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 study 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: Patients in Part 1 of the study were included at 4 sites (2 sites in Sweden and 2 sites in the US).	
Publication based on the study (reference): Manuscript submitted to scientific journal.	
Studied period (year-month-day): Date of first patient included in Part 1: 2013-02-06 Date of last data collection (follow-up) in Part 1: 2016-11-23	**Phase of development:** Phase II
Objectives: The present study aimed to determine whether pre-treatment with 2 different dose levels of PledOx lowers the frequency and severity of dose-limiting toxicity (DLT) from modified FOLFOX6 (mFOLFOX6) administration as first line therapy without interfering with the anti-tumor efficacy in patients with metastatic colorectal cancer. <u>Primary objective of Study Part 1 (dose escalation phase)</u> To characterize the safety, and severe adverse events; prevalence, severity, drug-relatedness and seriousness of adverse events (AEs) of PledOx in 2 doses	
Study design: The study was divided into 3 parts: an open dose escalation phase (Part 1), a randomized treatment phase (Part 2) and a 12-month follow-up period (Part 3) for all patients who were treated in Part 1 or Part 2 of the study. Data were also collected on overall survival (Part 3) after an additional 8 months, i.e. 20 months after End-of-Treatment. This report summarizes data from Part 1 of the study, including follow-up and overall survival of these patients. In the results, Part 3 data is referred to as follow-up data and overall survival data for Part 1 patients. Part 1 of the study was performed to assess safety of PledOx when administered together with mFOLFOX6 in Part 1a or mFOLFOX6 + Avastin (bevacizumab) in Part 1b. The planned doses for Part 1b were amended twice during the study. First, a lower start dose of PledOx was added for the first treatment cycle (2 µmol/kg) together with bevacizumab, followed by 10 µmol/kg PledOx for Cycles 2 to 8. Second, the highest dose was lowered in the study from 10 to 5 µmol/kg (and the first treatment cycle from 2 to 1 µmol/kg). This was based on pre-clinical data indicating that PledOx has a bell-shaped dose-response curve and preliminary efficacy data from Part 1a, indicating that the highest dose group, administered 10 µmol/kg PledOx, displayed more dose-limiting chemotherapy-related side-effects (neutropenia and thrombocytopenia) than the PledOx 2 µmol/kg group. There had been no safety issues with regard to PledOx. Eleven patients had been included in Phase I without any	

detectable toxicity to calmangafodipir. The decision to lower the highest dose level was made after dosing of 3 patients with 10 µmol/kg PledOx in Part 1a.

PledOx doses administered in Part 1a of the study (in combination with mFOLFOX6):

- Cohort 1: 2 µmol/kg PledOx
- Cohort 2: 10 µmol/kg PledOx

PledOx doses administered in Part 1b of the study (in combination with mFOLFOX6 and bevacizumab):

- 2 µmol/kg PledOx (first cycle) + 10 µmol/kg PledOx (remaining cycles)
- 1 µmol/kg PledOx (first cycle) + 5 µmol/kg PledOx (remaining cycles)

After screening, each patient enrolled in Part 1a or 1b received up to 8 cycles of PledOx plus mFOLFOX6 (plus bevacizumab in Part 1b), every 2 weeks, and were assessed at regular visits during treatment. At the end of the PledOx and chemotherapy treatment period, an End-of-Treatment visit was performed and each patient then entered the follow-up period (Part 3) of the study and had follow-up visits every 12 weeks for 12 months and additional data collection on overall survival 8 months later.

A Data Safety Monitoring Board (DSMB) reviewed data during the treatment period according to a regular schedule and decided if multiple cycles could proceed, if dosing of additional patients could proceed and if dose escalation could proceed. Dose modifications of chemotherapy were allowed according to the protocol.

All patients enrolled in Part 1 remained at the hospital on Day 1 of the first cycle until the 4-h pharmacokinetic (PK) sampling had been performed. The patients returned the next day for a 24-h PK sampling. For practical reasons and at the discretion of the investigator the patient could be hospitalized for 24 h. Prior to CSP Master Version 5.0 (dated 2013-05-20) and US Version 3.0 (dated 2013-05-27), patients also had PK sampling at 8 h and 12 h post-dose and these patients remained at the hospital for the first 24 h after PledOx dosing.

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

Assessments/procedures during the treatment cycles: assessment of cardiac function (3-lead ECG), prior and concomitant medications, hematology and clinical chemistry sampling, physical examination including WHO performance status, vital signs, blood Mn and exploratory Fe measurements, Leonard scale, cold allodynia test (and touch allodynia test – later removed from the study), physician observed oxaliplatin-related neuropathy, European Organization for Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ)-C30, PledOx administration, chemotherapy ± bevacizumab administration, assessment of DLTs, AEs, CT/MRI-scan and disease assessment every 8 weeks to assess response rate, neurological examination, MRI of central nervous system, 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, hematology and clinical chemistry sampling, prior and concomitant medications, blood Mn and exploratory Fe measurements, physical examination, vital signs, physician observed oxaliplatin-related neuropathy, Leonard scale, cold allodynia test, EORTC QLQ-C30, assessment of DLTs, AEs, neurological examination, MRI of CNS and blood Mn (if Parkinson-like symptoms)

Follow-up assessments: response rate (based on CT/MRI scan and disease assessment according to Response evaluation criteria in solid tumors [RECIST]), concomitant medications (anti-cancer treatment only), hematology and clinical chemistry sampling, physical examination, vital signs, EORTC QLQ-C30 questionnaire, optional assessment of Leonard scale 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.

Number of patients (planned and analyzed in Part 1 of the study):

		Part 1a		Part 1b	
	Total	PledOx 2 µmol/kg	PledOx 10 µmol/kg	PledOx 1+5 µmol/kg	PledOx 2+10 µmol/kg
No. planned:	**12**	3	3	3	3
No. included and treated:	**13**	5	3	3	2
Males/females:	**9/4**	5/0	1/2	2/1	1/1
Mean age (standard deviation):		68 (8) years	66 (12) years	62 (6) years	35 (3) years
No. completed 8 cycles (IMP and chemotherapy):	**8**	2	2	2	2
No. completed follow-up 1:	**11**	4	3	3	1
No. completed follow-up 2:	**10**	4	2	3	1
No. completed follow-up 3:	**10**	4	2	3	1
No. completed follow-up 4:	**7**	2	1	3	1
Overall survival data collected:	**12**	5	3	3	1
No. analyzed for safety:	**13**	5	3	3	2

Diagnosis and main criteria for inclusion:

Patients with biopsy-verified and measurable advanced metastatic colorectal cancer (stage IV), who were chemotherapy-naive or had completed chemotherapy at least 4 weeks prior to inclusion. Patients should previously not have been treated with oxaliplatin.

Test product, dose and mode of administration, batch number:

PledOx at doses of 2 and 10 µmol/kg in Part 1a, and 1, 2, 5 and 10 µmol/kg in Part 1b was administered as 0.2 mL/kg body weight (max. 20 mL) by intravenous infusion. PledOx was provided as 2 mM or 10 mM solutions (based on Mn [II]) in 20-mL vials, i.e. for the 1 and 5 µmol/kg doses administered in Part 1b, a dilution of the 2 or 10 mM solution was performed before administration. Batch numbers used: C12021 (2 mM) and C12025 (10 mM).

Reference product, dose and mode of administration, batch number:

None

Duration of treatment:

PledOx was given as a single pre-treatment infusion over approximately 5 minutes, starting 10 minutes (Part 1a) or 30 minutes (Part 1b) prior to oxaliplatin administration in the mFOLFOX6 regimen. The pre-treatment PledOx was given before each chemotherapy cycle for up to 8 treatment cycles, every 2 weeks.

Safety variables:

- AEs collected 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.
- Laboratory parameters measured at Screening, Day 1, Day 9 and Day 13 or Day 14 of Cycle 1, Day 9 and Day 13 of Cycles 2 to 7, Day 9 of Cycle 8, End-of-Treatment and FUP1 to FUP4
- Blood Mn, measured at Screening, Day 13 or Day 14 of Cycle 4, End-of-Treatment and in the event of any Parkinson-like symptoms
- Vital signs included weight and supine measurements of resting pulse (beats per minute), body temperature, and resting systolic and diastolic blood pressure, performed at Screening, Day 1 and Day 9 of Cycles 1 to 3, Day 1 of Cycles 4 to 8, End-of-Treatment and FUP1 to FUP4
- Physical examination including WHO performance status, performed at Screening, Day 1 and Day 9 of Cycles 1 to 3, Day 1 of Cycles 4 to 8, End-of-Treatment and FUP1 to FUP4
- A 3-lead ECG to assess cardiac function with calculation of heart rate and ECG intervals (P-wave duration, PQ, QRS, QT and QTc), performed at Day 1 of Cycles 1 to 3
- Assessment of Mn-associated neurotoxicity (Parkinson-like symptoms) at every visit
- Neurological examination, performed at Screening, Day 1 and Day 9 of Cycles 1 to 3, Day 1 of Cycles 4 to 8, End-of-Treatment, FUP1 to FUP4 and whenever any Parkinson-like symptoms occurred

Pharmacokinetic assessments:

- Blood sampling for analysis of zinc dipyridoxyl ethylenediamine diacetate (ZnPLED), zinc dipyridoxyl monophosphate (ZnDPMP), zinc dipyridoxyl diphosphate (ZnDPDP) in plasma, Mn and Zn in plasma at Cycle 1 Day 1: pre-dose and 0 min (i.e. immediately after PledOx infusion), 15 min, 30 min, 1 h, 4 h, (8 h, 12 h, not for all patients) and 24 h post infusion. Note that the samples at 8 h and 12 h post infusion were removed during the study due to protocol amendments.
- The following PK parameters were calculated for the analytes:
  - The 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_∞)

Efficacy variables:

Efficacy data were collected in Part 1 for exploratory purposes only. Selected variables were summarized for the CSR:

- Tumor response assessments 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 (complete response [CR], partial response [PR], stable disease [SD] or progressive disease [PD]), assessed after 4 and 8 cycles of treatment, at End-of-Treatment and at Follow-up visits 1 to 4
- Symptoms and signs of peripheral neuropathy:
  - Grading of oxaliplatin-induced neuropathy according to the Sanofi-NCI criteria for oxaliplatin-related paresthesia/dysesthesia, assessed at Cycle 1 Day 1 and at the end of each of the 8 treatment cycles
  - Cold allodynia test assessed at Day 1 to 4 of each treatment cycle (outcome summarized for Day 1 assessments)
- Overall survival, assessed 20 months after the End-of-Treatment visit

Statistical methods:
No statistical tests were performed on data from Part 1 of the study. Data were summarized by descriptive statistics using a single analysis set (Safety analysis set) consisting of all patients who had received at least one dose of PledOx.

SUMMARY OF SAFETY RESULTS

PledOx was generally well tolerated and no safety or tolerability concerns were identified. No toxicity that could be ascribed to calmangafodipir was detected among the patients who were still in the study after the first 3 cycles at 2 and 10 µmol/kg without (n=6) or with 5 and 10 µmol/kg with bevacizumab (n=5). Part 2 could be started after all patients had received 3 treatment cycles. One patient discontinued treatment due to an AE, but this event was judged by the investigator as unlikely to be related to PledOx (SAE: febrile neutropenia). Three other SAEs occurred in Part 1 of the study (nausea, constipation, and neutropenia), all of which were judged as unlikely to be related to PledOx. SAEs occurred both in Part 1a (1 SAE) and Part 1b (3 SAEs in 1 patient).

The most common PTs were in Part 1 overall were leukopenia, neutropenia, diarrhoea, thrombocytopenia, nausea, fatigue, and neuropathy peripheral.

The total number of AEs, relatedness to PledOx and severity of AEs were approximately the same with and without concomitant administration of bevacizumab (i.e. in Part 1a vs. Part 1b). Seven of eight patients reported 127 AEs in Part 1a (without bevacizumab) and 5 of 5 patients reported 108 AEs in Part 1b (with bevacizumab). Most AEs were judged to have an unlikely relationship to PledOx (121 of 127 AEs in Part 1a; 106 of 108 AEs in Part 1b). AEs with possible relationship to PledOx included 6 AEs in 4 patients in Part 1a (headache, fatigue [reported term: tired], nausea [2 AEs], diarrhoea [reported term: loose stools intermittent], and visual acuity reduced [reported term: impaired vision]) and 1 AE (anaemia) in Part 1b. One AE in Part 1b was assessed as probably related to PledOx: type IV hypersensitivity reaction [reported term: delayed hypersensitivity reaction on continuous 5-FU pump]). The majority of the AEs were mild in both Part 1a and Part 1b (91 and 87 AEs), followed by moderate (26 and 14 AEs) and severe (10 and 7 AEs). All AEs of severe intensity were judged by the investigator as unlikely to be related to PledOx. AE numbers are based on the data exported at the end of the treatment period.

No safety concerns were identified for PledOx based on laboratory parameters (including Mn and Fe), vital signs, cardiac function and Parkinson-like symptoms, physical examinations or neurological examinations.

OVERALL CONCLUSIONS:

No safety issues were identified to affect the theoretical benefit-risk considerations for any of the investigated doses of PledOx. Furthermore, there were no safety concerns administering PledOx as a pre-treatment to mFOLFOX6 alone or in combination with bevacizumab. Overall, the benefit-risk of PledOx was considered to be favorable and justified start of the double blinded Part 2 of the study.