2 SYNOPSIS

Name of Sponsor/Company: **PledPharma AB**			
Name of Finished Product: PledOx®			
Name of Active Ingredient: Calmangafodipir			
Study Title: A double-blinded, randomized, three armed, Phase II trial of PledOx® in two different doses in combination with FOLFOX6 compared to placebo + FOLFOX6 in patients with advanced metastatic colorectal (stage IV) cancer			
Coordinating /Principal Investigator: Bengt Glimelius, University Hospital, Uppsala, Sweden			
Study Centers: The study was performed at a total of 32 sites in 8 countries: Sweden (SE): 5 sites Denmark (DK): 2 sites Germany (DE): 3 sites Portugal (PT): 3 sites Georgia (GE): 5 sites Serbia (RS): 4 sites Bulgaria (BG): 5 sites USA (US): 5 sites			
Publication based on the study (reference): Accepted for publication in Acta Oncologica			
Studied period (year-month-day):			**Phase of development:**
Part 2a	Date of first enrolment in Part 2a:	2013-11-26	Phase II
	Date of last End-of-treatment visit in Part 2a:	2014-09-03	
Part 2b	Date of first enrolment in Part 2b:	2014-04-03	
	Date of last End-of-treatment visit in Part 2b:	2015-03-09	
Part 3	Last data collection on overall survival:	2016-12-22	

Abstract:

Oxaliplatin causes disabling acute and chronic peripheral neuropathy. The study 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 randomized phase II study (ClinicalTrials.gov, NCT01619423) in patients with metastatic colorectal cancer (mCRC).

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 hours) every fortnight for 8 cycles in first or second line were eligible. Calmangafodipir was given in a phase I dose-finding (Part 1) and in a phase II placebo-controlled part of the study (Part 2 and 3), as a 5 minutes infusion 10 minutes prior to oxaliplatin. Neurotoxicity was evaluated by the physician using the Oxaliplatin Sanofi Specific Scale (OSSS) and by the patient using the cold allodynia test (Ventzel cylinder) and the Leonard scale (oxaliplatin-associated neuropathy questionnaire, OANQ).

Eleven patients were included in phase I without any detectable toxicity to calmangafodipir. In the phase II study, 173 patients were randomized to placebo (n=60), calmangafodipir 2 µmol/kg (n=57) and calmangafodipir 5 µmol/kg (n=45, (part 2b) initially 10 µmol/kg, n=11 (part 2a)). Calmangafodipir-treated patients (all three doses pooled) had less physician graded neurotoxicity (odds ratio (90% confidence interval one-sided upper level) 0.615 (1.159), p=0.158), statistically significantly less problems with cold allodynia and fewer sensory symptoms in the Leonard scale during treatment and during follow-up 3 and 6 months after last dose. Response rate, progression-free and overall survival did not differ among groups.

Calmangafodipir at a dose of 5 µmol/kg appears to prevent the development of oxaliplatin-induced acute and delayed CIPN without apparent influence on tumor outcomes (see summary table below on key neuropathy efficacy endpoints for the full analysis set).

Table A Summary of key neuropathy efficacy endpoints (FAS)

Dose (Study Part)	2 µmol/kg (2a+2b)	5 µmol/kg (2b)	5+10 µmol/kg (2a+2b)	2+5+10 µmol/kg (2a+2b)
OSSS odds ratio over cycle 1 to 8[§] (p-value)	0.78 (p=0.31)	0.68 (p=0.25)	0.55 (p=0.15)	0.62 (p=0.16)
Leonard PRO, odds ratio at FU2* (p-value)	0.38 (p=0.15)	0.12 (p=0.018)	0.097[†] (p=0.007)	0.23 (p=0.014)

[§] *Investigator reported neuropathy grade 2 or higher vs. placebo*

** Proportion of patients scoring 3 or more on either numbness, tingling or burning pain/discomfort with cold in hands or feet at FU2 (6 months after last dose), which is approximately 10 months after the first dose for the majority of patients that reported follow-up 2.*

[†] *NB there was no patient randomized to the 10 µmol/kg dose contributing with data at FU2. Thus, the difference between results for the 5 µmol/kg dose and the 5+10 µmol/kg is due to differences in results in the placebo arm between Part 2b and Parts 2a+2b*

Objectives:

Primary objective

- To assess the efficacy of 2 different dose levels of PledOx when added to mFOLFOX6 chemotherapy as measured by its protection from mFOLFOX6 toxicity on peripheral neuropathy (Grade 2 or higher) as assessed using the oxaliplatin Sanofi specific scale (OSSS) criteria for paresthesia/dysesthesia

The primary objective was changed before the treatment code was broken, from assessment of efficacy as measured by neutropenia Grade 3 and 4 (National Cancer Institute-Common Terminology Criteria for Adverse Events [NCI-CTCAE] v4) to assessment of efficacy based on peripheral neuropathy Grade 2 or higher (OSSS). This change was made after a blinded data review which indicated that the incidence of neutropenia was only 4%, whereas the expected incidences were 40% for placebo and 20% for active treatment with PledOx. A blinded data review of the incidence of neuropathy showed that there were enough cases for these data to be evaluable.

Secondary/Exploratory objectives

- To assess PledOx effect on patient reported cold allodynia assessments (Ventzel metal rod) and patient reported Leonard scale (oxaliplatin-associated neuropathy questionnaire, OANQ)
- Assessment of anti-tumor effect of PledOx when added to mFOLFOX6 chemotherapy by Response Evaluation Criteria in Solid Tumors (RECIST) 1.1
- To assess PledOx effect on progression free survival
- To assess PledOx effect on overall survival
- To assess PledOx effect on neutropenia Grade 3 and 4 (NCI-CTCAE v4)
- To assess PledOx effect on febrile neutropenia
- To assess PledOx effects on other chemotherapy-induced AEs
- To assess PledOx effect on mFOLFOX6 delivered dose and dose intensity
- To assess PledOx effect on oral mucositis
- To assess PledOx effect on Quality of Life
- Characterize the PK profile of PledOx (metabolites ZnDPDP, ZnDPMP, ZnPLED) and the metals manganese (Mn) and zinc (Zn)
- Determine the PK parameters: plasma concentration half-life ($t_{½}$), the peak plasma concentration (C_{max}), area under the plasma concentration-time curve (AUC), time to peak plasma concentration (t_{max}) of PledOx (metabolites ZnDPDP, ZnDPMP, ZnPLED) and the metals Mn and Zn

Safety objective

- To determine the safety profile of PledOx when added to mFOLFOX6 chemotherapy, including:
  - Adverse drug reactions and serious adverse drug reactions
  - Safety Mn measurements in blood
  - Changes in hematology and clinical chemistry values, including those associated with hepatic and renal function
  - Assessment of physical examinations
  - Vital signs
  - Cardiac function (QT prolongation)
  - Assessment of Mn-associated neurotoxicity (Parkinson-like symptoms)

Study Design:

This Clinical Study Report (CSR) summarizes data from Parts 2a and 2b of the study and follow-up and overall survival (Part 3 of the study) for patients included in Parts 2a or 2b. In the results, Part 3 data is referred to as follow-up data for Part 2 patients. See text below for details on Part 2.

Parts 2a and 2b of the study (the treatment phase) was a double-blinded, randomized, multi-center, Phase II, parallel-group, placebo-controlled study. Patients were screened and concurrently randomized (1:1:1) into one of the following 3 treatment arms:

- Group A: mFOLFOX6 + PledOx 2 µmol/kg
- Group B: mFOLFOX6 + PledOx 5 µmol/kg (Part 2b). Note, during the study there was a change in dose level for the highest dose group. In Part 2a, patients in the highest dose group received 10 µmol/kg.
- Group C: mFOLFOX6 + placebo

It was originally planned to randomize approximately 126 patients in Part 2 of the study. However, due to the change in dose level for the highest dose group, the total number of randomized patients was expanded to allow an adequate sample size also for the new dose in Part 2b (i.e. at least 126 additional patients were planned after a new randomization had been performed). The patients randomized before the dose change were considered as randomized in Part 2a of the study. To change the highest dose was a futility decision based on preliminary efficacy data from Part 1 (open dose-escalation phase, reported in a separate CSR), where the first 3 patients administered 10 µmol/kg PledOx, displayed numerically more dose-limiting chemotherapy-related side-effects (neutropenia and thrombocytopenia) than the PledOx 2 µmol/kg group. There was no safety concern with regard to the 10 µmol/kg impacting the dose decision. Due to time lags in implementing the dose change, a total of 39 patients (11 to PledOx 10 µmol/kg) were randomized in Part 2a.

Each patient in Parts 2a and 2b was to receive treatment according to their randomization (A, B or C) every 2 weeks (if no dose delay) for up to 8 cycles unless unacceptable toxicity or disease progression occurred. The patients were hospitalized during the first 4 h of the first treatment cycle to allow PK sampling. Treatments were administered intravenously in a double-blinded manner. PledOx or placebo was given as pre-treatment 10 minutes before the oxaliplatin dose, as a 5-minute infusion. Treatment with mFOLFOX6 beyond the 8th cycle was left to the investigator's discretion. However, no patient continued beyond 8 cycles. The addition of bevacizumab to mFOLFOX6 was optional. After completion of the first mFOLFOX6 cycle, reduction and delays in chemotherapy dosing could be performed.

The duration of the treatment period of Parts 2a and 2b of the study for each patient was approximately 19 weeks, depending on the timing of the screening visit and if the patient completed all 8 cycles of treatment (treatment every 2 weeks). During this time, each patient performed up to 18 visits, including Screening, 2 visits per treatment cycle and an End-of-Treatment visit. In addition, the patients completed the cold allodynia test at home on Days 2 to 4 of each treatment cycle.

Response rate was evaluated at end of treatment. After the treatment with PledOx and chemotherapy had been ended (up to 8 cycles), patients entered a follow-up phase (Part 3 of the study) to collect data on progression-free survival, every 12 weeks (4 visits), for 12 months after End-of-Treatment and overall survival for an additional 8 months. During the follow-up period, neurotoxicity and other safety issues were also evaluated and recorded.

Assessments during the study are summarized below:

Screening assessments: informed consent, computed tomography (CT)/magnetic resonance imaging (MRI)-scan and disease assessment, assessment of cardiac function (3-lead ECG), pregnancy test (women of childbearing potential only), demography, eligibility criteria, medical history, prior and concomitant medications, hematology and clinical chemistry sampling, blood Mn and exploratory iron measurements, physical examination, WHO performance status, vital signs, neurological examination and randomization.

Assessments during the treatment cycles: assessment of cardiac function (3-lead ECG), prior and concomitant medications, physical examination including WHO performance status, vital signs, hematology and clinical chemistry, blood Mn and exploratory Fe measurements, Leonard scale questionnaire, cold allodynia test, European Organization for Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ)-C30 quality of life questionnaire, PledOx/placebo administration, chemotherapy ± bevacizumab administration and any dose modifications, assessment for dose-limiting toxicity (DLT), AEs, CT/MRI-scan and disease assessment after Cycle 4 to assess response rate according to Response evaluation criteria in solid tumors (RECIST 1.1) and pharmacokinetic (PK)-sampling. In case of Parkinson-like symptoms of central nervous system (CNS) toxicity: neurological examination, MRI of CNS and blood Mn.

End-of-treatment assessments: CT/MRI-scan and disease assessment according to RECIST 1.1, hematology and clinical chemistry, prior and concomitant medications, blood Mn and exploratory Fe measurements, physical examination (including WHO performance status), vital signs, oxaliplatin-induced peripheral neuropathy by OSSS, Leonard scale questionnaire, cold allodynia test, EORTC QLQ-C30 quality of life questionnaire, assessment for DLT, AEs, neurological examination, MRI of CNS and blood Mn (if Parkinson-like symptoms).

Follow-up assessments: response (based on CT/MRI scan and disease assessment according to RECIST 1.1), concomitant medications (anti-cancer treatment only), hematology and clinical chemistry, physical examination (including WHO performance status), vital signs, EORTC QLQ-C30 quality of life questionnaire, optional assessment of Leonard scale questionnaire and cold allodynia test every 12 weeks for 12 months after end-of-treatment, progression-free survival and overall survival after an additional 8 months.

Table B Number of patients in Part 2 and 3 of the study

	Total	PledOx 2 µmol/kg (N=57)	PledOx 5 µmol/kg (N=45)	PledOx 10 µmol/kg (N=11)	Placebo (N=60)
No. randomized (and treated):					
Parts 2a and 2b:	**173**	57	45	11	60
Part 2a:	**39**	13	-	11	15
Part 2b:	**134**	44	45	-	45
No. males/females					
Parts 2a and 2b:	**116/57**	41/16	24/21	5/6	46/14
Part 2a:	**27/12**	8/5	-	5/6	14/1
Part 2b:	**89/45**	33/11	24/21	-	32/13
Mean age (range in years)					
Parts 2a and 2b:	**62.7 (38 to 83)**	63.1 (42 to 83)	62.6 (38 to 82)	64.8 (51 to 80)	62.0 (41 to 80)
Part 2a:	**63.2 (50 to 80)**	64.5 (56 to 76)	-	64.8 (51 to 80)	61.0 (50 to 77)
Part 2b:	**62.5 (38 to 83)**	62.7 (42 to 83)	62.6 (38 to 82)	-	62.3 (41 to 80)
No. completed 8 cycles of IMP treatment:					
Parts 2a and 2b:	**126**	44	30	5	47
Part 2a:	**27**	9	-	5	13
Part 2b:	**99**	35	30	-	34
No. completed each follow-up visit (FUP):					
FUP1 (3 months after last dose)					
Parts 2a and 2b:	**133**	45	33	8	47
Part 2a:	**31**	11	-	8	12
Part 2b:	**102**	34	33	-	35
FUP2 (6 months after last dose)					
Parts 2a and 2b:	**107**	35	26	8	38
Part 2a:	**26**	8	-	8	10
Part 2b:	**81**	27	26	-	28
FUP3 (9 months after last dose)					
Parts 2a and 2b:	**87**	31	20	7	29
Part 2a:	**21**	6	-	7	8
Part 2b:	**66**	25	20	-	21
FUP4 (12 months after last dose)					
Parts 2a and 2b:	**72**	24	17	7	24
Part 2a:	**21**	6	-	7	8
Part 2b:	**51**	18	17	-	16

	Total	PledOx 2 µmol/kg (N=57)	PledOx 5 µmol/kg (N=45)	PledOx 10 µmol/kg (N=11)	Placebo (N=60)
Parts 2a and 2b:					
Randomized Population	173	57	45	11	60
Safety Population	173	57	45	11	60
Full Analysis Set Population	173	57	45	11	60
Per-Protocol Population	169	56	44	11	58
Part 2a:					
Randomized Population	39	13	-	11	15
Safety Population	39	13	-	11	15
Full Analysis Set Population	39	13	-	11	15
Per-Protocol Population	38	13	-	11	14
Part 2b:					
Randomized Population	134	44	45	-	45
Safety Population	134	44	45	-	45
Full Analysis Set Population	134	44	45	-	45
Per-Protocol Population	131	43	44	-	44

Main criteria for inclusion:
Patients with biopsy-verified and measurable advanced metastatic colorectal cancer (stage IV), who were chemotherapy-naive or had completed one line of chemotherapy for metastatic disease at least 4 weeks prior to signing consent. Patients should previously not have been treated with oxaliplatin.

Test product, dose and mode of administration:

PledOx was administered at doses of 2 and 10 µmol/kg in Part 2a, and 2 and 5 µmol/kg in Part 2b. The study drug was provided as 2 mM or 10 mM solutions (based on Mn [II]) in 20-mL vials, and administered as 0.2 mL/kg body weight by intravenous infusion. For the 5 µmol/kg dose a dilution of the 10 mM solution was performed before administration.

Reference product, dose and mode of administration:

Placebo (0.9% sodium chloride, provided in 50-mL vials), 0.2 mL/kg body weight administered by intravenous infusion.

Duration of treatment:

IMP (PledOx or placebo) was given as a single pre-treatment infusion over approximately 5 minutes, 10 minutes prior to oxaliplatin administration in the mFOLFOX6 regimen. The pre-treatment IMP was given before each chemotherapy cycle for up to 8 treatment cycles, every 2 weeks.

Efficacy Assessments:

Primary efficacy variable

- The primary efficacy variable was presence of neuropathy Grade 2 or higher (according to the investigator reported OSSS criteria for oxaliplatin-related paresthesia/dysesthesia). This was assessed during chemotherapy treatment up to End-of-Treatment.

The primary variable was changed from neutropenia to neuropathy before the treatment code was broken. This change was made after a blinded data review which indicated that the incidence of neutropenia was

only 4%, whereas the expected incidences were 40% for placebo and 20% for active treatment with PledOx. The blinded data review of the incidence of neuropathy showed that there were enough cases of these to be evaluable.

Secondary/exploratory efficacy variables (All efficacy variables were assessed at Baseline, i.e. at Cycle 1 Day 1 and /or at Screening. Post-dose assessments were made at the end of each of the 8 treatment cycles unless otherwise stated.)

- Symptoms and signs of peripheral neuropathy:
  - Outcome of patient reported cold allodynia test, assessed post-infusion at Day 1 to Day 4 of each of the 8 treatment cycles, at End-of-Treatment and optionally at FUP1 to FUP4
  - Patient reported oxaliplatin-associated sensory neuropathy scale (Leonard Scale questionnaire), assessed pre-infusion at Day 1 of each of the 8 treatment cycles, at End-of-Treatment and optionally at FUP1 to FUP4
- Objective and overall response – based on CT or MRI of thorax, abdomen and pelvis and characterization of target and non-target lesions, according to the RECIST 1.1 (CR, PR, SD or PD). Best overall response rate was calculated based on the assessments after 4 and 8 cycles
- Progression-free survival assessed at 12 months of follow-up
- Overall survival assessed 20 months after the End-of-Treatment visit
- Presence of febrile neutropenia
- Presence of oral mucositis
- Absolute neutrophil count, and other relevant laboratory test variables
- Neutropenia grade, and presence of neutropenia Grade 3 or higher
- Thrombocytopenia grade, and presence of thrombocytopenia Grade 1 or higher
- Frequency and intensity of other (predefined) chemotherapy-induced AEs
- Dose modification (dose frequency and intensity)
- Quality of life (EORTC QLQ-30C), assessed pre-infusion at the first day of treatment Cycle 2 to Cycle 8, at the end of Cycle 8 and at FUP 1 to FUP 4
- PK variables, assessed pre-dose and post-dose at treatment Cycle 1:
  - Pre-dose concentration (C_{pre})
  - Half-life of the terminal slope ($t_{½}$)
  - The observed maximum concentration (C_{max})
  - The observed time to reach maximum concentration (t_{max})
  - The area under the concentration-time curve from time zero to the last measured concentration (AUC_t)
  - The area under the concentration-time curve from time zero to infinity (AUC_∞)

Safety Assessments:

- AEs, adverse drug reactions and serious adverse drug reactions, collected from Cycle 1 Day 1 up to End-of-Treatment. Any AEs that were ongoing at the End-of-Treatment visit were followed up until resolution during the follow-up period.
- Changes in hematology and clinical chemistry values, including those associated with hepatic and renal function, measured at Screening, Cycle 1 Day 1, Day 13 or Day 14 of each of the 8 treatment cycles, End-of-Treatment and FUP1 to FUP4
- Assessment of physical examination, performed at Screening, Day 1 of each of the 8 treatment cycles, End-of-Treatment and FUP1 to FUP4
- Vital signs, performed at Screening, Day 1 of each of the 8 treatment cycles, End-of-Treatment and FUP1 to FUP4
- Cardiac function (QT prolongation), assessed at Screening and Cycle 1 Day 1
- Safety Mn measurements in blood, measured at Screening, Day 13 or Day 14 of Cycle 4, End-of-Treatment and in the event of any Parkinson-like symptoms
- Assessment of Mn-associated neurotoxicity (Parkinson-like symptoms) at every visit

Statistical methods:

General

The statistical analyses were described in a Statistical Analysis Plan (SAP) that was finalized prior to unblinding the study. In particular, the change of the primary objective/endpoint was made before unblinding the study. Additional exploratory analyses (planned after code break) were also performed and described separately (SAP Addendum 3 and SAP Addendum 4).

Continuous data were summarized using descriptive statistics, including the following parameters: Number of observations (n) and missing observations (nmiss), mean and standard deviation, median, first and third quartiles, extreme values (minimum and maximum). Categorical data were summarized using absolute frequency (n) and percentage (%).

Tests of primary endpoint were one-sided and performed at the 10% significance level if not otherwise specified. All confidence intervals were at the 90% level, and model-based where appropriate. In addition, we have performed hypothesis testing using a two-sided approach using $p<0.05$ for other endpoints, to be interpreted as exploratory evidence.

Patients were classified into the following 3 analysis sets before breaking the blind:

- The **Full analysis set (FAS)** for efficacy analysis: all randomized patients who had received at least 1 dose of the IMP
- The **Per-protocol analysis set (PPAS):** a subset of the FAS, consisted of patients who had also fulfilled the following:
    - Had sufficiently complied with the CSP (i.e., had no major protocol deviations)
    - Had available data for the assessment of the primary variable (i.e., at least 1 post-baseline assessment of oxaliplatin-induced neuropathy) during the treatment phase
- The **Safety analysis set:** all patients who received at least 1 dose of the IMP

The change of the high dose of PledOx, from 10 µmol/kg to 5 µmol/kg, implies some difficulties in the analysis and interpretation of some dose versus placebo comparisons in Part 2. Patients were randomized to placebo and 2 µmol/kg in both Part 2a and Part 2b, to 10 µmol/kg only in Part 2a and to 5 µmol/kg only in Part 2b. Most of the results are presented based on the complete data, Parts 2a+2b. For the comparison of 5 µmol/kg versus Placebo using data from Parts 2a+2b, such comparison hinges on the additional

assumption on homogeneity between Parts. In some cases, a separate analysis of 5 µmol/kg versus Placebo using data from Parts 2b only is also presented. In all Tables and Figures, the basis for any comparisons is clarified in footnotes.

Patient and baseline data
Descriptive summaries were provided for the following: patient disposition, treatment with bevacizumab, premature withdrawals from the study, protocol deviations, demographics, medical history, prior and concomitant medications.

Efficacy
The main efficacy analyses were performed on the FAS and the PPAS, with the focus on the FAS.

The SAP stipulated controlling the type I error across multiple analyses of the primary variable by using a hierarchical structure of analyses and a closed testing procedure. However, since the primary endpoint was not met, all tests of secondary endpoints are considered as exploratory and no adjustments for multiplicity are presented here.

Pharmacokinetic Data
The PK variables and plasma concentrations for PledOx (metabolites ZnDPDP, ZnPLED, ZnDPMP) and the metals Zn and Mn were summarized descriptively in tables and graphs.

Safety Evaluation
Safety data were summarized descriptively using the Safety analysis set. This included extent of exposure, AEs, hematology and clinical chemistry data, physical examination, vital signs, body weight and cardiac function.

Primary efficacy summary (grade 2 or higher, investigator reported neuropathy OSSS):

- For the primary variable, investigator-reported oxaliplatin-induced neuropathy (Grade 2 or higher, according to OSSS), no statistically significant superiority was shown. This was neither found for any of the treatment groups defined according to dose and part of the phase II study (Part 2). Although not statistically significant, a strong tendency was observed for a lower patient proportion with neuropathy of Grade 2 or higher when testing PledOx 2 µmol/kg, PledOx 5 µmol/kg and PledOx 10 µmol/kg compared to placebo. This was based on the odds ratio of 0.615, which indicated an approximately 40% lower proportion of patients with Grade 2 or higher neuropathy in the PledOx pooled group (PledOx 2 µmol/kg, PledOx 5 µmol/kg and PledOx 10 µmol/kg) compared to placebo (p=0.1579) in the FAS. The proportion of patients with oxaliplatin-related neuropathy symptoms (Grade 2 or higher) was consistently lower in the PledOx groups than in the placebo group from End of Cycle 3 to End of Cycle 8. The overall proportion of patients with Grade 2 or higher neuropathy during the treatment phase in the placebo group was 23.3% (compared to 14.1% in the combined PledOx group), which was lower than the 40% assumed for the sample size calculations, and may have contributed to the lack of statistical significance despite observed differences between the treatment arms in favor of PledOx.
- Country-specific variations in overall incidence were observed, with particularly low incidences in Serbia, Georgia and the US (up to only 7.5% across all treatment groups), which may furthermore have contributed to the lack of statistical significance.

Exploratory analyses of the primary variable

- In the exploratory analyses of neuropathy of Grade 2 or higher excluding Cycles 1 and 2, a statistically significant improvement was observed for the combined groups (PledOx 2 + 5 + 10) µmol/kg compared to placebo.
- The PledOx 5 µmol/kg and PledOx 10 µmol/kg groups combined appeared to have less and delayed appearance of Grade 2 or higher neuropathy that persisted for 2 consecutive cycles compared to the placebo or PledOx 2 µmol/kg group (i.e. investigator reported neuropathy symptoms that can trigger an oxaliplatin dose-reduction). The differences were however based on few events and the total number of patients affected in each treatment group were: n=6 placebo; n=5 PledOx 2 µmol/kg; n=2 PledOx 5 + 10 µmol/kg.

Secondary efficacy summary:
The results of all secondary variables should be interpreted with caution due to the large number of statistical tests performed without multiplicity adjustments, which may lead to chance findings.

Leonard scale questionnaire (patient-reported)

- In the exploratory analysis, statistically significant superiority over placebo was shown for the proportion of patients scoring 3 or more (moderate/severe sensory symptoms) for the PledOx 5 µmol/kg (Part 2b) group alone (FUP1 odds ratio: 0.163; p=0.0355, FUP2 odds ratio: 0.121; p=0.0180) and with the combined 5+10 µmol/kg PledOx group (Parts 2a+2b, FUP1 odds ratio: 0.115; p=0.0073, FUP2 odds ratio: 0.097; p=0.0072) for the 'sensory score' (tingling, numbness score or burning pain to cold score). For the combined 2+5+10 µmol/kg PledOx group the odds ratio at FUP2 was 0.23 (p=0.014), indicating an approximately 75% reduction of chronic CIPN.
- The proportions of patients with a symptom rating score of 3 or more for any of the combined symptom score ratings were higher at most time points in the placebo groups, compared to the PledOx groups.

Cold allodynia (patient-reported)

- The analysis of outcome of the cold allodynia test showed statistically significantly less cold allodynia in the combined PledOx group compared to the placebo group at the later time points (Cycle 4 to Cycle 8).
- The results from the exploratory analysis of the cold allodynia test using Part 2b only were similar to the results from Parts 2a and 2b combined.

Neutropenia

- Few patients reported neutropenia of higher grade (Grade 3 or Grade 4). Mainly neutropenia of lower grade (Grade 1 and Grade 2), if any, was reported.
- The proportion of patients with any grade neutropenia (Grade 1 or higher) was consistently lower in the PledOx 2 µmol/kg group compared to the placebo group, at all time points. The effect of the combined PledOx 5+10 µmol/kg group was less pronounced compared to the 2 µmol/kg group.
- When analyzing the AE-module, a statistically significant difference in incidence of neutropenia Grade 1 or higher was observed in the pooled 2+5+10 PledOx treated group vs. placebo, 30% vs. 49% ($p<0.05$) as well as for 2 µmol/kg dose PledOx alone 26% ($p<0.05$).

Febrile neutropenia

- There were no cases of febrile neutropenia in the study.

Thrombocytopenia

- Mainly thrombocytopenia of lower grade (Grade 1 and Grade 2), if any, was reported. With no consistent differences in frequency between the PledOx groups and the placebo group, however, see effect of PledOx on platelet count below.

Absolute neutrophil count and other relevant laboratory parameters

- Statistically significantly higher group mean ANC values across all cycles post baseline were found in the PledOx 2 µmol/kg group compared to the placebo group. The means and medians were lower in the placebo group compared to both the PledOx 2 µmol/kg and the PledOx 5 µmol/kg groups at most time points.
- Statistically significantly higher group mean platelet count values across all cycles post baseline were found in the PledOx 5 µmol/kg group compared to the placebo group, but also in the pooled group given PledOx 2 µmol/kg, PledOx 5 µmol/kg or PledOx 10 µmol/kg compared to the placebo group.
- Statistically significantly higher group mean white blood cell count values across all cycles post baseline were found in the PledOx 2 µmol/kg group compared to the placebo group, but also in the combinations of the groups compared to the placebo group. The mean white blood cell count values decreased over time in all the treatment groups, and there was a tendency for lower means (greater decreases) in the placebo group compared to the other treatment groups from Cycle 2 onwards.

Dose modifications

- There were no statistically significant differences in the mean dose of oxaliplatin or 5-fluorouracil per patient and cycle between PledOx and placebo.

Quality of life

- Based on the mean scores from the EORTC-questionnaire, quality of life was in general stable during the treatment and follow-up phases and differed very little between the treatment groups.

Objective response rate

- There were no statistically significant differences between the PledOx groups versus placebo groups in objective response rate based on the best overall response.

Progression-free survival

- No statistically significant difference in progression-free survival was shown for any of the comparisons.
- The median progression-free survival time in the PledOx 2 µmol/kg, PledOx 5 µmol/kg and PledOx 10 µmol/kg combined group was 214 days and in the placebo group 238 days, a non-significant difference. There was no tendency that any of the PledOx doses behaved differently.

Overall survival

- Of the 173 treated patients, 75 patients (43.4%) were alive at the time for the overall survival assessment (20 months after End-of-Treatment) and 98 patients (56.6%) were deceased before this time point. Disease progression was the main reason for death.
- No statistically significant difference in overall survival was shown for any of the comparisons.
- The median overall survival time was 539 days for the combined PledOx groups and 529 days in the placebo group.

Pharmacokinetic summary

- For ZnPLED, the median elimination half-life was approximately 2 hours. T_{max} appears shortly after the end of infusion (around 0.3 hours), as expected. Median AUCs and C_{max}'s are roughly proportional to dose. The blood sampling for PK analysis started at end of infusion (5 minutes). Most patients had an estimated T_{max} around the first 2 samples (5 and 15 minutes).
- Only a few patients had enough quantifiable ZnDPMP and ZnDPDP concentrations to enable a PK analysis. The pattern with low concentrations of ZnDPDP and ZnDPMP ligands compared with the ZnPLED ligand is in line with previous nonclinical experience according to the Investigator's Brochure for PledOx.
- As expected for an endogenous substance, all patients had pre-dose Mn and Zn concentrations. After PledOx infusion, there was a short Mn concentration peak, and within a few hours the concentration levels were approximately back to the pre-dose endogenous level again.
- The PledOx infusion in the PledOx 5 µmol/kg and PledOx 10 µmol/kg groups caused a short and slight decrease in Zn concentration, and thereafter the levels increased above base levels with a peak around 1 hour and finally the levels decreased slowly again towards base levels. For the patients in the lowest (PledOx 2 µmol/kg) dose group, levels decreased slightly initially but were approximately back to baseline after 30-60 minutes.

Safety Summary:
Adverse events

- All patients, except 2 patients in the PledOx 2 µmol/kg group, reported AEs. The average number of AEs per patient was approximately 8 AEs in the PledOx 10 µmol/kg group, 10 AEs in the PledOx 2 µmol/kg group, 11 AEs in the PledOx 5 µmol/kg group and 14 AEs per patient in the placebo group.
- Twenty patients reported 26 SAEs. The SAE reporting frequency was 14.0%, 8.9%, 18.2% and 10.0% in the PledOx 2 µmol/kg, PledOx 5 µmol/kg, PledOx 10 µmol/kg and placebo groups, respectively. All SAEs were assessed as unlikely related to treatment with IMP except 2 SAEs, neither of which were SUSARs: hypersensitivity (reported by patient SE0204 [PledOx 10 µmol/kg] Cycle 6, Day 1; probable relationship to treatment, expected) and diarrhea (reported by patient RS0404 [placebo], Cycle1, Day 1, possible relationship to treatment, expected). Overall, there was no apparent difference between treatment groups in terms of numbers or types of SAEs.
- A total of 105 patients died during the study, of which 98 patients died within 20 months after End-of-Treatment, the time point used as a cut-off in the overall survival analysis. The main reason for death was disease progression. There were 5 deaths during the treatment phase of the study of which 2 occurred in the placebo group (small intestinal obstruction and hemiparesis) and 1 each in the PledOx groups (subileus, disease progression and ileus).
- Five patients were withdrawn due to AEs, during the treatment phase of the study; 2 in the PledOx 10 µmol/kg group (hypersensitivity and musculoskeletal pain) and 1 in each of the

PledOx 2 µmol/kg group (intestinal perforation), PledOx 5µmol/kg group (thrombocytopenia) and the placebo group (vertigo).

- Most AEs were of toxicity Grade 1 or Grade 2 (based on NCI-CTCAE grading criteria). AEs of toxicity Grade 3 were reported in similar patient proportions in the PledOx 2 µmol/kg and PledOx 10 µmol/kg groups (19.3% and 9.1% of the patients, respectively) and in slightly larger proportions in the PledOx 5 µmol/kg and placebo groups (35.6% and 31.7% of the patients, respectively). AEs of toxicity Grade 4 or 5 were reported occasionally.
- The most common AEs in the study (reported by ≥10% of the patients in any single treatment group) were: thrombocytopenia, fatigue, neutropenia, nausea, neurotoxicity, leukopenia, diarrhea, peripheral sensory neuropathy, anemia, decreased appetite, paresthesia, neuropathy peripheral, vomiting, paresthesia oral and pyrexia. All of the AEs except pyrexia were expected chemotherapy induced AEs.
- Of the most common AEs related to hematology, leukopenia and neutropenia were less commonly reported in the PledOx groups than in the placebo group. The differences were most obvious in the PledOx 2 µmol/kg group where the proportion of patients reporting leukopenia (19.3%) and neutropenia (26.3%) were lower compared to the placebo group (leukopenia: 36.7%; neutropenia: 48.3%). For thrombocytopenia and anemia there were no consistent differences in reporting frequency between the PledOx groups and the placebo group.
- For the most common nervous system disorders (neurotoxicity, peripheral sensory neuropathy, paresthesia and neuropathy peripheral), Grade 2 or higher appeared to be less common in the combined PledOx 5+10 µmol/kg group compared to the placebo group (11% versus 23%).
- Of the gastrointestinal disorders, nausea was less frequently reported in the PledOx groups than in the placebo group (approximately 36% vs. 47%). Diarrhea was more common in the combined PledOx 5 and 10 µmol/kg group (approximately 46%) than in both the PledOx 2 µmol/kg group (29.8%) and the placebo group (33.3%) and there appeared to be more Grade 2 events in the PledOx 5 µmol/kg group than in the other groups, however, there were no grade 3 events in either the PledOx 5 or 10 µmol/kg groups. NB that these numbers are in contrast to the clearly lower frequencies reported in the DLT module, where the incidence was highest at End of Cycle 1 and ranged between 14.0% (2 µmol/kg PledOx) and 21.7% (Placebo). There were no major differences in the proportions of patients who reported vomiting.

Laboratory assessments

- Safety laboratory assessments confirmed the expected decrease in mean counts of white blood cells, neutrophils (ANC) and platelets over time in all treatment groups. The decreases occurred at approximately the same rate in all groups. All individual clinically significant abnormal hematology values detected during the treatment phase, were reported as AEs. The most common AEs related to hematology which occurred in up to 68.4% (thrombocytopenia, 2 µmol/kg dose vs 63.3% in the placebo group), 48.3% (neutropenia, placebo group), 36.7% (leukopenia, placebo group) and 28.9% (anemia, 5 µmol/kg dose vs 26.7% in the placebo group) of patients.
- Slight decreases from baseline also occurred in lymphocytes, basophils and eosinophils in all treatment groups. Monocyte counts generally increased, but the clinical significance of this finding, if any, is unclear.
- There were minor changes in all clinical chemistry parameters during the study, but no tendencies regarding treatment or dose-related effects. The parameters assessed were: creatinine, bilirubin, ALAT, ASAT, sodium, calcium, magnesium, or potassium. Few patients had AEs related to clinical chemistry values and there were no apparent differences between treatment groups regarding types or frequencies of such AEs.

- In the summaries of safety Mn measurements, there was a dose-dependent increase in blood Mn, from baseline to End of Cycle 8, in the PledOx groups, and a slight increase also in the placebo group. There was only one report of a clinically significant abnormal Mn level. No increases from baseline were observed in plasma Mn.
- In the exploratory summaries of the exploratory blood iron measurements, the placebo group had a mean change from baseline to End of Cycle 8 of 10.7%, while the PledOx groups showed only minor changes from baseline to End of Cycle 8. For the exploratory plasma iron, large increases from baseline were shown in all the PledOx groups from baseline to End of Cycle 8. There were however large variations in plasma iron changes between individuals within the groups.

Vital signs

- There was a slight decrease in mean body weight over time from Baseline to the End of Treatment, in the PledOx 2 µmol/kg group. In comparison, the PledOx 5 µmol/kg group and placebo group both showed a slight increase in mean weight over time during the treatment phase. At the end of the follow-up phase (FUP4), all PledOx groups showed a slight increase in mean body weight compared to baseline, which was not seen in the placebo group. For the other vital signs assessed (mean pulse, mean body temperature, systolic and diastolic blood pressure), there were no clear differences in the vital signs observed over time within the treatment groups or between the different treatment groups.
- There were a few vital sign-related AEs reported; weight decrease reported in 4 patients and hypotension reported in 1 patient.

Physical examination

- Overall, few patients in the study had abnormal findings in the physical examinations post-baseline. Clinically significant abnormalities were reported as AEs. Two patients had abnormal clinically significant neurological findings during the treatment phase (neurological changes and neurosensory neuropathy). It was not specified whether these findings were related to the CNS. During the follow-up phase, 3 patients had abnormal clinically significant neurological findings, all of which were peripheral.
- The WHO performance status was graded as 0-2 for all patients at baseline (as per the inclusion criteria) and remained within Grades 0-2 for all patients except 1 throughout the treatment phase. During the follow-up phase, a few patients had a deterioration in WHO performance status to Grade 3 or 4. This occurred both in the PledOx and placebo treatment groups.

Cardiac function

- According to the overall clinical judgement of the 3-lead ECGs, cardiac function was normal for the majority of patients both before and after dosing with IMP on Day 1 of Cycle 1. The data did not indicate an increase in cardiac abnormalities after dosing with PledOx compared to placebo.

Mn-associated neurotoxicity (Parkinson-like symptoms)

- All patients were evaluated at each visit about development of Parkinson-like symptoms, which was reported by about 10% of the patients. An MRI was recommended in these cases, however only considered indicated in **three placebo patients** (of which two had Parkinson-like symptoms). It showed brain metastases in two and no abnormalities in one patient.
- Blood manganese levels were normal in all but two patients where it was slightly but insignificantly elevated above 2xULN (one patient received 2 µmol/kg and one patient 5 µmol/kg).

OVERALL CONCLUSIONS:

Primary efficacy conclusion

- Pre-treatment of patients receiving mFOLFOX6 therapy for metastatic colorectal cancer with PledOx resulted in a lower incidence of peripheral neuropathy. A strong tendency of less neuropathy of Grade 2 or higher was observed for PledOx 2, 5 and 10 µmol/kg combined compared to placebo, based on the odds ratio (0.615), indicating an approximately 40% lower proportion in the PledOx treated group. Although no statistical significance was reached in the primary analysis, the observed relative reduction in the primary endpoint was approximately the same as assumed. The overall proportion of patients with Grade 2 or higher neuropathy during the treatment phase in the placebo group was 23%, which was lower than the 40% assumed for the sample size calculations, which may have contributed to the lack of statistical significance.
- In an exploratory analysis of neuropathy of Grade 2 or higher excluding Cycles 1 and 2, a statistically significant improvement was observed for the combined groups (PledOx 2 + 5 + 10) µmol/kg compared to placebo.

Secondary efficacy conclusions

Peripheral neuropathy

- Significant differences in the 2 patient-reported outcome forms, the cold allodynia test for acute neuropathy and the Leonard scale questionnaire for chronic CIPN, supported the primary endpoint by indicating a statistically significantly lower incidence and severity of patient reported peripheral neuropathy symptoms.
- The significant differences in the Leonard scale remained after treatment was finished for 3 (FU1) and 6 months (FU2) after end of treatment. The odds ratio against placebo for the proportion of patients scoring 3 or more on either numbness, tingling or burning pain/discomfort with cold at FU2, which is approximately 10 months after first dose was 0.38, p=0.15 for the 2 µmol/kg dose of PledOx (Part 2a+2b), 0.12, p=0.018 for the 5 µmol/kg dose PledOx (Part 2b) and 0.097 p=0.0072 for the 5+10 µmol/kg doses of PledOx (Part 2a+2b). For the combined 2+5+10 µmol/kg PledOx group the odds ratio at FUP2 was 0.23 (p=0.014), indicating an approximately 75% reduction of chronic CIPN.

Neutropenia

- A statistically significant difference in incidence of neutropenia Grade 1 or higher was observed in the pooled 2+5+10 PledOx treated group vs. placebo, 30% vs. 49% ($p<0.05$) as well as for the 2 µmol/kg dose PledOx alone 26% ($p<0.05$).

Dose modifications

- There was no statistically significant difference in the mean dose of oxaliplatin or 5-fluorouracil per patient and cycle between PledOx and placebo.

Quality of life

- Quality of life, based on the EORTC QLQ-C30 questionnaire scores, was in general stable during the treatment phase and the follow-up phase, and differed very little between treatment groups.

Objective response rate

- PledOx had no apparent negative impact on the likelihood of patients achieving an objective response to mFOLFOX6 therapy.

Progression-free survival

- No statistically significant difference in progression-free survival was observed between the groups.
- The median progression-free survival time was 214 days in PledOx treated patients and 238 in placebo treated patients.

Overall survival

- No statistically significant difference in overall survival was observed between the groups.
- The median overall survival time was 539 for PledOx treated patients and 529 for those who received placebo.

Pharmacokinetic conclusions

- For ZnPLED, the median half-life was approximately 2h. T_{max} appeared short after end of infusion (approximately 0.3 h). Median AUCs and C_{max} were roughly proportional to dose.

Safety conclusions

- PledOx was generally well tolerated with no safety or tolerability concerns identified.
- All patients, except 2 patients in the PledOx 2 µmol/kg group, reported AEs. Most AEs were of toxicity Grade 1 or Grade 2 in all groups. Of the most common AEs related to hematology (thrombocytopenia, neutropenia, leukopenia and anemia), leukopenia and neutropenia were less commonly reported in the PledOx groups than in the placebo group.
- There were 26 SAEs reported for 20 patients. All SAEs were assessed as unlikely related to treatment with IMP (probability of relationship to IMP was reported by the investigator), except 2 SAEs (hypersensitivity and diarrhea), however assessed as expected, i.e. no SUSAR was reported. Overall, there was no apparent difference between treatment groups in terms of numbers or types of SAEs.
- No safety concerns were identified for PledOx based on assessment of laboratory parameters (including Mn), vital signs, physical examination, cardiac function and assessment of Parkinson-like symptoms.