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Neurofilament light chain in plasma as a sensitive diagnostic biomarker of peripheral neurotoxicity: In Vivo mouse studies with oxaliplatin and paclitaxel - NeuroDeRisk project

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ABSTRACT

Identifying compounds that are neurotoxic either toward the central or the peripheral nervous systems (CNS or PNS) would greatly benefit early stages of drug development by derisking liabilities and selecting safe compounds. Unfortunately, so far assays mostly rely on histopathology findings often identified after repeated-dose toxicity studies in animals. The European NeuroDeRisk project aimed to provide comprehensive tools to identify compounds likely inducing neurotoxicity. As part of this project, the present work aimed to identify diagnostic non-invasive biomarkers of PNS toxicity in mice. We used two neurotoxic drugs in vivo to correlate functional, histopathological and biological findings. CD1 male mice received repeated injections of oxaliplatin or paclitaxel followed by an assessment of drug exposure in CNS/PNS tissues. Functional signs of PNS toxicity were assessed using electronic von Frey and cold paw immersion tests (oxaliplatin), and functional observational battery, rotarod and cold plate tests (paclitaxel). Plasma concentrations of neurofilament light chain (NF-L) and vascular endothelial growth factor A (VEGF-A) were measured, and histopathological evaluations were performed on a comprehensive list of CNS and PNS tissues. Functional PNS toxicity was observed only in oxaliplatin-treated mice. Histopathological findings were observed dose-dependently only in paclitaxel groups. While no changes of VEGF-A concentrations was recorded, NF-L concentrations were increased only in paclitaxel-treated animals as early as 7 days after the onset of drug administration. These results show that plasma NF-L changes correlated with microscopic changes in the PNS, thus strongly suggesting that NF-L could be a sensitive and specific biomarker of PNS toxicity in mice.

1. Introduction

The biomarkers of peripheral neuropathy identification is an increasingly interesting topic both for non-clinical and clinical research, aiming to improve the safety of therapeutic compound and the patients' quality of life [1]. Unfortunately, reliable and responsive biomarkers for peripheral neuropathies are still understudied and limited to few proteins, promising candidates, but often not specific among very different

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neurological disorders [1].

Among them, neurofilaments are gaining increasing attention as candidate biomarkers of neuro-axonal injury because they are abundant structural scaffolding proteins that are exclusively expressed in neurons and that reach abnormal levels both in cerebrospinal fluid (CSF) and in blood, as a result of axonal damage in neurodegenerative, inflammatory, vascular and traumatic diseases [2]. Neurofilaments are highly specific for neuronal cell damage and eventual neuronal cell death, offering a key advantage over other possible biomarkers [2]. Neurofilaments are classified as intermediate filaments according to their diameter (~10 nm): human neurofilament heavy chain (NF-H, 112.5 kDa), neurofilament medium chain (NF-M, 102.5 kDa and 145–160 kDa), and neurofilament light chain (NF-L, 61.5 kDa and 70–86 kDa) [2]. Neurofilaments, mainly NF-L but also NF-H, have been extensively studied in central nervous system (CNS) disorders (multiple sclerosis, dementia, stroke, traumatic brain injury, amyotrophic lateral sclerosis, Parkinson's disease, and Huntington's disease) [2]. More recently, blood NF-L concentrations have been explored in chemotherapy-induced peripheral neuropathy (CIPN), both in rodents (paclitaxel, cisplatin and vincristine) and humans (oxaliplatin and paclitaxel), demonstrating its significant relationship with peripheral nervous system (PNS) toxicity [3–6].

Vascular endothelial growth factor (VEGF) is associated to several neurological disorders (amyotrophic lateral sclerosis, Alzheimer's disease, Huntington's disease, Parkinson's disease [7], painful peripheral neuropathies [8], and persistent pain [9]). VEGF enhances neuronal regeneration after injury indirectly, via blood vessel-dependent mechanisms, and possibly also directly [7]. For example, plasma levels of VEGF-A were increased in rats treated by repeated injections of oxaliplatin in comparison to control animals [10].

The NeuroDeRisk (Neurotoxicity de-risking in preclinical drug discovery - neuroderisk.eu) project is an "Innovative Medicines Initiative" (IMI2) project aiming to provide novel validated integrated tools for improving the preclinical prediction of adverse effects of pharmaceuticals on the nervous system (CNS and PNS) and thus help to de-risk drug candidates earlier in the Research and Development phases.

The present study of the NeuroDeRisk project is focused on candidate blood soluble factors as PNS toxicity biomarkers in mice, based on the available scientific bibliography and on the NeuroDeRisk partners expertise. To improve knowledge about neuropathic mechanisms and to study biomarker candidates, biological assessments were performed on plasma samples of mice treated with neurotoxicants known to induce peripheral neuropathy in rodents and humans (oxaliplatin and paclitaxel [11,12]. Peripheral neuropathy was monitored using behavioral assays and histopathology of nervous tissues. A correlation among plasma biomarker concentrations, functional and histopathological alterations was the base for identifying the more relevant circulating biomarker of PNS toxicity.

2. Materials and methods

2.1. Study design

Study design is presented in Fig. 1. Studies have been conducted in two separate laboratories (oxaliplatin in Université Clermont Auvergne and paclitaxel in Sanofi). Animals received repeated injections of oxaliplatin or of paclitaxel (described in the section "Drugs"). Behavioral assays were performed throughout the duration of the study to explore functional disorders in animals (described in the section "Behavioral assays"). Drugs exposure in plasma and nervous tissues (brain, spinal cord, dorsal root ganglia (DRG), sciatic, tibial and saphenous nerves) were monitored on Days 23–25 for oxaliplatin, and on Day 16 for paclitaxel using dedicated animal groups described in the section "Drug exposure assessment". A final necropsy (oxaliplatin: Days 23–25 and paclitaxel: Day 21) was performed to monitor histopathological modifications in nervous tissues (brain, spinal cord, DRG, sciatic, tibial and saphenous nerves) described in the section "Histopathological evaluations". Plasma concentration of biomarkers (i.e. NF-L and VEGF-A) was monitored at Day 7 only for paclitaxel, and the time of the final necropsy for both drugs (described in the section "Plasma biomarkers assessment").

2.2. Animals

Experiments were conducted on 88 (24 for oxaliplatin, 64 for paclitaxel) male CD1 mice (5–7 weeks old upon arrival, Charles River Labs) according to the European Communities Council directive (86/609/EEC of 24 November 1986). Animals were housed 6 per cage, with water and food ad libitum, a 12:12 h light/dark cycle, and 40–75% hygrometry.

The experiment involving oxaliplatin received ethical approval from the Animal Care and Use Committee of Auvergne (C2EA-02) and from the French Ministry of Higher Education, Research and Innovation (Ministère de l'Enseignement supérieur, de la Recherche et de l'Innovation), with the following agreement number APAFIS#25284–2020050308076476 v2 (October 13, 2020). The experiment involving paclitaxel complied with the Directive 2010/63/EU of the European parliament and the related French transposition texts, and was approved by the local "Comité d'Ethique pour la Protection des Animaux de Laboratoire" (Animal Care and Use Committee) and is deemed compliant with the Sanofi Policy on the protection of animals, with the following agreement number APAFIS#3289–2015121823078043 v2 (January 6, 2016) and APAFIS#28441–2020112713518455 (November 27, 2020). The experiments were reporting according to the ARRIVE guidelines for animal research [13].

2.3. Drugs

Oxaliplatin (Leancare, UK) was solubilized in 5% glucose in a stock solution of 2 mg/mL, and in order to prepare extemporaneously dosing

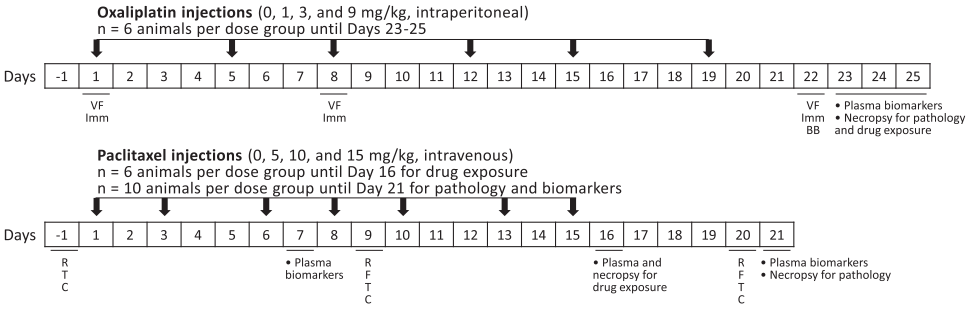


Fig. 1. Design of the studies. BB: Beam balance test; VF: von Frey test; Imm: Paw immersion test (10 °C); R: Rotarod test; F: functional observational battery (FOB); T: Body temperature; C: Cold plate test (4 °C).

solutions of 0.1, 0.3, and 0.9 mg/mL, in 5% glucose aqueous solution. Oxaliplatin was administered by intraperitoneal route at 1, 3, and 9 mg/kg/administration (10 mL/kg), twice a week (Monday and Friday) for 3 weeks, and a total of 6 injections (cumulative doses of oxaliplatin: 6, 18, and 54 mg/kg, respectively). Control animals received an equal amount of vehicle (5% glucose). Doses and methods derived from previous studies in mouse [14,15].

Paclitaxel (Sigma-Aldrich, France), was solubilized extemporaneously in a solution containing 4%/4%/92% Cremophor EL/Ethanol/0.9%NaCl respectively, in order to prepare dosing solutions of 0.5, 1, and 1.5 mg/mL. Paclitaxel was administered at 5, 10, and 15 mg/kg/administration, intravenously at the tail vein by slow bolus at 10 mL/kg, every 2 or 3 days for a total of 7 injections, corresponding to cumulative doses of paclitaxel: 35, 70, and 105 mg/kg, respectively. Injections were performed from Day 1 up to Day 15. Control animals received an equal amount of vehicle (4%/4%/92% Cremophor EL/Ethanol/0.9%NaCl respectively). Doses and methods derived from previous studies in mouse and internal feasibility study (data not shown) [16,17].

2.4. Behavioral assays

For oxaliplatin study, tactile and cold sensitivity assays were performed, thanks to a von Frey hair test (0.6 g hair, Bioseb France), (Days 1, 8, and 22) cold paw immersion test (10 °C) (Days 1, 8, and 22), and Beam balance test (Day 22) (Fig. 1). Experimenters were blinded to the treatment allocation, and experiments were performed in the morning (between 8 a.m. and 12 a.m.).

Mechanical allodynia was assessed using a 0.6 g bending force calibrated Von Frey hair filament (Bioseb, France), applied perpendicular to the plantar surface of the hind paw until it bent. Scores were expressed, averaging results from the left and right paws, as a number of responses elicited by five consecutive stimulations by a given filament was averaged between the left and right paw [14].

Cold thermal allodynia was assessed using the cold water (10 °C) paw immersion test. The paw of the animal was immersed in the temperature-controlled water bath until withdrawal was observed (cutoff time: 30 s). Two separate withdrawal latency time determinations were averaged [14].

Gross vestibulomotor function was evaluated with the beam-balance test which involves placing the mice on a suspended (50 cm from the floor) narrow wooden beam (5 mm diameter, 80 cm length). Performance on the beam was quantified by measuring the time needed by the mouse to cross the beam and the number of paw slips that occurred in the process [15].

For paclitaxel study, the functional assays performed were a functional observational battery (FOB, gross behavior profile including body temperature), a rotarod test (5–25 rpm, Imétronic, France) and a cold plate test (4 °C, Ugo Basile, Italy).

The FOB was performed on Days 9 and 20 in the morning, according to a method derived from that described by Moser [18] and Haggerty [19] and consisted of 32 non-blinded measures corresponding to the sensory, motor and autonomic functions. The first evaluation was performed while the animal was alone in the home cage. The observer evaluated each animal's posture and spontaneous activity, respiratory rate and skin color. Presence and/or absence of tremors and convulsions were also recorded and graded. Following this evaluation, the animal was removed from its home cage. The response to prehension and the reactivity to handling as well as eye watering, salivation, ptosis and body tone were recorded. The presence or absence of flexor and stretching reflexes and piloerection were also tested. The mouse was then placed on a flat surface (i.e. open field) covered with a clean absorbent pad. The mouse was observed for gait characteristics, the moving ease of the animal despite gait abnormalities (motility), arousal, its rearing and the presence and appearance of fecal boluses. Stereotypical movements were also recorded. Then, reflex testing consisted of recording each mouse's response to the approach of a blunt object (visio-olfactive response), to the touch with an object at the posterior flank (response to touch), and to an auditory stimulus. Pinna and palpebral reflexes and responsiveness to a pinch of the tail (nociceptive reflex) were also assessed. Finally, the mouse was tested for grip ability, catalepsy and righting reflex. All other clinical signs were also recorded as observations (vocalization, Straub tail reaction, etc.). The body temperature, considered as a part of the neurological parameter, was measured on Days 9 and 20, just after the FOB, using a scapula-localized subcutaneous electronic implant. An additional measurement was performed on Day − 1 to record individual baselines. FOB data were of two types:

- Graded data which were grouped according to functional domains. The results of each of these tests being graded to a severity scores (using a single 1-to-4 or 1-to-3 scale) were grouped and summed per domain, giving 6 global scores: abnormal/involuntary motor movements (tremors, clonic convulsion, tonic convulsion, stereotypy), activity (posture, spontaneous activity, motility, rearing), excitability (response to prehension, handling reactivity, arousal), neuromuscular (body tone, gait characteristic, righting reflex), and sensory-motor (visio-olfactive response, response to touch, auditory and nociceptive reflexes).
- Binary data which were considered as clinical signs. The results of these tests were noted as present or absent (and coded as 1 or 0, respectively) and comprised the following tests: flexor reflex, stretching reflex, piloerection, palpebral reflex, pinna reflex, grip ability and catalepsy.

The effect of paclitaxel on motor coordination and muscle tone was evaluated by the rotarod test according to a method derived from that described by Watzman et al. [20]. A substance that impairs motor coordination/muscle tone, decreases the time the animals remain on a rotating rod. The rotarod test was performed in mice in the morning on Day − 1 (for training), then on Day 9 and on Day 20 for all animals. The animals were tested in non-blinded conditions on the rotating rod for a maximum of 180 s (one acceleration ramp: starting speed of 5 rpm and acceleration up to the maximum speed of 25 rpm within 180 s).

Thermal hyperalgesia was assessed using the cold plate test, according to a method derived from that described by Sałat et al. [21]. Mice were taken in blinded conditions from home-cages and placed onto the surface of the cold-plate (Ugo Basile, Varese, Italy) maintained at a constant temperature of 4 °C. Ambulation was restricted by a cylindrical Plexiglas chamber (diameter: 10 cm, height: 15 cm), with an open top. The timing response started when the mouse was placed onto the cold-plate. Pain-related behavior (hind paw flinch and licking) was observed: the time (seconds) of the first sign was recorded as well as the total time duration of the hind paw flinches. The cut-off time was set at 60 s. All animals were tested first on Day − 1 to obtain baseline latencies (i.e. timing before the first hind paw flinch, and total duration of hind paw flinches), then the cold plate test was performed in the afternoon on Days 9 and 20, at least 2 h after the rotarod test (Fig. 1).

2.5. Histopathological evaluations

At necropsy, for each animal, tissues were collected for histopathological examination. PNS tissues were the sciatic and tibial (only for paclitaxel) sensorimotor nerves, the saphenous sensory nerve, and the DRG from lumbar spinal cord levels L3 to L5. CNS tissues were the brain and the cervical, thoracic and lumbar spinal cord. For oxaliplatin study, only the left side of each tissue was collected for histopathology evaluations, and the right side was collected for drug exposure evaluations. For paclitaxel study, right and left sides were collected for histopathology evaluations.

Collected tissues were immersion-fixed in 10% neutral buffered formalin for at least 24 h, processed and embedded in paraffin blocks, cut into 4–6 μm sections, and stained with hematoxylin and eosin.

Stained tissue sections were examined microscopically by pathologists, and histomorphological changes were qualitatively graded using a severity score scale of 0–5: 0 (no observable pathology), 1 (minimal or very slight), 2 (mild or slight), 3 (moderate), 4 (marked), or 5 (severe).

2.6. Drug exposure assessment

To better understand the relationship between drug exposure in plasma/target-sites and drug-induced peripheral neuropathy (histopathological findings and grade), drug exposure levels in CNS (brain and spinal cord) and in PNS (sciatic, tibial (only for paclitaxel), saphenous nerves, and pooled DRG from L3 to L5 levels) were assessed to confirm the drug exposures of nervous tissues. After collection, each specimen (tissues) was weighted (data not shown) and placed in appropriate polypropylene tubes before being frozen at − 80 °C until analysis.

Total platinum (Pt) concentrations (resulting from oxaliplatin and biological exposures) were determined by inductively coupled plasma mass spectrometry (ICP-MS) using a NexION 350xx ICP-MS (Perkin-Elmer, France). The sample introduction system was equipped with a glass concentric nebulizer, a glass cyclonic spray chamber and a peristaltic pump. The mineralization of total tissues was achieved in close glass vessels with 360 µL of HNO_3 (trace metal grade, 65% w/w, Sigma Aldrich) and 140 µL HCl (trace metal grade, 34% w/w, Sigma Aldrich) at room temperature overnight followed of 1 h at 90 °C in at hot block digestion system. After sample cooling at room temperature, the samples were diluted until 5 mL with milli-Q water and tubes were centrifuged at 4000 g for 10 min. Prior to analysis, samples were filtered (using a 0.2 µm microporous membrane, Pall Medical, France) and measured, after instrument tuning, in triplicate by ICP-MS. The Instrumental ICP-MS parameters for total Pt quantification were as follows: radio frequency power: 1500 W, nebulization gas flow rate: 0.92 mL/min, auxiliary gas flow rate: 1.2 mL/min, plasma gas flow: 13 mL/min, analytes: Y^{89} and Pt^{194} operating mode: standard, scanning mode: peak hopping, dwell time per AMU: 50 ms, and integration time: 1000 ms. Calibrations solutions between 0.1 and 10 µg/L Pt were prepared by diluting stock solutions of 1000 µg/L (TraceCERT, Sigma-Aldrich, France) in a blank rat brain digest. The linearity of the quantification method was designed for a concentration range from 0.05 to 10 µg/L (equation: $y = 0.01x + 0.002$, and $R^2 = 0.999927$). Background equivalent concentration was 0.052 ± 0.008 µg/L and detection limit was 0.011 ± 0.004 µg/L. Calibration standards were prepared freshly each day. Pt concentrations in the tissues were calculated as Pt amount per wet weight of the tissue.

Quantification of paclitaxel in plasma and nervous tissues was performed using a Waters ACQUITY ultra-performance liquid chromatography (UPLC) system coupled to a Waters Xevo TQ-S Micro triple quadrupole mass spectrometer (Waters, Milford, MA). Nervous tissues were individually weighed and homogenized with phosphate-buffered saline, pH 7.4 using a 4-Place Mini Beads Mill Homogenizer (VWR International B.V., Leuven, Belgium). Pretreatment of biological samples included protein precipitation with acetonitrile containing deuterated analog paclitaxel-D5 as the internal standard, followed by dilution with 0.1% formic acid in Milli-Q water. The chromatographic separation was achieved on a Waters ACQUITY UPLC BEH C18 column (50 × 2.1 mm, 1.7 µm) (Waters, Milford, USA) at a flow rate of 0.3 mL/min, using a gradient of aqueous (solvent A: 0.1% formic acid in Milli-Q water) and organic (solvent B: 0.1% formic acid in acetonitrile) mobile phases. Within a run time of 3.5 min, the gradient elution started from 5% B, then linearly increased to 95% B within 2.5 min and maintained at 95% B for 0.5 min, before returning to 5% B for equilibrium. The detection of the analyte and internal standard was carried out in a positive electrospray ionization mode using multiple reaction monitoring transitions of m/z 854.4 → 286.1 for paclitaxel and m/z 859.4 → 291.1 for paclitaxel-D5. The linearity ranges were 0.1–200 ng/mL for plasma samples and 0.1–500 ng/mL for nervous tissue samples, with a lower limit of quantification of 0.1 ng/mL. By using 1/x2 weighting, the coefficient of determination (R2) was higher than 0.992 for all the calibration curves.

2.7. Plasma biomarkers assessment

For oxaliplatin study, biomarker concentrations were measured in plasma on Days 23–25 (NF-L and VEGF-A). Plasma was recovered by cardiac puncture in K3EDTA vials. For paclitaxel study, biomarker concentrations were measured in K2EDTA-plasma on Day 7 (NF-L), and on Day 21 (NF-L and VEGF-A) (Fig. 1). All biomarkers concentrations were measured using an ECLIA (ElectroChemiluminescent Immuno-Assay) method from a Mesoscale Discovery platform (Quickplex SQ 120, Meso Scale Discovery, USA). Analyses were performed as recommended by the manufacturer using commercially available assays. The assays were based on capture and detection of mouse NF-L and VEGF-A using human anti-NF-L and mouse anti-VEGF-A antibodies. Based on the successful comp-letion of following validation items (assay variability, linearity and determination of the lower limit of quantification), the method for dosing NF-L in mouse plasma with the R-PLEX Human Neurofilament L Assay was considered valid for analysis of samples of the present studies.

2.8. Statistical analyses

All the quantitative variables are expressed by mean ± standard deviation. The comparisons of repeated and non-paired quantitative variables (electronic von Frey test, cold immersion test, and cold plate test) were performed using a repeated-measure ANOVA test and followed by a post hoc Tukey-Kramer test to compare differences between groups. For multiple comparisons with no repetition of non-paired quantitative variables (drug exposure and concentration of biomarkers), a Kruskal-Wallis non-parametric test was performed followed by a post hoc Dunn test. For comparison of paired quantitative variables (drug exposure between nervous tissues and for the same dose), a Friedman test was performed. Correlations between plasma concentrations of biomarkers and the histopathological grades, or the doses of neurotoxic drugs were assessed with the Spearman's rank correlation coefficient (Rho) and significance. For all these analyses, two-tailed tests were run and differences considered statistically significant for a p-value < 0.05. Statistical analyses were performed using XLSTAT (2022 v5.1, Addinsoft).

For the FOB test, a Cochran-Mantel-Haenszel test with Bonferroni-Holm correction was performed to compare each group versus control stratified by day, using SAS (v9.4 under Linux).

3. Results

On pretest day, then on tests days, responses of control animals were associated with a normal behavior, normal responses to tests and a normal body temperature (only assessed for paclitaxel) profile, without visible clinical signs.

3.1. Mortality

Regarding oxaliplatin study, mortality occurred in 2 oxaliplatin animals treated at 9 mg/kg, 1 on Day 15 and 1 on Day 19.

Regarding paclitaxel study, mortality occurred for the main study in 2 control animals out of 10 on Day 1 and on Day 3, as well as in 1 animal out of 10 on Day 15 in paclitaxel group at 15 mg/kg. Moreover, for the drug exposure dedicated groups, 1 animal out of 3 died in each paclitaxel group (2 animals on Day 6 and 1 animal Day 15, respectively). These mortalities appeared just after administration and their cause remained unclear.

3.2. Behavioral assays

For the oxaliplatin study, tactile thresholds (von Frey test) of

oxaliplatin-treated animals increased on Day 8 for the 3 and 9 mg/kg groups in comparison to control animals, and on Day 22 for the three treated groups (1, 3 and 9 mg/kg) (Fig. 2A). Paw withdrawal latencies of oxaliplatin-treated animals decreased on Day 8 and 22 for the three dose groups (1, 3 and 9 mg/kg), in comparison to control animals (Fig. 2B). No modification of the duration to cross the beam and of the number of failures was recorded between animals (Fig. 2C).

For the paclitaxel study, no effect of paclitaxel at dose levels of 5, 10, and 15 mg/kg versus control animals was noted on Day 9 and on Day 20 during the FOB on any functional domain (Table 1). Only transient clinical signs were observed on Day 9 (i.e. crawling gate and dragging hind paw for a few animals). These clinical signs were no more observed on Day 20 and were considered as minor.

For the paclitaxel study, no obvious effect of paclitaxel at dose levels of 5, 10 and 15 mg/kg versus control animals was noted on Day 9 and on Day 20 for the cold plate test (latency Fig. 2D and paw flinch duration Fig. 2E), whatever the paclitaxel-treated groups. Only a minimal decrease of paw flinch duration was recorded on Day 9 for the 10 mg/kg dose in comparison to 0 and 5 mg/kg doses ($p < 0.05$, both). This change was not considered as biologically relevant. Moreover, no licking of the hind paw was observed all over the duration of the cold plate test. Global analysis of the FOB functional domains reported no modification between paclitaxel-treated animals (whatever the dose) and control ones (Table 1). Regarding body temperature measured during FOB, a difference was detected on Day 9 for paclitaxel group treated at 10 mg/kg in comparison to control animals but this effect was due to a difference of the pretest values between the control group and the others groups. This change was therefore considered as non-biologically relevant (Fig. 2F). For rotarod test, all animal durations were equal or possibly higher than 180 s (cut-off) in all groups and for Day 9 and Day 20 (data not shown).

Table 1
Global analysis (FOB) of functional domains.

Domain	Index (0–20D)	Vehicle	Paclitaxel			p-value
			5 mg/kg	10 mg/kg	15 mg/kg	
Abnormal movement	Mean	7	7	7	7	0.49
	Max	7	7	7	7	-
Activity	Mean	3.5	4	3.5	3.5	0.33
	Max	4	4	4	4	0.45
Autonomic	Mean	6	6	6	6	0.33
	Max	6	6	6	6	0.33
Excitability	Mean	3	3	3	3	0.44
	Max	3	3	3	3	0.42
Neuromuscular	Mean	3	3	3	3	0.30
	Max	3	4.5	3	3	0.29
Sensory motor	Mean	4	4.5	4	4	0.32
	Max	4	5	4	4	0.32

3.3. Histopathological evaluations

For the oxaliplatin study, mild axonal degenerations in DRG and as well as in sciatic nerve of one control animal were reported (Table 2). No histopathological finding was reported for any of the oxaliplatin doses

Table 2
Incidence of microscopic changes in the nervous system of animals treated with oxaliplatin.

Tissues	Findings	Grade	Oxaliplatin (mg/kg)			
			0	1	3	9
			Number of animals			
			6	6	6	4
DRG L4	Degeneration, axonal	Mild	1	0	0	0
Sciatic nerve	Degeneration, axonal	Mild	1	0	0	0

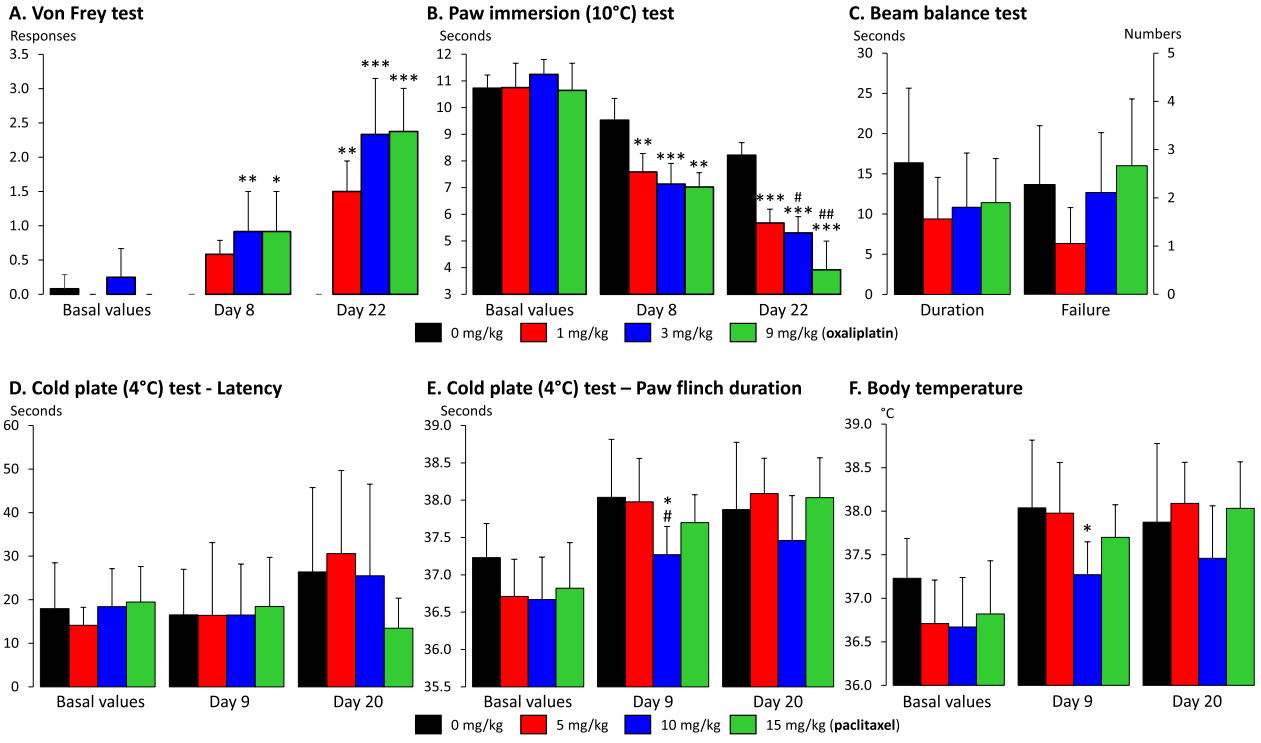


Fig. 2. Results of behavioral assays of animals treated by oxaliplatin or paclitaxel. Results are presented by mean + standard deviation. Von frey test (A.), paw immersion (10 °C) test (B.) and beam balance test (C.) were done before (basal values), at Day 8 and Day 22 during oxaliplatin administrations. Cold plate (4 °C) test latency (D.) & paw flinch duration (E.) and body temperature (F.) were done before (basal values), at Day 9 and Day 20 during paclitaxel administrations. * $p < 0.05$, * * $p < 0.01$, and * ** $p < 0.001$ vs. 0 mg/kg; # $p < 0.05$ and ## $p < 0.01$ vs. 1 mg/kg (oxaliplatin) or 5 mg/kg (paclitaxel).

(Table 2).

There were no paclitaxel-related macroscopic observations at the end of the study. Paclitaxel-related microscopic changes were noted in both CNS and PNS. These were observed in the brain of occasional animals treated at 15 mg/kg and consisted of minimal single neuronal cell necrosis mainly in the olfactive lobes, cortex and/or in the neuropile or molecular of the cerebellum (Table 3). Additional changes in the brain were noted in the cerebellum in areas adjacent to the fourth ventricle; these consisted of minimal neuropile vacuoles containing eosinophilic flocculent material. In the spinal cord, paclitaxel-related changes were only observed in the cervical and thoracic regions of occasional animals at 15 mg/kg (Table 3). These consisted of minimal to mild vacuolar degeneration in the dorsal funiculi of the white matter and were mainly characterized by myelin or neuronal cell debris within vacuolar spaces. In lumbar DRG, minimal to mild axonal degeneration (i.e. axonal dilatation and disruption and myelin ovoids within vacuolar spaces) was noted in several animals at 10 mg/kg upwards (Table 3). Minimal to moderate axonal degeneration was observed in sciatic, saphenous and/or tibial nerves with increasing incidence and severity at 5 mg/kg upwards (Table 3).

3.4. Drug exposure

After repeated oxaliplatin injections and blood sampling at Days 23–25, Pt concentrations in nervous tissues increased accordingly to oxaliplatin doses (1, 3, and 9 mg/kg) (Fig. 3A). Pt concentrations were higher in the saphenous nerve than in brain and cervical spinal cord (Fig. 3C).

After repeated intravenous dosing every 2 or 3 days at 5, 10 or 15 mg/kg, paclitaxel concentrations in nervous tissues increased accordingly to doses (5, 10, and 15 mg/kg) (Fig. 3B). Paclitaxel concentrations were higher in PNS than in CNS tissue (Fig. 3D). The tissue-to-plasma concentration ratios were assessed for paclitaxel-treated animals and revealed no biologically relevant change in comparison to tissue exposure (data not shown).

Table 3
Incidence of microscopic changes in the nervous system of animals treated with paclitaxel.

Tissues	Findings	Grade	Paclitaxel (mg/kg)			
			0	5	10	15
			Number of animals			
			10	9	10	10
Brain	Single cell necrosis	Minimal	0	0	0	3
	Vacuolation: floor of the 4th ventricle	Minimal	0	0	0	1
Spinal cord cervical	Vacuolation: dorsal funiculi	Minimal	0	0	0	2
		Mild	0	0	0	1
Spinal cord thoracic	Vacuolation: dorsal funiculi	Minimal	0	0	0	1
DRG L3 left	Degeneration, axonal	Minimal	0	0	0	3
		Mild	0	0	0	1
DRG L3 right	Degeneration, axonal	Minimal	0	0	1	3
DRG L4 left	Degeneration, axonal	Minimal	0	0	0	5
DRG L4 right	Degeneration, axonal	Minimal	0	0	0	2
DRG L5 left	Degeneration, axonal	Minimal	0	0	1	2
		Mild	0	0	1	1
DRG L5 right	Degeneration, axonal	Minimal	0	0	0	2
		Mild	0	0	0	2
Sciatic nerve left	Degeneration, axonal	Minimal	0	1	1	5
		Mild	0	0	0	1
		Moderate	0	0	0	2
Sciatic nerve right	Degeneration, axonal	Minimal	0	0	3	7
		Moderate	0	0	0	1
Saphenous nerve left	Degeneration, axonal	Minimal	0	0	0	5
Saphenous right	Degeneration, axonal	Minimal	0	0	0	4
Tibial nerve left	Degeneration, axonal	Minimal	0	0	1	4
		Moderate	0	0	0	2
Tibial nerve right	Degeneration, axonal	Minimal	0	0	0	6
		Mild	0	0	0	1

3.5. Plasma concentrations of biomarkers

For the oxaliplatin study, no modification of NF-L concentrations was recorded whatever the oxaliplatin dose (Fig. 4A).

For the paclitaxel study, NF-L concentrations started to increase of 4.8-fold in the high-dose on Day 7 (range 1.1–29.3-fold). On Day 21, NF-L values were below the lower limit of quantification in half of the control animals (Fig. 4B). NF-L plasma concentrations were treatment- and dose-related and consisted of NF-L increases of 2.2-fold in the mid-dose (range 1.2–4.8-fold) and 19-fold (range 5.5–46-fold) in the high-dose when compared to the control group mean value (Fig. 4B). Moreover, plasma concentrations of NF-L were correlated with the highest grades of histopathological findings in the PNS assessed on Day 21 (Day 7, Rho: 0.51 and $p = 0.002$; Day 21, Rho: 0.69 and $p < 0.001$), as well as with the paclitaxel doses (Day 7, Rho: 0.39 and $p = 0.02$; Day 21, Rho: 0.55 and $p = 0.001$) (Fig. 4B).

For both studies, VEGF-A was detectable, but, even if a statistically significant difference was observed for paclitaxel for treated groups at 5 and 15 mg/kg, no remarkable changes were noted between groups for this biomarker and whatever the drug assessed (Fig. 4C and D).

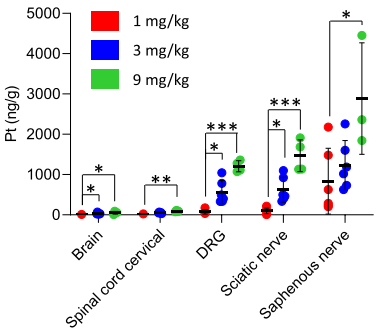
4. Discussion

While being among the most common neurological diseases, peripheral neuropathies are usually not recognized as an expected and persistent consequence of drug therapy. Nevertheless, antiproliferative anticancer drugs and antiretroviral drugs above all, consistently elicit these serious adverse effects, most often characterized by the development of a sub-acute or chronic polyneuropathy with predominant sensory involvement such as sensory loss, paresthesia, dysesthesia and numbness, sometimes associated with neuropathic pain, leading possibly to comorbidities such as depression, insomnia, falls and decreases of health-related quality of life [12]. Moreover, a number of other drugs, such as the antibiotic linezolide or the antiarrhythmic amiodarone, have been connected with these types of adversities [22]. Finally, neuropathies of peripheral origin have repeatedly been reported with certain newer drug classes characterized by exceptionally frequent chronic use, such as statins, thus imparting additional societal consequences [23]. This further emphasizes the need for developing better preclinical models and to investigate biomarkers of drug-induced peripheral neurotoxicity.

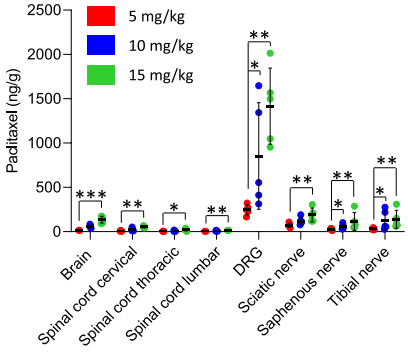
Neurofilaments (NF-H, NF-M, and NF-L) are specific and abundant in the neuro-axonal compartments. NF-L is the most abundant intermediate filament in neurons and axons and plays a substantial role in the assembly and maintenance of the axonal cytoskeleton. Disruption of the axonal membrane releases neurofilaments into the interstitial fluid, and ultimately into both CSF and blood. Since, NF-L has the lowest molecular weight and the highest solubility compared to other neurofilaments, therefore it diffuses more easily from the parenchyma into the CSF after axonal degeneration or neuronal death or disruption. Consequently, elevated blood NF-L concentrations would reflect an axonal degeneration [24].

The development of an assay for NF-L measurement that is sensitive enough to detect NF-L in plasma will allow its rapid and expanding use in the research for neurotoxicity biomarker. The main focus is neurological diseases like multiple sclerosis, amyotrophic lateral sclerosis or Parkinson's disease, but NF-L increases are observed with stroke and traumatic brain injury as well [25,26]. Recently, studies in large population of healthy volunteers established reference ranges and defined influences of aging and body mass index on NF-L levels [27,28]. Research into NF-L utility for drug induced nervous system injury is also of a great interest since there are currently no inexpensive, easy-to-use biomarkers of drug-induced nervous system injury. NF-L is one of the

A. Oxaliplatin (Pt) exposure – Days 23-25



B. Paclitaxel exposure – Day 16



C. Comparisons of nervous tissue exposure to Pt

Doses	P-values				
1 mg/kg	Brain	SCC	DRG	ScN	SaN
Spinal cord cervical (SCC)	0.99	1			
DRG	0.38	0.52	1		
Sciatic nerve (ScN)	0.52	0.67	0.99	1	
Saphenous nerve (SaN)	**0.02**	**0.03**	0.67	0.52	1
3 mg/kg					
Spinal cord cervical (SCC)	0.93	1			
DRG	0.09	0.44	1		
Sciatic nerve (ScN)	0.09	0.44	1	1	
Saphenous nerve (SaN)	**0.002**	**0.03**	0.75	0.75	1
9 mg/kg					
Spinal cord cervical (SCC)	0.94	1			
DRG	0.37	0.84	1		
Sciatic nerve (ScN)	0.24	0.70	0.99	1	
Saphenous nerve (SaN)	**0.02**	0.14	0.70	0.84	1

D. Comparisons of nervous tissue exposure to paclitaxel

Doses	P-values							
5 mg/kg	Brain	SCC	SCT	SCL	DRG	ScN	SaN	TN
Spinal cord cervical (SCC)	0.99	1						
Spinal cord thoracic (SCT)	0.66	0.99	1					
Spinal cord lumbar (SCL)	0.74	0.99	1	1				
DRG	0.22	**0.02**	**0.001**	**0.002**	1			
Sciatic nerve (ScN)	0.62	0.12	**0.01**	**0.02**	0.99	1		
Saphenous nerve (SaN)	0.99	0.78	0.25	0.32	0.62	0.94	1	
Tibial nerve (TN)	0.99	0.62	0.15	0.19	0.78	0.99	1	1
10 mg/kg								
Spinal cord cervical (SCC)	0.94	1						
Spinal cord thoracic (SCT)	0.44	0.99	1					
Spinal cord lumbar (SCL)	0.36	0.97	1	1				
DRG	0.44	**0.03**	**0.001**	**0.001**	1			
Sciatic nerve (ScN)	0.97	0.36	**0.045**	**0.03**	0.97	1		
Saphenous nerve (SaN)	1	0.90	0.36	0.29	0.53	0.99	1	
Tibial nerve (TN)	0.998	0.62	0.12	0.09	0.85	1	1	1
15 mg/kg								
Spinal cord cervical (SCC)	0.70	1						
Spinal cord thoracic (SCT)	0.22	0.99	1					
Spinal cord lumbar (SCL)	0.09	0.94	1	1				
DRG	0.78	**0.03**	**0.002**	**<0.001**	1			
Sciatic nerve (ScN)	0.99	0.29	**0.045**	**0.01**	0.996	1		
Saphenous nerve (SaN)	0.99	0.97	0.62	0.36	0.36	0.90	1	
Tibial nerve (TN)	1	0.85	0.36	0.17	0.62	0.99	1	1

Fig. 3. Oxaliplatin (platinum - Pt) and paclitaxel exposures of nervous tissues. Drug exposures of nervous tissues (A. Pt and B. paclitaxel) are presented by mean + standard deviation. Comparisons of nervous tissue exposures to drug (C. Pt and D. paclitaxel) are presented by the p-values (bold ones: $p < 0.05$) of the comparison between tissues and for each oxaliplatin and paclitaxel doses. * $p < 0.05$, * * $p < 0.01$, and * ** $p < 0.001$.

candidate biomarkers of drug-induced nervous system injury in IMI international consortia Transbioline, that is focusing on regulatory qualification of blood-based biomarkers with Federal Drug Administration and European Medicine Agency to enable decisions in early clinical trials with a neurotoxicity risk. Taken together, NF-L is well positioned to become a sensitive diagnostic and safety translational biomarker of nervous system injury both in the nonclinical and clinical space during drug development.

The aim of this study was to assess the relevance of two plasma biomarkers of peripheral neuropathy in mice exposed to neurotoxic drugs, which are known to induce CIPN, oxaliplatin and paclitaxel [12]. The peripheral neuropathy was assessed and validated with both behavioral and histopathological assessments. While repeated administrations of oxaliplatin induced tactile and cold thermal allodynia and no histopathological finding, repeated paclitaxel administrations induced no nociceptive disorders but strong dose-related histopathological findings in the PNS (axonal degenerations of DRG and nerves). The exposure of nervous tissues to these neurotoxic compounds has been confirmed, underlining a higher exposure of PNS tissues than CNS ones.

Plasma concentrations of NF-L were significantly increased in animals treated with the highest dose of paclitaxel (15 mg/kg), as soon as day 7 after the beginning of the study, in comparison to controls and animals treated with 5 and 10 mg/kg doses, whereas no modification was reported for oxaliplatin-treated animals whatever the dose received. Moreover, this increase of plasma NF-L concentrations for paclitaxel-treated animals was correlated with the grade of histopathological findings of PNS tissues, underlining the strong link between NF-L concentrations and PNS lesions. It has to be noted that the four animals of the highest dose of paclitaxel (15 mg/kg) reported with CNS findings were also concerned by PNS findings.

No clear incidental modification of VEGF-A concentrations was reported for both oxaliplatin and paclitaxel-treated animals.

These results are in accordance with a growing number of data linking the blood concentrations of NF-L and the occurrence of peripheral neuropathy, both in animal and human studies. In rat (female Wistar rats) models of CIPN, serum NF-L quickly increased after paclitaxel (10 mg/kg, intravenous injections once a week for 4 weeks), cisplatin (2 mg/kg, intraperitoneal injections twice a week for 4 weeks), and vincristine (0.2 mg/kg, intravenous injection once/week for 4 weeks) administrations. For these three animal models of CIPN, serum NF-L concentrations were significantly increased from the first week of treatment until the end of the experiments, in comparison to control animals. The peripheral neuropathy was confirmed by morphological and neurophysiological alterations of caudal nerves [3,4].

Very recently, Lucarini et al. demonstrated an increase in NF-L plasma concentrations in mice after repeated injections of paclitaxel (2 mg/kg, intraperitoneal injections on days 1, 3, 5, and 8) compared to control animals (vehicle) [29]. NF-L concentrations were assessed on day 18. Importantly, on day 18, the authors reported intraepidermal nerve fiber loss (hind limb paw skin), a decrease in the myelin area of the

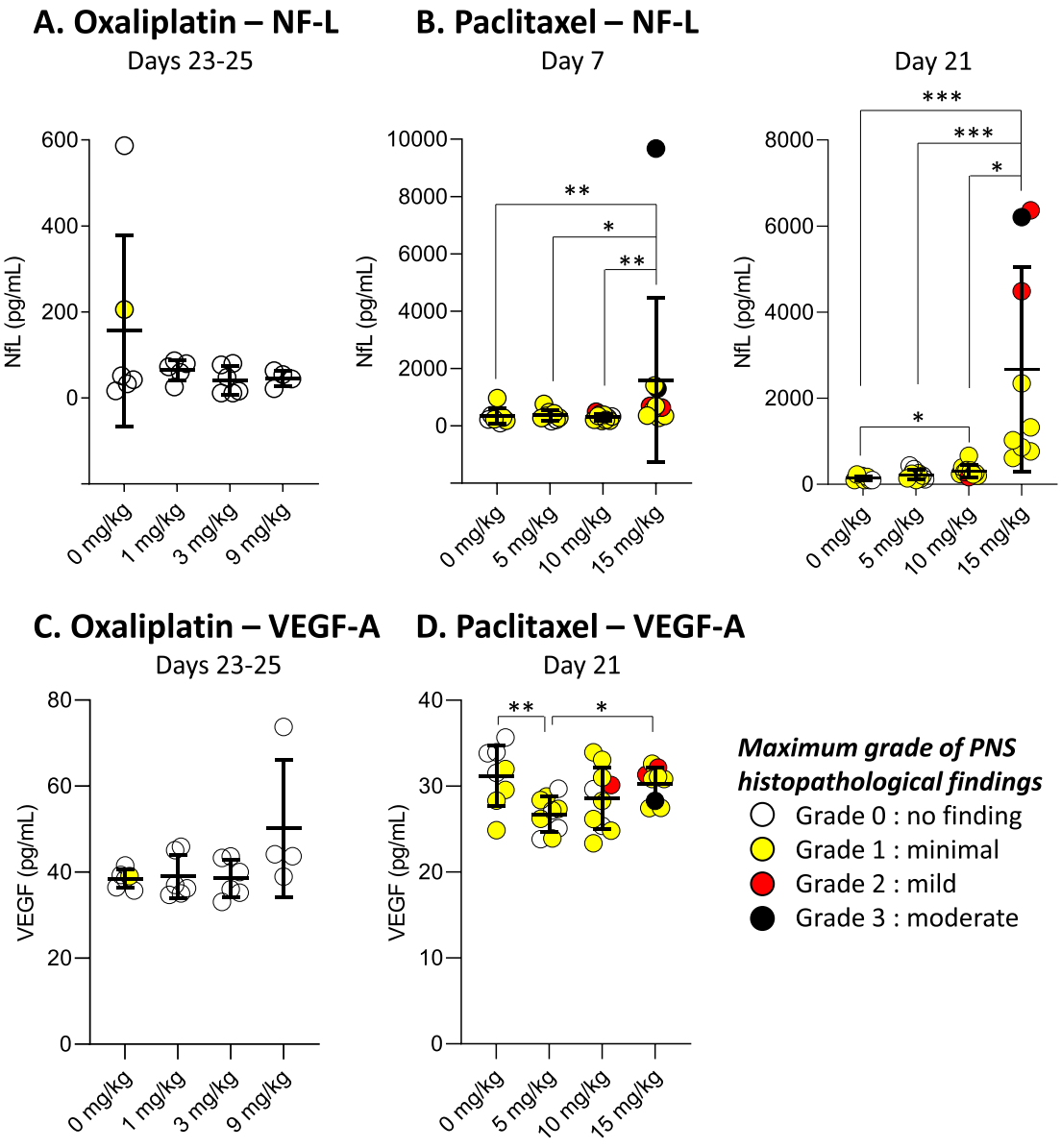


Fig. 4. Plasma concentrations of NF-L and VEGF-A in animals treated by oxaliplatin or paclitaxel. Results are presented by mean ± standard deviation for neurofilament light chain (NF-L) and vascular growth factor A (VEGF-A). Plasma concentrations of NF-L and VEGF-A are reported for each animal according to the maximal grade of peripheral nervous system (PNS) histopathological findings assessed at Days 23–25 for oxaliplatin and Day 21 for paclitaxel. * $p < 0.05$, * * $p < 0.01$, and * ** $p < 0.001$.

sciatic nerve, and an increase in multinucleolated neurons in the DRG in paclitaxel-treated mice compared to control mice. Moreover, paclitaxel-treated mice developed cold and mechanical allodynia, as well as mechanical hyperalgesia [29].

In patients, serum NF-L concentrations have been related to the oxaliplatin-related CIPN in colorectal cancer patients, and associated with the CIPN grade and electrophysiological disorders [5]. Similarly, serum NF-L concentrations have been related to the paclitaxel (± carboplatin)-related CIPN in breast, ovarian or endometrial cancer patients, and correlated with the scores of CIPN questionnaires [6,30,31], the CIPN grade [6,32,33], and the nerve conduction disorders [31,34].

The present study has been conducted using two different neurotoxic anticancer drugs well-known to promote CIPN in patients, characterized by paresthesia (tingling, numbness) and dysesthesia (evoked or spontaneous neuropathic pain) with a stocking and glove distribution that can last several years after the end of treatment and altering the health-related quality of life of patients [12,35]. In a metanalysis, CIPN prevalence has been estimated to 68.1% (57.7–78.4) in the first month after chemotherapy end, 60.0% (36.4–81.6) at 3 months and 30.0% (6.4–53.5) after 6 months [36]. More precisely, the prevalence of oxaliplatin-related CIPN ranged from 50% to 93.7% and paclitaxel-related CIPN from 59.2% to 92.8%.

4.1. Limitations of the study

NF-L is not specific of PNS lesions (i.e. peripheral neuropathy), but is a biomarker of neurological disorders since several studies reported a significant relation between the increase of NF-L concentrations in blood or CSF and CNS disorders [2]. However, in animals treated with the highest dose of paclitaxel, the neurological lesions with the highest grade were reported in PNS tissues in comparison to CNS tissues, and highly correlated with tissue exposure.

This study has been conducted only in male mice. However, blood

NF-L does not appear to be influenced by sex either in healthy individuals or patients with neurologic disease. Only studies on amyotrophic lateral sclerosis reported higher blood NF-L concentrations in females than in males, but this can be explained by a higher severity of the disease in females than in males [25]. In patients, oxaliplatin or paclitaxel-related CIPN are more severe in females than in males [35, 37], but results in animals (mice) for oxaliplatin-related CIPN are less clear with some specific alterations more pronounced in male animals than in female ones and conversely [38].

5. Conclusion

This study is one of the first to describe and to relate plasma NF-L concentrations with histopathological findings in mouse models of CIPN assessed with paclitaxel and with oxaliplatin. The changes showed a consistent correlation between plasma NF-L increases and histopathological findings in PNS tissues observed on Day 21 with paclitaxel, as well as the absence of modification in NF-L blood concentration was well correlated with the absence of histopathological findings with oxaliplatin. Regarding functional tests (FOB, Rotarod and cold plate), clinical observations were not able to highlight peripheral neuropathy induced by paclitaxel administrations.

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CRediT authorship contribution statement

Conceptualization: D.B., J.B., O.P., M.S. Data curation: D.B., O.P., M. S. Formal analysis: D.B., O.P., D.R., M.Q., C.B., Y.U., I.L., O.P., M.S. Funding acquisition: D.B., C.G., Y.U., I.L., W.E.G., L.M., C.G., L.D.C.M., O.P., M.S. Investigation: D.B., J.B., C.B., C.D., L.P., S.L., D.R., M.Q., A.H., S.H., Y.U., I.L., O.P., M.S. Methodology: D.B., J.B., C.B., O.P., M.S. Project administration: D.B., J.B., O.P., MS. Resources: D.B., J.B., Y.U., I. L., O.P., M.S. Software: D.B., J.B., Y.U., I.L., O.P., M.S. Supervision: D.B., J.B., O.P., M.S. Validation: D.B., J.B., O.P., M.S. Visualization: D.B., J.B., C.B., Y.U., I.L., W.E.G., O.P., M.S. Roles/Writing – original draft: D.B., J. B., O.P., M.S. Writing – review & editing: D.B., J.B., C.B., Y.U., I.L., W.E. G., L.D.C.M., O.P., M.S.

Declaration of Competing Interest

The authors declare no competing interests. Some authors (C.B., A. H., S.H., Y.H., W.E.G., O.P. and M.S.) are employees in the companies Sanofi, Merck and Boehringer Ingelheim, and may have access to stock/share options.

References

[1] L. Wieske, D. Smyth, M.P. Lunn, F. Eftimov, C.E. Teunissen, Fluid biomarkers for monitoring structural changes in polyneuropathies: their use in clinical practice and trials, Neurother. J. Am. Soc. Exp. Neurother. 18 (2021) 2351–2367, https://doi.org/10.1007/s13311-021-01136-0.

[2] M. Khalil, C.E. Teunissen, M. Otto, F. Piehl, M.P. Sormani, T. Gattringer, C. Barro, L. Kappos, M. Comabella, F. Fazekas, A. Petzold, K. Blennow, H. Zetterberg, J. Kuhle, Neurofilaments as biomarkers in neurological disorders, Nat. Rev. Neurol. 14 (2018) 577–589, https://doi.org/10.1038/s41582-018-0058-z.

[3] C. Meregalli, G. Fumagalli, P. Alberti, A. Canta, V.A. Carozzi, A. Chiorazzi, L. Monza, E. Pozzi, Å. Sandelius, K. Blennow, H. Zetterberg, P. Marmiroli, G. Cavaletti, Neurofilament light chain as disease biomarker in a rodent model of chemotherapy induced peripheral neuropathy, Exp. Neurol. 307 (2018) 129–132, https://doi.org/10.1016/j.expneurol.2018.06.005.

[4] C. Meregalli, G. Fumagalli, P. Alberti, A. Canta, A. Chiorazzi, L. Monza, E. Pozzi, V. A. Carozzi, K. Blennow, H. Zetterberg, G. Cavaletti, P. Marmiroli, Neurofilament light chain: a specific serum biomarker of axonal damage severity in rat models of Chemotherapy-Induced Peripheral Neurotoxicity, Arch. Toxicol. 94 (2020) 2517–2522, https://doi.org/10.1007/s00204-020-02755-w.

[5] S.-H. Kim, M.K. Choi, N.Y. Park, J.-W. Hyun, M.Y. Lee, H.J. Kim, S.K. Jung, Y. Cha, Serum neurofilament light chain levels as a biomarker of neuroaxonal injury and severity of oxaliplatin-induced peripheral neuropathy, Sci. Rep. 10 (2020), 7995, https://doi.org/10.1038/s41598-020-64511-5.

[6] S. Karteri, J. Bruna, A.A. Argyriou, S. Mariotto, R. Velasco, M. Alemany, F. Kalofonou, P. Alberti, A. Dinoto, D. Velissaris, A. Stradella, G. Cavaletti, S. Ferrari, H.P. Kalofonos, Prospectively assessing serum neurofilament light chain levels as a biomarker of paclitaxel-induced peripheral neurotoxicity in breast cancer patients, J. Peripher. Nerv. Syst. Jpns. 27 (2022) 166–174, https://doi.org/10.1111/jns.12493.

[7] C. Lange, E. Storkebaum, C.R. de Almodóvar, M. Dewerchin, P. Carmeliet, Vascular endothelial growth factor: a neurovascular target in neurological diseases, Nat. Rev. Neurol. 12 (2016) 439–454, https://doi.org/10.1038/nrneurol.2016.88.

[8] L. Micheli, C. Parisio, E. Lucarini, A. Vona, A. Toti, A. Pacini, T. Mello, S. Boccella, F. Ricciardi, S. Maione, G. Graziani, P.M. Lacal, P. Failli, C. Ghelardini, L. Di Cesare Mannelli, VEGF-A/VEGFR-1 signalling and chemotherapy-induced neuropathic pain: therapeutic potential of a novel anti-VEGFR-1 monoclonal antibody, J. Exp. Clin. Cancer Res. 40 (2021), 320, https://doi.org/10.1186/s13046-021-02127-x.

[9] D. Selvaraj, V. Gangadharan, C.W. Michalski, M. Kurejova, S. Stösser, K. Srivastava, M. Schweizerhof, J. Waltenberger, N. Ferrara, P. Heppenstall, M. Shibuya, H. G. Augustin, R. Kuner, A functional role for VEGFR1 expressed in peripheral sensory neurons in cancer pain, Cancer Cell 27 (2015) 780–796, https://doi.org/10.1016/j.ccell.2015.04.017.

[10] L. Di Cesare Mannelli, B. Tenci, L. Micheli, A. Vona, F. Corti, M. Zanardelli, A. Lapucci, A.M. Clemente, P. Failli, C. Ghelardini, Adipose-derived stem cells decrease pain in a rat model of oxaliplatin-induced neuropathy: Role of VEGF-A modulation, Neuropharmacology 131 (2018) 166–175, https://doi.org/10.1016/j.neuropharm.2017.12.020.

[11] S. Gadgil, M. Ergün, S.A. van den Heuvel, S.E. van der Wal, G.J. Scheffer, C. R. Hooijmans, A systematic summary and comparison of animal models for chemotherapy induced (peripheral) neuropathy (CIPN), PloS One 14 (2019), e0221787, https://doi.org/10.1371/journal.pone.0221787.

[12] N. Kerckhove, A. Collin, S. Condé, C. Chaleteix, D. Pezet, D. Balayssac, Long-term effects, pathophysiological mechanisms, and risk factors of chemotherapy-induced peripheral neuropathies: a comprehensive literature review, Front. Pharmacol. 8 (2017) 86, https://doi.org/10.3389/fphar.2017.00086.

[13] C. Kilkenny, W.J. Browne, I.C. Cuthill, M. Emerson, D.G. Altman, Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research, PLoS Biol. 8 (2010), e1000412, https://doi.org/10.1371/journal.pbio.1000412.

[14] S. Lamoine, M. Cumenal, D.A. Barriere, V. Pereira, M. Fereyrolles, L. Prival, J. Barbier, L. Boudieu, E. Brasset, B. Bertin, Y. Renaud, E. Miot-Noirault, M.-A. Civiale, D. Balayssac, Y. Aissouni, A. Eschalier, J. Busserolles, The Class I HDAC inhibitor, MS-275, prevents oxaliplatin-induced chronic neuropathy and potentiates its antiproliferative activity in mice, Int. J. Mol. Sci. 23 (2021) 98, https://doi.org/10.3390/ijms23010098.

[15] L. Poupon, S. Lamoine, V. Pereira, D.A. Barriere, S. Lolignier, F. Giraudet, Y. Aissouni, M. Meleine, L. Prival, D. Richard, N. Kerckhove, N. Authier, D. Balayssac, A. Eschalier, M. Lazdunski, J. Busserolles, Targeting the TREK-1 potassium channel via riluzole to eliminate the neuropathic and depressive-like effects of oxaliplatin, Neuropharmacology 140 (2018) 43–61, https://doi.org/10.1016/j.neuropharm.2018.07.026.

[16] J. Ruiz-Medina, A. Baulies, S.A. Bura, O. Valverde, Paclitaxel-induced neuropathic pain is age dependent and devolves on glial response, Eur. J. Pain. Lond. Engl. 17 (2013) 75–85, https://doi.org/10.1002/j.1532-2149.2012.00172.x.

[17] S.B. Smith, S.E. Crager, J.S. Mogil, Paclitaxel-induced neuropathic hypersensitivity in mice: responses in 10 inbred mouse strains, Life Sci. 74 (2004) 2593–2604, https://doi.org/10.1016/j.lfs.2004.01.002.

[18] V.C. Moser, Screening approaches to neurotoxicity: a functional observational battery, J. Am. Coll. Toxicol. 8 (1989) 85–93, https://doi.org/10.3109/10915818909009095.

[19] G.C. Haggerty, Strategy for and experience with neurotoxicity testing of new pharmaceuticals, J. Am. Coll. Toxicol. 10 (1991) 677–688, https://doi.org/10.3109/10915819109078660.

[20] N. Watzman, H. Barry, W.J. Kinnard, J.P. Buckley, Influence of certain parameters on the performance of mice on the rotarod, Arch. Int. Pharmacodyn. Ther. 169 (1967) 362–374.

[21] K. Sałat, A. Furgała, N. Malikowska-Racia, Searching for analgesic drug candidates alleviating oxaliplatin-induced cold hypersensitivity in mice, Chem. Biol. Drug Des. 93 (2019) 1061–1072, https://doi.org/10.1111/cbdd.13507.

[22] H. Manji, Drug-induced neuropathies, Handb. Clin. Neurol. 115 (2013) 729–742, https://doi.org/10.1016/B978-0-444-52902-2.00042-4.

[23] H.J. Koslik, A.H. Meskimen, B.A. Golomb, Physicians' experiences as patients with statin side effects: a case series, Drug Saf. - Case Rep. 4 (2017), 3, https://doi.org/10.1007/s40800-017-0045-0.

[24] Z. Alirezaei, M.H. Pourhanifeh, S. Borran, M. Nejati, H. Mirzaei, M.R. Hamblin, Neurofilament light chain as a biomarker, and correlation with magnetic resonance imaging in diagnosis of CNS-related disorders, Mol. Neurobiol. 57 (2020) 469–491, https://doi.org/10.1007/s12035-019-01698-3.

[25] C. Barro, T. Chitnis, H.L. Weiner, Blood neurofilament light: a critical review of its application to neurologic disease, Ann. Clin. Transl. Neurol. 7 (2020) 2508–2523, https://doi.org/10.1002/acn3.51234.

[26] N.J. Ashton, S. Janelidze, A. Al Khleifat, A. Leuzy, E.L. van der Ende, T.K. Karikari, A.L. Benedet, T.A. Pascoal, A. Lleó, L. Parnetti, D. Galimberti, L. Bonanni, A. Pilotto, A. Padovani, J. Lycke, L. Novakova, M. Axelsson, L. Velayudhan, G. D. Rabinovici, B. Miller, C. Pariante, N. Nikkheslat, S.M. Resnick, M. Thambisetty, M. Schöll, G. Fernández-Eulate, F.J. Gil-Bea, A. López de Munain, A. Al-Chalabi, P. Rosa-Neto, A. Strydom, P. Svenningsson, E. Stomrud, A. Santillo, D. Aarsland, J. C. van Swieten, S. Palmqvist, H. Zetterberg, K. Blennow, A. Hye, O. Hansson, A multicentre validation study of the diagnostic value of plasma neurofilament light, Nat. Commun. 12 (2021), 3400, https://doi.org/10.1038/s41467-021-23620-z.

[27] A. Ladang, S. Kovacs, L. Lengelé, M. Locquet, J.-Y. Reginster, O. Bruyère, E. Cavalier, Neurofilament light chain concentration in an aging population, Aging Clin. Exp. Res. 34 (2022) 331–339, https://doi.org/10.1007/s40520-021-02054-z.

[28] A. Manouchehrinia, F. Piehl, J. Hillert, J. Kuhle, L. Alfredsson, T. Olsson, I. Kockum, Confounding effect of blood volume and body mass index on blood neurofilament light chain levels, Ann. Clin. Transl. Neurol. 7 (2020) 139–143, https://doi.org/10.1002/acn3.50972.

[29] E. Lucarini, L. Micheli, R. Rajagopalan, C. Ciampi, J.J.V. Branca, A. Pacini, M. Leandri, P. Rajagopalan, C. Ghelardini, L. Di Cesare Mannelli, Broad-spectrum neuroprotection exerted by DDD-028 in a mouse model of chemotherapy-induced neuropathy, Pain (2023), https://doi.org/10.1097/j.pain.0000000000002963.

[30] P. Huehnchen, C. Schinke, N. Bangemann, A.D. Dordevic, J. Kern, S.K. Maierhof, L. Hew, L. Nolte, P. Körtvelyessy, J.C. Göpfert, K. Ruprecht, C.J. Somps, J.-U. Blohmer, J. Sehouli, M. Endres, W. Boehmerle, Neurofilament proteins as a potential biomarker in chemotherapy-induced polyneuropathy, JCI Insight 7 (2022), e154395, https://doi.org/10.1172/jci.insight.154395.

[31] S.-H. Kim, K.H. Kim, J.-W. Hyun, J.H. Kim, S.-S. Seo, H.J. Kim, S.-Y. Park, M. C. Lim, Blood neurofilament light chain as a biomarker for monitoring and predicting paclitaxel-induced peripheral neuropathy in patients with gynecological cancers, Front. Oncol. 12 (2022), 942960, https://doi.org/10.3389/fonc.2022.942960.

[32] C. Mortensen, K.D. Steffensen, E. Simonsen, K. Herskind, J.S. Madsen, D.A. Olsen, D.B. Iversen, T.K. Bergmann, A. Pottegård, T.B. Stage, Neurofilament light chain as a biomarker of axonal damage in sensory neurons and paclitaxel-induced peripheral neuropathy in patients with ovarian cancer, Pain 164 (2022) 1502–1511, https://doi.org/10.1097/j.pain.0000000000002840.

[33] B.L. Burgess, E. Cho, L. Honigberg, Neurofilament light as a predictive biomarker of unresolved chemotherapy-induced peripheral neuropathy in subjects receiving paclitaxel and carboplatin, Sci. Rep. 12 (2022), 15593, https://doi.org/10.1038/s41598-022-18716-5.

[34] R. Velasco, A.A. Argyriou, C. Marco, S. Mariotto, A. Stradella, J. Hernández, S. Pernas, S. Ferrari, J. Bruna, Serum neurofilament levels correlate with electrodiagnostic evidence of axonal loss in paclitaxel-induced peripheral neurotoxicity, J. Neurol. 270 (2023) 531–537, https://doi.org/10.1007/s00415-022-11377-4.

[35] M. Selvy, B. Pereira, N. Kerckhove, C. Gonneau, G. Feydel, C. Pétorin, A. Vimal-Baguet, S. Melnikov, S. Kullab, M. Hebbar, O. Bouché, F. Slimano, V. Bourgeois, V. Lebrun-Ly, F. Thuillier, T. Mazard, D. Tavan, K.E. Benmammar, B. Monange, M. Ramdani, D. Péré-Vergé, F. Huet-Penz, A. Bedjaoui, F. Genty, C. Leyronnas, J. Busserolles, S. Trevis, V. Pinon, D. Pezet, D. Balayssac, Long-term prevalence of sensory chemotherapy-induced peripheral neuropathy for 5 years after adjuvant FOLFOX chemotherapy to treat colorectal cancer: a multicenter cross-sectional study, J. Clin. Med. 9 (2020), E2400, https://doi.org/10.3390/jcm9082400.

[36] M. Seretny, G.L. Currie, E.S. Sena, S. Ramnarine, R. Grant, M.R. MacLeod, L. A. Colvin, M. Fallon, Incidence, prevalence, and predictors of chemotherapy-induced peripheral neuropathy: a systematic review and meta-analysis, Pain 155 (2014) 2461–2470, https://doi.org/10.1016/j.pain.2014.09.020.

[37] D. Mizrahi, S.B. Park, T. Li, H.C. Timmins, T. Trinh, K. Au, E. Battaglini, D. Wyld, R. D. Henderson, P. Grimison, H. Ke, P. Geelan-Small, J. Marker, B. Wall, D. Goldstein, Hemoglobin, body mass index, and age as risk factors for paclitaxel- and oxaliplatin-induced peripheral neuropathy, JAMA Netw. Open. 4 (2021), e2036695, https://doi.org/10.1001/jamanetworkopen.2020.36695.

[38] U.O. Warncke, W. Toma, J.A. Meade, A.J. Park, D.C. Thompson, M. Caillaud, J. W. Bigbee, C.D. Bryant, M.I. Damaj, Impact of dose, sex, and strain on oxaliplatin-induced peripheral neuropathy in mice, Front. Pain. Res. 2 (2021), 683168, https://doi.org/10.3389/fpain.2021.683168.