

# **First Clinical Experience with the Magnetic Resonance Imaging Contrast Agent and Superoxide Dismutase Mimetic Mangafodipir as an Adjunct in Cancer Chemotherapy-A Translational Study**

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## First Clinical Experience with the Magnetic Resonance Imaging Contrast Agent and Superoxide Dismutase Mimetic Mangafodipir as an Adjunct in Cancer Chemotherapy—A Translational Study<sup>1,2</sup>

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### Abstract

Preclinical research suggests that the clinically approved magnetic resonance imaging contrast agent mangafodipir may protect against adverse events (AEs) caused by chemotherapy, without interfering negatively with the anti-cancer efficacy. The present translational study tested if pretreatment with mangafodipir lowers AEs during curative (adjuvant) FOLFOX6 chemotherapy in stage III colon cancer (Dukes' C). The study was originally scheduled to include 20 patients, but because of the unforeseen withdrawal of mangafodipir from the market, the study had to be closed after 14 patients had been included. The withdrawal of mangafodipir was purely based on commercial considerations from the producer and not on any safety concerns. The patients were treated throughout the first 3 of 12 scheduled cycles. Patients were randomized to a 5-minute infusion of either mangafodipir or placebo (7 in each group). AEs were evaluated according to the National Cancer Institute's (NCI) Common Terminology Criteria for Adverse Events and the Sanofi-NCI criteria. The primary end points were neutropenia and neurosensory toxicity. There were four AEs of grade 3 (severe) and one AE of grade 4 (life threatening) in four patients in the placebo group, whereas there were none in the mangafodipir group ( $P < .05$ ). Of the grade 3 and 4 events, two were neutropenia and one was neurosensory toxicity. Furthermore, white blood cell count was statistically, significantly higher in the mangafodipir group than in the placebo group ( $P < .01$ ) after treatment with FOLFOX. This small feasibility study seems to confirm what has been demonstrated preclinically, namely, that pretreatment with mangafodipir lowers AEs during adjuvant 5-fluorouracil plus oxaliplatin-based chemotherapy in colon cancer patients.

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<sup>2</sup>Jan Olof G. Karlsson owns shares in NORSWEDAL Holding AS (one of the main owners of PledPharma AB). Karlsson is inventor on two granted patent families (e.g., US6258828 and US6147094) and patent applications (WO2009078794 and WO2011004323) covering the therapeutic use of mangafodipir in cancer. Rolf G.G. Andersson is a board member of PledPharma AB and has received research funding (less than 100,000 USD during the last 5 years) from PledPharma AB. Andersson is inventor on patent applications (WO2009078794 and WO2011004323) covering the therapeutic use of mangafodipir in cancer. Per Jynge owns shares in NORSWEDAL Holding AS (one of the main owners of PledPharma AB). Jynge is inventor on two granted patent families (e.g., US6258828 and US6147094) covering the therapeutic use of mangafodipir in cancer. The other authors declare no competing interests.

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## Introduction

Cancer of the colon or rectum (colorectal adenocarcinomas) is the second most common cancer in the United States and Europe. About half of the colorectal cancer patients will ultimately die of the disease, corresponding annually to approximately 50,000 people in the United States [1] and 200,000 people in Europe [2].

The chance to survive from colorectal cancer depends on the stage of the disease, generally being high in patients with a cancer confined to the primary site (Dukes' A and B) and low in advanced metastatic disease (Dukes' D) [1]. In approximately one third of the diagnosed colorectal cancers, the disease is locally advanced to one or more lymph nodes (Dukes' C). Postoperative adjuvant chemotherapy in Dukes' C colon cancer patients, for many years with 5-fluorouracil/leovorin (5-FU/LV) alternatively capecitabine alone and now in combination with oxaliplatin, has increased survival substantially in this group from approximately 48% to 73%. However, still more than 25% of the patients going through this regimen will die within 5 years because of relapse.

The efficacy of oxaliplatin in combination with 5-FU/LV is compromised because of a substantial risk of severe toxicity, in particular, neutropenia and neurotoxicity. Toxicity presents in more than half of the patients an intolerable burden and causes dose reductions, delays, or, in a worst-case scenario, complete discontinuation of therapy. The ability to deliver the planned dose and intensity of chemotherapy (the amount of drug administered/unit of time) is considered important for tumor control, survival, and quality of life. In clinical practice, as evident from, for example, the MOSAIC study [3], neutropenia is the main general limiting factor toward achieving this aim. Peripheral neurotoxicity is, however, the main cause to complete discontinuation of chemotherapy. Residual functional neuropathy is, of course, a serious problem in patients who have had a potentially curative resection. Numerous methods to prevent neurotoxicity, thus far, have been proven unsuccessful [4].

Mangafodipir is an approved magnetic resonance imaging (MRI) contrast agent for use in humans that entered the market in 1997 but has been withdrawn from the market by the manufacturer in 2010. To the best of our knowledge, the withdrawal was purely based on commercial reasons. During the development of mangafodipir, it was accidentally discovered that it had profound antioxidant properties [5]—properties that may, for instance, be used to protect the heart from oxidative stress during acute percutaneous coronary intervention [6,7].

During the past decades, it has been demonstrated that increase in oxidative stress, unrelated to known drug metabolism pathways, usually occurs after exposure to a series of structurally unrelated anticancer agents, including oxaliplatin, 5-FU, and paclitaxel [8]. The mechanisms of initiation of reactive oxygen production during exposure by different cancer chemotherapeutic agents are, however, unclear. In preclinical models, mangafodipir has been demonstrated to protect various healthy cells against injuries caused by drug-induced oxidative stress, for example, paracetamol-induced liver necrosis and apoptosis [9,10], doxorubicin-induced cardiotoxicity (Kurz et al., accepted for publication in *Translational Oncology*), and myelosuppressive effects of cytostatic/cytotoxic drugs, including oxaliplatin and 5-FU [11–13]. The cytoprotective effect has been obtained without diminishing the anticancer effects of oxaliplatin and 5-FU. On the contrary, mangafodipir enhances the anticancer effects of oxaliplatin and 5-FU [11,13]. These results are of interest because they suggest that at least some of the toxic effects of secondary reactive oxygen production after exposure to anticancer agents can be pharmacologically ameliorated [8], without diminishing the anticancer efficacy.

Because mangafodipir has been used clinically as a contrast agent, this drug was suggested in an 2006 editorial in the *Journal of the National Cancer Institute* by James H. Doroshow [8] to be appropriate as a chemoprotective compound in human trials.

The present small translational study was designed to find out whether pretreatment with the compound mangafodipir lowers the frequency and severity of AEs during adjuvant chemotherapy according to the FOLFOX6 regimen in patients operated on for carcinoma of the colon (stage Dukes' C). To the best of our knowledge, this is the first placebo-controlled study to report results from mangafodipir treatment in cancer patients on chemotherapy.

## Materials and Methods

### *Ethical Conduct of the Study*

The final study protocol, patient information, and consent form were reviewed and approved by the Regional Ethics Committee at the Linköping University and by the Swedish Competent Authorities (Läkemedelsverket; EudraCT 2007-002905) before inclusion of patients. The study was registered at ClinicalTrials.gov (NCT00671996). The study was conducted in accordance with the protocol, regulatory requirements, and the ethical principles of the Declaration of Helsinki.

### *Patient Population, Inclusion Criteria, and Randomization*

The study encompassed patients continuously enlisted to the FOLFOX6 regimen and included 14 patients who were followed up throughout three treatment cycles (of 12 scheduled FOLFOX6 cycles). Each of the first three cycles was preceded by a 5-minute intravenous infusion of 2  $\mu\text{mol/kg}$  mangafodipir or a corresponding volume of 0.9% sodium chloride (placebo) in two groups each consisting of seven patients. The only treatment variable was adjunct pretreatment with mangafodipir. Patients received mangafodipir or placebo throughout three cycles of chemotherapy, and accordingly, they were randomized to a mangafodipir treatment group and a non-treated placebo group.

### *Baseline Evaluation*

Pretreatment (baseline) evaluation were performed within 1 week before the start of chemotherapy and included (i) medical history; (ii) physical examination; (iii) vital signs including weight and height; (iv) hematology including white blood cell (WBC) count, absolute neutrophil count (ANC), platelet count, and hemoglobin (Hb); (v) serum chemistry including creatinine, bilirubin, alanine aminotransferase, and aspartate aminotransferase.

### *Treatment Evaluation after Each of the Three Placebo or Mangafodipir-Treated FOLFOX6 Cycles*

Evaluation was performed within 24 hours before the start of the second, third, and fourth FOLFOX6 chemotherapy cycles and included (i) physical examination; (ii) hematologic assessments including WBC count, ANC, platelet count, and Hb (venous blood samples for hematology were taken between 8:00 and 10:00 A.M. the day before the start the FOLFOX6 cycle); (iii) signs and symptoms of adverse events (AEs) according to National Cancer Institute's (NCI) Common Terminology Criteria for Adverse Events (CTCAE) Version 3 or Sanofi-NCI criteria (no effect = 0, mild = 1, moderate = 2, severe = 3, and life threatening = 4) with emphasis on hematology and neurosensory toxicity; oral mucositis, vomiting, diarrhea, fever, and infection; and

(iv) serum chemistry including creatinine, bilirubin, alanine aminotransferase, and aspartate aminotransferase.

The hematologic and serum biochemical analyses were performed according to clinical routine at accredited laboratories in Jönköping County (Swedish Board for Accreditation and Conformity Assessment, 2007-06-20).

### Follow-up

After the end of treatment with mangafodipir or placebo, patients were scheduled to go through nine more FOLFOX6 cycles. This means that the patients were followed up for at least 4 months after the end of mangafodipir or placebo treatment, as long as they did not interrupt chemotherapy for any reason. Delays and dose reduction due to dose-limiting toxicity (DLT) were compared in the two groups during the follow-up period.

### Study Treatment

Two groups, each consisting of seven patients, received either mangafodipir 2  $\mu\text{mol/kg}$  body weight or placebo accompanying the first three cycles of FOLFOX6. All patients received 5-FU, calcium-LV, and oxaliplatin according to the FOLFOX6 regimen throughout 12 cycles, and pretreatment with antiemetics and corticosteroids was given, as specified in Table 1. Mangafodipir treatment was undertaken with a ready-to-use investigative drug formulation identical with what was in the diagnostic use as a contrast medium for MRI (ATC code V08CA05):

- Formulation content: mangafodipir 10 mmol/ml and ascorbic acid 6 mmol/ml
- Administered dose per cycle: 2  $\mu\text{mol/kg}$  body weight
- Administration form: ready-to-use formulation (solution)
- Placebo: 0.9% NaCl
- Mangafodipir or placebo, 0.2 ml/kg body weight, was administered as an intravenous (IV) infusion for 5 minutes approximately 30 minutes before the start of chemotherapy.

Vials containing either yellow-colored mangafodipir or noncolored placebo and the corresponding infusion set were hidden for the patient during administration.

### Evaluable Patients, End Point Analyses, and Statistics

All patients who received at least the first FOLFOX6 cycle as specified in Table 1 and showed up for the second cycle according to the FOLFOX6 schedule were considered evaluable for end point analysis with regard to mangafodipir or placebo treatment. For patients going through all three treatment cycles and appearing for the fourth (non-treated) FOLFOX6 cycle, the last cycle is defined as the third cycle. However, for patients who, for some reasons, for example, changes in FOLFOX6-reg (dose reduction or delay), were not able to go through

**Table 1.** Chemotherapy Schedule.

Drug	Dose	Administration	Infusion Agent	Time (h)
Ondansetron	8 mg	IV injection		0.5 before chemotherapy
$\beta$ -Methasone	8 mg	IV injection		0.5 before chemotherapy
Oxaliplatin	85 mg/m <sup>2</sup>	IV infusion	0.5 L of glucose 5%	0-2
Ca-LV	200 mg/m <sup>2</sup>	IV infusion	0.5 L of glucose 5%	0-2
5-FU	400 mg/m <sup>2</sup>	IV bolus	Chase infusion 0.5 L of NaCl 0.9%	2
5-FU	2400 mg/m <sup>2</sup>	IV continued infusion	Chase infusion	2-48

all three cycles as scheduled in the protocol, the last cycle was defined as the first or the second. In such a case, mangafodipir or placebo administration was stopped.

The present study was designed as a small translational/feasibility study in which it was expected from preclinical findings to find a positive influence of the test substance particularly on hematologic toxicity, but in which we had no prior data in man to support our hypothesis, except for a published case report [14]. We also expected to see positive effects on other AEs. The primary end points were FOLFOX-related DLT, e.g., neutropenia and neurotoxicity. The secondary end points were the frequency and severity of other FOLFOX6-related AEs.

It was presumed from preclinical data that a sample of 20 patients (10 placebo treated and 10 mangafodipir treated) would be enough for detecting a statistically significant difference regarding the primary end points between the placebo group and the mangafodipir group. Unfortunately, during the course of the study, the producer of the MRI contrast agent mangafodipir decided to withdraw it from the market. This decision was—to the best of our knowledge—purely based on commercial considerations and *not* on any safety concerns. However, the withdrawal of mangafodipir resulted in that the study had to be prematurely closed after 14 patients instead of 20 patients had been enrolled. Reduction in the number of patients in such a small feasibility study influenced the probability of showing a statistically significant difference between the groups, and accordingly, an ad hoc statistical analysis was performed on the number of patients who experienced severe AEs (grades 3 and 4) in the two groups. A Fisher exact test (one-sided) was used to test the statistical difference between placebo and mangafodipir-treated patients.  $P < .05$  was considered statistically significant.

Regarding the hematologic data and where appropriate, a non-paired, one-sided Student's  $t$  test was used to test statistical differences between the placebo and the mangafodipir groups.  $P < .05$  was considered statistically significant. Hematologic data are presented in graph as means  $\pm$  SEM.

## Results

### Study Patients

The patients were enrolled between June 2008 and March 2010 at the Oncology Clinic, at the County Hospital in Jönköping, Sweden. Ten were men (six were treated with mangafodipir and four were treated with placebo) and four were women (three were treated with placebo and one was treated with mangafodipir). The mean age of patients in the mangafodipir and placebo groups was 60.7 and 64.3 years, respectively.

As discussed in the Materials and Methods section, the present study was originally scheduled to enroll 20 patients, but because of the withdrawal of mangafodipir from the market, the study had to be closed after 14 patients had been enrolled.

### Adverse Events

Except for one event, all other AEs seen in the study were considered to be related to chemotherapy and/or the disease. On one occasion, a mangafodipir patient reported moderate respiratory problems during the initial part of the 5-minute mangafodipir infusion, which may have been indicative of a light hypersensitivity reaction. The same patient experienced later on, during the follow-up period (cycle 8), a more serious hypersensitive reaction to oxaliplatin, which resulted in the omission of oxaliplatin in the subsequent chemotherapy.

**Table 2.** AEs Occurring in the Placebo Group and the Mangafodipir Group, According to NCI-CTCAE Version 3 or Sanofi-NCI Criteria (No Effect = 0, Mild = 1, Moderate = 2, Severe = 3 and Life Threatening = 4).

AE	Placebo (Seven Patients; 18 Cycles)				Mangafodipir (Seven Patients; 20 Cycles)			
	Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade 3	Grade 4
Neutropenia	3	1	1	1	4	1*	0	0
Other hematologic toxicity	15	1	0	0	13	0	0	0
Neurosensory toxicity	9	0	1	0	9	0	0	0
Oral mucositis	2	2	0	0	0	0	0	0
Nausea	7	3	0	0	9	1*	0	0
Vomiting	1	1	1	0	2	0	0	0
Diarrhea	0	0	0	0	8	0	0	0
Fatigue	4	2	0	0	4	0	0	0
Other AE	3 <sup>†,‡,§</sup>	1 <sup>¶</sup>	1 <sup>#</sup>	0	1 <sup>**</sup>	0	0	0
ΣAE	44	11	4	1	50	2	0	0

\*Patient received a 10% higher dose during the first FOLFOX6 cycle.

<sup>†</sup>Chest wall pain.

<sup>‡</sup>Dyspnea.

<sup>§</sup>Skin reaction (face).

<sup>¶</sup>Pain in lower extremities.

<sup>#</sup>Ileus.

\*\*Nasal mucositis.

Eleven grade 2 (moderate) AEs were seen in the placebo group, whereas two were found in the mangafodipir group (Table 2). Four grade 3 (severe) AEs were observed in the placebo group and none in the mangafodipir group. One patient in the placebo group experienced a grade 4 AE (life threatening). Severe or life-threatening AEs were seen in three women and in one man. A one-sided Fisher exact test indicated a statistically significant difference ( $P = .035$ ) in the number of patients who experienced grade 3 or 4 events in the two groups. In 3 of 21 scheduled cycles in the placebo group, treatment was delayed because of grade 3 or 4 AEs, whereas one cycle was delayed in the mangafodipir group because of a grade 1 thrombocytopenia, in accordance with clinical routine in colon cancer chemotherapy.

There were eight events of mild (grade 1) diarrhea in the mangafodipir group (four patients), whereas there were none in the placebo group. This was the only AE, although mild, where it was a clear tendency of a higher frequency in the mangafodipir than in the placebo group. Whether this is a coincidence, or not, remains to be clarified in forthcoming studies. When mangafodipir is used as an MRI contrast agent, although at a higher dose level (5-10  $\mu\text{mol/kg}$ ) in comparison to the present study (2  $\mu\text{mol/kg}$ ), diarrhea has been reported to occur at a low frequency.

### Hematologic Observations

WBC (leukocytes), ANC (neutrophils), and platelet count in general decreased in both groups during the first three FOLFOX6 cycles, whereas no decrease was seen in Hb (Figure 1). Although there were two events of severe/life-threatening (grade 3/4) neutropenia in the placebo group, but none in the mangafodipir group, there were no statistically significant differences between these two groups regarding the mean fall in neutrophil counts between the first and last mangafodipir/placebo-treated cycle (Figure 2). However, the WBC count in the mangafodipir group was statistically, significantly higher than in the placebo group after the last treatment cycle (Figure 2).

### Serum Biochemical Findings

There were only minor effects on the results obtained from the biochemical assays of the blood serum in both groups during the first three cycles of chemotherapy (not shown).

### Follow-up

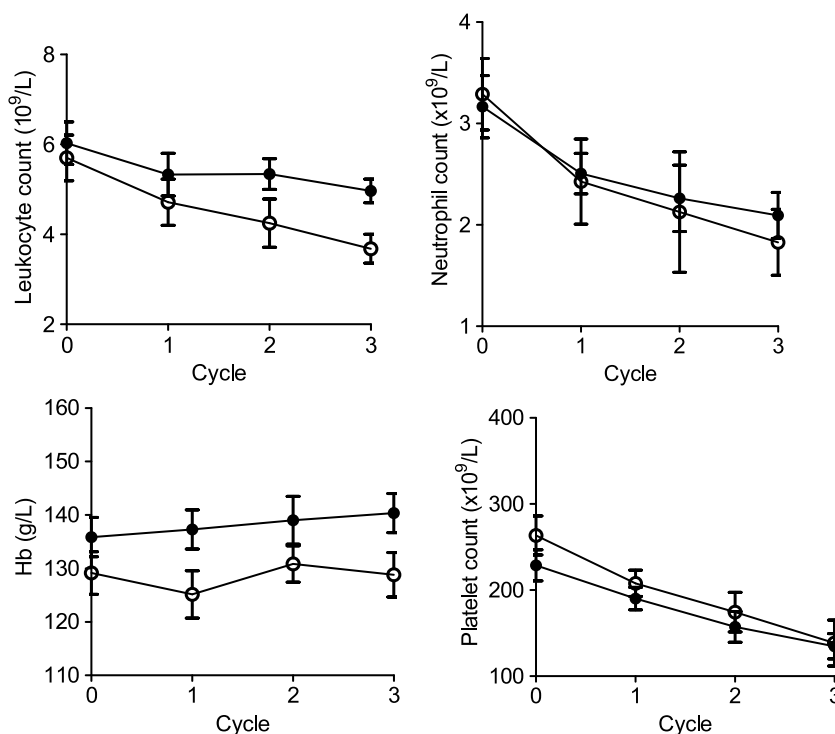
One patient in the placebo group experienced serious edema of the papillary of the eye during follow-up, an AE probably related to FOLFOX6 treatment. Because the patients were scheduled to be treated with placebo or mangafodipir during the first three cycles, according to the study design, any influence of treatment was expected to be seen immediately before the start of cycles 2, 3, and 4. During the placebo or mangafodipir treatment period (i.e., the first three FOLFOX cycles), there were four patients who experienced DLT delays corresponding to a total of 24 days, whereas there were two patients in the mangafodipir group, corresponding to a total of 12 days of delay. Importantly, during the placebo/mangafodipir nontreated period, i.e., from cycle 5 to 12, there were no more delays in the placebo group than in the mangafodipir group. The total number of delays during that period was 121 days in the placebo group and 156 days in the mangafodipir group, corresponding to approximately 15.4% and 19.8%, respectively, of the scheduled treatment days during that period. This observation indicates that the baseline sensitivity toward DLT to FOLFOX6 in the mangafodipir group was at least as high as that in the placebo group. The most common cause of delays was neutropenia, followed by thrombocytopenia.

Reduction in the oxaliplatin dose due to DLT taking place over time in the two groups was similar, which may further indicate similar baseline DLT sensitivity in the groups.

### Discussion

The cytoprotective effect of mangafodipir is probably related to its superoxide dismutase (SOD) mimetic activity [15] and its strong iron-chelating properties [16]. The SOD mimetic activity in combination with iron chelation makes mangafodipir extremely effective in arresting production of the most toxic reactive oxygen species, namely hydroxyl radicals and peroxynitrite.

During pathologic oxidative stress, the production of superoxide anions exceeds the endogenous protective potential. Moreover, superoxide reacts readily with nitric oxide to form highly toxic peroxynitrite, which nitrates tyrosine residues of the MnSOD enzyme and irreversibly inactivates the enzyme. Many years ago, this mechanism was suggested to participate in the chronic rejection of human renal allografts [17],



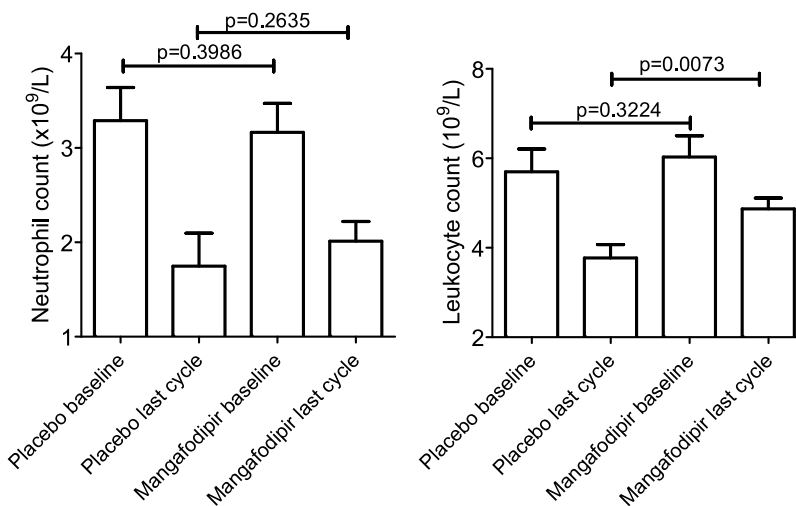
**Figure 1.** Hematologic data in the placebo group (○) and in the mangafodipir group (●) during the first three FOLFOX6 cycles: cycle 0 represents pretreatment value. *n* = 7 in each group (mean ± SEM).

and recent results indicate nitration of MnSOD to be an early step in paracetamol (acetaminophen)-induced liver failure [18], a condition known to be ameliorated by mangafodipir [9,10]. Whether this mechanism is also applicable for cytotoxic drugs remains to be shown.

Antimyelosuppressive, in particular antineutropenic, effects of mangafodipir were demonstrated in mice at a dose of approximately 15 μmol/kg [11], corresponding to a dose of 1 to 2 μmol/kg in man (when expressed as μmol/m<sup>2</sup>). The same dose has also been shown by the same authors to significantly increase survival in *Staphylococcus aureus*-infected mice treated with paclitaxel. Contrary to mangafodipir,

two other well-known SOD mimetics, MnTBAB and CuDIPS, did not protect against myelosuppressive effects [11].

When it comes to the other serious DLT, namely oxaliplatin-induced neurotoxicity, no preclinical data exist, to the best of our knowledge, showing protective effects of mangafodipir. However, a case report [14] described a patient who received 15 palliative cycles of oxaliplatin plus 5-FU/LV (“Nordic FLOX” regimen), suggesting that mangafodipir protects against peripheral neurotoxicity. In 14 of the cycles, the patient received pretreatment with mangafodipir. The patient received an accumulated dose of 1275 mg/m<sup>2</sup> oxaliplatin, which is a dose likely



**Figure 2.** Neutrophil and leukocyte counts at baseline and after the last placebo- or mangafodipir-treated cycle.

to give neurotoxic symptoms. No neurotoxic symptoms were detected except during the fifth cycle, when mangafodipir was deliberately left out and the patient experienced peripheral sensory neuropathy. After five cycles, the performance status for the patient was drastically improved, and the demand for analgesics was significantly reduced. Neutropenia did not occur during any of the chemotherapy cycles.

The present small translational study was designed to find out whether pretreatment with the compound mangafodipir lowers the frequency and severity of AEs during chemotherapy, as preclinical data and the above-mentioned case report suggest. For that purpose, it was decided to test mangafodipir in patients operated on for carcinoma of the colon, Dukes' C, and who went through adjuvant/curative chemotherapy according to the FOLFOX6 regimen. The main reason for selecting that group of patients was to achieve a small population as homogenous as possible. After recovery from surgery, most patients are generally in a good healthy condition, which often distinguishes them from patients with stage Dukes' D. To make the population even more homogenous, it was also decided to test mangafodipir during the first three cycles of FOLFOX6 chemotherapy. We expected that most patients should be able to go through the first three cycles without any changes in FOLFOX6 dosing. We anticipated effects of FOLFOX6 on the hematologic data, particularly on neutropenia, during the first three cycles. Furthermore, we anticipated statistically significant smaller effect in the mangafodipir group in comparison to the placebo group. However, regarding the primary parameters (neutropenia and neurotoxicity), the unforeseen and premature closing of study, after enrollment of 14 patients—instead of the scheduled 20 patients—of course, interfered negatively on the probability of showing a statistically significant difference between placebo- and mangafodipir-treated patients.

Nevertheless, there was a statistically significant less effect of FOLFOX6 on the leukocytes in the mangafodipir group than in the placebo group. Moreover, two patients in the placebo group but none in the mangafodipir group experienced severe/life-threatening (grade 3/4) neutropenia during the first three cycles of chemotherapy. This difference did not reach statistical significance, probably because of the small sample size.

Furthermore, there was no statistically significant difference in neutrophils between the mangafodipir and placebo group after FOLFOX. However, there was a tendency, although weak, of a larger fall over time in the placebo group than in the mangafodipir group. Preclinical studies have shown statistically significant difference in both leukocyte and neutrophil counts in balb/c mice receiving placebo pretreatment and mice receiving mangafodipir pretreatment followed by chemotherapy [11]. In an animal model of leukopenia, as the one used by Alexandre et al. [11], leukopenia may be expected to occur in every animal receiving chemotherapy (without any adjunct pretreatment), and the process governing leukopenia is assumed to be a continuous function. Balb/c mice are highly inbred and hence genetically homogenous. Therefore, they are expected to display a homogenous response toward chemotherapy. In genetically heterogeneous patients, however, it is often impossible to predict when and if a particular patient will experience chemotherapy-induced leukopenia. For instance, in one placebo patient in the present study, the neutrophil count decreased from approximately  $5 \times 10^9/L$  down to less than  $0.8 \times 10^9/L$  between the second and third cycles. In that particular patient, there was no effect of chemotherapy on the neutrophils between the first and the second cycle. A few other patients in the study went through the three first cycles without any major change in neutrophils.

Such heterogeneity makes it much more difficult to demonstrate the efficacy of mangafodipir in a clinical setting than in a preclinical setting. Another factor to take in consideration is the mangafodipir dose. In balb/c mice, pretreatment with approximately  $15 \mu\text{mol/kg}$  mangafodipir was shown to more or less normalize the leukocyte count. The mangafodipir dose in the present study, i.e.,  $2 \mu\text{mol/kg}$ , was selected from the assumption that the efficacy of mangafodipir correlates with the dose per squared meter. Whether  $2 \mu\text{mol/kg}$  is a somewhat low dose in man has to be sorted out. In the case report by Yri et al. [14], that patient received in fact  $10 \mu\text{mol/kg}$  per chemotherapy cycle. Furthermore, the dose was divided into two doses each consisting of  $5 \mu\text{mol/kg}$ ; the first dose was given immediately before starting chemotherapy and the second dose was given the day after.

As expected from the fact that circulating erythrocytes normally live for several months, in comparison to only hours to days for circulating leukocytes, no negative effect of chemotherapy on Hb was seen during the first three cycles. The difference in mean Hb between the placebo and the mangafodipir group probably relates to the below-described gender mismatch and, in general, the higher Hb value in men; six men and one woman in the mangafodipir group *versus* four men and three women in the placebo group.

There are reports suggesting that AEs on 5-FU/LV-based chemotherapy may be more common among females than males [19,20]. The accidental mismatch in the number of male and female patients included in the present study and the number of males and females randomized into the mangafodipir group, therefore, have to be taken in consideration. However, when delays in dosing and dose reductions are compared in the two groups from cycle 5 onward, that is, when no influence of mangafodipir or placebo is expected, there was in fact more delays in the mangafodipir group and no difference in dose reductions, suggesting that the baseline sensitivity toward dose-limiting toxicity of chemotherapy was at least as high in the mangafodipir group as in the placebo group.

According to the literature, toxicity presents in approximately half of the patients receiving 5-FU/LV plus oxaliplatin an intolerable burden and causes dose reductions, delays, or, in a worst-case scenario, complete discontinuation of therapy. For instance, in the MOSAIC study, discontinuation of treatment occurred in 25% and the patients received, on average, approximately 80% of the planned dose of FOLFOX [3]. However, the present study shows that dose-limiting toxicity may even be more problematic. The follow-up in the present study shows that none of the 14 patients included in the study could go through FOLFOX6 chemotherapy as scheduled. The follow-up period may be considered as nontrial situation for the patients; during that time, the patients did not receive any other treatment than FOLFOX6. That dose-limiting toxicity may even be more problematic in nontrial patients has been suggested by Fernández-Lobato et al. [21]. They reported that of 30 patients, only 16 completed the 12 planned FOLFOX4 cycles; 14 patients stopped their treatment (after an average of 8.1 cycles) because of dose-limiting toxicity in 10 patients, clinical progression in 3 patients, and death in 1 patient. Of the total 368 cycles administered, 68 suffered administration delays and 22 underwent dosage reduction.

Every patient in the present study experienced some grade of FOLFOX-related toxicity during the first three cycles. The patient in the mangafodipir group who experienced one event of grade 2 neutropenia and vomiting accidentally received a 10% higher FOLFOX6 dose during the first cycle, which may have contributed to both neutropenia and vomiting. However, none of the mangafodipir-treated

patients experienced any severe or life-threatening (grade 3/4) toxicity, whereas four patients in the placebo group experienced grade 3/4 toxicity. Although it should be stressed that the present study is small and only included a limited number of chemotherapeutic cycles, it is interesting to see that two (28%) of seven patients in the placebo group experienced grade 3/4 neutropenia, which fits into the relative number of patients reported to experience grade 3/4 neutropenia in the MOSAIC study, including 1108 patients on FOLFOX treatment. Furthermore, it is reported that neutropenia occurs most often early in the course of chemotherapy and is often underreported in clinical trials [22]. It may be further argued, based on retrospective reviews of large clinical trials, that myelosuppression and neutropenia are surrogates for delivered dose intensity with patients encountering neutropenic events uniformly experiencing better survival [22,23].

The overall conclusion from the present small translational study is that mangafodipir lowers the dose-limiting toxicity of chemotherapy, in this case FOLFOX6, that is, the study seems to confirm in man what has been shown already in animal studies. The promising result of the present study has encouraged the Swedish company PledPharma to start producing a therapeutic brand of mangafodipir for forthcoming larger clinical trials.

## References

- [1] O'Neil BH and Goldberg RM (2008). Innovations in chemotherapy for metastatic colorectal cancer: an update of recent clinical trials. *Oncologist* **13**, 1074–1083.
- [2] Ferlay J, Autier P, Boniol M, Heanue M, Colombet M, and Boyle P (2007). Estimates of the cancer incidence and mortality in Europe in 2006. *Ann Oncol* **18**, 581–592.
- [3] André T, Boni C, Mounedji-Boudiaf L, Navarro M, Tabernero J, Hickish T, Topham C, Zaninelli M, Clingan P, Bridgewater J, et al. (2004). Oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment for colon cancer. *N Engl J Med* **350**, 2343–2351.
- [4] McWhinney SR, Goldberg RM, and McLeod HL (2009). Platinum neurotoxicity pharmacogenetics. *Mol Cancer Ther* **8**, 10–16.
- [5] Asplund A, Grant D, and Karlsson JOG (1994). Mangafodipir (MnDPDP) and MnCl<sub>2</sub>-induced endothelium-dependent relaxation in bovine mesenteric arteries. *J Pharmacol Exp Ther* **271**, 609–618.
- [6] Karlsson JOG, Brurok H, Eriksen M, Towart R, Toft KG, Moen O, Engebretsen B, Jynge P, and Refsum H (2001). Cardioprotective effects of the MR contrast agent MnDPDP and its metabolite MnPLED upon reperfusion of the ischemic porcine myocardium. *Acta Radiol* **42**, 540–547.
- [7] Smith HJ (2001). Contrast-enhanced MR imaging in the diagnosis and preservation of cardiac viability [editorial]. *Acta Radiol* **42**, 539.
- [8] Doroshov JH (2006). Redox modulation of chemotherapy-induced tumor cell killing and normal tissue toxicity [editorial]. *J Natl Cancer Inst* **98**, 223–225.
- [9] Bedda S, Laurent A, Conti F, Chéreau C, Tran A, Tran-Van Nhieu J, Jaffray P, Soubrane O, Goulvestre C, Calmus Y, et al. (2003). Mangafodipir prevents liver injury induced by acetaminophen in the mouse. *J Hepatol* **39**, 765–772.
- [10] Karlsson JOG (2004). Antioxidant activity of mangafodipir is not a new finding [letter to the editor]. *J Hepatol* **40**, 872–873.
- [11] Alexandre J, Nicco C, Chéreau C, Laurent A, Weill B, Goldwasser F, and Bateau F (2006). Improvement of the therapeutic index of anticancer drugs by the superoxide dismutase mimic mangafodipir. *J Natl Cancer Inst* **98**, 236–244.
- [12] Karlsson JOG, Brurok H, Towart R, and Jynge P (2006). The magnetic resonance imaging contrast agent mangafodipir exerts antitumor activity via a previously described superoxide dismutase mimetic activity [letter to the editor]. *Cancer Res* **66**, 598.
- [13] Laurent A, Nicco C, Chéreau C, Goulvestre C, Alexandre J, Alves A, Lévy E, Goldwasser F, Panis Y, Soubrane O, et al. (2005). Controlling tumor growth by modulating endogenous production of reactive oxygen species. *Cancer Res* **65**, 948–956.
- [14] Yri OE, Vig J, Hegstad E, Hovde O, Pignon I, and Jynge P (2009). Mangafodipir as a cytoprotective adjunct to chemotherapy—a case report. *Acta Oncol* **48**, 633–635.
- [15] Brurok H, Ardenkjaer-Larsen JH, Hansson G, Skarra S, Berg K, Karlsson JO, Laursen I, and Jynge P (1999). Manganese dipyriddyloxyl diphosphate: MRI contrast agent with antioxidative and cardioprotective properties. *Biochem Biophys Res Commun* **254**, 768–772.
- [16] Rocklage SM, Cacheris WP, Quay SC, Hahn FE, and Raymond KN (1989). Manganese(II) *N,N'*-dipyridoxylethylenediamine-*N,N'*-diacetate 5,5'-bis (phosphate). Synthesis and characterization of a paramagnetic chelate for magnetic resonance imaging enhancement. *Inorg Chem* **28**, 477–485.
- [17] MacMillan-Crow LA, Crow JP, Kerby JD, Beckman JS, and Thompson JA (1996). Nitration and inactivation of manganese superoxide dismutase in chronic rejection of human renal allografts. *Proc Natl Acad Sci USA* **93**, 11853–11858.
- [18] Agarwal R, Macmillan-Crow LA, Rafferty TM, Saba H, Roberts DW, Fifer EK, James LP, and Hinson JA (2010). Acetaminophen-induced hepatotoxicity in mice occurs with inhibition of activity and nitration of mitochondrial manganese superoxide dismutase. *J Pharmacol Exp Ther* **337**, 110–118.
- [19] Díaz R, Aparicio J, Molina J, Palomar L, Giménez A, Ponce J, Segura A, and Gómez-Codina J (2006). Clinical predictors of severe toxicity in patients treated with combination chemotherapy with irinotecan and/or oxaliplatin for metastatic colorectal cancer: a single center experience. *Med Oncol* **23**, 347–357.
- [20] Sloan JA, Goldberg RM, Sargent DJ, Vargas-Chanes D, Nair S, Cha SS, Novotny PJ, Poon MA, O'Connell MJ, and Loprinzi CL (2002). Women experience greater toxicity with fluorouracil-based chemotherapy for colorectal cancer. *J Clin Oncol* **20**, 1491–1498.
- [21] Fernández-Lobato B, Díaz-Carrasco MS, Pareja A, Marín M, Vila N, and de la Rubia A (2009). Therapeutic use and profile of toxicity of the FOLFOX4 regimen. *Farm Hosp* **33**, 89–95.
- [22] Lyman GH (2006). Chemotherapy dose intensity and quality cancer care. *Oncology* **20**(suppl 9), 16–25.
- [23] Lyman GH (2009). Impact of chemotherapy dose intensity on cancer patient outcomes. *J Natl Compr Canc Netw* **7**, 99–108.