A Randomized, Double-Blinded, Placebo-Controlled Phase II Trial of Recombinant Human Leukemia Inhibitory Factor (rhuLIF, Emfilermin, AM424) to Prevent Chemotherapy-Induced Peripheral Neuropathy

Ian D. Davis,[1,3] Lynette Kiers,[2] Lachlan MacGregor,[2] Michael Quinn,[4] Joseph Arezzo,[5] Michael Green,[2,3,6] Mark Rosenthal,[2,3] Michael Chia,[7] Michael Michael,[3,8] Peter Bartley,[2] Leonie Harrison,[9] and Michael Daly[9]

[1]Austin Health, Heidelberg, Victoria, Australia; [2]Royal Melbourne Hospital and [3]Cancer Trials Australia, Inc., Parkville, Victoria, Australia; [4]Royal Women's Hospital, Carlton, Victoria, Australia; [5]Albert Einstein College of Medicine, New York, New York; [6]Western Hospital, Footscray, Victoria, Australia; [7]Burnside Hospital, Toorak Gardens, South Australia, Australia; [8]Peter MacCallum Cancer Centre, East Melbourne, Victoria, Australia; and [9]Amrad Operations Pty Ltd., Richmond, Victoria, Australia

ABSTRACT

Purpose: **To determine whether recombinant human leukemia inhibitory factor (rhuLIF, AM424, emfilermin) can prevent or ameliorate the development of chemotherapy-induced peripheral neuropathy (CIPN) after treatment with carboplatin (AUC 6) and paclitaxel (175 mg/m^2 over 3 hours).**

Experimental Design: **Randomized double-blind placebo-controlled phase II clinical trial. Eligible patients had solid tumors for which treatment with carboplatin/paclitaxel was appropriate. The primary end point was a standardized composite peripheral nerve electrophysiology (CPNE) score, based on nerve velocities and amplitudes, measured at baseline and after four cycles of chemotherapy. Secondary efficacy end points included CPNE score at last cycle and at exit evaluation, vibration perception threshold, H-reflex latency, symptom scores, and quantitative assessment of neurologic signs. Study drug was given s.c. daily for 7 days starting the day before chemotherapy. Patients were randomized to receive low-dose rhuLIF (2 μg/kg), high-dose rhuLIF (4 μg/kg), or placebo.**

Results: **Patients (n = 117) were randomized across seven neurology test centers. Thirty-six patients received low dose rhuLIF (2 μg/kg), 39 received high dose rhuLIF (4 μg/kg) and 42 received placebo. rhuLIF was well tolerated with 95% compliance and no adverse effects on quality of life. No differences between groups in CPNE or any of the individual neurologic testing variables were observed between baseline and cycle 4 or by the secondary efficacy variables.**

Conclusions: **rhuLIF is not effective in preventing CIPN caused by carboplatin and paclitaxel. CPNE is a reliable and valid tool that was sensitive to the onset and progression of CIPN.**

INTRODUCTION

Chemotherapy-induced peripheral neuropathy (CIPN) is a common and often dose-limiting side effect of several cytotoxic agents, including *Vinca* alkaloids, platinum analogues, and taxanes (1). For each of these drug classes, the severity of neurotoxicity is determined by the dose per cycle, the cumulative dose, and the dose intensity (2). Platinum compounds affect sensory fibers with little or no involvement of motor fibers (3). At doses between 50 and 75 mg/m^2 and a cumulative dose exceeding 300 mg/m^2, the incidence of neuropathy with cisplatin is 24% to 92% (2). Neurotoxicity due to carboplatin is considerably less than that seen with cisplatin, although when high doses of carboplatin are used, neurotoxicity is qualitatively similar to cisplatin (4).

Paclitaxel is commonly combined with a platinum-based drug and the combined neurotoxicity is greater than for each agent individually. Paclitaxel causes a distal axonal neuropathy affecting both sensory and motor fibers with a decrease in sensory action potential and combined muscle action potential amplitudes, with normal distal latencies and conduction velocities. The severity of paclitaxel neuropathy is related to both the single and cumulative dose. It usually occurs at cumulative doses in excess of 1,400 mg/m^2 (5). With lower doses of paclitaxel (135-200 mg/m^2) or with weekly regimens, neuropathy is less common (6).

Symmetrical polyneuropathy is the most common form of CIPN. Initial symptoms tend to be sensory in nature, symmetrical in distribution, progressive, and especially evident in the lower limbs. Paresthesia is the major early symptom, but numbness can usually also be detected if properly evaluated. Motor symptoms develop as neuropathy progresses. If pain develops, it can be severe and difficult to treat.

There are currently no active measures available to treat CIPN. After cessation of neurotoxic chemotherapy, CIPN tends towards improvement over a time frame of several months. In the case of cisplatin and carboplatin, the symptoms can worsen before they begin to improve ("coasting"). Long-term neuropathy can be found in 60% of patients surviving >5 years after their chemotherapy (7). Preventative strategies include dose reduction of neurotoxic agents or changing to nonneurotoxic

Received 8/17/04; revised 9/23/04; accepted 10/5/04.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Requests for reprints: Ian D. Davis, Austin Health Studley Road, Heidelberg, Victoria 3084, Australia. Phone: 61-3-9496-5726; Fax: 61-3-9457-6698; E-mail: Ian.Davis@ludwig.edu.au.

agents once neuropathy has become clinically evident. However, this may lead to a suboptimal clinical outcome. Amifostine (Ethyol) may reduce the incidence of severe CIPN, but its use is limited by cost, toxicity, and limited evidence of clinical efficacy for neuropathy (8).

Leukemia inhibitory factor (LIF; ref. 9) is a member of the gp130 group of cytokines that includes ciliary neurotrophic factor, interleukin-6, interleukin-11, cardiotrophin-1, and oncostatin-M. Signaling through the LIF receptor leads to changes in gene expression, stem cell differentiation, proliferation, and regeneration of many cell types, including myocytes and neurons (10). LIF expression is rapidly up-regulated in response to neural insult (11, 12). LIF has been shown to mediate a number of potential therapeutic effects in models of neurologic dysfunction or loss and there is also evidence to suggest that LIF is neuroprotective in models of peripheral neuropathy (Table 1). Recombinant human LIF (rhuLIF, emfilermin, AM424) has undergone formal preclinical development and has been evaluated in a phase I safety study in healthy volunteers, as well as in a phase 1b safety study in oncology patients (13). These studies show that rhuLIF is safe and generally well tolerated either as a single daily dose, given for 7 days before chemotherapy and 14 days post chemotherapy, or when given at a dose of 4 μg/kg thrice daily for a total of 7 days, commencing 24 hours before chemotherapy. Doses of 2 or 4 μg per kg per day by single s.c. injection in cancer patients showed evidence of biological effects with acceptable toxicity; these doses were chosen for the current study.

Attempts to develop neuroprotective agents have been hampered by the lack of consensus end points. Until recently, there have been few validated measures that have shown sensitivity and specificity for the detection of the onset of neuropathy and the monitoring of its progression. Several consensus panels including those of the American Neurologic Association (14), the American Diabetic Association (15), and the Peripheral Nerve Society (16) have supported nerve electrophysiology as part of an efficacy evaluation in clinical trials of peripheral neuropathy. In a multicenter study of diabetic neuropathy, nerve electrophysiology was shown to be highly reliable with a coefficient of variation of 3% to 4% across many study sites (17). Maximal nerve conduction velocities and peak sensory amplitudes provide a highly standardized, sensitive, and reliable index of the functional integrity of precisely these nerve fibers (18), and these measures have been used to trace the improvement or deterioration in neuropathy in previous multicenter clinical trials (19). Although electrophysiology is a "surrogate end point" for clinical progression, it provides an accurate measure of early change in nerve function and is especially useful for tracking induced neurotoxic deficits in multiple nerves. A recent study confirmed that Taxol-induced neuropathy in cancer patients was principally characterized by pronounced functional impairment in large diameter, heavily myelinated axons (i.e., A-β fibers; ref. 20). Change in electrophysiologic parameters in distal sensory nerves have been shown to correlate strongly ($r = -0.405$; $P = 0.001$) with the severity of CIPN in patients exposed to cisplatinin and paclitaxel-based chemotherapy (21). However, the electrophysiologic changes may occur earlier and are true parametric values. Therefore, in this trial, electrophysiology was selected as the primary indicator of the effect of rhuLIF in the prevention and/or amelioration of peripheral nerve damage following chemotherapy. In addition, efficacy was examined by a battery of direct clinical assessments of change in neuropathy status.

Table 1 Interactions of LIF with the nervous system

Effect	Reference
Neural LIF expression upregulated with nerve injury	(11, 12)
Muscle LIF expression upregulated with nerve injury	(25)
Neuroprotective effects in rat axotomy model	(11, 26, 27)
Prevents spinal motor neuron death after axotomy	(26)
Retards progression of motor neuron axonopathy in wobbler mouse	(28)
Prevents denervation induced muscle atrophy	(29, 30)
Prevents paclitaxel-induced large sensory nerve fiber loss in mice and prevents development of sensory impairment	(29, 31)

The objectives of the current study were to determine whether rhuLIF could reduce the decline in peripheral nerve function induced by multiple cycles of paclitaxel and carboplatin and to determine the safety of rhuLIF when given in conjunction with multiple cycles of chemotherapy. A once-daily dosing regimen was chosen for this study based on data derived from the previous phase 1b study (13).

MATERIALS AND METHODS

Study Protocol. The present study was a prospective, randomized phase II, double-blind, placebo-controlled, parallel group, dose-finding design, using standardized neurologic assessment end points. The primary objective was to determine whether rhuLIF will reduce, by at least 25%, the decline in peripheral nerve function induced by multiple cycles of paclitaxel and carboplatin. The end point for this objective was the change in a composite peripheral nerve electrophysiology (CPNE) score (described below). The secondary objective was to determine the safety of rhuLIF when given in conjunction with multiple cycles of chemotherapy. The end point for safety was toxicity according to National Cancer Institute Common Toxicity Criteria, and production of rhuLIF antibodies.

Patients. Patients were eligible if they satisfied the following criteria: solid tumors requiring chemotherapy and for which the combination of carboplatin/paclitaxel was appropriate; able to receive a minimum of four cycles of chemotherapy with carboplatin/paclitaxel; age ≥18 years; Eastern Cooperative Oncology Group performance status ≤1 (≤2 if ovarian cancer to allow for early postoperative therapy); absolute neutrophil count $\geq 1.5 \times 10^9$/L; hemoglobin ≥90 g/L; platelets $\geq 100 \times 10^9$/L; creatinine clearance ≥0.8 mL/s; bilirubin <1.5 × upper limit of normal; negative pregnancy test; and provision of written informed consent. Patients were excluded if any of the following applied: uncontrolled clinically significant medical condition; >1 prior course of chemotherapy for metastatic disease; any prior neurotoxic chemotherapy requiring modification due to neuropathic side effects; leptomeningeal disease; known allergy to

Escherichia coli–derived pharmaceutical; treatment with an investigational agent, hematopoietic growth factor or anticancer therapy within 30 days before initial dose of study drug; treatment with amifostine, interleukin-11, carbamazepine, sodium valproate, phenytoin or cimetidine (other than as premedication) within 30 days before the initial dose of study drug or at any time during the study period; prior irradiation to >30% of estimated red marrow volume; radiotherapy within 14 days before the initial dose of study drug (Amendment 2); uncontrolled brain metastases; and current alcoholism (Amendment 2).

Patients were also excluded if they had evidence of a preexisting polyneuropathy detected by the following variables: physical signs of neuropathy as determined by an Einstein Neurologic Examination score of ≥3 at screening; absent sural, ulnar, or median sensory nerve action potentials or peroneal motor nerve action potential (on nerve conduction studies) at screening; proximal asymmetrical neuropathy, plexopathies, acute or active mononeuropathies, the presence of which might obscure accurate assessment of severity of the chemotherapy-induced neuropathy, with the exception of carpal tunnel syndrome; previous bilateral sural nerve biopsies; presence of symptomatic neuropathy as defined by a total score of ≥12, or a score of ≥3 on any single item (confirmed by neurologist) for questions 1 to 16 inclusive in the chemotherapy-induced peripheral neuropathy survey 32 assessment (CIPNS-32; Amendment 2); and presence of pes cavus. The fields covered by the Einstein Neurologic Examination and CIPNS-32 are shown in Appendix 1.

Treatment Regimens. The study schema is shown in Fig. 1. After provision of signed informed consent, patients deemed eligible on initial screening underwent formal neurologic evaluation at a designated test center. Eligible patients who passed this screening then commenced treatment. Patients were randomized 2:2:1:1 to receive rhuLIF at one of two doses (2 or 4 μg per kg per day) or one of two volumes of placebo (sterile solution of sorbitol, polysorbate 80, and adjusted to pH 5.0 with citrate buffer). The randomization of placebo patients ensured that treatment blinding was intact. Treatment with study drug commenced on the day prior to chemotherapy. rhuLIF or placebo was given by daily s.c. injection after a premedication with acetaminophen 1 g orally, because such premedication had been shown previously to reduce rhuLIF toxicity (13). The first injection was given at hospital and was followed by a 2-hour period of observation. The second injection was given the following day, 2 hours before chemotherapy. Injections done outside of hospital were given by the patient, a nurse, or a suitable trained relative. Chemotherapy consisted of paclitaxel (175 mg/m^2 i.v. over 3 hours) followed by carboplatin (AUC 6 i.v. over 0.5-1 hour) and was scheduled every 21 days. Study drug was continued by daily s.c. injection for a total of seven consecutive doses per treatment cycle. Patients were to receive a minimum of four cycles and up to a maximum of six treatment cycles of chemotherapy. Following completion of up to six cycles of chemotherapy, patients continued to be observed for a further 3 months to determine whether rhuLIF was associated with any long-term or delayed effects on the progression of neuropathy.

Neurologic Assessments. Neurologic assessments were done before study drug administration, following the fourth cycle of treatment (primary end point), last cycle of chemotherapy (usually cycle 6), and at exit evaluation (3 months following last cycle). The primary efficacy variable was the change in CPNE score calculated at baseline and after four cycles of chemotherapy. Cycle 4 was chosen for this evaluation to ensure patients had received sufficient chemotherapy to elicit the predicted effect on the peripheral nerves but also to ensure a high percentage of randomized patients were still participants in the trial. The CPNE score includes antidromic maximal conduction velocities and amplitudes of three sensory nerves

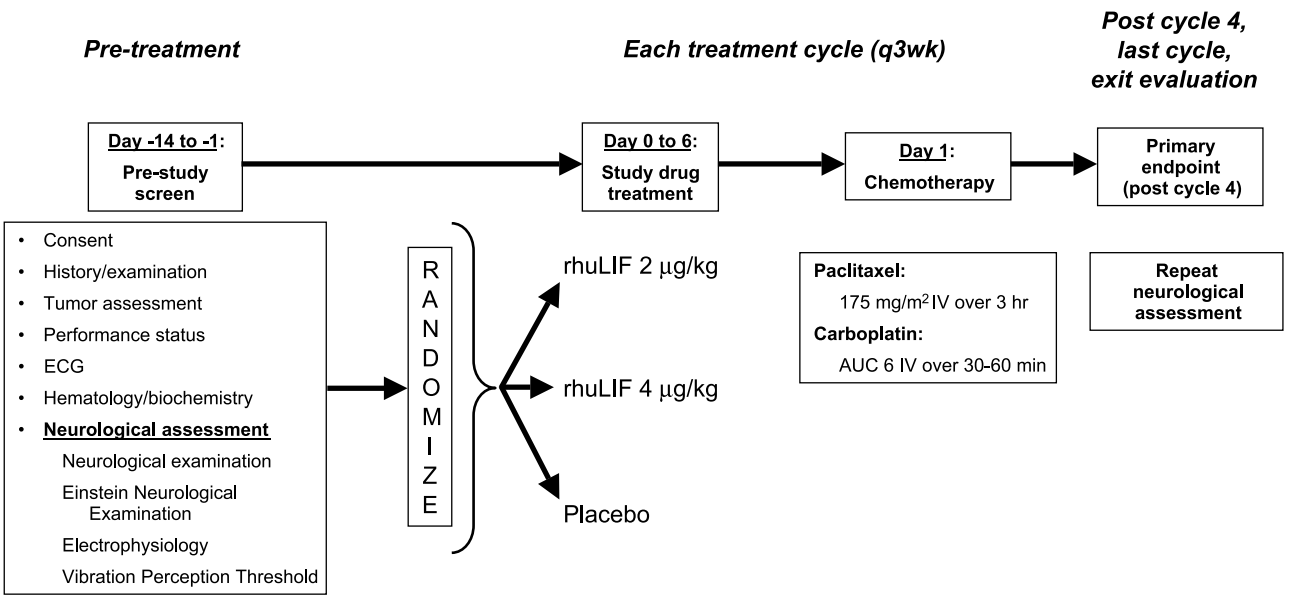

Fig. 1 Study schema.

(sural, median, and ulnar) and one motor nerve (peroneal). The CPNE score was calculated as follows. For each of the four nerves, the difference between values at two time points was determined, separately for amplitude and velocity, and ranked across all subjects (the subject with the maximum positive change received the highest rank and the average rank was taken for tied values). The rank scores were then summed across all eight measures (four velocities and four amplitudes) and scaled by the number of subjects to obtain composite scores that ranged between 0 and 1.

The change from baseline to the end of cycle 4 was considered the primary end point. If cycle 4 data were not available for a particular subject, data for the last cycle were used in its place for the intent-to-treat analysis. The primary per-protocol analysis included patients in whom valid neurologic data were available for cycle 3 as the final cycle. A separate per-protocol analysis was determined for each time point before analysis. If conduction was correctly measured but not detectable (physiologically absent), the value was set to that corresponding to the first percentile of the distribution of all valid measures of the same nerve in the present study. Values that were not recorded or were technically unsatisfactory were regarded as missing and were not included in further calculations. In addition to the CPNE, separate composite measures for velocity and amplitude, as well as change scores for each individual nerve and for the tibial H-reflex latency, were determined. Vibration perception thresholds were assessed from the ventral surface of the great toe on the nondominant side, using a Vibratron II device (Physitemp, Inc., Clifton, NJ) and a "two alternative forced choice" testing algorithm (22).

Change in clinical status was assessed using standard measures known to be affected by chemotherapy. Neurologic signs were explored using the Einstein Neurologic Examination (see Appendix 1). The neurologic examination focused on distal function but also included an evaluation of symmetry and progression along a distal to proximal gradient. Each modality was assigned a categorical value ranging from 0 (absence of deficit) to 3 (severe, bilateral deficit, involving proximal sites); the entire examination was scored on a range of 0 to 15. Change in symptoms was examined using a subset of questions from a chemotherapy induced peripheral neuropathy survey (CIPNS-32) that focused on distal sensory motor function. These end points were selected because of their sensitivity to CIPN and their direct clinical relevance. The severity of each symptom and the extent that it interfered with normal function were determined separately on a 0 to 4 scale, with a total score for symptoms ranging from 0 to 100. To our knowledge, there is no standard, reliable, and validated set of measures for quality of life or neurologic signs and symptoms in patients with CIPN. The CPNE score was to be validated in this study by correlation with conventional neurologic assessments and quality-of-life measures.

The study was conducted in accordance with the International Conference on Harmonization Guideline for Good Clinical Practice (CPMP/ICH/135/95, January 1997), the Declaration of Helsinki (Amendment of Somerset West, South Africa, October, 1996), National Health and Medical Research Council National Statement on Ethical Conduct in Research involving Humans (June 1999), and the laws and regulations of the countries in which the protocol was being conducted; whichever afforded greater protection to the individual. Amrad Operations Pty Ltd. sponsored the trial. The study was conducted under the Australian Clinical Trial Notification Scheme 21 July 1999 and a US IND (BB-IND 9198, 13 November 2000), and the trial safety was monitored by an independent Drug Safety Monitoring Board. The trial was approved by the Human Research Ethics Committees of the hospitals involved, and all patients provided written informed consent prior to any study-specific procedures.

Statistical Analysis. Comparisons across treatment groups for evidence of efficacy for each neurologic end point was determined using an analysis of covariance (ANCOVA) procedures that controlled for covariates such as sex, age, and tumor type. The dose-by-treatment term was used to estimate and test for a dose-response effect. Continuous variables were summarized using number of observations, minimum, maximum, median, and lower and upper quartiles. Summary statistics for continuous variables analyzed with an ANOVA model included least-square means and their SEs. Summary statistics for categorical variables included frequency counts and percentages. Unless otherwise stated, all percentages were calculated using the sample size for the relevant study population as the denominator. All statistical tests were considered exploratory, two sided, and done at the 5% significance level.

The initial sample size calculations were based on the power required for a two-group study to detect a difference between placebo and active treatment of a 1 to 2 m/s change from baseline in conduction velocity after six cycles of chemotherapy. This change is the smallest difference that is considered clinically meaningful based on discussions with regulatory agencies in the United States and Europe. Approximately 40 patients per treatment arm are required to have >90% statistical power to detect a 1.0 m/s difference in maximal conduction velocity between placebo and active treatment using an α level of 0.05. The proposed four-group study aimed to recruit 180 subjects to obtain 120 evaluable subjects.

Review of blinded patient data during the course of the study showed that the SD for the change from baseline to the end of cycle 4 in the conduction velocity for the median nerve was likely to be about 1.5 m/s. Review of the statistical methods changed the focus of the primary analysis from a comparison between the four treatment groups to a more specific hypothesis comparing rhuLIF and placebo. In addition, the power was changed to 80%. These changes in assumptions for the power calculation reduced the required number of evaluable patients to a total of 80 patients, which entails recruitment of 120 patients.

All subjects who received at least one dose of study drug were included in the safety analysis. Subjects were included in the intent-to-treat efficacy analysis if they received at least one dose of study drug (i.e., were included in the safety analysis) and had a record of baseline and post-baseline efficacy data. Subjects were also included in a per-protocol analysis if they were not in violation of the major protocol criteria, completed cycle 4 assessments and had baseline and end cycle 4 efficacy data. Subjects with baseline and end cycle 3 efficacy data were also included in the per-protocol analysis if not in violation of major protocol criteria. An independent evaluation committee assessed eligibility for the per-protocol analysis after examining blinded

data and before the study blind was broken. Patients who were not compliant with study medication were withdrawn from per-protocol analysis as protocol violators.

The per-protocol analysis was considered to be the primary analysis. Where appropriate, exploratory analysis of efficacy data was done for subgroups of the study sample (such as groups defined by number of chemotherapy cycles and the cumulative dose of chemotherapy received).

Health-related quality-of-life outcomes were included as an exploratory end point. The questionnaire included a validated general cancer survey (European Organization for Research and Treatment of Cancer QLQ-C30; ref. 23) and a CIPNS-32 (see Appendix 1) developed specifically for this study. CIPNS-32 included 32 questions referring to specific symptoms or problems in the hand, foot, sensory function, and specific activities that might be affected by peripheral neuropathy. The objectives of the quality-of-life analyses were to (a) establish the scale structure and evaluate the psychometric properties of CIPNS-32; (b) confirm the psychometric properties of European Organization for Research and Treatment of Cancer QLQ-C30 in this patient population; and (c) explore the impact of rhuLIF on overall functioning and well-being (European Organization for Research and Treatment of Cancer QLQ-C30) and symptoms, problems, and activities associated with peripheral neuropathies (CIPNS-32).

RESULTS

Patients. The patient characteristics are shown in Table 2. Patients (n = 117) were randomized across seven neurology centers. In general, the treatment groups remained comparable in relation to primary diagnosis. Twenty-three subjects (64%) in the 2 μg/kg rhuLIF group, 26 subjects (67%) in the 4 μg/kg rhuLIF group, and 30 subjects (72%) in the placebo groups were treated per-protocol up to cycle 4. Thirty-three percent of study subjects (n = 12) in the rhuLIF low dose group completed both the cycle 6 evaluation and the exit cycle evaluation (study completion), whereas 46% (n = 18) of patients in the rhuLIF high dose group, 27% (n = 6) of patients in the placebo low-dose group, and 45% (n = 9) of patients in the placebo high-dose group completed both cycle 6 and exit cycle evaluations. The exit evaluation was completed by 64% of patients in the low-dose rhuLIF group, 74% in the rhuLIF high-dose group, 36% in the placebo low-dose group, and 60% in the placebo high-dose group.

Table 2 Patient characteristics

Total patients	117
rhuLIF, 2 μg/kg	36
rhuLIF, 4 μg/kg	39
Placebo	42
Male/female	64:53
Median age (range)	58 (22-78)
Diagnosis	
Non–small-cell lung cancer	36
Ovary	25
Adenocarcinoma of unknown primary	13
Small cell lung cancer	8
Mesothelioma	7
Bladder	5
Other	23

Table 3 Adverse events with significant difference between treatment groups (Fisher's exact test, $P < 0.05$)

Adverse event	rhuLIF, 2 μg per kg per d (n = 36), %	rhuLIF, 4 μg per kg per d (n = 39), %	Combined placebo (n = 42), %
Injection site reaction (not otherwise specified)	28	36	5
Injection site erythema	11	23	0
Rigors	8	28	2
Vomiting	33	44	52

NOTE. Only events reported by ≥13 patients are listed.

rhuLIF Is Well Tolerated. In general, treatment with rhuLIF was well tolerated, with at least 95% compliance with rhuLIF treatment. All patients reported at least one adverse event, usually related to the chemotherapy or to the underlying disease. Adverse events with statistically significant differences between treatment groups are listed in Table 3. The most common adverse events related to rhuLIF were injection site reactions and rigors. Interestingly, vomiting occurred significantly less frequently in the rhuLIF treatment groups than with placebo.

Only five subjects receiving rhuLIF experienced serious adverse events thought to be possibly, probably, or definitely related to study drug. These serious adverse events included lightheadedness, rigors/chills, myocardial ischemia, and hypotension. No significant differences were observed between treatment groups in the proportion of subjects suffering serious adverse events or in the proportion of subjects withdrawing from the study. No adverse effects of rhuLIF on tumor progression or response to chemotherapy were observed. Eight patients died during the course of the study (3 receiving rhuLIF, 5 receiving placebo); in no case was death attributable to study drug treatment.

The European Organization for Research and Treatment of Cancer QLQ-30 and CIPNS-32 quality-of-life measures used were assessed in 90 patients in the intention-to-treat population and were shown to have good reliability and validity in the study population. There were no significant differences between the treatment groups from baseline to cycle 4, baseline to final cycle, or baseline to exit evaluation. Patients receiving rhuLIF reported significantly greater improvements in global health status and significantly greater reductions in levels of fatigue relative to patients receiving placebo. Quality of life measures correlated with CPNE scores.

Deficits Associated with CIPN. The sensitivity of the neurologic end points to CIPN was confirmed. Each neurologic end point showed deficits for the population as a whole following exposure to carboplatin/paclitaxel. The CPNE measure, which included four nerve velocities and four nerve amplitudes, showed a small but consistent decrement (i.e., higher values on a 0-1 scale) between baseline and the end of cycle 4 (Fig. 2*A*). For the velocity of the median nerve, the mean deficit was 2.2 m/s, which was consistent with the assumptions used for sample size calculation. Thus, neuropathy did occur during the first four cycles of chemotherapy and was clearly detectable by our primary measures. The magnitude of change in CPNE was only slightly less than that predicted at the study onset.

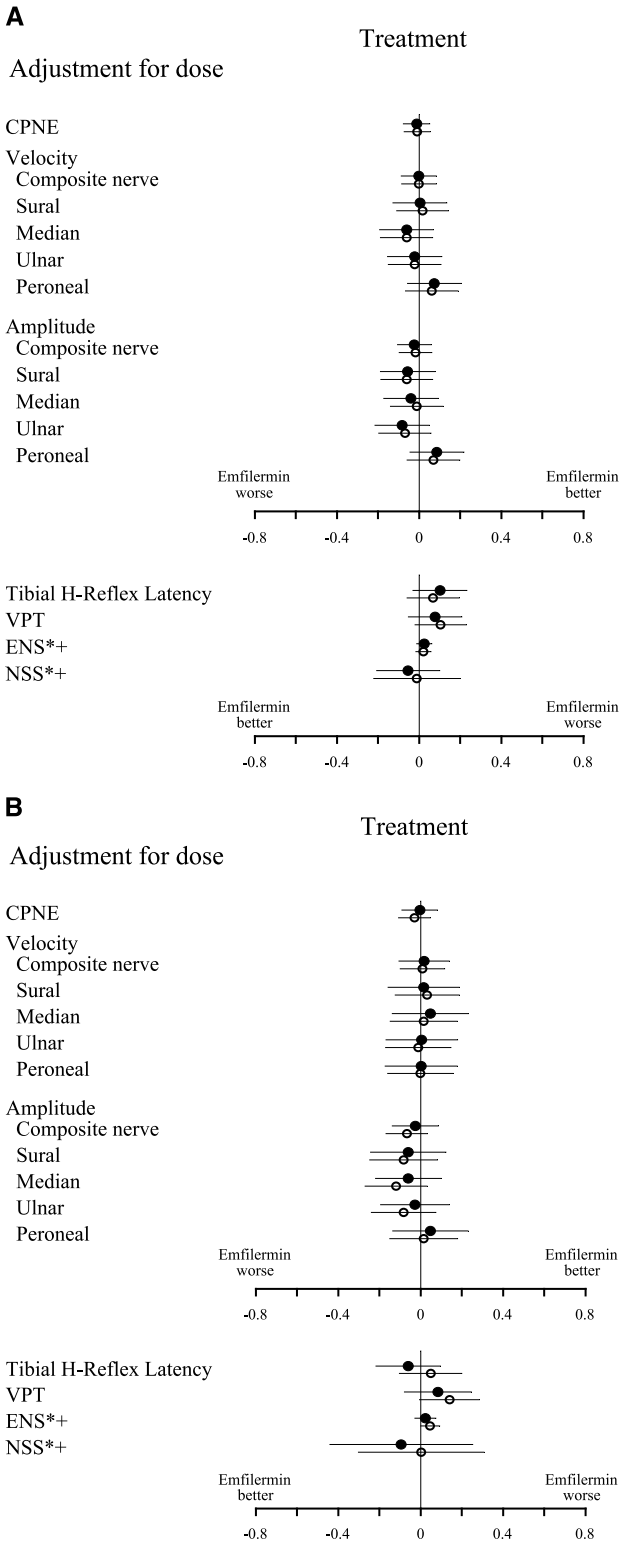

Fig. 2 Change in CPNE and secondary variable results. *A,* baseline to treatment cycle 4. *B,* baseline to treatment cycle 5 or 6. Protocol population (●), intent-to-treat population (○). Point estimates and 95% confidence intervals are derived from ANOVA and analysis of covariance modelling. *, scores have been scaled by a factor of 20. +, includes baseline score as additional covariate.

These results are consistent with a recent report of the monitoring of CIPN in cancer patients receiving paclitaxel (175 mg/m^2) + cisplatin (75 mg/m^2) with or without ifosfamide (5 mg/m^2; ref. 21). That study used a composite neurologic test battery similar to the Einstein Neurologic Examination to document a deterioration in distal sensory function and showed strong correlation of the clinical assessment of deficits with toxicity and electrophysiologic measures. In the present study, each of the secondary clinical and electrophysiologic end points also confirmed the development of neuropathy. Quantitative sensory testing of vibration threshold at the great toe was strongly altered by chemotherapy, providing a second "objective" measure of the developing neuropathy.

Neurologic deterioration was already evident by cycle 4, and generally declined further for those patients whose last cycle was assessed following cycle 5 or 6. In the majority of patients, the neurologic variables had substantially returned to baseline at the exit evaluation, confirming substantial recovery from the magnitude and nature of the CIPN induced by the chemotherapy program used in this study. The exit evaluation data also suggests that there was little or no "coasting," defined as a worsening of the neurotoxic effect following cessation of treatment.

Somewhat surprisingly, the peroneal motor amplitude and velocity declined by a similar degree to the sensory nerves. In the per-protocol population, for all patient groups combined, the velocity in the peroneal nerve declined by 2.3 m/s at cycle 4, comparable with the 2.2 m/s decline in median sensory velocity.

The decline in electrophysiologic variables between baseline and cycle 4 correlated well with the change in clinical neurologic assessments. Analyses of the signs and symptoms were based on raw changes from baseline. These analyses were conducted adjusting for the covariates of age, gender, tumor type, and baseline Einstein neurologic examination and neuropathy symptom scores. The mean neurologic examination score increased (indicating a worsening of neuropathy) by 1.68 points for all patients combined, whereas the mean score for symptoms increased by 2.85 units. These values had returned to baseline by the exit evaluation.

rhuLIF Does Not Protect against CIPN Caused by Carboplatin/Paclitaxel. CPNE was shown to be a sensitive measure of subclinical CIPN and correlated well with quality-of-life measurements (data not shown). Similar CPNE changes were seen in all treatment groups. No significant differences were seen in CPNE between baseline and cycle 4, last cycle, or exit evaluation, for any treatment group, either per-protocol or by intent-to-treat (Figs. 2*A*, *B* and 3). In addition, no significant differences were seen for the secondary efficacy variables. The proportion of subjects suffering from clinically evident neuropathy at cycle 4 was not statistically different between the two treatment groups.

For the change from baseline to the end of cycle 4 in the velocity of the median nerve, placebo-treated study subjects had an average of 0.8 m/s better nerve conduction than rhuLIF-treated patients. The 95% confidence interval for this difference was 2.7 m/s better conduction to 1.2 m/s worse conduction with placebo than with rhuLIF. For the sample size calculation, a difference of >1 m/s in favor of rhuLIF was to

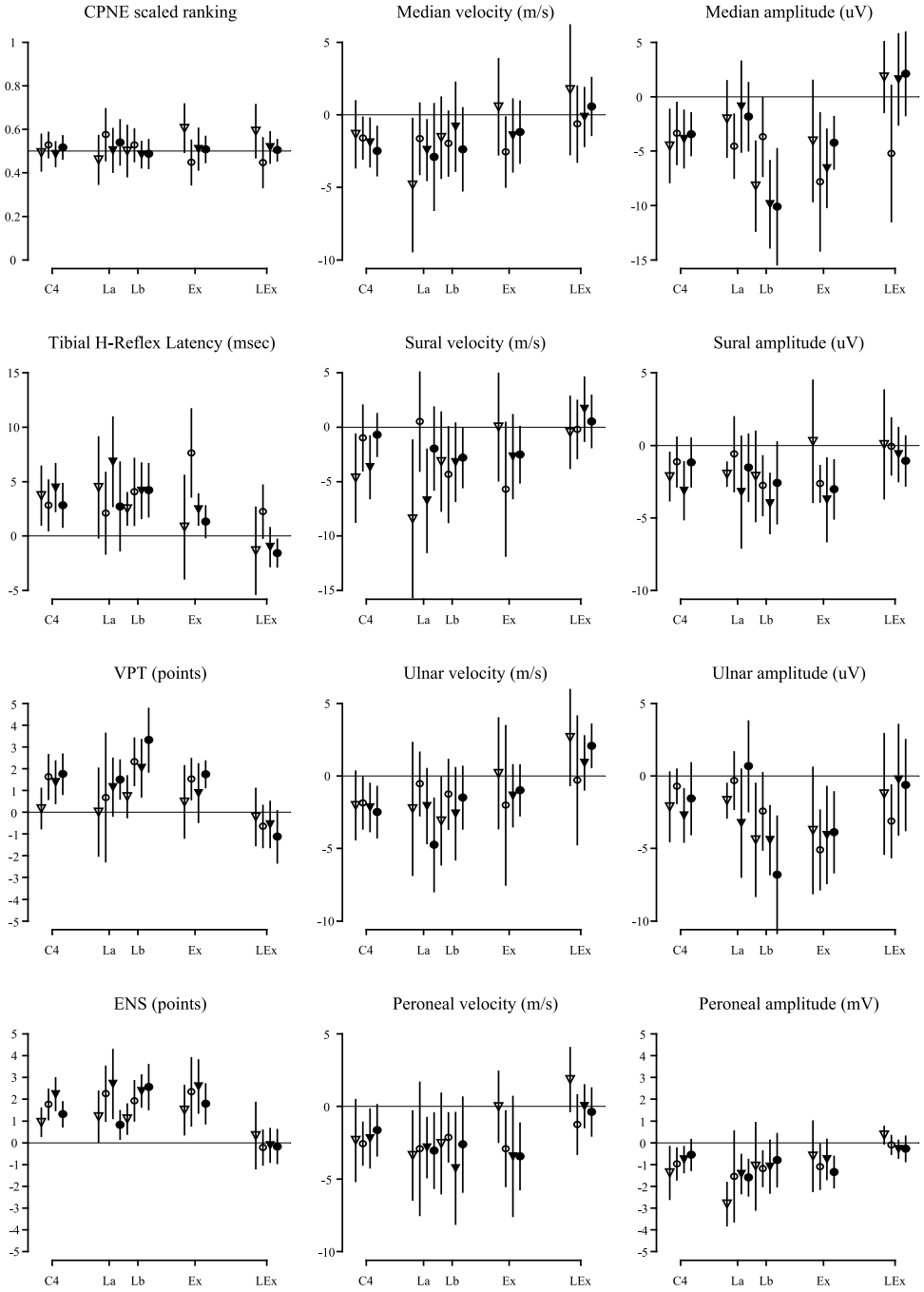

Fig. 3 Change in efficacy endpoints from baseline of results for individual treatment groups, various time points, and separate neurological endpoints. y-axis, units of change from baseline. ○ and ▽, placebo (two dose levels); ▼, rhuLIF 2 μg/kg; ●, rhuLIF 4 μg/kg. C4, cycle 4; La, last cycle (1-4); Lb, last cycle (5-6); Ex, exit cycle; Lex, last cycle to exit cycle; VPT, vibration perception threshold; ENS, Einstein neurologic score. *Bars,* ±2 SE.

be considered clinically relevant. Thus, the confidence interval, although wide, suggests that placebo-treated patients are unlikely to have clinically significantly less neuropathy than rhuLIF patients.

DISCUSSION

The principal hypothesis, that rhuLIF could protect against CIPN induced by carboplatin/paclitaxel, was not able to be proved using this study design, as there was no evidence that rhuLIF prevented, delayed, or diminished the magnitude of CIPN. The CPNE is expected to have greater power than any of its individual components. Our results indicate that the CPNE score is sufficiently sensitive to detect even subclinical levels of neurotoxicity and it is therefore unlikely that a small benefit of rhuLIF has been missed. This means that this study was likely to have found a clinically relevant difference between placebo and rhuLIF-treated study subjects, had one existed. It is possible that the design of the study meant that such a difference could not be detected, particularly with the chemotherapy regimen used or the scheduling or route of administration of rhuLIF. However, based on these results, rhuLIF is not planned to undergo further

development as an agent for prevention of CIPN or other neurologic diseases.

The combination of carboplatin/paclitaxel was chosen for this trial as it is a commonly used regimen with activity in a variety of cancers. Cisplatin/paclitaxel is also a highly active combination but is less commonly used because of the higher incidence of neurotoxicity (1). CIPN did occur with carboplatin/paclitaxel and was evident by cycle 4 both electrophysiologically and on clinical scores. With the chemotherapy regimen used in this trial, the majority of abnormalities had resolved by the exit evaluation (3 months after the last dose of chemotherapy). It is possible that rhuLIF may be of benefit in the setting of more neurotoxic chemotherapy where clinically evident neuropathy rather than subclinical effects might be observed. We could not justify this approach on ethical grounds, because although use of higher doses or alternative regimens could potentially afford greater therapeutic benefit against the cancer, this would probably be associated with more severe and permanent neurotoxic effects. The identification of an effective neuroprotective toxic agent that could either allow an increase in the total dose of chemotherapy or improve the quality of life for patients during treatment is still a cardinal goal of clinical oncology.

There is no clear explanation for the lack of efficacy of rhuLIF in this clinical setting, particularly given the encouraging preclinical data supporting the use of LIF as a neuroprotective agent. The available animal models may not have been sufficiently predictive of the human clinical situation. There may have also been too little consideration of differences in the effects of this class of cytokines in developing neurons versus fully mature cells. It is also possible that the route of administration of rhuLIF did not provide adequate activity at the key target sites (i.e., distal sensory nerve) or that the dose was simply insufficient. Alternatively, the system may compensate for the administration of exogenous neurotrophins and down-regulate endogenous LIF, as well as other related cytokines. Finally, the disease process treated by the chemotherapy may have affected the distribution and interaction of various cytokines, including the administered rhuLIF. A recent review (24) summarizes similar problems encountered in a multicenter clinical trial of nerve growth factor.

This study has shown that sensitive and quantitative neurologic assessments can be done in cancer patients receiving neurotoxic chemotherapy. The CPNE score has been validated against conventional neurologic criteria, including clinical assessment, neurophysiologic testing, and quality-of-life measure. This method can be standardized across multiple testing centers and provides a valuable system for assessment of subclinical neuropathy and for testing of future neuroprotective agents.

ACKNOWLEDGMENTS

We thank the work contributed by all of the investigators and study coordinators at the participating clinical sites listed alphabetically as follows. New South Wales: Albury Wodonga Neurology, Mater Misericordiae Hospital, Murray Valley Private Hospital, Nepean Hospital, Royal North Shore Hospital, Westmead Hospital. South Australia: Burnside Hospital, Queen Elizabeth Hospital, and Royal Adelaide Hospital. Queensland: Wesley Hospital. Victoria: Austin Health, Box Hill Hospital, Mercy Hospital for Women, Monash Medical Centre Peter MacCallum Cancer Centre, Royal Melbourne Hospital, Royal Women's Hospital, and Western Hospital. United States of America: Volunteer Research Group University of Tennessee Medical Center.

APPENDIX 1: CIPNS-32

Symptoms: Hand
Paresthesia
Numbness
Altered perception of texture
Warm perception
Cold perception
Allodynia
Pain
Object identification
Involuntary distal movements
Symptoms: Foot
Paresthesia
Numbness
Altered perception of texture
Warm perception
Cold perception
Allodynia
Pain
Involuntary distal movements
Central nervous system deficits
Blurred vision
Tinnitus
Loss of hearing
Unsteady posture
Activity deficits
Dressing
Washing, drying or brushing hair
Brushing teeth
Opening a jar
Cutting food
Writing
Using a key
Carrying heavy object
Walking
Climbing stairs
Standing for long periods

NOTE. Each item scored on a 0 to 4 scale for the magnitude of the symptom or deficit and on a 0 to 5 scale for "how bothered were you" by the problem.

Einstein Neurologic Examination	
Modality tested	Method
Light touch	Monofilaments (Semmes-Weinstein)
Cold detection	NTE-2a Thermal Tester (Physitemp, Inc.)
Sharp perception	Neurotips (Owen Mumford)
Distal strength	Finger spread, great toe extension, ankle dorsiflexion
Reflexes	Biceps brachii, quadriceps femoris, Achilles tendon

NOTE. Each item scored on a 0 to 5 scale, considering magnitude, symmetry, and distal-to-proximal gradient of the deficit (if present).

REFERENCES

1. Chaudhry V, Rowinsky EK, Sartorius SE, Donehower RC, Cornblath DR. Peripheral neuropathy from taxol and cisplatin combination chemotherapy: clinical and electrophysiological studies. Ann Neurol 1994;35:304–11.

2. Hilkens PH, ven den Bent MJ. Chemotherapy-induced peripheral neuropathy. J Peripher Nerv Syst 1997;2:350–61.

3. Pace A, Bove L, Aloe A, et al. Paclitaxel neurotoxicity: clinical and neurophysiological study of 23 patients. Ital J Neurol Sci 1997;18:73–9.

4. Cavaletti G, Bogliun G, Zincone A, et al. Neuro- and ototoxicity of high-dose carboplatin treatment in poor prognosis ovarian cancer patients. Anticancer Res 1998;18:3797–802.

5. van Gerven JM, Moll JW, van den Bent MJ, et al. Paclitaxel (Taxol) induces cumulative mild neurotoxicity. Eur J Cancer 1994;30A:1074–7.

6. Markman M. Management of toxicities associated with the administration of taxanes. Expert Opin Drug Saf 2003;2:141–6.

7. van der Hoop RG, van der Burg ME, ten Bokkel Huinink WW, van Houwelingen C, Neijt JP. Incidence of neuropathy in 395 patients with ovarian cancer treated with or without cisplatin. Cancer 1990;66: 1697–702.

8. Openshaw H, Beamon K, Synold TW, et al. Neurophysiological study of peripheral neuropathy after high-dose paclitaxel: lack of neuroprotective effect of amifostine. Clin Cancer Res 2004;10:461–7.

9. Gearing DP, Gough NM, King JA, et al. Molecular cloning and expression of cDNA encoding a murine myeloid leukaemia inhibitory factor (LIF). EMBO J 1987;6:3995–4002.

10. Hilton DJ. LIF: lots of interesting functions. Trends Biochem Sci 1992;17:72–6.

11. Thoenen H. The changing scene of neurotrophic factors. Trends Neurosci 1991;14:165–70.

12. Curtis R, Scherer SS, Somogyi R, et al. Retrograde axonal transport of LIF is increased by peripheral nerve injury: correlation with increased LIF expression in distal nerve. Neuron 1994;12:191–204.

13. Gunawardana DH, Basser RL, Davis ID, et al. A phase I study of recombinant human leukemia inhibitory factor in patients with advanced cancer. Clin Cancer Res 2003;9:2056–65.

14. Report and recommendations of the San Antonio conference on diabetic neuropathy. Ann Neurol 1988;24:99–104.

15. Proceedings of a consensus development conference on standardized measures in diabetic neuropathy. Summary and recommendations. Muscle Nerve 1992;15:1167–70.

16. Diabetic polyneuropathy in controlled clinical trials: consensus report of the Peripheral Nerve Society. Ann Neurol 1995;38:478–82.

17. Bril V, Ellison R, Ngo M, et al. Electrophysiological monitoring in clinical trials. Roche Neuropathy Study Group. Muscle Nerve 1998;21: 1368–73.

18. Arezzo JC, Zotova E. Electrophysiologic measures of diabetic neuropathy: mechanism and meaning. Int Rev Neurobiol 2002;50: 229–55.

19. Greene DA, Arezzo JC, Brown MB. Effect of aldose reductase inhibition on nerve conduction and morphometry in diabetic neuropathy. Zenarestat Study Group. Neurology 1999;53:580–91.

20. Dougherty PM, Cata JP, Cordella JV, Burton A, Weng HR. Taxol-induced sensory disturbance is characterized by preferential impairment of myelinated fiber function in cancer patients. Pain 2004; 109:132–42.

21. Cavaletti G, Bogliun G, Marzorati L, et al. Grading of chemotherapy-induced peripheral neurotoxicity using the Total Neuropathy Scale. Neurology 2003;61:1297–300.

22. Shy ME, Frohman EM, So YT, et al. Quantitative sensory testing: report of the Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology. Neurology 2003;60:898–904.

23. Aaronson NK, Ahmedzai S, Bergman B, et al. The European Organization for Research and Treatment of Cancer QLQ-C30: a quality-of-life instrument for use in international clinical trials in oncology. J Natl Cancer Inst 1993;85:365–76.

24. Apfel SC. Nerve growth factor for the treatment of diabetic neuropathy: what went wrong, what went right, and what does the future hold? Int Rev Neurobiol 2002;50:393–413.

25. Kurek JB, Austin L, Cheema SS, Bartlett PF, Murphy M. Up-regulation of leukaemia inhibitory factor and interleukin-6 in transected sciatic nerve and muscle following denervation. Neuromuscul Disord 1996;6:105–14.

26. Ikeda K, Iwasaki Y, Shiojima T, Kinoshita M. Neuroprotective effect of various cytokines on developing spinal motoneurons following axotomy. J Neurol Sci 1996;135:109–13.

27. Cheema SS, Richards L, Murphy M, Bartlett PF. Leukemia inhibitory factor prevents the death of axotomised sensory neurons in the dorsal root ganglia of the neonatal rat. J Neurosci Res 1994;37: 213–8.

28. Ikeda K, Iwasaki Y, Tagaya N, Shiojima T, Kinoshita M. Neuroprotective effect of cholinergic differentiation factor/leukemia inhibitory factor on wobbler murine motor neuron disease. Muscle Nerve 1995;18: 1344–7.

29. Kilpatrick TJ, Phan S, Reardon K, Lopes EC, Cheema SS. Leukaemia inhibitory factor abrogates paclitaxel-induced axonal atrophy in the Wistar rat. Brain Res 2001;911:163–7.

30. Finkelstein DI, Bartlett PF, Horne MK, Cheema SS. Leukemia inhibitory factor is a myotrophic and neurotrophic agent that enhances the reinnervation of muscle in the rat. J Neurosci Res 1996;46:122–8.

31. Boyle FM, Beatson C, Monk R, Grant SL, Kurek JB. The experimental neuroprotectant leukaemia inhibitory factor (LIF) does not compromise antitumour activity of paclitaxel, cisplatin and carboplatin. Cancer Chemother Pharmacol 2001;48:429–34.

Clinical Cancer Research

A Randomized, Double-Blinded, Placebo-Controlled Phase II Trial of Recombinant Human Leukemia Inhibitory Factor (rhuLIF, Emfilermin, AM424) to Prevent Chemotherapy-Induced Peripheral Neuropathy

Ian D. Davis, Lynette Kiers, Lachlan MacGregor, et al.

Clin Cancer Res 2005;11:1890-1898.