Lack of neurotoxicity of the vascular targeting agent ZD6126 following repeated i.v. dosing in the rat

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Abstract

The vascular targeting agent ZD6126 is a water-soluble prodrug of N-acetylcolchinol that acts by disrupting the cytoskeleton of tumor endothelial cells. It is currently undergoing clinical evaluation in man. As peripheral neuropathy is a major dose-limiting toxicity associated with tubulin binding agents, the neurotoxic potential of ZD6126 was investigated in male and female Wistar rats. ZD6126 was administered i.v. at up to maximum tolerated doses using subacute (0 to 20 mg/kg/d for 5 days) and chronic (0 to 10 mg/kg/d for 5 days, repeated monthly for 6 months) dosing regimens. A separate study examined a combination of ZD6126 (three cycles of ZD6126 given as in the chronic dosing regimen) and paclitaxel (12 mg/kg/wk for 9 weeks) to assess whether coadministration of ZD6126 altered the time course or magnitude of a paclitaxel-induced neuropathy. Neurotoxic potential was examined using a comprehensive series of tests including a functional observation battery, measurements of muscle strength (forelimb and hind limb grip strength), nociception (tail flick test), locomotor activity, neuropathology, and whole nerve electrophysiology. There was no evidence that ZD6126 induced neurotoxicity in the rat following either subacute or chronic i.v. dosing. In a chronic electrophysiology study, ZD6126 produced a slight slowing of the maturational increase of caudal nerve amplitude, with some evidence of reversibility. However, this was not associated with any changes in caudal nerve conduction velocity, motor nerve conduction velocity or amplitude, functional observation battery behavioral and function parameters (including no effects on tail flick latency), and neuropathology. As expected, paclitaxel administration was associated with a significant decrease in caudal nerve conduction velocity (P = 0.0001). Coadministration of ZD6126 did not increase the neurotoxicity of paclitaxel. These studies suggest that ZD6126 should not induce the peripheral neuropathy associated with other antitubulin chemotherapeutic agents and that ZD6126 may not exacerbate the neurotoxicity of other agents with dose-limiting neuropathies. [Mol Cancer Ther 2004;3(7):783–91]

Introduction

ZD6126, the water-soluble phosphate prodrug of N-acetylcollchinel, is a vascular targeting agent that is being developed for its tubulin binding properties and ability to disrupt the tubulin cytoskeleton of neoendothelial cells (1-3). It is currently undergoing clinical evaluation for the treatment of solid tumors (4-6). ZD6126 is one of a new class of antitumor agents that include the tubulin binding combretastatin A4-phosphate (7) and the flavonoid 5,6-dimethylxanthenone-4-acetic acid (8). These drugs target the proliferating, fragile neoendothelial cells found in tumor vasculature and differ conceptually from antiangiogenic approaches that are designed to prevent the formation of new blood vessels (9). ZD6126 destroys selectively the vasculature of tumors by inducing changes in tumor endothelial morphology, leading to vessel occlusion and massive central tumor necrosis (1, 2, 10). As with other vascular targeting agents, a characteristic peripheral rim of viable tumor cells remains from which tumor regrowth occurs (9). The antitumor effects of ZD6126 are increased following multiple daily dosing or when given in combination with paclitaxel (1), cisplatin (2, 11), radiation (12), the antiangiogenic agent ZD6474 (13), and the epidermal growth factor receptor tyrosine kinase inhibitor gefitinib (14).

Peripheral neuropathy is a major dose-limiting toxicity associated with antitubulin agents such as taxanes and Vinca alkaloids (15, 16). The neuropathy is predominantly sensory and manifests as a range of clinical symptoms in man, including tingling and numbness of hands and feet, pain, paresthesia, painful dysesthesia, and loss of deep tendon reflexes (15, 16). Animal models are available for studying peripheral neuropathy in vivo. In these models, antitubulin agents can induce peripheral neuropathies similar to those seen in humans. Studies have used a range of behavioral and functional (17-23), neuropathologic (20-25), and electrophysiologic (18-23, 25, 26) assessments to obtain useful insights into the mechanisms underlying the neurotoxicity induced. Vincristine administration is associated with reductions in sensory nerve conduction velocity and amplitude, which are consistent

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with the demyelination and axonopathy observed morphologically (15). The underlying mechanism is thought to involve the abundance of tubulin in neurons and the role of microtubules in axonal transport (27).

As the development of neurotoxicity is drug and dose dependent, new generation antitubulin agents have been developed, which are less neurotoxic or nonneurotoxic in animal models (28, 29) and in man (30). Therefore, as part of the preclinical safety evaluation of ZD6126, we have assessed its neurotoxic potential following subacute and chronic i.v. dosing in rats at doses associated with toxicity in other tissues. Neurotoxicity was assessed using a comprehensive battery of behavioral and functional tests, whole nerve electrophysiology procedures, and neuropathology.

Endothelial cells play a key role in the integrity of the blood-brain and blood-nerve barriers, thus protecting the nervous system from circulating agents capable of disturbing neural function (31-33). Disruption of these barriers may enhance the neurotoxicity of concomitantly administered chemical agents. For example, central nervous system toxicity is rare following paclitaxel administration but has been reported in patients with a disrupted blood-brain barrier due to brain metastases or following brain surgery or irradiation (34). Chen et al. (35) have also demonstrated an increased permeability of the blood-nerve barrier during endothelial cell turnover, which could be altered by anti-vascular agents leading to a disruption of the cytoskeleton in neoendothelial cells. Therefore, the investigation of potential changes in the blood-brain and blood-nerve barriers, as well as the possible exacerbation of the neurotoxicity of coadministered compounds, is an important aspect of the safety evaluation of antivascular agents. This is particularly important for vascular targeting agents, which are likely to be most effective as part of combination therapies (9).

Therefore, as part of the preclinical safety evaluation of ZD6126, we have also assessed its potential to alter the time course or magnitude of a paclitaxel-induced neuropathy measured using whole nerve electrophysiology.

**Materials and Methods**

**Animals and Treatments**

The neurotoxic potential of ZD6126 was assessed in (1) subacute neurotoxicity, (2) chronic neurotoxicity, and (3) chronic electrophysiology studies. A fourth study examined the potential for ZD6126 to alter the time course or magnitude of a paclitaxel-induced neuropathy assessed using whole nerve electrophysiology. Wistar-derived Alpk:APfSD rats (AstraZeneca, Macclesfield, Cheshire, United Kingdom) were used for the neurotoxicity studies, and Wistar rats were used for the electrophysiology studies. Animals were 5 to 6 weeks old at the start of dosing. Sterile solutions of ZD6126 (AstraZeneca) in saline were administered i.v. via the tail vein. Paclitaxel (Sigma Chemical Co., St. Louis, MO) was administered i.p. as a 12 mg/kg solution in Cremophor [50:50 volume of Cremophor EL (Sigma Chemical) and 100% ethanol diluted 1:4 in saline on the day of injection]. Saline was used as the vehicle control for the ZD6126 studies, while saline and Cremophor were used in the ZD6126-paclitaxel study control. In all studies, ZD6126 was administered as single or multiple dosing cycles, each consisting of five daily doses followed by up to 23 days off-dose. Table 1 summarizes the experimental design of the studies. Different maximum dose levels of ZD6126 for males and females were used because of gender differences found in previous studies.

**Table 1. Study design**

<table>
<thead>
<tr>
<th>Study</th>
<th>Dosing Schedule</th>
<th>Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subacute</td>
<td>0 (saline control), 5, 10 (females only), or 20 (males only) mg/kg/d i.v. for 5 days followed by 16 days of observation.</td>
<td>5</td>
</tr>
<tr>
<td>Chronic neurotoxicity</td>
<td>0 (saline control), 0.5, 2.5, 5 (females only), or 10 (males only) mg/kg/d i.v. for 5 days repeated every 28 days for six cycles followed by a further 5 days’ dosing. Half the animals were killed 24 hours after the final dose. Remaining animals were observed for a further 13 weeks to assess recovery.</td>
<td>20</td>
</tr>
<tr>
<td>Chronic electrophysiology</td>
<td>0 (saline control), 0.5, 2.5, 5 (females only), or 10 (males only) mg/kg/d i.v. for 5 days repeated every 28 days for five cycles followed by a further 5 days’ dosing. Animals were retained for a further 53 days to assess recovery.</td>
<td>10</td>
</tr>
<tr>
<td>ZD6126-paclitaxel</td>
<td>12 mg/kg/wk i.p. paclitaxel for 9 weeks. Paclitaxel i.p (as above) plus 1, 5 (females only), or 10 (males only) mg/kg ZD6126 i.v. for 5 days every 28 days for two cycles followed by a further 5 days’ dosing. ZD6126 alone as above. Saline and Cremophor (vehicle controls) given using the same dosing schedule as for ZD6126 and paclitaxel.</td>
<td>6</td>
</tr>
</tbody>
</table>

NOTE: Animals, number of animals of each gender and dose group.
toxicity studies in rats. The higher doses of ZD6126 evaluated (i.e., 5, 10, and 20 mg/kg/d equivalent to ~33, 66, and 132 mg/m²/d) approached the limit of tolerability when given as five daily doses. In all studies, animals were observed for any changes in their clinical condition, and body weight was recorded.

**Functional Observation Battery and Locomotor Activity**

A functional observation battery was conducted, and locomotor activity was assessed at baseline (day −1) and on days 5 (~30 minutes after dosing), 8, 15, and 21 of the subacute neurotoxicity study. In the chronic neurotoxicity study, the tests were carried out at baseline (week −1), on day 5 of dosing cycles 2, 4, and 6 (in weeks 5, 13, and 21), and at the end of the 13-week recovery phase (week 37). The functional observation battery comprised detailed clinical observations and quantitative assessments. Clinical observations were made both in the home cage and within a standard open arena. Observations were made of autonomic function (e.g., lachrymation, salivation, piloerection, exophthalmus, urinary incontinence, diarrhea, and papillary response to light and ptosis), convulsions, tremors, abnormal motor movements, reactivity to stimuli, arousal/alertness, abnormal posture/gait, hearing deficits, unusual or abnormal behavior, excessive or repetitive actions (stereotypes), emaciation, dehydration, hypotonia, hypertonia, or other changes in appearance. The presence and/or absence of clinical observations were recorded, and the degree of each condition was noted (e.g., slight, moderate, or extreme) where appropriate. Quantitative assessments were made of landing foot splay, muscle weakness (forelimb and hind limb grip strength), and sensory perception (tail flick test). All observations were made blind with respect to treatment. As soon as possible after completion of the functional observation battery clinical observations, locomotor activity was measured using an automated activity recording apparatus (Coulbourn Lab Linc Infrared Motion Activity System, Coulbourn Instruments, Allentown, PA), which recorded small and large movements as an activity count. Data were analyzed as the total number of large and small movements over 50 minutes. Each 50-minute observation period was divided into 10 scans of 5 minutes. Locomotor activity was assessed in a separate room from the study to minimize disturbances.

**Neuropathology**

At the end of the dosing and recovery phases, designated rats from the subacute and chronic neurotoxicity studies were anesthetized with barbiturate i.p. and killed by perfusion fixation with modified Karnovsky’s fixative. A comprehensive list of neurologic tissues was taken from the controls and highest dose group of each gender. The following tissues were taken from both studies: brain (examined at seven levels), vertebral column including the dorsal root ganglia and dorsal and ventral spinal roots, gastrocnemius muscle, spinal cord (at cervical and lumbar swellings), sciatic nerve, and tibial nerve. In addition, eyes (with optic nerve and retina) were taken from animals on the chronic study, while sural nerve and Gasserian ganglia of the trigeminal nerve were taken in the subacute study. Brain, gastrocnemius muscle, decalcified vertebral column, and eyes were embedded in paraffin wax, and 5 μm sections were cut and stained with H&E. Spinal cord and peripheral nerves were embedded in araldite, and semithin sections stained with toluidine blue. Tissues were examined by light microscopy.

**Whole Nerve Electrophysiology**

Electrophysiology measurements were made on animals in the chronic electrophysiology study at baseline (week −1), monthly (after completion of the 5-day dosing phase in weeks 1, 5, 9, 13, 17, and 21), and at the end of the recovery phase (week 29). In the ZD6126-paclitaxel combination study, measurements were made at baseline (week −1) and following ZD6126 dosing in weeks 1, 5, and 9. All recordings were made using a portable electrophysiology instrument (BioPac MP100, Acknowledge version 3.5.7 software, BioPac Systems, Santa Barbara, CA). Animals were sedated with halothane and placed on a warm heating pad, and rectal temperature was monitored. Subdermal platinum needle electrodes were used for both recording and stimulation. Caudal nerve conduction velocity and amplitude were recorded orthodromically, with the active recording electrode positioned 10 mm below the hairline on the tail and the stimulating cathode 60 mm distal. Tibial nerve motor latency and amplitude were recorded orthodromically at the midline of the distal plantar surface of the foot, with stimulating electrodes placed at the ankle. Supramaximal stimulation was achieved using a constant voltage square pulse (0.1 millisecond duration) isolated from ground.

**Statistical Analysis**

All statistical tests were two sided. Male and female animal data were analyzed separately. Body weights were considered by analysis of variance on initial body weight. Locomotor activity, time to tail flick (in the sensory function test), landing foot splay, and grip strength were considered by ANOVA. Differences in electrophysiology measurements were determined by quantitative analysis of waveform characteristics using a Student’s t test.

**Results**

**Clinical Condition and Body Weight**

In all studies, there were no mortalities, adverse effects, or signs suggesting any neurotoxicity related to the administration of ZD6126. In the subacute neurotoxicity study, body weight for males dosed at 20 mg/kg/d and females at 10 mg/kg/d was slightly lower than concurrent controls by 6% to 8% from days 2 to 6 (P < 0.01), with evidence of recovery from days 8 to 22. In the chronic neurotoxicity study, no effects on body weight were seen. In the chronic electrophysiology study, there were minor, nonsignificant reductions in the rate of body weight gain over the dosing period in males only, with subsequent evidence of recovery. In the ZD6126-paclitaxel combination combination study, measurements were made at baseline (week −1), monthly (after completion of the 5-day dosing phase in weeks 1, 5, 9, 13, 17, and 21), and at the end of the recovery phase (week 29). In the ZD6126-paclitaxel combination study, measurements were made at baseline (week −1) and following ZD6126 dosing in weeks 1, 5, and 9. All recordings were made using a portable electrophysiology instrument (BioPac MP100, Acknowledge version 3.5.7 software, BioPac Systems, Santa Barbara, CA). Animals were sedated with halothane and placed on a warm heating pad, and rectal temperature was monitored. Subdermal platinum needle electrodes were used for both recording and stimulation. Caudal nerve conduction velocity and amplitude were recorded orthodromically, with the active recording electrode positioned 10 mm below the hairline on the tail and the stimulating cathode 60 mm distal. Tibial nerve motor latency and amplitude were recorded orthodromically at the midline of the distal plantar surface of the foot, with stimulating electrodes placed at the ankle. Supramaximal stimulation was achieved using a constant voltage square pulse (0.1 millisecond duration) isolated from ground.

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study, all changes seen during the treatment period, including significant reductions in body weight gain ($P < 0.05$) following a cumulative dose of 60 to 84 mg/kg paclitaxel, were consistent with the expected toxicity associated with paclitaxel, and there was no ZD6126-induced exacerbation of these effects.

**Functional Observation Battery**

The functional observation battery assessments during the subacute and chronic studies showed no signs indicative of any ZD6126-induced neurotoxicity. Any observations recorded were consistent with a mild, general toxicity. In addition, no consistent changes were seen in any of the quantitative functional observation battery parameters (including landing foot splay, forelimb and hind limb grip strength, and tail flick latency; Figs. 1 and 2). Although isolated minor statistically significant differences were occasionally seen in the quantitative functional observation battery parameters, in the absence of changes in other functional observation battery parameters or at later time points, they were considered to be incidental to treatment with ZD6126.

**Locomotor Activity**

There were no consistent ZD6126 effects on locomotor activity following subacute or chronic administration (Fig. 3). During week 21 of the chronic study, locomotor activity was statistically significantly lower than controls in males dosed with ZD6126. However, there was no dose relationship, and the small differences were considered unrelated to treatment. A review of individual values for these animals over the period 6 to 45 minutes, when occasional statistically significant differences for group mean values were observed, also confirmed that the range of movements within each 5-minute period were similar across all groups (control: 0 to 86 movements per 5-minute

![Figure 1](https://example.com/figure1.png)

Lack of ZD6126 neurotoxicity following subacute administration: quantitative functional observation battery measurements in males (left) and females (right). The highest dose levels were 10 mg/kg/d (females) or 20 mg/kg/d (males). Columns, mean from five animals; bars, SD. *, $P < 0.01$, **, $P < 0.001$, significant differences from control data.
period, 0.5 mg/kg/d ZD6126: 0 to 97 movements per 5-
minute period, 2.5 mg/kg/d ZD6126: 0 to 93 movements per 5-minute period, and 5 mg/kg/d ZD6126: 0 to 84 movements per 5-minute period).

**Neuropathology**

Comprehensive neuropathologic evaluation of tissues from control and high-dose group animals in the subacute and chronic neurotoxicity studies revealed no changes that were related to the administration of ZD6126. A summary of all histopathologic findings from these studies is given in Tables 2 and 3.

**Whole Nerve Electrophysiology**

During the chronic electrophysiology study, caudal nerve conduction velocity increased (~7 m/s per month) in control and ZD6126-treated animals throughout the 21-week study period (Fig. 4). This finding was consistent with maturation of both axon and myelin in growing animals (36). There were no significant intergroup differences in caudal nerve conduction velocity following chronic dosing with ZD6126. ZD6126 produced a slight, dose-related slowing of the maturational increase of caudal nerve amplitude, which was first observed following dosing in week 13, principally in females (Fig. 4). There was some evidence of recovery in amplitude in the low-dose group at the end of the recovery phase (week 29). However, caudal nerve amplitude in high-dose group animals remained significantly lower (P < 0.001) than values in age-matched control subjects at the end of the recovery period. No significant differences in tibial nerve conduction velocity and amplitude, estimated by distal motor latencies and maximal amplitudes, were seen throughout the study period following dosing with ZD6126.

Figure 2. Lack of ZD6126 neurotoxicity following chronic administration: quantitative functional observation battery measurements in males (left) and females (right). The highest dose levels were 5 mg/kg/d (females) or 10 mg/kg/d (males). Columns, mean from 19 of 20 animals (first four time points) or 9 of 10 animals (week 37); bars, SD. *, P < 0.01, **, P < 0.001, significant differences from control data.
ZD6126–Paclitaxel Electrophysiology Study
All animals had an increase in caudal nerve conduction velocity, which was consistent with the maturation of both axon and myelin (Fig. 5). During week 9, a paclitaxel-induced deficit in velocity was observed. Caudal nerve conduction velocity in the group receiving paclitaxel alone decreased by 13 m/s (20%) in comparison with age-matched controls ($P < 0.0001$) following a cumulative dose of 108 mg/kg. The coadministration of ZD6126 did not alter the timing or exacerbate the magnitude of the observed paclitaxel-induced neuropathy. Caudal nerve amplitude demonstrated maturational changes throughout the study period; however, there were no significant group differences in this measure as a result of either paclitaxel or paclitaxel-ZD6126 administration. Tibial nerve conduction and amplitude (data not shown) was not significantly altered by treatment.

Discussion
Rodent models have proved useful in studying the clinical neuropathies induced by tubulin binding agents such as vincristine (17) and paclitaxel (18-26). Assessment batteries have included behavioral, functional, electrophysiologic, and/or pathologic assessments. However, whole nerve electrophysiology had emerged as a particularly valuable, objective, sensitive, and valid index of experimentally induced neuropathies (23, 25, 37). For example, studies in rats showed that paclitaxel produced a significant reduction in caudal nerve conduction velocity after 9 weeks of treatment with 12 mg/kg/week (cumulative dose 108 mg/kg). These published changes were seen in rats after cumulative doses of 36 to 40 mg/kg (37) and 40 to 80 mg/kg (25). In the study by Cavaletti et al. (25), a cumulative dose of 80 mg/kg reduced nerve conduction velocity by $>25\%$ compared with age-matched controls and by $\sim15\%$ compared with rats given a cumulative dose of 40 mg/kg. The difference in nerve conduction velocity across the two doses was supported by a greater degree of axon degeneration and/or atrophy at the higher dose (25). In both dose groups, paclitaxel-induced neuropathy was characterized by the linear aggregation of neurotubules within the axoplasm of myelinated fibers. Other studies

Table 2. Neuropathology findings following subacute administration of ZD6126

<table>
<thead>
<tr>
<th>Tissue Examined</th>
<th>Male</th>
<th></th>
<th>Female</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>20 mg/kg</td>
<td></td>
<td>10 mg/kg</td>
</tr>
<tr>
<td>Gasserian ganglia demyelination</td>
<td>1/5</td>
<td>0/5</td>
<td>2/5</td>
<td>0/5</td>
</tr>
<tr>
<td>Gastrocnemius muscle myofiber degeneration</td>
<td>0/5</td>
<td>0/5</td>
<td>1/5</td>
<td>0/5</td>
</tr>
<tr>
<td>Sciatic nerve demyelination</td>
<td>1/5</td>
<td>2/5</td>
<td>1/5</td>
<td>2/5</td>
</tr>
<tr>
<td>Spinal cord demyelination</td>
<td>0/5</td>
<td>0/5</td>
<td>1/5</td>
<td>0/5</td>
</tr>
<tr>
<td>Spinal roots demyelination</td>
<td>0/5</td>
<td>1/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
</tbody>
</table>

NOTE: All changes were of minimal or slight severity. Only tissues for which findings were seen are included.
have also documented a paclitaxel-induced peripheral neuropathy in rats (23, 38, 39). These findings mirror the reductions in nerve conduction velocity that have been reported clinically following the administration of both paclitaxel (40) and vincristine (41) and support the choice of the rat as a good model for studying experimentally induced neuropathy.

During the preclinical safety evaluation of ZD6126, a battery of neurologic assessments was used to assess the neurotoxic potential of this novel vascular targeting agent in rats. The dose levels administered to the animals approached the level of tolerability using a five daily dosing schedule and were in the range (based on mg/m²) reported to induce antivascular effects detected by magnetic resonance imaging in clinical studies (42). In the studies reported here, both subacute and chronic i.v. administration of ZD6126 over a period of up to 6 months produced no evidence of neurotoxicity in the rat. The only notable change in ZD6126-treated animals was a slight slowing of the expected (36) maturational increase in caudal nerve amplitude, which was detected after 13 weeks in the chronic electrophysiology study with some evidence of reversibility. This minor change in amplitude was not associated with slowing of conduction velocity in the caudal or tibial nerves, reduction in tibial motor amplitude, functional observation battery behavioral or function parameters (including no effects on tail flick), or neuropathology. A reduction in compound nerve amplitude without associated change in maximal conduction velocity is consistent with a distal sensory axonopathy that spares the largest diameter myelinated fibers. However, the lack of morphologic, behavioral, or additional electrophysiologic evidence of dysfunction in the present study makes this interpretation unlikely. Previous studies with taxol have reported reduced caudal nerve amplitudes, but these findings have been strongly associated with some effects on conduction velocities, with severe reductions in the density of myelinated fibers in the dorsal roots and with deficits in sensorimotor coordination in behavioral tests (23). The isolated reduction in the growth rate of the caudal nerve amplitude in the present study associated with high doses of ZD6126 is of unknown clinical and functional significance. This observation may be related to the effects of ZD6126 on maturation rather than direct neurotoxic effects in the adult rat.

There was no evidence that coadministration of ZD6126 exacerbated the effect of paclitaxel on caudal nerve conduction velocity and amplitude.

### Table 3. Neuropathology findings following chronic administration of ZD6126

<table>
<thead>
<tr>
<th>Tissue Examined</th>
<th>Male</th>
<th>Control</th>
<th>10 mg/kg</th>
<th>Female</th>
<th>Control</th>
<th>5 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain: hydrocephalus</td>
<td>0/9</td>
<td>1/10</td>
<td></td>
<td>1/10</td>
<td>0/10</td>
<td>0/10</td>
</tr>
<tr>
<td>Distal tibial nerve demyelination</td>
<td>0/9</td>
<td>2/10</td>
<td></td>
<td>1/10</td>
<td>2/10</td>
<td></td>
</tr>
<tr>
<td>Proximal tibial nerve demyelination</td>
<td>4/9</td>
<td>4/10</td>
<td></td>
<td>0/10</td>
<td>1/10</td>
<td></td>
</tr>
<tr>
<td>Proximal sciatric nerve demyelination</td>
<td>3/9</td>
<td>3/10</td>
<td></td>
<td>0/10</td>
<td>0/10</td>
<td></td>
</tr>
<tr>
<td>Eye: retinal rosettes</td>
<td>1/9</td>
<td>0/10</td>
<td></td>
<td>0/10</td>
<td>0/10</td>
<td></td>
</tr>
<tr>
<td>Eye: retinal degeneration</td>
<td>0/9</td>
<td>0/10</td>
<td></td>
<td>3/10</td>
<td>1/10</td>
<td></td>
</tr>
<tr>
<td>Eye: focal retinal dysplasia</td>
<td>1/9</td>
<td>0/10</td>
<td></td>
<td>0/10</td>
<td>0/10</td>
<td></td>
</tr>
<tr>
<td>Gastrocnemius muscle: focal myopathy</td>
<td>5/9</td>
<td>2/10</td>
<td></td>
<td>3/10</td>
<td>0/10</td>
<td></td>
</tr>
<tr>
<td>Spinal roots: focal gliosis</td>
<td>0/9</td>
<td>0/10</td>
<td></td>
<td>0/10</td>
<td>1/10</td>
<td></td>
</tr>
</tbody>
</table>

**NOTE:** All changes were of minimal or slight severity. Only tissues for which findings were seen are included.

During the preclinical safety evaluation of ZD6126, a battery of neurologic assessments was used to assess the neurotoxic potential of this novel vascular targeting agent in rats. The dose levels administered to the animals approached the level of tolerability using a five daily dosing schedule and were in the range (based on mg/m²) reported to induce antivascular effects detected by magnetic resonance imaging in clinical studies (42). In the studies reported here, both subacute and chronic i.v. administration of ZD6126 over a period of up to 6 months produced no evidence of neurotoxicity in the rat. The only notable change in ZD6126-treated animals was a slight slowing of the expected (36) maturational increase in caudal nerve amplitude, which was detected after 13 weeks in the chronic electrophysiology study with some evidence of reversibility. This minor change in amplitude was not associated with slowing of conduction velocity in the caudal or tibial nerves, reduction in tibial motor amplitude, functional observation battery behavioral or function parameters (including no effects on tail flick), or neuropathology. A reduction in compound nerve amplitude without associated change in maximal conduction velocity is consistent with a distal sensory axonopathy that spares the largest diameter myelinated fibers. However, the lack of morphologic, behavioral, or additional electrophysiologic evidence of dysfunction in the present study makes this interpretation unlikely. Previous studies with taxol have reported reduced caudal nerve amplitudes, but these findings have been strongly associated with some effects on conduction velocities, with severe reductions in the density of myelinated fibers in the dorsal roots and with deficits in sensorimotor coordination in behavioral tests (23). The isolated reduction in the growth rate of the caudal nerve amplitude in the present study associated with high doses of ZD6126 is of unknown clinical and functional significance. This observation may be related to the effects of ZD6126 on maturation rather than direct neurotoxic effects in the adult rat.

There was no evidence that coadministration of ZD6126 exacerbated the effect of paclitaxel on caudal nerve conduction velocity and amplitude.

### Figure 4. Caudal nerve conduction velocity and amplitude at baseline, during chronic ZD6126 dosing (weeks 1 to 21), and following recovery (week 29) in males (top) and females (bottom). *, P ≤ 0.02, **, P < 0.001, significant differences from control data.

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nerve conduction velocity (i.e., no evidence of increased peripheral neuropathy). The latter finding is consistent with the lack of neurotoxicity seen in the subacute and chronic ZD6126 alone dosing studies. In addition, the data support the study of combined modality approaches in the clinic and provide some preliminary evidence suggesting that ZD6126 does not interfere with the blood-nerve barrier and exacerbate the neurotoxicity of coadministered agents.

The finding of a lack of neurotoxicity in rats is consistent with the emerging clinical findings with ZD6126 at doses of up to 112 mg/m². Preliminary clinical experience suggests that neurotoxicity may not appear to be of significant concern with ZD6126 (5, 43). In contrast, the dose-limiting toxicities following administration of combretastatin A4-phosphate included reversible ataxia and motor neuropathy, with the neurologic toxicity predicted from dog toxicology studies (44). Acute neurologic toxicity was also considered the dose-limiting effect for the vascular targeting agent 5,6-dimethylxanthenone-4-acetic acid (45).

Several mechanisms are involved in the neurotoxicity of anticancer agents. The neurotoxicity associated with antitubulin agents is thought to involve the abundance of tubulin in neurons and at least partially reflects a disruption of the role of microtubules in axonal transport. Microtubules accumulate in axons following the administration of paclitaxel, whereas Vinca alkaloids interfere with axonal transport (27). It has been suggested that, for Vinca alkaloids, the overall drug affinity for tubulin may contribute to the severity of neuropathy observed clinically (46). Similarly, Ogawa et al. (28) suggested that the lack of neurotoxicity with the dolastatin 10 derivative TZT-1027 might relate to antitubulin agent differences in their affinity for post-translational modified microtubules. Whether the same applies for ZD6126 remains to be established.

In summary, the studies described here found no consistent evidence of neurotoxicity in rats following the chronic administration of the vascular targeting agent ZD6126 at clinically relevant doses. The work also found that ZD6126 did not increase the neurotoxicity of coadministered paclitaxel. These studies suggest that ZD6126 should not induce the peripheral neuropathy associated with other antitubulin chemotherapeutic agents and suggest that ZD6126 may not exacerbate the neurotoxicity of other agents with dose-limiting neuropathies.

References

Figure 5. Caudal nerve conduction velocity and amplitude in the ZD6126-paclitaxel combination study. *, P < 0.01, significant differences from control data.
Lack of neurotoxicity of the vascular targeting agent ZD6126 following repeated i.v. dosing in the rat
