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Intratumoral Comparison of Nanoparticle Entrapped Docetaxel (CPC634) with Conventional Docetaxel in Patients with Solid Tumors

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ABSTRACT

Purpose: CPC634 is a novel nanoparticle entrapping docetaxel, developed to enhance the intratumoral chemotherapy exposure. This randomized cross-over study compared the intratumoral and plasma pharmacokinetics of CPC634 with conventional docetaxel.

Patients and Methods: Adult patients with solid tumors were randomized to receive CPC634 (75 mg/m^2) in cycle 1, and conventional docetaxel (75 mg/m^2) in cycle 2 or *vice versa*. The study was powered to identify a 25% increase of intratumoral total docetaxel exposure after CPC634 infusion compared with conventional docetaxel. Four patients were allocated per tumor sampling time point, that is, 24, 48, 72, and 96 hours, 7 and 14 days after infusion during both cycles. Total docetaxel and released docetaxel from the nanoparticle were determined in tumor tissue derived from a metastatic lesion and in plasma. Pharmacokinetic data were analyzed using linear mixed modeling.

Results: In total, 24 evaluable patients were included. In the tumor, CPC634 exhibited a 461% higher total docetaxel ($P < 0.001$) and a comparable released docetaxel concentration ($P = 0.43$). Plasma AUC_{inf} was 27% higher ($P = 0.001$) and C_{max} was 91% lower ($P < 0.001$) for CPC634 released docetaxel. The median observed neutrophil count nadir after conventional docetaxel treatment was lower (0.50×10^9/L) compared with CPC634 (4.30×10^9/L; $P < 0.001$).

Conclusions: Here, we demonstrated that CPC634 enhanced the intratumoral total docetaxel exposure compared with conventional docetaxel. The lower incidence of neutropenia during CPC634 treatment is presumably related to lower plasma C_{max} of released docetaxel. The unique pharmacokinetic profile of CPC634 nanoparticles has the potential to improve docetaxel treatment. A phase II efficacy trial of CPC634 is currently ongoing.

Introduction

Docetaxel is a highly potent antimitotic agent, used within multiple settings (1). Most anticancer drugs targeting mitosis like docetaxel are not tumor specific, and thus also affect healthy tissues where cell division frequently occurs (2). This results in adverse events such as neutropenia, neuropathy, stomatitis, and alopecia (3). Neutropenia is the most important dose-limiting toxicity of docetaxel because it may lead to potentially life-threatening neutropenic fever (4, 5). Different regimens and schedules of docetaxel have been explored to improve the clinical outcome, but the interpatient toxicity and efficacy of docetaxel remains heterogeneous and largely unpredictable (6). Suboptimal systemic and intratumoral concentrations of docetaxel are likely to be (partly) responsible for adverse events and treatment failure, respectively.

Docetaxel has a low molecular weight and a high hydrophobicity accompanied with low solubility (7, 8). The very low aqueous solubility of less than 5 μg/mL requires the addition of relatively toxic solubilization enhancers such as Polysorbate 80 to administer docetaxel intravenously (7–9). Polysorbate 80 has been implicated to induce hypersensitivity systemic reactions with an incidence rate ranging from 5% to 40% (9). From a pharmacologic point of view, a prolonged (and higher) drug exposure within the tumor is therefore desired, with limited—or ideally no—drug exposure in the healthy tissues and without the need of a detrimental solvent. A strategy to achieve this is the development of nanomedicines. Water-soluble nanoparticles loaded with chemotherapy are closed reservoirs of the native anticancer drug, which increases the molecular weight and solubility whereas it simultaneously decreases the distribution volume of the native anticancer drug. As a result of the leaky vasculature and poor lymphatic drainage of the tumor microenvironment, these loaded nanoparticles may accumulate in the tumor. Healthy tissues are (relatively) spared because of normal endothelial fenestrations which limit nanoparticle penetration (10). This selective transport to the tumor is called the "enhanced permeability and retention (EPR) effect" (**Fig. 1**).

Twenty-five years ago, the PEGylated liposome loaded with doxorubicin was the first FDA-approved nanomedicine (11) followed by nab-paclitaxel (12) and liposomal irinotecan (13). Although nab-paclitaxel has shown clinical superiority over the native drug paclitaxel,

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Translational Relevance

Nanoparticles aim to improve the systemic pharmacokinetic profile and enhance intratumoral exposure to the effective drug. CPC634 is a novel nanoparticle entrapping docetaxel. We demonstrated for the first time in a clinical study, by comparing the nanoparticle formulation CPC634 to its conventional formulation, that the nanoparticle leads to an enhanced and sustained intratumoral total docetaxel concentration. Moreover, the incidence of high-grade neutropenia was lower after CPC634 infusion compared with the same dose of conventional docetaxel. The hypothesis of nanoparticles leading to an accumulated intratumoral exposure and favorable systemic pharmacokinetics suggested in preclinical studies is now observed in this clinical setting. This may lead the way forward to a more efficient docetaxel treatment, if improved efficacy is shown during further phases of drug development. A recently conducted phase I study already proved its safety, and currently a phase II trial in ovarian cancer is ongoing.

the incidence of grade 3 to 4 toxicity was higher than seen with conventional paclitaxel (12). Several other nanoparticles have been under development but none of the novel formulations demonstrated an improved circulation and target site efficacy in the clinical setting which compared the native drug head-to-head with the nanoformulation (14–16). The most important limitation of nanomedicine is the premature drug release (17). An approach to overcome this is by stabilizing the nanoparticles via crosslinking so that prolonged circulation time and efficient EPR-mediated target site accumulation can be achieved (18). CPC634 is a novel nanoparticle of 65 nm size containing docetaxel. The nanoparticle has the morphology of a polymeric micelle, namely consisting of a hydrophobic core and a hydrophilic shell. The hydrophobic core solubilizes docetaxel, whereas both the polymers and docetaxel are cross-linked to prevent premature drug release, improving the systemic stability of the docetaxel entrapped nanoparticles (19). In preclinical experiments, CPC634 resulted in improved plasma pharmacokinetics and enhanced intratumoral drug exposure (20). A phase I trial (NCT02442531; the NAPOLY trial) was performed to investigate the plasma pharmacokinetic profile and the recommended phase II dose of CPC634. The recommended phase II dose of CPC634 was established at 60 mg/m^2 every 3 weeks (21). To address whether CPC634 improves the pharmacokinetic profile of the native compound docetaxel in a clinical setting, we initiated this study. Here, we investigated in a randomized cross-over study in patients with solid tumors, the plasma and the intratumoral drug exposure of CPC634, and compared this with conventional docetaxel pharmacokinetics.

Patients and Methods

Clinical study design

We performed a randomized two-arm pharmacokinetic study in patients with solid tumors. The primary objective was to demonstrate a 25% increase in total docetaxel concentration in the tumor after intravenous administration of CPC634 compared with conventional docetaxel whereas the secondary objectives were to compare the systemic pharmacokinetic and toxicity profile. Patients were randomized to receive CPC634 in cycle 1 and conventional docetaxel in cycle 2 (arm A) or *vice versa* (arm B). Tumor biopsies, to explore the intratumoral drug exposure over time, were taken in four patients per time point. More specifically, the time points were 24, 48, 72, 96 hours (±6 hours); 7 days (±1 day); or 14 days (±1 day) after administration of both study drugs, requiring (6 × 4) 24 evaluable patients. Patients were evaluable for the primary endpoint after completion of the second cycle and two successful tumor biopsies. Patients went off study after the second cycle, and continued conventional docetaxel treatment until progression or excessive toxicity was noticed. The study was approved by the Medical Research and Ethics Committee, and the board of directors of the Erasmus Medical Center. The trial was conducted in accordance with the International Conference on Harmonization Good Clinical Practice guidelines, applicable regulations, guidelines governing clinical study conduct, and ethical principles which have their origin in the Declaration of Helsinki. Signed informed consent was obtained from all participating patients, and the trial was registered in the Netherlands Trial Register (www.trialregister.nl; number NL6229).

Patients

Adult patients (≥18 years) with an advanced solid tumor with measurable disease according to Response Evaluation Criteria in Solid Tumors (RECIST 1.1; ref. 22) accessible for biopsy for whom no standard therapy existed, and for whom treatment with a taxane was an appropriate treatment option, were included. Eligibility criteria included an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, and an adequate hematological, liver, and renal function. Patients with unresolved toxicities [>grade 2 according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.03] related to previous anticancer therapy were excluded. Prohibited herbal or medicinal products included strong inducers or inhibitors of CYP3A4.

Treatment

CPC634 and conventional docetaxel were both administered intravenously at 75 mg/m^2 during a 1-hour infusion. At the start of our cross-over study, the phase I trial of CPC634 (NCT02442531) was still ongoing. In that trial, CPC634 was dosed at 70 mg/m^2 and could be administered safely without occurrence of dose-limiting toxicity during cycle 1. For this reason, a single dose of 75 mg/m^2 CPC634 was regarded safe and chosen to perform an optimal comparison between CPC634 and conventional docetaxel pharmacokinetics, because a 75 mg/m^2 every 3 weeks dose of docetaxel is most often used in clinical practice. According to local guidelines, 3 × 8 mg oral dexamethasone [respectively 12, 3, and 1 hour(s) before infusion] premedication was given prior to the administration of conventional docetaxel. CPC634 was given without premedication. One cycle of CPC634 lasted 4 weeks compared with 3 weeks for conventional docetaxel to ensure an optimal wash-out of CPC634 before the next cycle of chemotherapy was administered. Patients who were allocated to have a tumor biopsy at day 7 (±1 day) also received prophylactic granulocyte–colony stimulating factor (G-CSF) at 24 hours after the administration of conventional docetaxel to reduce the risk of neutropenia on the day of biopsy. During the CPC634 cycle, no G-CSF was administered because a neutrophil count decrease was not expected on the basis of the data of our phase I study with CPC634 (21). The conventional docetaxel solution contained polysorbate 80, citric acid, and 50% water-free ethanol as a solvent. CPC634 solution contained 20 mmol/L ammonium acetate pH 5 buffer supplemented with sodium chloride as an iso-osmotic solution (Cristal Therapeutics). The drug product CPC634 contained less than 2% released docetaxel and 98% chemically bound docetaxel to the nanoparticles. The content of

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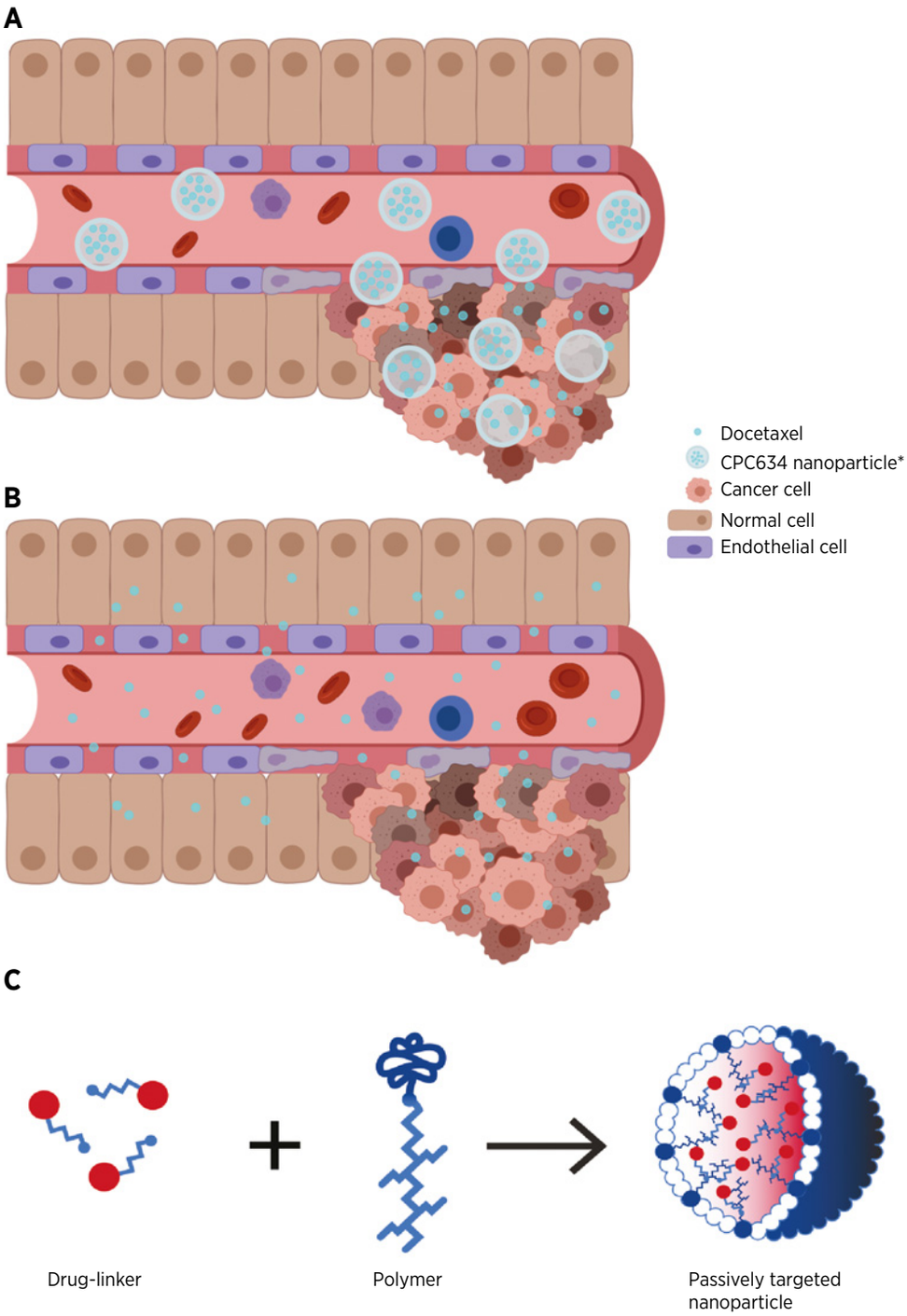

Figure 1.

Illustration of the "enhanced permeability and retention" effect by nanomedicines after entering the circulation. **A,** Proposed mechanism of intratumoral drug accumulation by docetaxel entrapped nanoparticles (CPC634). Because of leaky blood vessels in the tumor as a result of enlarged endothelial fenestrations, the nanoparticles can diffuse mainly to the tumor, and release the native anticancer drug (docetaxel) locally. **B,** Proposed mechanism of drug accumulation by conventional docetaxel. The small molecular size of conventional docetaxel enables diffusion through the endothelial fenestrations of tumor and normal vessels to healthy tissue, which can result in toxicity. This figure was created by BioRender.com. *, The size of nanoparticle in this figure is chosen to illustrate the concept of CPC634 and is not to scale. In reality, CPC634 is 50 to 100× smaller than the size of a human cell. **C,** Simplified illustration of the core-crosslinked polymeric micelles with covalently entrapped drugs (derived from ref. 20).

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released docetaxel in CPC634 solution was determined by liquid chromatography as previously described and according to standard quality control guidelines (19).

Pharmacokinetic assessments

Image-guided histologic tumor biopsies were taken of a safely accessible metastatic lesion by a radiologist. Lidocaine 2% was used for local anesthesia, and an 18-gauge large-core needle (22 mm true-cut) was used to perform the biopsy (23). Biopsies were taken from the same lesion at the same time point after each treatment infusion. Biopsy samples were directly (i.e., within seconds) frozen in liquid nitrogen and stored at $T < -70°C$ until analysis. Plasma samples were taken before and at 5 minutes, 15 minutes, 30 minutes, 1 hour, 1.5 hours, 2 hours, 4 hours, 6 hours, 24 hours, day 7 (±1 day), and day 14 (±1 day) after infusion, and at the time of tumor biopsy for pharmacokinetic analysis. Biopsy and plasma samples were processed and measured using a validated LC/MS method as described previously (24). The lower limit of quantification was 0.25 ng/mL for released docetaxel from CPC634 and 2.00 ng/mL for total docetaxel (24). Released docetaxel is defined as the free molecule in plasma either bound or unbound to plasma proteins. Total docetaxel is defined as the sum of released plus docetaxel which is covalently bound to the nanoparticles. Pharmacokinetic (and exposure) parameters were derived from the measured plasma concentration–time curve using noncompartment pharmacokinetic analysis in WinNonlin version 8.1 Phoenix. These parameters included the maximum plasma concentration (C_{max}), elimination half-life ($T_{1/2}$), and exposure from pre-infusion to infinity, expressed as area under the curve (AUC_{inf}).

Statistical analysis

A 25% intrapatient increase of total docetaxel concentration by CPC634 in the tumor relative to conventional docetaxel was considered clinically relevant, and a 25% within-patient variability was assumed for intratumoral pharmacokinetic parameters. A total of 14 patients was required to show a difference given 80% power with a one-sided alpha of 5% (25). The one-sided alpha was selected on the basis of preclinical data, which showed an increase in total docetaxel for CPC634 compared with conventional docetaxel (19). To explore the tumor drug concentration over time, biopsies were taken at six different time points (one time point per patient). Four patients were included per time point. Initially, four time points were defined which was amended during the study to six time points to also include day 7 (±1 day) and day 14 (±1 day). As a result, 24 evaluable patients were needed in total. For all the mentioned pharmacokinetic parameters, an intrapatient comparison (CPC634 relative to conventional docetaxel) was performed. All the analyses were performed on log-transformed data, except for the $T_{1/2}$. The mean differences in pharmacokinetic parameters were determined using a linear mixed effect model with treatment, sequence, and period as fixed effects, and patients within sequence as a random effect. Variance components were estimated on the basis of restricted maximum likelihood methods, and the Kenward–Roger method for computing the denominator degrees of freedom was used (26). For the primary objective, the mean difference in intratumoral concentration of docetaxel, and its two-sided 90% confidence interval (CI) boundaries, were exponentiated to estimate the geometric means (GEM) ratio, and 90% CI for this ratio. These can be interpreted as the relative difference in percentages. For the secondary objectives (AUC_{inf}, C_{max}), the mean differences, and their two-sided 95% CI boundaries, were exponentiated to estimate the GEMs ratios and 95% CIs for these ratios, which again can be interpreted as relative differences in percentages. Plasma $T_{1/2}$ of CPC634 and conventional docetaxel, and the absolute neutrophil nadir during each cycle were analyzed by means of the Wilcoxon signed rank test, and described with medians and interquartile ranges (IQR). Descriptive statistics were used for toxicity data.

Results

Patient demographics

From April 4, 2017, to April 9, 2019, a total of 39 patients were screened for study participation of whom 33 patients completed screening and received at least one cycle of chemotherapy. Eighteen patients were randomized into arm A, and 21 patients into arm B. In arm A, 8 patients were not evaluable for the primary endpoint due to screen failure ($n = 1$), withdrawal of informed consent ($n = 2$), nonevaluable tumor biopsy ($n = 2$), early termination of the study due to toxicity ($n = 2$), and early clinical progression ($n = 1$; Supplementary Fig. S1). In arm B, 7 patients were not evaluable for the primary endpoint due to screen failures ($n = 2$), withdrawal of informed consent ($n = 1$), nonevaluable tumor biopsy ($n = 2$), early termination of the study related to toxicity ($n = 1$), and early clinical progression ($n = 1$; Supplementary Fig. S1). Baseline characteristics are displayed in **Table 1**.

Intratumoral pharmacokinetics

For the intratumoral pharmacokinetic comparison analysis, patients with paired tumor biopsies evaluable for analysis were included ($n = 24$). The majority of the tumor biopsies were from liver metastases ($n = 13$). Other biopsy sites were lymph nodes ($n = 5$),

Table 1. Baseline characteristics.

	No. (%)
Patients	
Screened	39
Enrolled	33
Evaluable for the primary endpoint	24
Randomization	
Arm A	18 (46)
Arm B	21 (54)
Number of patients receiving treatment	
CPC634 + conventional docetaxel	26
CPC634	29
Conventional docetaxel	30
Age [years (IQR)]	
Median	64 (16.5)
Gender	
Female	12 (31%)
Male	27 (69%)
ECOG performance status	
0	10 (26%)
1	29 (74%)
Previous lines of systemic treatment	
≤2	33 (85%)
>2	6 (15%)
Primary tumor	
Pancreatic carcinoma	9 (23%)
Esophageal carcinoma	6 (15%)
Carcinoma of unknown primary	4 (10%)
Cholangio carcinoma	4 (10%)
Urothelial carcinoma	3 (8%)
Other tumor types	13 (33%)

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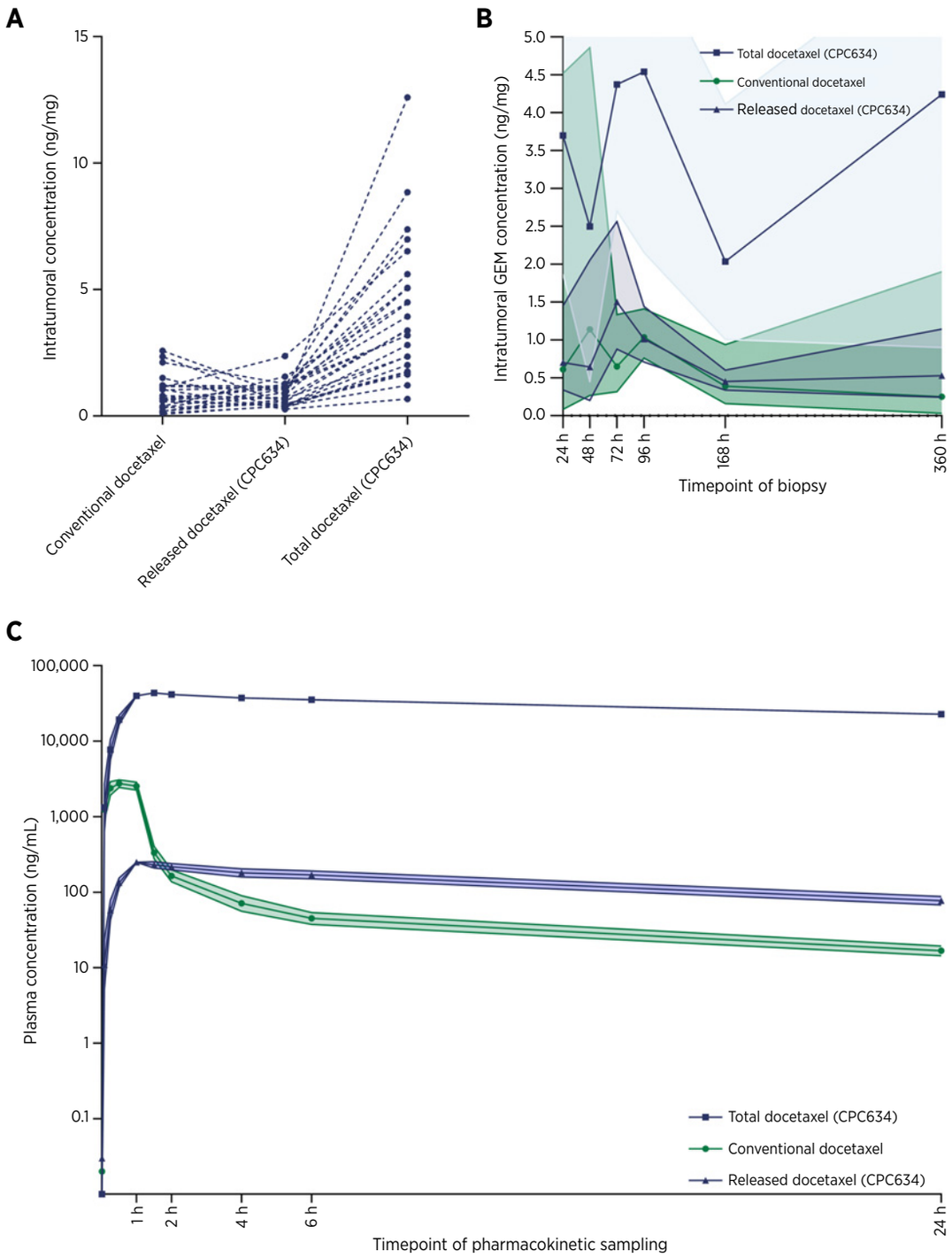

Figure 2.

Absolute intratumoral concentration of conventional docetaxel, released docetaxel, and total docetaxel for CPC634 per patient (**A**). Intratumoral GEM concentration per timepoint with the 95% CI as shade (**B**). Plasma GEM concentration time curves of conventional docetaxel, released and total docetaxel for CPC634 ($n = 26$; **C**). Shading represents 95% CI of the measurement time point.

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abdominal wall ($n = 2$), adrenal gland ($n = 2$), retroperitoneal cavity ($n = 1$), and an intrathoracic lesion ($n = 1$). **Figure 2A** represents the intratumoral docetaxel concentration after administration of conventional docetaxel and CPC634 for all individual patients. The GEM concentration of total docetaxel was significantly higher after administration of CPC634 (3.41 ng/mg; 95% CI, 2.56–4.55 ng/mg) compared with conventional docetaxel (0.599 ng/mg; 95% CI, 0.403–0.892 ng/mg) with a relative difference of 461% (95% CI, 243%–816%; $P < 0.001$). The intratumoral released docetaxel concentration after CPC634 administration was 0.74 ng/mg (95% CI, 0.582–0.941 ng/mg), which was not significantly different from the 0.599 ng/mg (95% CI, 0.403–0.892 ng/mg) intratumoral released docetaxel measured after conventional docetaxel administration (relative difference of 17%; 95% CI, −22% to 77%; $P = 0.43$). **Figure 2B** illustrates the intratumoral GEM concentration with the 95% CI of docetaxel for each biopsy time point. Although the sample size at each individual time point was too small to perform statistical testing, **Figure 2B** suggests that the intratumoral T_{max} of conventional docetaxel could be earlier (∼24–48 hours) compared with T_{max} of released and total docetaxel by CPC634 (∼72–96 hours).

Plasma pharmacokinetics

Patients who received at least one treatment cycle during the study with accurate blood sampling ($n = 26$) were included for plasma pharmacokinetic analysis. Pharmacokinetic parameters are described in **Table 2**. CPC634 revealed a 27% (95% CI, 12%–44%; $P < 0.001$) higher AUC_{inf}, and a 91% (95% CI, −92% to −90%; $P < 0.001$) lower C_{max} for released docetaxel by CPCP634 relative to conventional docetaxel. The AUC_{inf} and C_{max} of total docetaxel by CPC634 were 250- and 15-fold higher compared with conventional docetaxel, respectively (**Table 2**; **Fig. 2C**). The $T_{1/2}$ of CPC634 was estimated at 59 hours (IQR = 56.5–66.9) for released docetaxel, and 38 hours (IQR = 35.8–41.6) for total docetaxel. This was lower than the $T_{1/2}$ of conventional docetaxel [100 hours (IQR = 84.8–139; $P < 0.001$)].

Treatment-emergent adverse events

Table 3 summarizes the treatment-emergent adverse events (TEAE) present in 3 or more patients during each treatment cycle. Grade 1 to 2 skin toxicity occurred in 76% of the patients during CPC634 treatment, and in 50% during conventional docetaxel treatment. Sensory neuropathy (grade 1–2) was more present during the CPC634 cycle (21%) compared with the conventional docetaxel cycle (3%). Because almost all patients experienced neutropenia at day 8 after infusion of conventional docetaxel without G-CSF—which is a contra indication for taking biopsies—it was decided to administer G-CSF 24 hours after infusion of conventional docetaxel in the cohort of patients undergoing a tumor biopsy at day 7 (±1 day). Because of the influence of G-CSF administration on the neutrophil count, this cohort of patients was excluded from all analyses regarding neutrophil count. Grade ≥3 neutropenia occurred more frequently after conventional docetaxel treatment (70% vs. 3% for CPC634; **Fig. 3**). The median neutrophil count nadir after conventional docetaxel treatment was significantly lower (0.50×10^9/L) compared with CPC634 (4.30×10^9/L; $P < 0.001$). Consequently, 3 patients with neutropenia during conventional docetaxel treatment also developed febrile neutropenia grade ≥3. During the study 23 serious adverse events (SAE) were reported. Of these, 11 SAEs were considered related to the treatment (seven related to conventional docetaxel and four related to CPC634). Related SAEs were diarrhea ($n = 1$), hyponatremia ($n = 1$), nausea and vomiting ($n = 5$), and (febrile) neutropenia ($n = 4$). All SAEs are summarized in Supplementary Table S1.

Discussion

In this first randomized study in humans, which directly compared the intratumoral and plasma pharmacokinetics of conventional docetaxel with CPC634, the nanomedicine entrapping docetaxel, resulted in more than 4-fold higher total docetaxel concentration in the tumor compared with conventional docetaxel. In addition, the plasma exposure of released docetaxel by CPC634 was promising, with a significantly higher AUC, and a lower C_{max}, resulting in a lower incidence of grade 3 or 4 neutropenia.

The hypothesis that nanoparticles may have a better safety and efficacy profile compared with conventional cytotoxic agents, is based on the EPR effect. However, this effect may be oversimplified since multiple chemical and biological steps are involved in the biodistribution, and on/off-target release of the anticancer drug from the nanoparticles (27). The question whether nanoparticles with entrapped anticancer drugs lead to improved tumor and plasma pharmacokinetic profiles in humans—compared with the native anticancer drug—is

Table 2. Pharmacokinetic data.

Plasma released docetaxel ($n = 26$)	GEM CPC634	95% CI	GEM conventional docetaxel	95% CI	RD[a]	95% CI	*P* value
AUC_{inf} (mg*h/mL)	7.41	6.63–8.29	5.74	5.09–6.48	+27%	12–44	0.001
C_{max} (mg/mL)	0.262	0.241–0.284	2.88	2.59–3.20	−91%	−92 to −90	<0.001
Plasma total docetaxel ($n = 26$)							
AUC_{inf} (mg*h/mL)	1,530	1,450–1,630	5.74	5.09–6.48	26,500%	23,100–30,400	<0.001
C_{max} (mg/mL)	44.4	42.1–46.8	2.88	2.59–3.20	1,440%	1,280–1,610	<0.001
Intratumoral concentrations ($n = 24$)							
Intratumoral released docetaxel (ng/mg)	0.74	0.582–0.941	0.599	0.403–0.892	+17%	−22 to 77	0.43
Intratumoral total docetaxel (ng/mg)	3.41	2.56–4.55	0.599	0.403–0.892	+461%	243–816	<0.001
Half-life ($T_{1/2}$) ($n = 26$)	**Median CPC634**	**IQR**	**Median conventional docetaxel**	**IQR**			***P* value**
Released docetaxel (hours)	59.05	56.5–66.9	100.09	84.8–139	—	—	<0.001
Total docetaxel (hours)	38.03	35.8–41.6	100.09	84.8–139	—	—	<0.001

Abbreviation: RD, relative difference.
[a]Relative difference of CPC634 compared with conventional docetaxel.

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Table 3. TEAE according to CTCAE 4.03 occurring in ≥3 patients during conventional docetaxel (Cd) and CPC634 cycle.

	Grade 1–2		Grade ≥3	
TEAE	**Cd (%)**	**CPC634 (%)**	**Cd (%)**	**CPC634 (%)**
Nausea	9 (30)	11 (38)	1 (3)	2 (7)
Vomiting	2 (7)	6 (21)	1 (3)	1 (3)
Anorexia	14 (47)	12 (38)	1 (3)	1 (3)
Stomatitis	9 (30)	10 (35)	1 (3)	2 (7)
Constipation	7 (23)	6 (21)	1 (3)	0
Diarrhea	9 (30)	8 (28)	1 (3)	1 (3)
Pain	9 (30)	20 (69)	6 (20)	1 (3)
Skin toxicity	14 (47)	22 (76)	1 (3)	0
Fatigue	16 (53)	10 (34)	3 (10)	4 (14)
Edema	4 (13)	2 (7)	0	0
Dyspnea	7 (23)	4 (14)	0	1 (3)
Alopecia	11 (37)	7 (24)	0	0
Dizziness	3 (10)	6 (21)	0	0
Sensory neuropathy	1 (3)	6 (21)	1 (3)	0
Chills	1 (3)	3 (10)	0	0
Infection	1 (3)	2 (7)	7 (23)	1 (3)
Fever	4 (13)	2 (7)	0	0
Neutropenia[a]	6 (21)	9 (30)	20 (70)	1 (3)
Febrile neutropenia	0	0	3 (10)	0
Anemia	4 (14)	6 (20)	0	0
Thrombocytopenia	6 (21	7 (23)	0	0
Increased creatinine	8 (28)	9 (30)	0	0
Increased AST	5 (17)	11 (37)	0	1 (3)
Increased ALT	3 (10)	5 (17)	0	0
Increased GGT	5 (17)	1 (3)	3 (10)	5 (17)
Increased ALP	4 (14)	1 (3)	2 (7)	2 (7)

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase.
[a]No correction on the incidence of neutropenia was performed for G-CSF administration.

therefore not fully answered yet. For conventional docetaxel, the intratumoral exposure in a clinical setting has only been assessed by nuclear imaging up to 80 minutes after infusion (28, 29). Here, we assessed the intratumoral docetaxel exposure at different time points, and we demonstrated that docetaxel could be quantified with an LC/MS method (24) up to 14 days in tumor biopsies after the administration of both conventional docetaxel and CPC634. CPC634 persistently resulted in a higher total docetaxel concentration at all biopsy time points compared with conventional docetaxel. This increased intratumoral concentration of total docetaxel was measured in metastatic lesions. Nevertheless, the heterogeneity of a tumor, and differences in vasculature of the primary tumor and that of a metastatic lesion may be factors that could have an effect on drug penetration and exposure inside the primary tumor versus a metastatic lesion. However, addressing intratumoral concentrations of metastatic lesions are most relevant to investigate since these lesions usually determine the overall survival of a patient.

In contrast to the large difference in intratumoral total docetaxel concentrations between the two compounds, the intratumoral released docetaxel concentration after CPC634 and conventional docetaxel did not significantly differ up to 14 days. On the basis of this, it could be questioned if CPC634 will exert a higher therapeutic effect compared with conventional docetaxel. Nevertheless, pharmaceutical data demonstrated that more than 80% of the docetaxel entrapped inside the nanoparticle is expected to be released over time (19). Because of the high levels of total docetaxel, it is expected that CPC634 nanoparticles will continue to release docetaxel even later than 14 days after drug infusion. Thus, it can be speculated that this continued release of docetaxel from CPC634 will ultimately result in an increased intratumoral drug exposure in terms of AUC. The measured intratumoral drug concentrations suggest a longer intratumoral T_{max} of total and released docetaxel after CPC634 administration.

Premature drug release from nanoparticles in general in the systemic circulation may result in comparable systemic exposure as the native drug, and this is probably the reason why most of the nanomedicines in clinical development have comparable toxicity profiles with marginal improvement in efficacy (14–16). However, in this study, the increased plasma AUC and decreased plasma C_{max} of released docetaxel by CPC634 compared with conventional docetaxel, indicate that docetaxel is efficiently conserved by the nanoparticles in the systemic circulation resulting from the cross-linked network. In addition, the polymeric micelle structure significantly increases the water solubility of docetaxel, and thus no additional solubilizing solvent is required in the final drug formulation. This can prevent the frequent occurring (severe) infusion reactions related to polysorbate 80 solvent by the conventional docetaxel formulation (30). To prevent the aforementioned infusion reaction during the infusion of conventional docetaxel and fluid retention as severe side effect of conventional docetaxel, dexamethasone is given as premedication. Dexamethasone is a moderate CYP3A4 inducer which theoretically may influence the systemic exposure of docetaxel. However, data from

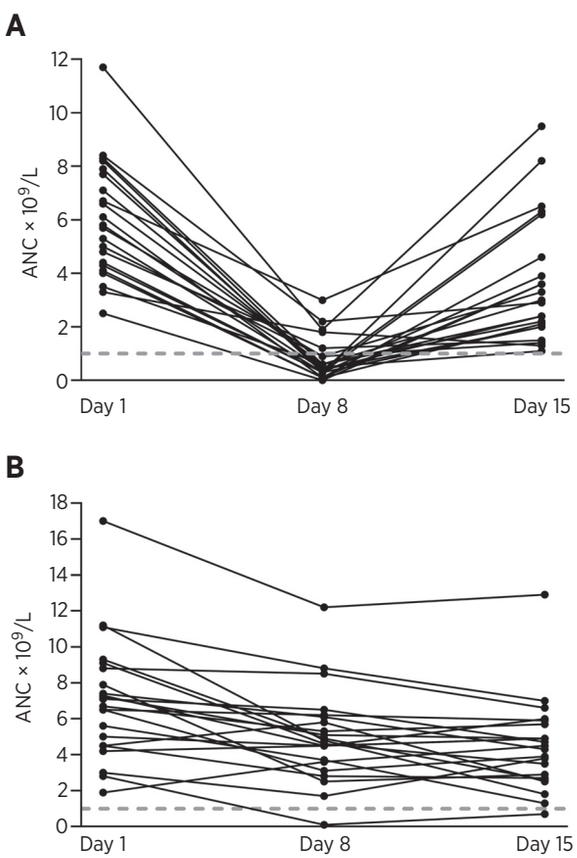

Figure 3.
Neutrophil count (ANC) during conventional docetaxel (**A**) and CPC634 cycle (**B**). Patients receiving G-CSF administration were excluded from this analysis.

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population pharmacokinetics of docetaxel has shown that dexamethasone premedication has no effect on docetaxel pharmacokinetics (31, 32). Several retrospective studies suggest that the C_{max} of docetaxel is the driver of neutropenia (6, 33–35). This is supported by the fact that, despite the lower plasma AUC of conventional docetaxel compared with CPC634, the incidence of grade 3 or 4 neutropenia in this study was significantly higher during conventional docetaxel treatment (70% for conventional docetaxel vs. 3% for CPC634). The estimated elimination half-life of conventional docetaxel was higher (i.e., ∼100 hours) compared with the half-life according to Summary of Product Characteristics (i.e., ∼11 hours; ref. 36). A likely explanation for this is the longer sampling time during the terminal elimination phase of the concentration–time curve, which is supported by Baker and colleagues who estimated the half-life of conventional docetaxel to be 86 hours after prolonged sampling up to 3 weeks (37).

Recently, a phase I trial to study the safety of CPC634 (the NAPOLY trial; Clinicaltrials.gov number NCT02442531) was finalized and showed that this drug is safe to be administered in the therapeutic dosing range (21). A phase II efficacy study of CPC634 monotherapy in patients with ovarian cancer (the CINOVA trial) is currently ongoing (Clinicaltrials.gov number NCT03742713).

In conclusion, we have demonstrated that CPC634 results in a sustained high and prolonged exposure of docetaxel in the tumor. These sustained high intratumoral drug levels support the hypothesis that this nanoparticle formulation of docetaxel may increase docetaxel efficacy. Furthermore, we observed a lower incidence of grade 3 or 4 neutropenia after administration of CPC634 compared with conventional docetaxel, which could be explained by lower systemically released docetaxel C_{max} concentrations after CPC634 administration. This study is the first clinical study which—by a head to head intrapatient comparison—demonstrates that the hypothesis of nanomedicine leading to an accumulated, and therefore increased, concentration of an anticancer drug in the tumor, is correct.

Disclosure of Potential Conflicts of Interest

M.P. Lolkema is an employee/paid consultant for Amgen, Jansen Cilag, Bayer, Servier, Roche, Pfizer, Sanofi Aventis, Astellas, AstraZeneca, MSD, and Novartis, and reports receiving other commercial research support from JnJ, Sanofi, Astellas, and MSD. C.J. Rijcken, R. Hanssen, and S.L.W. Koolen are employees/paid consultants for Cristal Therapeutics. No potential conflicts of interest were disclosed by the other authors.

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