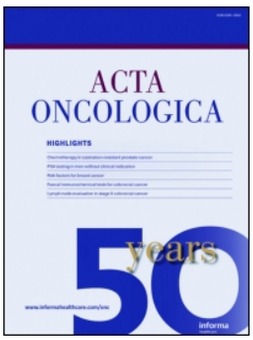

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ORIGINAL ARTICLE

Persistent prevention of oxaliplatin-induced peripheral neuropathy using calmangafodipir (PledOx®): a placebo-controlled randomised phase II study (PLIANT)

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ABSTRACT

Purpose: Oxaliplatin causes disabling acute and chronic peripheral neuropathy. We explored the preventive effects of calmangafodipir, mimicking the mitochondrial enzyme manganese superoxide dismutase, thereby protecting cells from oxidative stress, in a placebo-controlled, double-blinded randomised phase II study (ClinicalTrials.gov.NCT01619423) in patients with metastatic colorectal cancer (mCRC).

Patient and methods: mCRC patients treated with modified FOLFOX-6 (folinic acid 200 mg/m^2, 5-fluorouracil bolus 400 mg/m^2, oxaliplatin 85 mg/m^2 and 5-fluorouracil 2400 mg/m^2 continuous infusion for 46 h) every fortnight for 8 cycles in first or second line were eligible. Calmangafodipir was given in a phase I dose-finding and in a phase II placebo-controlled study, as a 5-min infusion 10 min prior to oxaliplatin. Neurotoxicity was evaluated by the physician using the Oxaliplatin Sanofi Specific Scale and by the patient using the cold allodynia test and the Leonard scale.

Results: Eleven patients were included in phase I without any detectable toxicity to calmangafodipir. In the phase II study, 173 patients were randomised to placebo ($n = 60$), calmangafodipir 2 μmol/kg ($n = 57$) and calmangafodipir 5 μmol/kg ($n = 45$, initially 10 μmol/kg, $n = 11$). Calmangafodipir-treated patients (all three doses pooled) had less physician graded neurotoxicity (odds ratio (90% confidence interval one-sided upper level) 0.62(1.15), $p = .16$), significantly less problems with cold allodynia (mean 1.6 versus 2.3, $p < .05$) and significantly fewer sensory symptoms in the Leonard scale (cycle 1–8 mean 1.9 versus 3.0, $p < .05$ and during follow-up after 3 and 6 months, mean 3.5 versus 7.3, $p < .01$). Response rate, progression-free and overall survival did not differ among groups.

Conclusions: Calmangafodipir at a dose of 5 μmol/kg appears to prevent the development of oxaliplatin-induced acute and delayed CIPN without apparent influence on tumour outcomes.

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Introduction

In colorectal cancer (CRC), chemotherapy has established effects as adjuvant treatment to prevent recurrences and in metastatic disease to prolong survival, convert non-resectable disease to resectable and alleviate symptoms and improve quality-of-life [1].

Oxaliplatin is together with a fluoropyrimidine used as adjuvant treatment in patients with stage II and III CRC [2]. In metastatic CRC (mCRC) oxaliplatin is used, again with a fluoropyrimidine, in first- or second-line [2]. Oxaliplatin induces peripheral neuropathy (chemotherapy-induced peripheral neuropathy, CIPN) that may be problematic. The acute oxaliplatin-induced CIPN (OIPN), seen in most patients, is characterised by tingling, numbness and allodynia in hands and feet, sometimes together with oropharyngeal dysesthesia, often induced by cold. It is usually reversible, and therefore clinically less important [3]. The chronic OIPN with sensory impairment, tingling, numbness and pain, particularly in the feet, can be troublesome and often long-lasting [4–9].

There are no effective preventive or therapeutic treatments for OIPN. Several agents have been tested, but they have so far failed [6,7,10,11]. For therapy, the American Society of Clinical Oncology [11] states that duloxetine may be used, whereas no recommendations can be given for other agents.

Calmangafodipir [$Ca_4Mn(DPDP)_5$, PledOx®] has shown promising activities in model systems in preventing

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Supplemental data for this article can be accessed here.

oxaliplatin-induced adverse effects [12]. Calmangafodipir, developed from mangafodipir, extensively used as a magnetic resonance imaging contrast agent [13], mimics the activity of the mitochondrial enzyme manganese superoxide dismutase (MnSOD), and thereby helps degrade reactive oxygen species (ROS) and cells to survive oxidative stress. Work in animal models indicates that mitochondrial dysfunction in afferent sensory neurons is essential in the chronic peripheral neuropathies seen in patients receiving chemotherapeutic drugs [14]. Abnormally vacuolated mitochondria in peripheral nerve sensory axons were first seen in rats with paclitaxel-induced painful peripheral neuropathy [15], and subsequently after oxaliplatin or bortezomib [16–19]. These nerves contain significantly raised levels of nitrated MnSOD [14,17], reducing MnSOD activity.

MnSOD catalyses dismutation of superoxide and forms hydrogen peroxide and molecular oxygen [20,21]. MnSOD limits the reaction of superoxide with nitric oxide to form the reactive nitrogen species peroxynitrite [22,23]. During pathologic oxidative stress, superoxide reacts readily with nitric oxide to form highly toxic peroxynitrite, which nitrates the tyrosine residue of MnSOD and irreversibly inactivates the enzyme [24].

There is a great need to prevent OIPN due to the long-standing problems in many patients. A dose-escalating phase I study followed by a placebo-controlled, double-blinded randomised phase II study explored whether the acute and chronic OIPN could be decreased using calmangafodipir and to get an indication that it does not reduce the antitumor efficacy of oxaliplatin-containing chemotherapy.

Patient and methods

Patients

Eligible patients had biopsy-proven mCRC with measurable disease according to RECIST 1.1. The disease should not be eligible for curatively intended metastasectomy, even if tumour regression was seen. They should also be candidates for oxaliplatin-based chemotherapy in first- or second-line, i.e. have symptomatic or progressive disease (ESMO group 2 [2]). The patients may have received up to two previous treatments, either adjuvant or for metastatic disease, but no previous oxaliplatin. They should be above 18 years old, have a WHO performance status $\leq$2, adequate haematological, renal and hepatic functions, no other malignancy within the previous 5 years, no signs of neuropathy, no evidence of central nervous system metastases, severe heart problems, history of stroke or cerebrovascular accident in the past six months or major psychiatric disorder. Welders, miners or workers with occupational high manganese exposure were excluded, as were those with a baseline blood manganese level above the upper limit normal (ULN) of 18.3 μg/L (at US sites, 1.5 × ULN or 27.5 μg/L). Manganese levels were analysed by inductively coupled plasma mass spectrometry (ICP-MS) at ALS Scandinavia, Luleå, Sweden.

All patients gave written informed consent and the study was approved by the Regional Ethics committees at participating sites. The study was registered at ClinicalTrials.gov.NCT01619423.

Treatment

The chemotherapy was modified FOLFOX6 [25] containing oxaliplatin 85 mg/m^2 during 2 h followed by calcium-levofolinate (100 mg/m^2) or calcium folinate (200 mg/m^2) as a 2 h infusion followed by 5-FU 400 mg/m^2 i.v. bolus and 5-FU continuous infusion 2400 mg/m^2 during 46 h. The chemotherapy was repeated every fortnight and planned for up to eight cycles after which a break was recommended. Tumour evaluations were performed after 4 and 8 cycles. Bevacizumab (5 mg/kg) was added at the discretion of the physician.

Conventional dose reductions for haematological and non-haematological toxicities were applied. For neurotoxicity grade 2, a 25% reduction in oxaliplatin dose was described if it persisted until the next cycle. For grade 3 toxicity, the dose should be reduced by 25% if it lasted for more than 7 days and stopped if it persisted. If toxicity reappeared, a second reduction was described. Grade 4 toxicity meant that oxaliplatin administration was stopped.

Calmangafodipir/placebo administration

Calmangafodipir/placebo was administrated as a 5-min infusion, 10 min prior to oxaliplatin. In the phase I study, performed at one European and two US sites, the starting dose of calmangafodipir was 2 μmol/kg. After the first patient had received the second cycle without grade 3–4 related toxicity (immediate blood pressure fall requiring medical intervention or hospitalisation, any life-threatening event requiring urgent intervention or severe diarrhoea or vomiting in excess of what can be expected with mFOLFOX6 alone), the next patient could start. Dose escalation to the next dose 10 μmol/kg could occur when the first patient had received three cycles of mFOLFOX6 + calmangafodipir without related toxicity and the next two patients at least 1 cycle. In case of related toxicity at the 10 μmol/kg dose, the dose should be lowered to 5 μmol/kg. If the first six patients had no grade 3–4 related toxicity, bevacizumab was added (only at US sites), using calmangafodipir at the tolerable dose, in this case 10 μmol/kg and, if related toxicity was seen, the dose below, or 5 μmol/kg (the dose of calmangafodipir with the first cycle of bevacizumab was 20% of the full planned dose, i.e. 2 and 1 μmol/kg, respectively).

In the phase II study, patients were randomised between placebo and two doses of calmangafodipir, 2 or 5 μmol/kg (initially 10 μmol/kg, see below). Placebo was produced by B. Braun Meslungen AG Spte Hospital Care Pharma, Meslungen, Germany. Since the colour of calmangafodipir is yellow, calmangafodipir/placebo was transferred to a yellow syringe by the pharmacist (not blinded), so that the administrating nurse/doctor and the patient were blinded to the content.

Evaluation of neurotoxicity

During treatment, neurologic toxicity was evaluated by the physician according to the NCI-CTCAE versus 4.03 scale and

the Oxaliplatin Sanofi Specific grade 0–4 Scale (OSSS) for oxaliplatin-related paraesthesia's/dysesthesias in advanced colorectal cancer (used for the primary endpoint, Supplementary Appendix S1). Two patient-reported outcome measures (PROs) were used. The Leonard Scale Questionnaire (LSQ, in a revision of several tools for evaluation of CIPN, it was designated the oxaliplatin-associated neuropathy questionnaire, OANQ, Supplementary Appendix S1) [26,27] should be filled out prior to the start of infusion day 1 of each treatment cycle and at follow-up visits every third month during the first year. The cold allodynia test, using a metal rod called the Ventzel cylinder [28] was used and the level of pain was rated on a 0–10 numerical scale prior to infusion day 1, at the end of oxaliplatin infusion day 1 and at days 2–4 of each cycle.

Statistical methods

The study was a three-armed, double-blinded, randomised, placebo-controlled, multicentre phase II study. Randomisation (electronically, blocks of 6) was stratified by site in a 1:1:1 ratio to FOLFOX6 + calmangafodipir 2 μmol/kg, calmangafodipir 5 μmol/kg or placebo. The study remained blinded until all patients had been followed for at least 1 year post-randomisation and completed all follow-up visits. The data was handled according to Good Clinical Practise and the data base was set up by an independent Contract Research Organisation (CRO) and all procedures were compliant with the FDA 21 CFR Part 11 for handling electronic records.

The primary endpoint was presence of neuropathy grade 2 or higher assessed by the physician using the OSSS after eight cycles of mFOLFOX6. Secondary endpoints were overall response rate (ORR, RECIST 1.1), progression-free survival (PFS), overall survival (OS), neurotoxicity according to the cold allodynia test and LSQ, other toxicity, particularly neutropenia and pharmacokinetics (chiefly described in Supplementary Appendix S2). PFS and OS were calculated from the day of randomisation.

Based upon the assumptions that 40% of the patients had neurotoxicity in the placebo arm and 20% on calmangafodipir, using a one-sided test at 0.10 for the type I error, and a power of 80%, 42 patients were required in each group. Since the highest dose of calmangafodipir was lowered after 39 randomised patients (Part IIA), additionally 3×42 patients were randomised in the second part (Part IIB), increasing power to 88%. The results for the primary endpoint are presented as odds ratio, OR, with corresponding upper limit of the one-sided 90% confidence interval and *p*-values. Since the purpose was to determine whether calmangafodipir appears effective to proceed with further development, i.e. if there are any or several doses of the active drug superior to placebo, only superiority to placebo (OR <1) is of interest, and a one-sided testing for the primary endpoint is appropriate. This is in agreement with the regulatory guidelines for designing dose-response studies. Therefore, results are not used to claim confirmatory evidence of efficacy such as in a pivotal study using a two-sided test controlled at a type-I error rate of 0.05. In addition, we have performed hypothesis testing using a two-sided approach using $p < .05$ for other endpoints, to be interpreted as exploratory evidence.

The analyses of the primary efficacy endpoint were done on all randomised individuals who had started treatment using a proportional odds model for repeat measures. The generalised estimation equations method estimated the parameters of the model. Missing data was handled in the statistical analysis of the model. The same approach was used for categorical endpoints if not otherwise specified. For secondary endpoints, observed cases were analysed, i.e. no imputation for missing data was made.

The time to cycle with grade 2 or more neuropathy and PFS/OS are presented using the Kaplan–Meier curves and corresponding hazard ratio (HR) and two-sided log-rank test and were used to compare treatment arms descriptively to illustrate results by time. Continuous endpoints are presented using the median and range or mean and standard error of the mean by treatment. Active treatment arms were compared to placebo using a two-sided Mann–Whitney *U* test if not otherwise stated. The between treatment arms comparison with regard to mean changes from baseline in the natural logarithm (ln) normalised blood manganese levels was done using the ANOVA model with treatment as a fixed factor in the model.

Since the primary endpoint was not met, all tests are considered as exploratory and no adjustments for multiplicity were made. All statistical analyses were performed using SAS® (version 9.3 or later), IBM SPSS Statistics (v22) or GraphPad Prism (v6).

Results

Phase I

No toxicity that could be ascribed to calmangafodipir was detected among the included patients ($n = 6$) after the first three cycles at 2 and 10 μmol/kg without bevacizumab. The second part of the phase I study, adding bevacizumab, was then initiated (US sites only). Again, no toxicity that could be ascribed to calmangafodipir was seen after the first three cycles using 10 (two patients, reasons for that the third patient was not initiated is described below in the next paragraph) or 5 μmol/kg (three patients)

The initial active drug dosing for 1:1:1 randomisation were 2 and 10 μmol/kg, initiated without bevacizumab in parallel to the second part of the phase I study, since no safety concerns were detected during phase I when all patients had received at least three cycles. However, after the three patients treated with 10 μmol/kg had received at least seven courses each, neutropenia was seen, but not in those treated with 2 μmol/kg. For this reason, the last patient allocated to be treated with 10 μmol/kg together with bevacizumab was never started; instead the dose was as described in the protocol lowered to 5 μmol/kg. When all 11 patients in the phase I study had been treated to the planned number of eight cycles (reached by nine patients), numerically more grade 3 toxicity other than neurotoxicity was also seen in patients treated with 10 μmol/kg (three out of the five patients got totally eight episodes). Of these eight episodes

seen in the three patients treated with 10 μmol/kg, six were ascribed to leukopenia with necessity for dose delay and dose reductions. No grade 3 or 4 toxicity was seen in the three patients treated with 2 μmol/kg and 1 episode of grade 3 febrile neutropenia after the seventh cycle in one of the three patients treated with 5 μmol/kg with bevacizumab. For these reasons, the highest calmangafodipir dose was lowered in the phase II study to 5 μmol/kg (phase IIB part). The 10 μmol/kg dose did also not appear more efficacious in reducing neurotoxicity, nor neutropenia. This was later confirmed by how the 10 μmol/kg dose behaved in the cold allodynia test, where it showed good efficacy during the first treatment cycles but then gradually became less efficient (data not shown) and using the Leonard scale, where again the efficacy appeared less (data not shown).

The physician-rated neurotoxicity was low in the 11 patients included in the phase I study compared to what could be expected [8] with two patients developing transient grade 2 and no patient grade 3 toxicity after median six (range 3–8) cycles.

Phase II study

Totally 173 patients were included between December 2013 and October 2014, from 32 hospitals in eight countries, 39 patients when 10 μmol/kg was the highest dose (part IIA) and 134 patients when 5 μmol/kg was the highest dose (part IIB) (Figure 1). During part IIA + B, patients who were randomised to placebo and 2 or 5(+10) μmol/kg were used in the evaluation of the primary outcome. An analysis of the primary outcome was also performed in part IIB separately to estimate the efficacy of the 5 μmol/kg dose.

Patient characteristics are provided in Table 1. Most (83%) patients received therapy in first-line and 126 (73%) patients received all eight cycles with no statistically significant differences between groups. The main reason for not receiving eight cycles was progressive disease (24/47). Six patients withdrew consent and 6 patients were lost to follow-up during the course of the disease, but all patients received at least one dose of study drug and were thus analyzsed.

Peripheral neuropathy during treatment and follow-up

The primary outcome, the physician-assessed neuropathy (OSSS) showed no difference between groups after the first two cycles, but from then on, calmangafodipir treated patients (2 + 5 + 10 μmol/kg, $n = 113$) had less grade 2 + toxicity than placebo treated patients ($n = 60$) (OR (90% CI one-sided upper level, UL) 0.62 (1.15), $p = .16$. The physician-assessed neuropathy by time (OSSS) is presented in

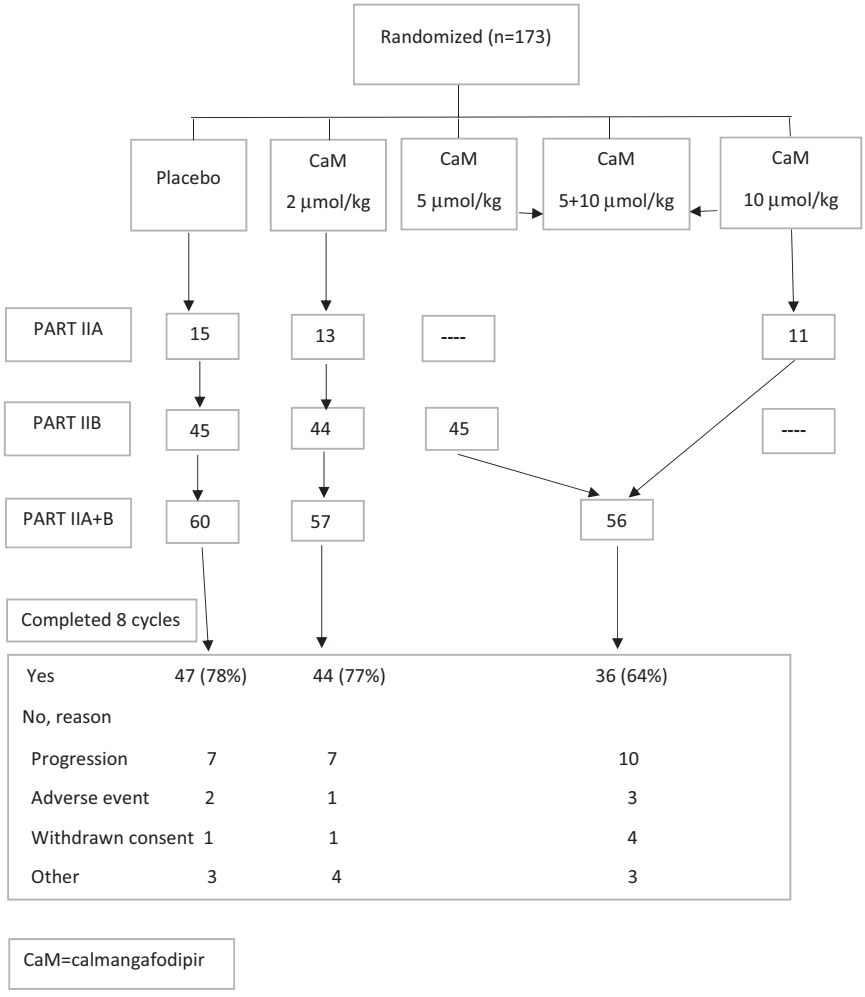

Figure 1. Consort diagram. Number of patients randomised in the phase II study. The highest dose of calmangafodipir was lowered after inclusion of 39 patients for reasons described in the text. All randomised patients received at least one dose of chemotherapy and study drug and all patients were analysed.

Figure 2 using the Kaplan–Meier plot of the development during the eight treatment cycles together with the HR and 95% confidence limits. The same clinical differences were seen when the analyses were restricted to part IIB (2 + 5 μmol/kg, $n = 89$, placebo, $n = 45$; data not shown).

During treatment, 14 (23%) patients had, according to the physician, grade 2 + 3 neurotoxicity at any time in the placebo group compared with 16 (14%) patients in the calmangafodipir groups. These numbers were nine (16%) in the 2 μmol/kg dose and seven (13%) in the 5 + 10 μmol/kg dose. The reduction in neuropathy after cycles 3–5 was similar in the 2 and 5 μmol/kg calmangafodipir doses but less using 2 μmol/kg after cycles 6–8; the few patients treated with 10 μmol/kg do not allow firm conclusions, although they numerically initially behaved similar to the 5 μmol/kg dose and after cycle 7–8 slightly worse. Persistent neuropathy (≥ grade 2 for 2 or more cycles) were seen more often and occurred earlier in placebo treated patients (10%, cycle 3) than in calmangafodipir-treated patients (2 μmol/kg, 9%, cycle 3; 5 + 10 μmol/kg, 4%, cycle 6). During treatment, there were thus some indications (statistically insignificant) of a dose response relationship [OR (90%CI) 0.78(UL1.50), $p = .31$ for 2 μmol/kg versus placebo and 0.55(1.16), $p = .15$ for 5 + 10 μmol/kg versus placebo].

Dose reductions due to neurotoxicity were uncommon (5–10%) and similar between groups.

During treatment, patients receiving calmangafodipir reported from cycle 3 and onwards significantly less cold allodynia (mean 1.6 versus 2.3, $p < .05$) using the Ventzel cylinder and less problems in the sensory symptoms of the Leonard scale (cycles 1–8 mean 1.9 versus 3.0, $p < .05$) (Figure 3(A,B)).

During follow-up, the sensory symptoms of the Leonard scale remained at the same levels in calmangafodipir-treated patients, whereas they were initially significantly higher in the placebo group (after 3 and 6 months, mean 3.5 versus 7.3, $p < .01$) (Figure 3(C)). The 5 + 10 μmol/kg group had less problems during the first 6 months than the 2 μmol/kg group (part IIA + B, data not shown) and the 5 μmol/kg group similarly less than the 2 μmol/kg group (part IIB, data not shown).

The few patients treated with bevacizumab did not behave differently from those who did not receive bevacizumab (data not shown). Similarly, the few patients with diabetes behaved like non-diabetic patients (data not shown).

Toxicity other than neuropathy

Neutropenia of any grade or of grade 3–4 was less frequently seen in the 2 μmol/kg calmangafodipir-treated patients (Table 2). Mean neutrophil values after eight cycles were reduced in all groups, but less in the calmangafodipir-treated groups than in the placebo group. The results did not differ if the analyses were restricted to part 2B only. The myeloprotective effect of calmangafodipir appeared less in 10 than in 5 μmol/kg, consistent with a bell-shaped dose-response curve, but based upon few patients treated with 10 μmol/kg, and not randomised (data not shown).

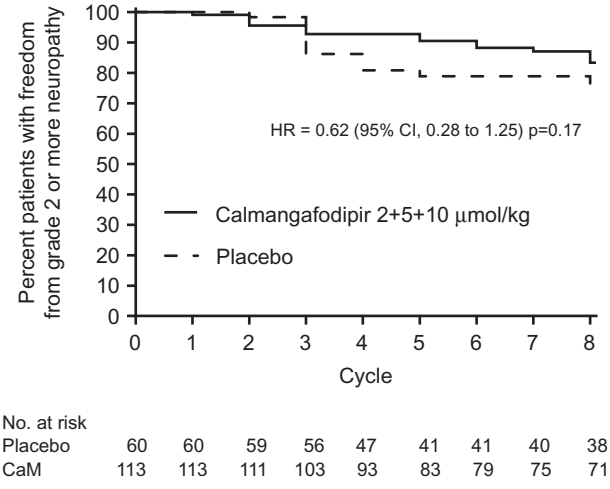

Figure 2. Freedom from grade 2 or more neuropathy evaluated by the physician using the OSSS in part IIA + B.

Table 1. Patient characteristics and treatment in the phase II study.

Characteristics	Part IIB			Part II A + B		
	Placebo ($N = 45$)	2 μmol/kg ($N = 44$)	5 μmol/kg ($N = 45$)	Placebo ($N = 60$)	2 μmol/kg ($N = 57$)	5 + 10 μmol/kg ($N = 56$)
	Number of patients[a]					
Age, median (range) years	63	64	63	63	65	63
Male	32	33	24	46	41	29
Female	13	11	21	14	16	27
Performance status						
0	22	18	23	29	27	31
1	22	25	22	30	28	25
2	1	1	0	1	2	0
Removed primary tumour	19	14	21	31	20	21
Synchronous metastases	31	28	24	41	39	37
Diabetes	4	7	4	5	8	6
Prior adjuvant therapy	4	2	4	8	9	4
1st line	38	35	35	52	47	45
2nd line	7	9	10	8	10	11
Treatment + bevacizumab	8	6	6	9	6	7
Number of cycles received						
Median, range	8, 1–8	8, 1–8	8, 2–8	8, 1–8	8, 1–8	8, 2–8

[a]Unless otherwise indicated.

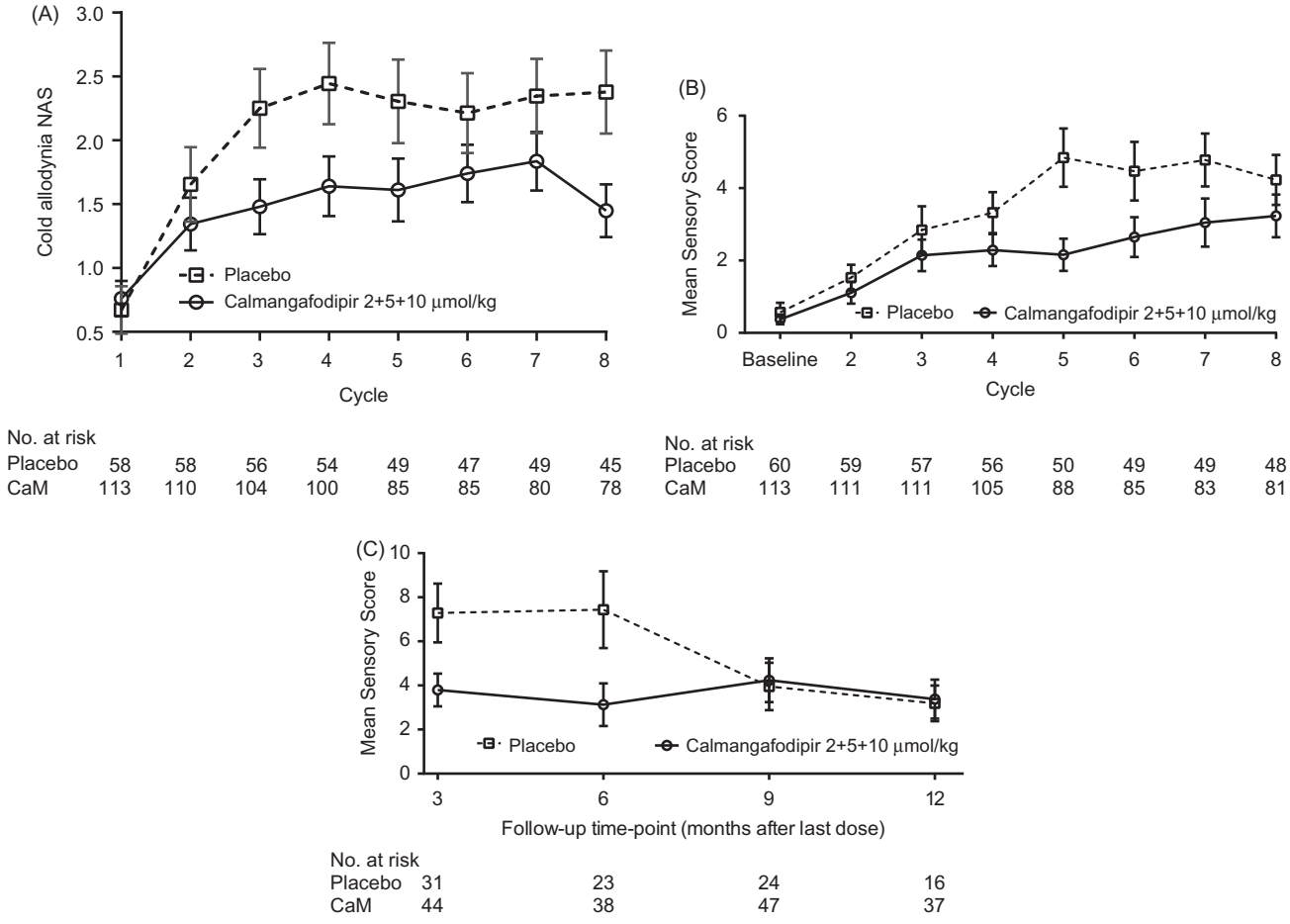

Figure 3. Patient reported neuropathy during treatment with placebo or calmangofodipir (CaM, 2 + 5 + 10 μmol/kg, part IIA + B) evaluated using a metal rod (cold allodynia, A, on a numeric analog scale (NAS) 0–10 on day 2 after each chemotherapy cycle), and during treatment (B) and follow-up every third month (C) for 1 year using the Leonard scale questionnaire ($p < .05$ after 3 months of follow-up and $p < .01$ after 6 months). Mean sensory score is the average sum of tingling, numbness and burning pain to cold in hands and feet. Data plotted as mean ± SEM. The same results were seen if the analyses were restricted to part IIB (data not shown).

Table 2. Worst toxicity in the PLIANT study, phase IIA + B, number of patients (percent).

Type	Grade	Placebo ($N = 60$)	2 + 5 + 10 μmol/kg ($N = 113$)	2 μmol/kg ($N = 57$)	5 + 10 μmol/kg ($N = 56$)
Anaemia	2–4	5 (8)	9 (8)	3 (5)	6(11)
Thrombocytopenia	2–4	6 (10)	7 (6)	5 (9)	2 (4)
Neutropenia	1–4	29 (49)	34 (30) ($p < .05$)	15 (26) ($p < .05$)	19 (34)
	2–4	17 (28)	20 (18)	8 (14) ($p = .07$)	12 (21)
	3–4	7 (12)	11 (10)	3 (5)	8 (14)
Neutrophils $\times 10^9$/L (Mean ± SEM)	Baseline	4.8 ± 0.3	5.1 ± 0.2	5.2 ± 0.3	5.1 ± 0.3
	Cycle 8	2.9 ± 0.3 ($p < .01$)[a]	3.6 ± 0.2 ($p < .01$)[a]	3.6 ± 0.2 ($p < .01$)[a]	3.7 ± 0.4 ($p < .05$)[a]
Diarrhea	3–4	2 (3)	2 (2)	2 (3)	0
Nausea/vomiting	3–4	1 (2)	1 (1)	0	1 (2)
Fatigue	3–4	1 (2)	2 (2)	0	2 (4)
Fever	3–4	1 (2)	0	0	0
Peripheral neuropathy	2–4	14 (23)	17 (15)	11 (19)	6 (11)
Parkinson-like symptoms	Any[b]	6 (10)	11 (10)	6 (10)	5 (9)
Chest pain, cardiac abnormalities[c]	3	1 (2)	0	0	0

[a]Statistically significant reduction from baseline to cycle 8, two-way ANOVA followed by Tukey's multiple comparisons test.

[b]All patients were asked at each visit about development of Parkinson like symptoms, reported by about 10% of the patients. An MRI was recommended, however only considered indicated in 3 patients. It showed brain metastases in 2 and no abnormalities in one patient (placebo group). Blood manganese levels were normal in all but two patients where is was slightly but in-significantly increased (one patient received 2 μmol/kg and one patient 5 μmol/kg).

[c]A three-lead ECG was measured during the first cycle at three time-points, before premedication, immediately prior to infusion of calmangafodipir/placebo and immediately after (prior to FOLFOX). No cardiac abnormalities occurred after dosing with calmangafodipir/placebo. All except three patients had normal QTc-intervals at all ECG recordings. The abnormalities were seen in two patients in the calmangafodipir 2 μmol/kg group and in one patient in the placebo group.

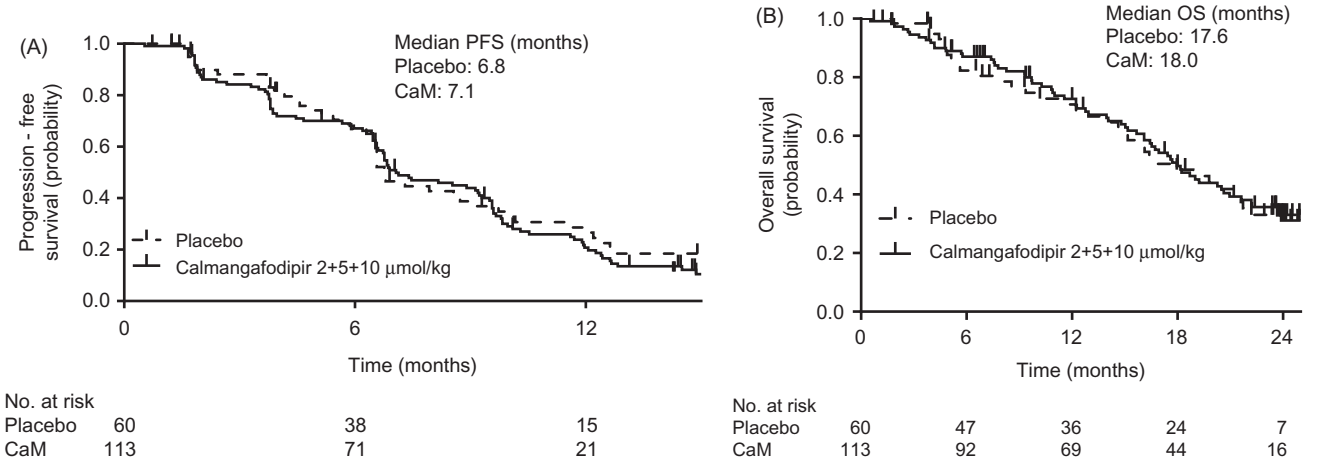

Figure 4. Progression-free (A) and overall (B) survival in patients treated with placebo or calmangofodipir (CaM, 2 + 5 or 10 µmol/kg (part IIA + B). Neither were any differences between 2 and 5 + 10 µmol/kg, or in part IIB between placebo, 2 and 5 µmol/kg seen.

Besides neutropenia (and neuropathy), haematological and non-haematological toxicity did not differ between groups (Table 2). Dose-reductions or dose delays due to toxicity occurred in between 30 and 40% of the patients and similar between groups, except that it was numerically higher (55%) in the few ($n = 11$) patients treated with 10 µmol/kg.

Tumour outcomes

PFS and OS did not differ between groups whether they were analysed in all patients (Figure 4) or only in part IIB (data not shown). Median PFS and OS were about 7 and 18 months, respectively. Overall response rates (ORR) did neither differ between groups (43% in the placebo group and 46% in the calmangafodipir (2 + 5 + 10 µmol/kg) group). Progressive disease (PD) as best response was also similar between groups (18–20%), i.e. disease control rates did not differ between groups. The results were virtually identical if restricted to phase IIB only (data not shown).

Manganese accumulation

The median baseline blood manganese level was 7.6 µg/L and all values were below the exclusion level set at the ULN of 18.3 µg/L (1.5 × ULN or 27.5 in the US). There was a statistically significant increase of blood manganese levels after both cycles 4 and 8 for calmangafodipir 5 µmol/kg ($p < .001$, one-way ANOVA, Figure 5) compared to the placebo group. However, the median blood manganese level after cycle 8 was 13.9 µg/L, and thus still below ULN. According to the Mayo Medical Laboratories, normal whole blood values for manganese are 4.7–18.3 µg/L. Values between 1 and 2 times the ULN may represent biologic variation or related to haematocrit levels while values greater than twice the ULN correlate with toxicity. Only one of the calmangafodipir 5 µmol/kg patients had levels of blood manganese above twice ULN. Median plasma manganese levels remained unaltered in the calmangafodipir 5 µmol/kg group during treatment (0.72, 0.72 and 0.82 µg/L for baseline and after cycles 4 and cycle 8, respectively).

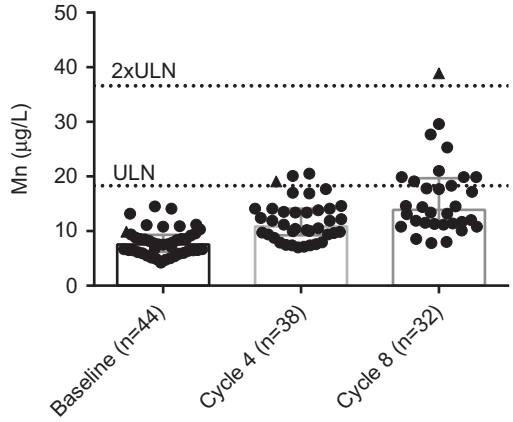

Figure 5. Blood manganese levels in the 5 µmol/kg calmangafodipir group. The triangle identifies the single patient with the largest increase in blood manganese levels over eight cycles and above 2 × ULN at cycle 8. Box plot and error bars indicate median ± interquartile range.

The disposition of calmangafodipir was further studied by measuring the plasma concentrations of Zn and fodipir as its metabolites ZnDPDP, ZnDPMP and ZnPLED (Supplementary Appendix S2).

Discussion

This randomised placebo-controlled clinical study shows a clear decrease in OIPN. A few randomised trials have previously reported significant protection against peripheral neuropathy from an intervention perspective (intravenous calcium-magnesium, xaliproden, venlaflaxine, reduced glutathione and oral glutathione) [29–33]. However, a meta-analysis including 42 trials recently concluded that no intervention has favourably influenced the rate of CIPN, including that caused by oxaliplatin [11]. The neuroprotective effects seen here appear clinically meaningful, delaying both the onset and reducing the intensity, and were seen both in the acute and in the early chronic phase. Longer follow-up times are needed in future trials since the OIPN may remain for many years. At the same time, no toxicity could be registered to the active agent calmangafodipir and there were no

signs of any tumour protective effect to the chemotherapy. A decrease in acute toxicity may, however, potentially mean that oxaliplatin dose reductions or actual termination are delayed, increasing late toxicity.

The neuroprotective effect was recorded by the physician, selected as the primary endpoint (not statistically significant) and by the patients using two PROs (secondary endpoints, both statistically significant). The optimal PRO measure for recording the specific symptoms caused by oxaliplatin in the acute or chronic phases is not known. When the trial was planned, the Leonard scale [26], the Fact/GOG-Ntx [34] and the EORTC-CIPN20 scales [35] were published. All scales measure tingling and numbness, the two most common sensory symptoms, and pain, being less frequently seen, but when present, very disturbing [5]. It is not likely that the choice of another scale would have modified the findings of a protective effect from calmangafodipir. Also the cold allodynia test revealed a significant difference favouring actively treated patients.

The physician-graded neurotoxicity after up to eight cycles of a regimen with an oxaliplatin dose of 85 mg/m^2 (like FOLFOX-4, some mFOLFOX and FLOX) was less than originally anticipated (grade 2 + 3 23% versus 40% expected), decreasing power, but similar to recent studies [8,36,37]. However, intertrial comparisons are difficult, particularly since the risk estimates vary between observers and between geographical regions [9,38,39]. The different studies have also used different scales or different versions of the scales, sometimes also with different wording. In the PLIANT study, the grading scale specially developed by Sanofi for use in metastatic colorectal cancer trials and reported in the label of Eloxantin® (https://www.accessdata.fda.gov/drugsatfda_docs/label/2011/021759s012lbl.pdf) was used. In comparison with some other used scales, it includes loss of function, although not influencing daily living, already in grade 2 as opposed to grade 3. This could be an explanation why less neurotoxicity grade 2 + was reported by the physicians. The study is too small to allow separate analyses of patient characteristics of relevance for the development of neuropathy. However, besides age and diabetes mellitus, few clinical factors predict neuropathy [40]. The few diabetic patients behaved similar to non-diabetic patients.

Besides preventing neurotoxicity, less neutropenia was also seen in calmangafodipir-treated patients, although again, neutropenia was not as common as has been reported [41]. Animal experiments have noted that calmangafodipir and the related mangafodipir prevent haematological toxicity [12,42].

As always, when drugs protecting normal tissues from adverse effects of cytotoxic drugs, or radiation, are explored, concerns of tumour cell protection arise. It is reassuring that we could not detect any protective effects on ORR, PFS or OS. The trial, including 173 patients, is too small to exclude clinically meaningful tumour protective effects. Multiple animal studies, reviewed in [12,42–44], support that calmangafodipir and the related mangafodipir do not protect tumour cells, rather the opposite. There are several potential mechanisms for their cytotoxic effect on cancer cells. *In vitro* it is attributed to the fodipir part of the molecules [12]. Fodipir compounds are potent iron and other transition metal chelators [45,46], which may contribute to their antioxidative effect in general and to their cytotoxic effect on cancer cells in particular [13,47]. However, *in vivo,* the cytotoxic effect is dependent on an intact immune system and it could be speculated that the cytotoxic effect of calmangafodipir is associated with an immunogenic cell death reported with oxaliplatin [48].

ORRs of 40–50%, a median PFS of 7 months and a median OS of 18 months are lower than reported in recent trials in mCRC [49]. It should then be noted that also second-line patients were included. Further, only patients with symptomatic or progressive disease and having disease manifestations that could never be resected, even if excellent tumour response was seen, were eligible. No molecular selection was made. Thus, the group of patients constitutes an unfavourable prognostic group, and the ORRs, PFS and OS are as can be expected.

In conclusion, a randomised placebo-controlled phase II trial gives indications that calmangafodipir prevents the development of CIPN during and after treatment, at least during the first 6 months, with a commonly used oxaliplatin combination for patients with mCRC. The effects are sufficiently strong and the lack of any indications of a tumour protective effect reassuring, motivating initiation of conclusive phase III trials. The dose of 5 μmol/kg appears optimal, at least during the follow-up. It is not likely that the effects are restricted to patients with CRC or to a FOLFOX-like regimen using a dose of about 85 mg/m^2, whereas it remains to be explored whether calmangafodipir also protects neuropathy from other cytostatic drugs like taxanes.

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Disclosure statement

Bengt Glimelius, Consulting or advisory role, PledPharma AB; Devalingham Mahalingam, Honoraria, Consulting, PledPharma AB; Peter Buhl Jensen, Employment, Leadership, Stock, Honoraria, Buhl Pharma and PledPharma AB; Jan Kowalski, Consulting, PledPharma AB; Marie Bengtson, Employment, Stock, PledPharma AB; Malin Nittve, Employment, Stock, PledPharma AB; Jacques Näsström, Employment, Leadership, Stock, Patent, PledPharma AB. Remaining authors have declared no conflicts of interest.

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