated hNGF-His, mNGF, or rNGF-His with different concentrations of DS002 was added to the plates. The binding was subsequently detected with streptavidin-HRP followed by TMB substrate.

Biolayer interferometry

Binding affinities were detected by BLI using Octet RED96 (ForteBio, Pall). DS002 (10 μg/mL) was coupled to protein A biosensors and incubated with different concentrations of NGF solution (100 nM, 50 nM, 25 nM, 12.5 nM, 6.25 nM, 3.125 nM, and 1.5625 nM). The NGF protein and DS002 were diluted in 0.05% *v/v* Tween 20. The biosensors were washed in 0.05% *v/v* Tween 20 before association and dissociation and were regenerated in glycine buffer (10 mM, pH 1.5) after dissociation. The K_D values of binding were determined using Data Analysis 9.0 (PALL/ForteBio).

TF-1 cell proliferation assay in vitro

For DS002-induced inhibition of TF-1 cell proliferation, 10% fetal bovine serum (FBS) medium was prepared by combining 500 mL of RPMI with 50 mL of FBS. After three washes with PBS, 60 μL of TF-1 cells was seeded in a 96-well U-bottom plate at 1.5×10^4 cells/well. Then, 20 μL of serially diluted antibodies (DS002, tanezumab, and isotype) in a 10% FBS medium was added to the cells and incubated for 30 min at 37 ℃. Then, 20 μL of NGF solution (25 ng/mL) was added to each well, and the cells were cultured in a 5% CO_2 incubator for 5–6 days at 37 ℃. Subsequently, 20 μL of CCK8 (mixed 1:1 with PBS) was added to each well and incubated for 4 h. The proliferation of TF-1 cells was measured by monitoring the absorbance signal at 450 nm. Six replicates were set for each treatment, and the experiments were repeated twice for confirmation.

Chemotherapy-induced peripheral neuropathy model

All animal experiments were performed under the approval of Institutional Animal Care and Use Committee protocols at Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

Paclitaxel (dissolved in polyoxyethylene castor oil) was administered i.p. to the rats at a 4 mg/kg dose for 4 days (d 0, d 2, d 4, d 6) between 1:00 and 3:00 PM. The test compounds were administered s.c. on d-3, d 3, and d 9, according to the grouping schedule. Von Frey tests and acetone tests were performed separately on d-5, d 7, and d 14; and d-4, d 8, and d 15. The results on d-5 and d-4 were used as baselines. The test ended after d 15.

Cisplatin (dissolved in normal saline) was administered i.v. to the rats at a 4 mg/kg dose for 2 days (d 0, d 6) between 1:00 and 3:00 PM. The test compounds were administered s.c. on d-3, d 3, d 9, and d 16, according to the grouping schedule. Von Frey tests and acetone tests were performed separately on d-5, d 7, d 14, and d 21; and d-4, d 8, d 15, and d 22. The results on d-5 and d-4 were used as baselines. The test ended after d 22.

Vincristine (dissolved in normal saline) was administered i.p. to the rats at a 0.1 mg/kg dose for 1 day (d0) between 1:00 and 3:00

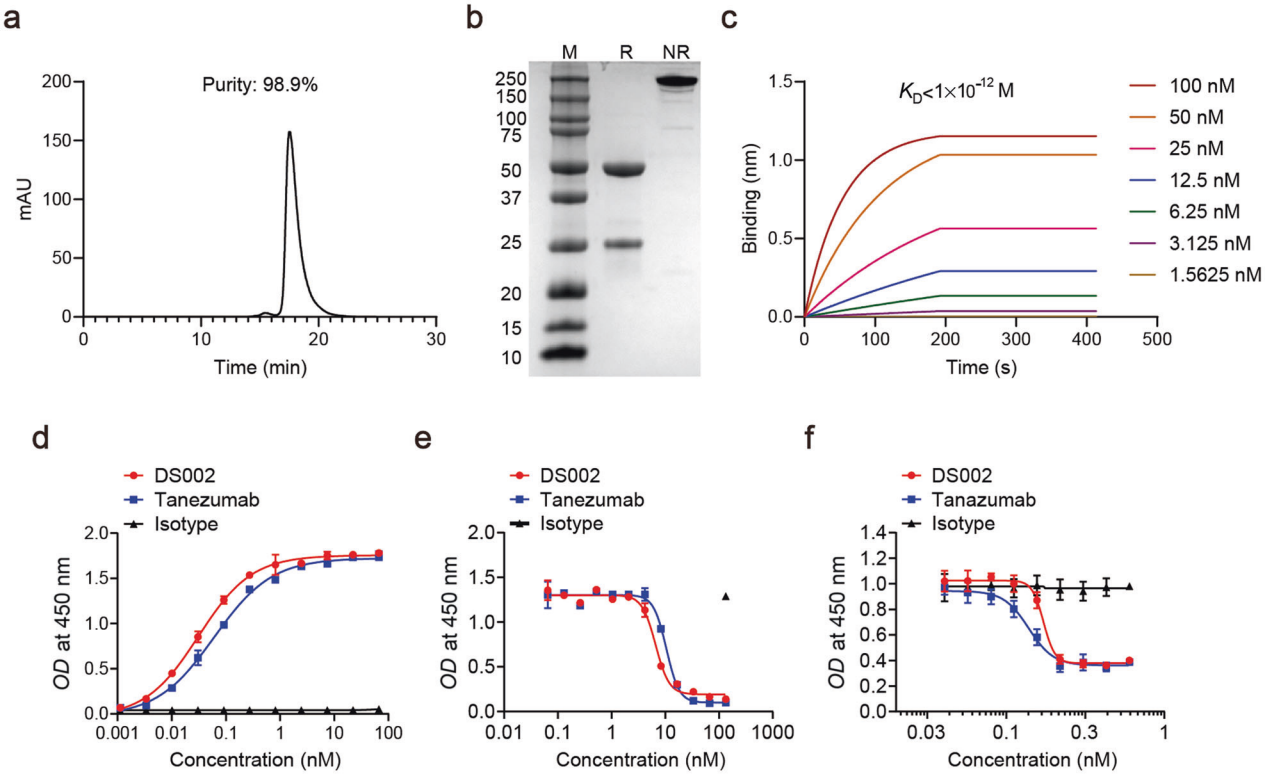

Fig. 1 DS002 has high affinity for human NGF and is an inhibitor of its function. Tanezumab was used as a positive control, and isotype was used as a negative control. **a** SEC-HPLC chromatogram of DS002 on the MAbPac SEC-1 column. The flow rate was 0.2 mL/min, the mobile phase was PBS buffer, and the monomer retention time was 17.54 min. **b** SDS-PAGE gels of the DS002. R: reduced; NR: nonreduced. M: marker. **c** Binding of DS002 to human NGF-His as measured by BLI. **d** Binding curves of DS002 against human NGF-His as measured by ELISA. **e** Binding of human NGF-His to human TrkA-Fc was blocked by DS002 as measured by ELISA. **f** Detection of DS002 inhibiting NGF-dependent TF-1 cell survival signaling.

Table 1. Binding and blocking effects of NGF mAbs as determined by ELISA.

	Target	NGF mAbs	
		DS002	Tanezumab
EC_{50} (nM)	hNGF	0.031	0.061
	rNGF	0.086	0.080
	mNGF	0.036	0.044
IC_{50} (nM)	hNGF	6.60	10.49
	rNGF	1.98	3.75
	mNGF	8.46	8.82

EC_{50} 50% effective concentration, *IC_{50}* 50% inhibitory concentration
hNGF human NGF-His, *rNGF* rat NGF-His, *mNGF* mouse βNGF.

PM. The test compounds were administered s.c. on d-3 and d 3, according to the grouping schedule. Von Frey tests and acetone tests were performed separately on d-5, d 7; and d-4, d 8. The results on d-5 and d-4 were used as baselines. The test ended after d 8.

Von Frey tests

Tests were performed according to previous reports [28]. Before the test, the SD rats were placed in a single transparent plexiglass container (20.5 cm × 10.5 cm × 11 cm) on a metal wire mesh support for approximately 30–60 min until they stopped prominent exploratory activities and were in a quiet state. The mechanical paw withdrawal threshold (PWT) was measured with von Frey filaments that can produce different strengths [ranging from 3.61 (0.4 g) to 5.18 (15 g)] according to the up and down method. Each von Frey filament was tested five times, with an interval of 3–5 s. If the positive reaction of paw withdrawal (rapid foot withdrawal, hind paw licking, paw withdrawal, or evasion of stimulation) was less than three times in five stimuli, the rat was switched to a more powerful filament. In contrast, if the positive reaction of paw withdrawal was more than three times, the rat was switched to less powerful cilia, and the measurement was repeated until the response mode could be used to calculate the 50% PWT.

Acetone test

As described in a previously published protocol [29, 30], acetone drops were used to stimulate the plantar surface of the hind paws of the rats, and each rat was tested 5 times with an interval of 5 min. The scores of the different degrees of foot withdrawal responses at the corresponding time points and the frequency of foot withdrawal reactions were recorded in five tests. The foot withdrawal responses of rats induced by acetone can be divided into 4 levels: 0, no response; 1, quick paw withdrawal and slapping; 2, repeated slapping (≥2); and 3, repeated slapping accompanied by paw licking.

Statistical analysis

GraphPad Prism 8 was used for graphics production and statistical analysis. The test data are shown as the mean ± standard error (mean ± SEM), and the differences between the control and experimental groups were analyzed by Bonferroni multiple comparison test. When $P < 0.05$, the corresponding experimental results were considered to have significant differences.

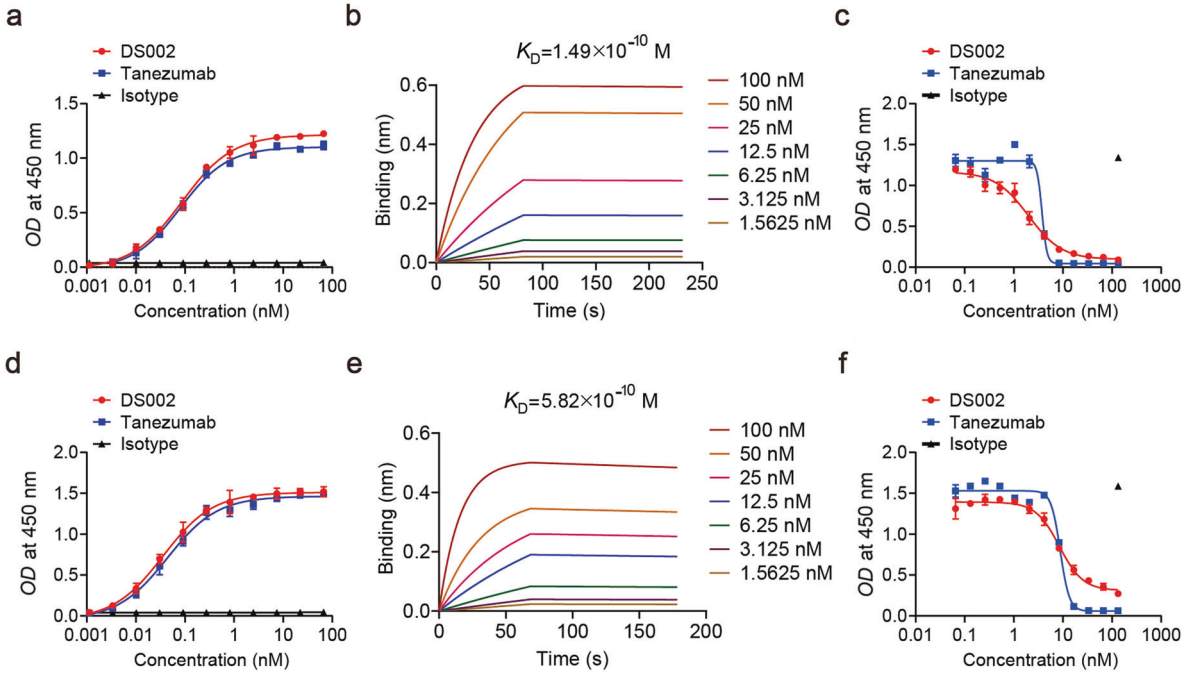

Fig. 2 Species cross-reactivity of DS002. Tanezumab was used as a positive control, and isotype was used as a negative control. **a** Binding of DS002 to His-tagged recombinant rat NGF protein as measured by ELISA. **b** Binding of DS002 to rat NGF-His as measured by BLI. **c** Binding of rNGF to the human TrkA protein was blocked by DS002 as measured by ELISA. **d** Binding of DS002 to mouse-βNGF as measured by ELISA. **e** Binding of DS002 to mouse-βNGF as measured by BLI. **f** Binding of mNGF to human TrkA protein was blocked by DS002 as measured by ELISA.

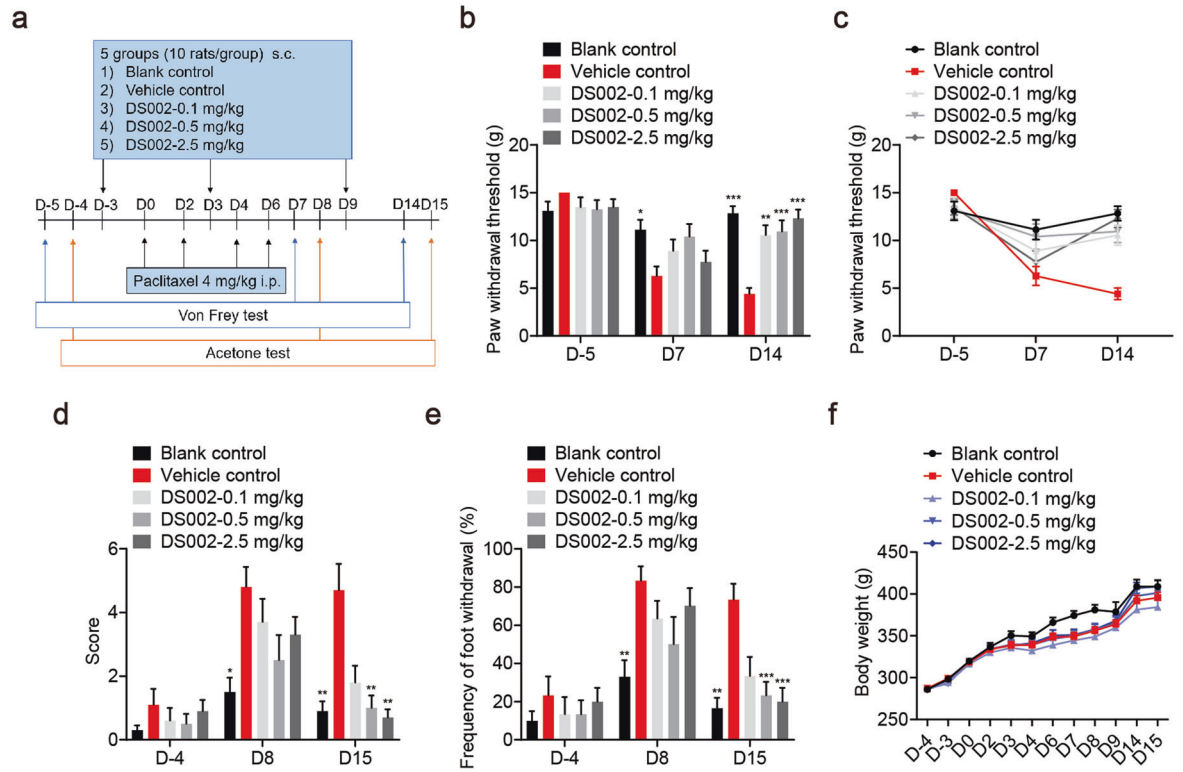

Fig. 3 Analgesic effect of DS002 in a paclitaxel-induced CIPN model. a Schematic diagram of the design of the experiment. The Von Frey test was used to evaluate paclitaxel-induced mechanical allodynia, and the acetone test was used to evaluate paclitaxel-induced cold allodynia. **b**, **c** Paclitaxel-induced mechanical allodynia was inhibited by subcutaneous administration of DS002 at doses of 0.1 mg/kg, 0.5 mg/kg, and 2.5 mg/kg on D14. Data are represented as the mean ± SEM. *P < 0.05; **P < 0.01; ***P < 0.001. **d**, **e** Paclitaxel-induced cold allodynia was relieved by subcutaneous administration of DS002 at 0.5 mg/kg and 2.5 mg/kg on D15. Data are represented as the mean ± SEM. *P < 0.05; **P < 0.01; ***P < 0.001. **f** Changes in body weight of the treated rats during the experiment. Data are represented as the mean ± SEM.

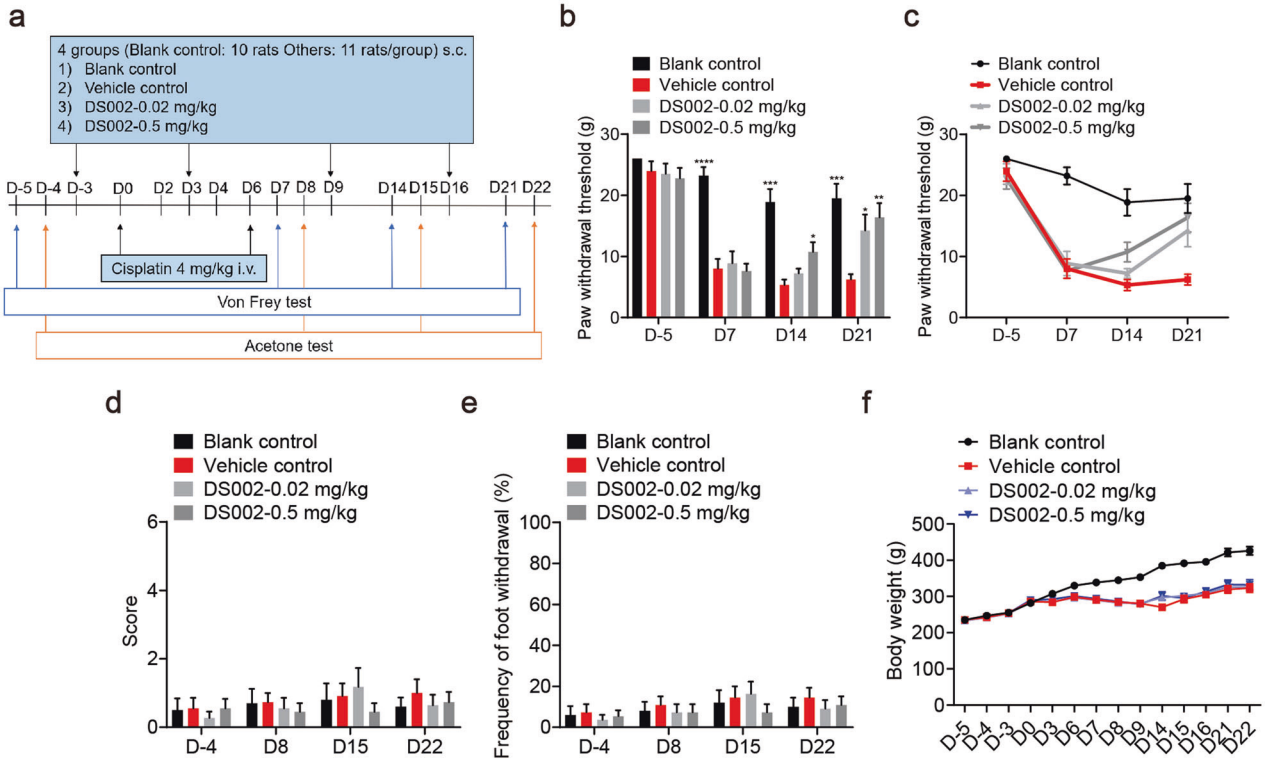

Fig. 4 Analgesic effect of DS002 in a cisplatin-induced CIPN model. a Schematic diagram of the design of the experiment. The Von Frey test was used to evaluate cisplatin-induced mechanical allodynia, and the acetone test was used to evaluate cisplatin-induced cold allodynia. **b**–**c** Cisplatin-induced mechanical allodynia was inhibited by subcutaneous administration of DS002 at doses of 0.02 mg/kg and 0.5 mg/kg on D21. Data are represented as the mean ± SEM. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$. **d**, **e** Cisplatin-induced cold allodynia failed to be established using acetone because no significant difference was observed between the blank control and vehicle control groups. **f** Changes in body weight of the treated rats during the experiment. Data are represented as the mean ± SEM.

RESULTS

DS002 is a potent blocker of the NGF–TrkA interaction

We determined the ability of DS002 to block NGF binding to TrkA and found that DS002 blocked NGF from binding to TrkA in a dose-dependent manner, and the IC_{50} value was 6.6 nM (Fig. 1e, Table 1). A TF-1 cell proliferation assay was used to assess the ability of DS002 to functionally inhibit NGF signaling. The results showed that the addition of DS002 inhibited the proliferation of TF-1 cells by blocking the downstream signaling pathway mediated by TrkA expressed on TF-1 cells (Fig. 1f). Tanezumab, the first NGF-targeting antibody to enter clinical development, was used here as a positive control for NGF inhibition. The isotype control did not show inhibition.

To determine the species cross-reactivity of DS002, we measured the binding affinity of DS002 to rNGF and mNGF, and the ability of DS002 to block the binding of rNGF and mNGF to TrkA with ELISAs. DS002 had high affinities for both rNGF and mNGF, with EC_{50} values of 0.086 nM and 0.036 nM, respectively (Fig. 2a, d, Table 1). The K_D values were 1.49×10^{-10} M and 5.82×10^{-10} M, respectively (Fig. 2b, e). Moreover, DS002 effectively blocked the binding of rNGF and mNGF to TrkA, with IC_{50} values of 1.98 nM and 8.46 nM, respectively (Fig. 2c, f, Table 1). There was no noticeable species difference in the binding and blocking abilities of DS002.

Analgesic effects of DS002 in the paclitaxel-induced rat model of CIPN

Mechanical and cold allodynia are common characteristics of CIPN [1]. First, we evaluated the preventive effects of DS002 on mechanical allodynia by measuring the PWT with von Frey filaments in a paclitaxel-induced rat model of CIPN (Fig. 3a). On d 7 and d 14, there was a significant difference between the vehicle and blank control groups, indicating that the model was successfully established (Fig. 3b, c). On d 14, the PWTs of the DS002 group at three different doses were significantly increased compared with that of the vehicle control group (Fig. 3b, c). Thus, DS002 had a prophylactic effect on paclitaxel-induced mechanical allodynia. Next, we investigated the analgesic effect of DS002 on cold allodynia in the same rat model by measuring the score and frequency of acetone-induced foot withdrawal behaviors (Fig. 3a). On d 8 and d 15, there was a significant difference between the vehicle and blank control groups, indicating that the model was successfully established (Fig. 3d, e). On d 15, DS002 treatment drastically decreased the pain score and response frequency, compared to the vehicle control group (Fig. 3d, e). Thus, DS002 showed an analgesic effect on paclitaxel-induced cold allodynia. No weight loss was observed in any of the treated rats (Fig. 3f). Thus, the application of DS002 achieved a prophylactic effect on paclitaxel-induced peripheral neuropathy.

Analgesic effect of DS002 in the cisplatin-induced rat model of CIPN

We next tested the analgesic effect of DS002 in a cisplatin-induced rat model of CIPN (Fig. 4a). In the von Frey test on d 21, the mean PWT of the blank control group was significantly higher than that of the vehicle control group, indicating that the model was successfully established (Fig. 4b, c). Moreover, the mean PWT of the DS002 group with two different doses was notably increased compared to that of the vehicle control group (Fig. 4b, c), indicating analgesia by DS002 on mechanical allodynia. However, for the acetone test, there was no significant difference in acetone-induced foot withdrawal behavior between the blank and vehicle

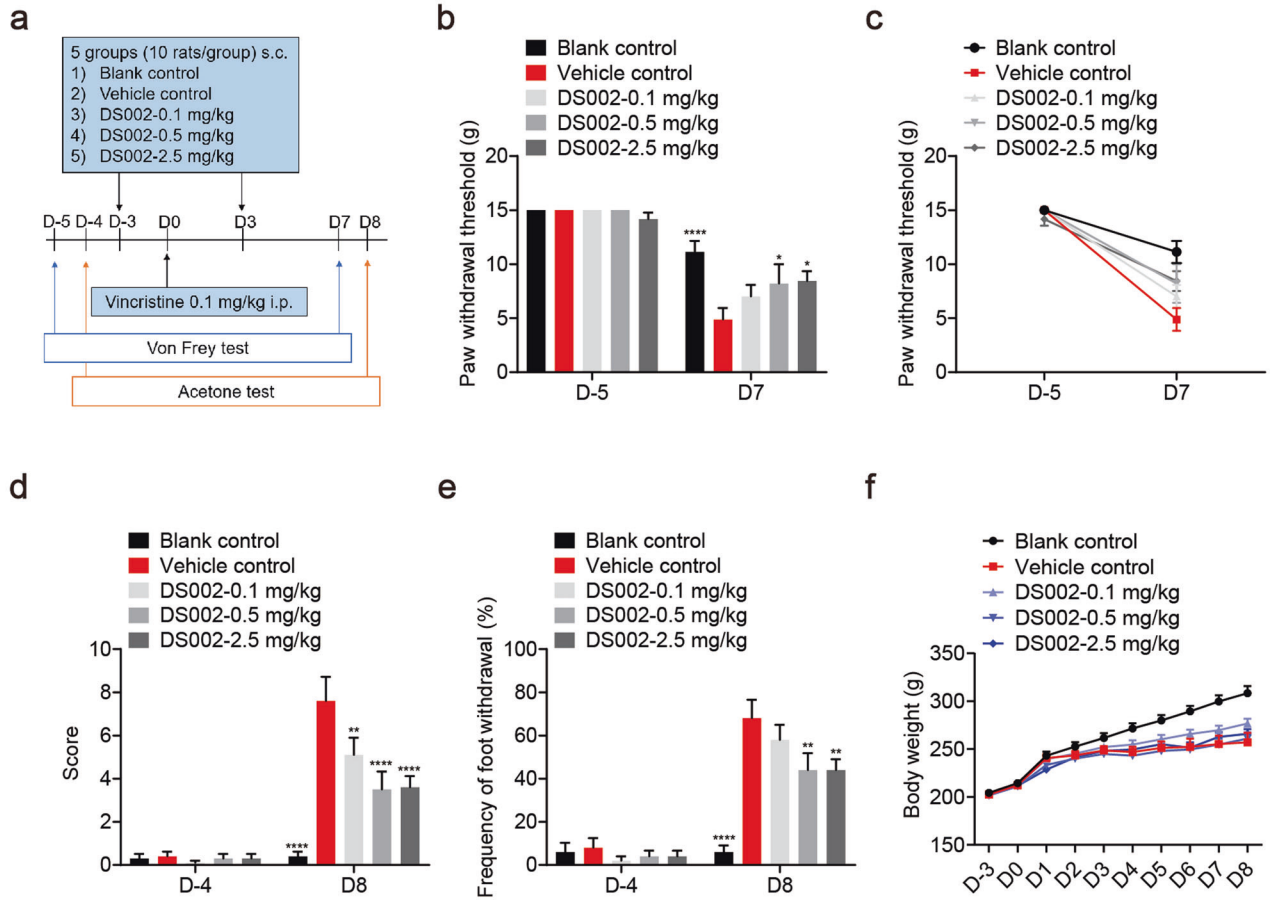

Fig. 5 Analgesic effect of DS002 in a vincristine-induced CIPN model. a Schematic diagram of the design of the experiment. The Von Frey test was used to evaluate vincristine-induced mechanical allodynia, and the acetone test was used to evaluate vincristine-induced cold allodynia. **b**–**c** Vincristine-induced mechanical allodynia was inhibited by subcutaneous administration of DS002 at doses of 0.5 mg/kg and 2.5 mg/kg on D7. Data are represented as the mean ± SEM. $^{*}P < 0.05$; $^{****}P < 0.0001$. **d**–**e** Vincristine-induced cold allodynia was relieved by subcutaneous administration of DS002 at doses of 0.5 mg/kg and 2.5 mg/kg on D8. Data are represented as the mean ± SEM. $^{**}P < 0.01$; $^{****}P < 0.0001$. **f** Changes in body weight of treated rats during the experiment. Data are represented as the mean ± SEM.

control groups until d 22. (Fig. 4d, e), indicating that the cisplatin-induced cold allodynia model was not successfully established. In all DS002-treated rats, there was no weight loss (Fig. 4f).

Analgesic effect of DS002 in the vincristine-induced rat model of CIPN

We also evaluated the analgesic effects of DS002 in CIPN by monitoring the sensitivity of rats to mechanical and cold allodynia in a vincristine-induced rat model of CIPN (Fig. 5a). In the von Frey test on d 7, the mean PWT of the blank control group was significantly higher than that of the vehicle control group, indicating that the model was successfully established (Fig. 5b, c). Moreover, the mean PWTs of the DS002 group with two of three different doses were significantly higher than that of the vehicle control group (Fig. 5b, c), indicating that DS002 lowered the sensitivity of rats to mechanical allodynia. In the acetone test on d 8, the pain score and response frequency of the blank control group were significantly lower than those of the vehicle control group, indicating that the model was successfully established (Fig. 5d, e). The total pain score and response frequency of the DS002 group with two of three doses were reduced compared to those of the vehicle control group (Fig. 5d, e), indicating that DS002 lowered the sensitivity of rats to cold allodynia. Additionally, there was no weight loss in the DS002-treated rats (Fig. 5f). In summary, DS002 showed analgesic effects in a vincristine-induced rat model of CIPN.

DISCUSSION

At present, CIPN pain severely affects the quality of life in cancer patients who receive chemotherapies. It is common to reduce the dose of, extend the treatment cycle or discontinue chemotherapies to relieve CIPN pain due to a lack of effective means to control this condition.

Tanezumab is the first NGF-targeting antibody to enter clinical research and was jointly developed by Pfizer and Eli Lilly in the United States. Clinical evidence has shown that the analgesic effects of high-dose tanezumab are comparable to those of morphine in relieving cancer pain and chronic low back pain (CLBP) [31]. Recently, in a phase III clinical trial of patients with moderate to severe bone metastatic cancer pain who did not respond well to the highest dose of morphine, tanezumab significantly alleviated pain compared to the placebo [32]. Tanezumab also showed positive analgesic effects in osteoarthritis (OA) [19], CLBP [18], and diabetic peripheral neuropathic pain (DPNP) [20]. Fasinumab is another NGF antibody that was shown effective in OA and CLBP trials [21, 22], but neither tanezumab nor fasinumab has been tested in clinical trials or animal models for the treatment of CIPN.

Our studies showed that DS002, a humanized mAb targeting human NGF, had remarkable analgesic effects in rat models of CIPN induced by paclitaxel, cisplatin, or vincristine, three common chemotherapeutics known to cause CIPN. This study thus provided a new option for the prevention and treatment of CIPN.

Due to its potent analgesic effects and lack of addictive risk, DS002 has advantage over opioids and NSAIDs in controlling CIPN.

To our knowledge, this study is the first time that an NGF inhibitor was demonstrated to effectively alleviate pain in animal models of CIPN. This finding may provide the necessary foundation for testing DS002 in clinical trials of CIPN.

ACKNOWLEDGEMENTS

This work was supported by the China National Major Scientific and Technological Special Project [2019ZX09732002-006]; the National Natural Science Foundation of China [81872785 and 81673347]; the Shanghai Municipal Commission of Science and Technology of China [17431904400, 19YF1457400 and 21S11904500]; the Institutes for Drug Discovery and Development, Chinese Academy of Sciences [CASIMM0120202007 and CASIMM0120202008]; and the Major Scientific and Technological Special Project of Zhongshan City [191022172638719 and 210205143867019].

AUTHOR CONTRIBUTIONS

CHW, LT and YLC conceived the study and designed the experiments. ZJL, ZBX, JT, GJC, GJL, LY, KXW, HPD, HQ, QW, and GFW conducted the experiments. ZJL and CHW wrote the manuscript. All authors read and approved the final manuscript.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41401-022-00904-8.

Competing interests: Jie Tan and Yi-li Chen received stipends from Shanghai Mabstone Biotechnologies, Ltd, and Lei Tang, Gan-jun Chen, Guo-jian Liu, Lin Yuan, Kai-xin Wang, Hua-ping Ding, Zuo-bin Xie, and Chun-he Wang from Dartsbio Pharmaceuticals, Ltd. The remaining authors declare no conflicts of interest.

REFERENCES

1. Bae EH, Greenwald MK, Schwartz AG. Chemotherapy-induced peripheral neuropathy: mechanisms and therapeutic avenues. Neurotherapeutics. 2021;18:2384–96.
2. Han Y, Smith MT. Pathobiology of cancer chemotherapy-induced peripheral neuropathy (CIPN). Front Pharmacol. 2013;4:156.
3. Quasthoff S, Hartung HP. Chemotherapy-induced peripheral neuropathy. J Neurol. 2002;249:9–17.
4. Gutiérrez-Gutiérrez G, Sereno M, Miralles A, Casado-Sáenz E, Gutiérrez-Rivas E. Chemotherapy-induced peripheral neuropathy: clinical features, diagnosis, prevention and treatment strategies. Clin Transl Oncol. 2010;12:81–91.
5. Hu S, Huang KM, Adams EJ, Loprinzi CL, Lustberg MB. Recent developments of novel pharmacologic therapeutics for prevention of chemotherapy-induced peripheral neuropathy. Clin Cancer Res. 2019;25:6295–301.
6. Tonello R, Lee SH, Berta T. Monoclonal antibody targeting the matrix metalloproteinase 9 prevents and reverses paclitaxel-induced peripheral neuropathy in mice. J Pain. 2019;20:515–27.
7. Hirayama Y, Ishitani K, Sato Y, Iyama S, Takada K, Murase K, et al. Effect of duloxetine in Japanese patients with chemotherapy-induced peripheral neuropathy: a pilot randomized trial. Int J Clin Oncol. 2015;20:866–71.
8. Loprinzi CL, Lacchetti C, Bleeker J, Cavaletti G, Chauhan C, Hertz DL, et al. Prevention and management of chemotherapy-induced peripheral neuropathy in survivors of adult cancers: ASCO guideline update. J Clin Oncol. 2020;38:3325–48.
9. Smith EM, Pang H, Cirrincione C, Fleishman S, Paskett ED, Ahles T, et al. Effect of duloxetine on pain, function, and quality of life among patients with chemotherapy-induced painful peripheral neuropathy: a randomized clinical trial. JAMA. 2013;309:1359–67.
10. Levi-Montalcini R, Hamburger V. Selective growth stimulating effects of mouse sarcoma on the sensory and sympathetic nervous system of the chick embryo. J Exp Zool. 1951;116:321–61.
11. Levi-Montalcini R. The nerve growth factor 35 years later. Science. 1987;237:1154–62.
12. Lewin GR, Rueff A, Mendell LM. Peripheral and central mechanisms of NGF-induced hyperalgesia. Eur J Neurosci. 1994;6:1903–12.
13. Pezet S, McMahon SB. Neurotrophins: mediators and modulators of pain. Annu Rev Neurosci. 2006;29:507–38.
14. Bibel M, Barde YA. Neurotrophins: key regulators of cell fate and cell shape in the vertebrate nervous system. Genes Dev. 2000;14:2919–37.
15. Lewin GR, Barde YA. Physiology of the neurotrophins. Annu Rev Neurosci. 1996;19:289–317.
16. Wiesmann C, Ultsch MH, Bass SH, de Vos AM. Crystal structure of nerve growth factor in complex with the ligand-binding domain of the TrkA receptor. Nature. 1999;401:184–8.
17. Schnitzer TJ, Easton R, Pang S, Levinson DJ, Pixton G, Viktrup L, et al. Effect of tanezumab on joint pain, physical function, and patient global assessment of osteoarthritis among patients with osteoarthritis of the hip or knee: a randomized clinical trial. JAMA. 2019;322:37–48.
18. Konno SI, Nikaido T, Markman JD, Ohta M, Machida T, Isogawa N, et al. Tanezumab for chronic low back pain: a long-term, randomized, celecoxib-controlled Japanese Phase III safety study. Pain Manag. 2022;12:323–35.
19. Zhang B, Tian X, Qu Z, Liu J, Yang L. Relative efficacy and safety of tanezumab for osteoarthritis: a systematic review and meta-analysis of randomized-controlled trials. Clin J Pain. 2021;37:914–24.
20. Bramson C, Herrmann DN, Carey W, Keller D, Brown MT, West CR, et al. Exploring the role of tanezumab as a novel treatment for the relief of neuropathic pain. Pain Med. 2015;16:1163–76.
21. Dakin P, DiMartino SJ, Gao H, Maloney J, Kivitz AJ, Schnitzer TJ, et al. The efficacy, tolerability, and joint safety of fasinumab in osteoarthritis pain: a phase iib/iii double-blind, placebo-controlled, randomized clinical trial. Arthritis Rheumatol. 2019;71:1824–34.
22. Dakin P, Kivitz AJ, Gimbel JS, Skrepnik N, DiMartino SJ, Emeremni CA, et al. Efficacy and safety of fasinumab in patients with chronic low back pain: a phase II/III randomised clinical trial. Ann Rheum Dis. 2020;80:509–17.
23. Hefti FF, Rosenthal A, Walicke PA, Wyatt S, Vergara G, Shelton DL, et al. Novel class of pain drugs based on antagonism of NGF. Trends Pharmacol Sci. 2006;27:85–91.
24. Mantyh PW, Koltzenburg M, Mendell LM, Tive L, Shelton DL. Antagonism of nerve growth factor-TrkA signaling and the relief of pain. Anesthesiology. 2011;115:189–204.
25. Youk J, Kim YS, Lim JA, Shin DY, Koh Y, Lee ST, et al. Depletion of nerve growth factor in chemotherapy-induced peripheral neuropathy associated with hematologic malignancies. PLoS One. 2017;12:e0183491.
26. De Santis S, Pace A, Bove L, Cognetti F, Properzi F, Fiore M, et al. Patients treated with antitumor drugs displaying neurological deficits are characterized by a low circulating level of nerve growth factor. Clin Cancer Res. 2000;6:90–5.
27. Velasco R, Navarro X, Gil-Gil M, Herrando-Grabulosa M, Calls A, Bruna J. Neuropathic pain and nerve growth factor in chemotherapy-induced peripheral neuropathy: prospective clinical-pathological study. J Pain Symptom Manag. 2017;54:815–25.
28. Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL. Quantitative assessment of tactile allodynia in the rat paw. J Neurosci Methods. 1994;53:55–63.
29. Yoon C, Wook YY, Sik NH, Ho KS, Mo CJ. Behavioral signs of ongoing pain and cold allodynia in a rat model of neuropathic pain. Pain. 1994;59:369–76.
30. Flatters SJ, Bennett GJ. Ethosuximide reverses paclitaxel- and vincristine-induced painful peripheral neuropathy. Pain. 2004;109:150–61.
31. Halvorson KG, Kubota K, Sevcik MA, Lindsay TH, Sotillo JE, Ghilardi JR, et al. A blocking antibody to nerve growth factor attenuates skeletal pain induced by prostate tumor cells growing in bone. Cancer Res. 2005;65:9426–35.
32. Fallon M, Sopata M, Dragon E, Brown MT, Viktrup L, West CR, et al. LBA62 efficacy and safety of tanezumab in subjects with cancer pain predominantly due to bone metastasis receiving background opioid therapy. Ann Oncol. 2021;32:S1339.