Towards prediction of efficacy of chemotherapy: a proof of concept study in lung cancer using $^{11}$C-docetaxel and positron emission tomography


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ABSTRACT

Background: Pharmacokinetics of docetaxel can be measured in vivo using positron emission tomography (PET) and $^{11}$C-docetaxel. The objective of this study was to investigate whether a tracer $^{11}$C-docetaxel PET study could predict tumor uptake of unlabeled (cold) docetaxel during a therapeutic infusion.

Methods: Docetaxel-naïve lung cancer patients underwent two $^{11}$C-docetaxel PET scans; one after bolus injection of $^{11}$C-docetaxel and another during combined infusion of $^{11}$C-docetaxel and a therapeutic dose of docetaxel (75 mg·m$^{-2}$). Compartmental and spectral analyses were used to quantify $^{11}$C-docetaxel tumor kinetics. $^{11}$C-docetaxel PET measurements were used to estimate the area under the curve (AUC) of cold docetaxel in tumors. Tumor response was evaluated using computed tomography scans.

Results: Net rates of influx ($K_i$) of $^{11}$C-docetaxel in tumors were comparable during microdosing and therapeutic scans. $^{11}$C-docetaxel AUC$_{tumor}$ during the therapeutic scan could be predicted reliably using an impulse response function derived from the microdosing scan together with the plasma curve of $^{11}$C-docetaxel during the therapeutic scan. At 90 min, the accumulated amount of cold docetaxel in tumors was < 1% of the total infused dose of docetaxel. $^{11}$C-docetaxel $K_i$ derived from the microdosing scan correlated with AUC$_{tumor}$ of cold docetaxel (Spearman’s $\rho = 0.715$; $P = 0.004$) during the therapeutic scan and with tumor response to docetaxel therapy (Spearman’s $\rho = -0.800$; $P = 0.010$).

Conclusions: Microdosing data of $^{11}$C-docetaxel PET can be used to predict tumor uptake of cold docetaxel during chemotherapy. The present study provides a framework for investigating the PET microdosing concept for radiolabeled anticancer drugs in patients.

INTRODUCTION

Docetaxel belongs to the class of taxanes, which act by disrupting the microtubular network that is essential for mitosis (1). Initially, the drug was approved as single agent for the treatment of anthracycline refractory advanced breast cancer (2). Thereafter, docetaxel has been approved both as single agent and in combination therapy to treat several advanced malignancies including gastric, head and neck, prostate and non-small cell lung cancer (3). Nevertheless, docetaxel fails to exert antitumor activity in a substantial number of patients and it is associated with potentially severe toxicities. Hence, there is a need for a tool to predict efficacy of docetaxel in individual patients.

The response to docetaxel treatment is thought to be directly related to drug concentrations in tumor tissue. As a direct relationship between plasma concentration
and efficacy is assumed, it is usual to determine pharmacokinetic profiles in blood (4). Plasma concentrations, however, do not necessarily reflect drug concentrations in tumors (5). In addition, efficacy of docetaxel may strongly depend on maintaining sufficiently high drug concentrations in tumor tissue. In animal studies, sacrifice experiments are commonly performed to measure drug concentrations in tumors (5). Direct assessment of tumor drug concentrations in cancer patients, however, is more challenging, as it requires accessibility to tumors that are usually deeply seated within the body. In addition, as docetaxel shows nonspecific binding to numerous materials, assessment of docetaxel tumor concentrations by microdialysis is not feasible (6).

Positron emission tomography (PET) is an imaging technique that can be used to monitor drug pharmacokinetics and pharmacodynamics in vivo non-invasively (7). Previously, docetaxel has been labeled with the positron emitting radionuclide carbon-11 (8,9) and it has been shown that a dynamic PET scan, following a tracer dose of \[^{11}\text{C}]\text{docetaxel}, provides a quantitative measurement of \[^{11}\text{C}]\text{docetaxel} kinetics in lung cancer patients (10). In the latter study, tumor kinetics of \[^{11}\text{C}]\text{docetaxel} were found to be highly variable. This, in turn, seemed to be associated with differences in tumor response and, although the number of patients was small, \[^{11}\text{C}]\text{docetaxel} PET studies may hold promise in selecting cancer patients for docetaxel therapy.

A potential caveat is the fact that pharmacokinetics of \[^{11}\text{C}]\text{docetaxel} at tracer doses may be different from those at therapeutic doses. The main aim of this study was to investigate whether an \[^{11}\text{C}]\text{docetaxel} PET study could predict tumor uptake of unlabeled docetaxel (further referred to as cold docetaxel) during a therapeutic infusion. To this end, therapeutic tumor uptake of \[^{11}\text{C}]\text{docetaxel} as predicted by spectral analysis of a tracer study was compared to actual measured \[^{11}\text{C}]\text{docetaxel} uptake during an infusion of a therapeutic dose of docetaxel. Furthermore, plasma kinetics of \[^{11}\text{C}]\text{docetaxel} were compared to those of the drug docetaxel during therapeutic infusion. Finally, it was assessed whether simplified analysis of a tracer study with \[^{11}\text{C}]\text{docetaxel} was in itself sufficient for prediction of tumor uptake of cold docetaxel during therapy.

**PATIENTS AND METHODS**

**Patient selection**
Nine patients with histologically confirmed advanced-stage lung cancer were prospectively enrolled. Inclusion criteria were the following: age ≥ 18 years, a malignant lesion ≥ 1.5 cm in diameter within the chest, scheduled for docetaxel containing chemotherapy, life expectancy of at least 12 weeks, Eastern Cooperative Oncology Group
(ECOG) performance status < 3, hemoglobin ≥ 6.0 mmol-L⁻¹, and absolute neutrophil count ≥ 1.5·10⁹-L⁻¹. Exclusion criteria were the following: prior treatment with taxanes, concurrent treatment with other anticancer agents or experimental drugs, pregnancy or lactation, metal implants (e.g. pacemakers), and claustrophobia. The study was approved by the Medical Ethics Review Committee of the VU University Medical Center. All patients gave written informed consent prior to study enrollment.

**Study design**

Patients underwent two [¹¹C]docetaxel PET scans on a single day: a tracer alone [¹¹C]docetaxel scan in the morning (further referred to as microdosing scan), and in the afternoon a second tracer [¹¹C]docetaxel scan during an infusion of docetaxel in a therapeutic dose (further referred to as therapeutic scan). The therapeutic scan was performed approximately four hours after the microdosing scan. Each [¹¹C]docetaxel scan was combined with a [¹⁵O]H₂O scan to measure tumor perfusion. Computed tomography (CT) scans were performed at baseline and after one or two cycles of treatment for evaluation of tumor response to docetaxel therapy.

**Synthesis of radiopharmaceuticals**

[¹¹C]docetaxel and [¹⁵O]H₂O were synthesized according to good manufacturing practice (GMP) standards as described previously (8,9,11). Docetaxel was obtained from Green PlantChem Company Ltd (Hangzhou, China). Docetaxel was chemically modified and used as precursor in the synthesis of [¹¹C]docetaxel. The tracer [¹¹C]docetaxel has an identical molecular structure as the drug docetaxel. [¹¹C]docetaxel was obtained with a decay corrected radiochemical yield of 10 ± 2% and a radiochemical purity of > 98%. The identity of [¹¹C]docetaxel was confirmed by comparison of retention times on high performance liquid chromatography (HPLC) with authentic docetaxel.

**Docetaxel therapy**

Premedication consisted of oral dexamethasone, 8 mg twice daily, for three days starting the day before the PET scans, and 8 mg ondansetron given as an intravenous infusion within 1 hour prior to docetaxel infusion. Docetaxel was dissolved in normal saline and was administered as a 1 hour intravenous infusion. [¹¹C]docetaxel was co-infused with the therapeutic dose of docetaxel, simultaneously starting a dynamic PET scan. The nuclear medicine ward and all personnel involved fulfilled criteria required for administration of chemotherapy according to established safety guidelines.

**Scanning protocol**

Scans were performed on a PET-CT scanner (Gemini TF-64, Philips Medical Systems,
Best, The Netherlands) (12), which has an axial field of view of 18 cm, divided into 45 contiguous planes. Patients were asked to fast from midnight before scanning. A light breakfast at 08.00 a.m. and a light lunch at 12.00 p.m., and water and tea were allowed until PET scanning. All patients received two venous catheters, one for tracer injection and infusion of therapeutic docetaxel, the other for blood sampling. Patients were positioned supine on the scanner bed, with both tumor and aortic arch located inside the axial field of view of the scanner. Movement during scanning was minimized using elastic body restraining bandages.

Prior to the microdosing scan with $[^{11}\text{C}]$docetaxel, a $[^{15}\text{O}]\text{H}_2\text{O}$ scan was carried out. This 10 min dynamic scan was started simultaneously with an intravenous injection of 370 MBq $[^{15}\text{O}]\text{H}_2\text{O}$ (5 mL at a rate of 0.8 mL s$^{-1}$), followed by a 35 mL saline flush (at a rate of 2 mL s$^{-1}$). Thereafter, a 50 mAs low-dose CT scan was performed for attenuation correction. At least 20 min after administration of $[^{15}\text{O}]\text{H}_2\text{O}$, a 60 min dynamic scan was started simultaneously with an intravenous injection of $[^{11}\text{C}]$docetaxel (dissolved in a maximum volume of 12 mL saline, at a rate of 0.8 mL s$^{-1}$), followed by 35 mL saline (at a rate of 2 mL s$^{-1}$). During the microdosing scan, the median injected dose of $[^{11}\text{C}]$docetaxel was 348 MBq (range 230-367 MBq) with a median specific activity of 10.1 GBq Â· mol$^{-1}$ (range 2.0-37.3 GBq Â· mol$^{-1}$). As a result, administration of a typical tracer dose of 370 MBq $[^{11}\text{C}]$docetaxel with a specific activity of ~ 10 GBq Â· mol$^{-1}$ contained a total of ~ 30 Â· mol $[^{11}\text{C}]$docetaxel. Sequential scans using $[^{15}\text{O}]\text{H}_2\text{O}$ and $[^{11}\text{C}]$docetaxel were possible because of the short half-lives of oxygen-15 and carbon-11, which are 2.0 and 20.3 minutes, respectively. Using the three-dimensional (3D) row action maximum likelihood reconstruction algorithm (3D RAMLA), $[^{15}\text{O}]\text{H}_2\text{O}$ and $[^{11}\text{C}]$docetaxel scans were reconstructed into 26 (1x10, 8x5, 4x10, 2x15, 3x20, 2x30 and 6x60 s) and 36 (1x10, 8x5, 4x10, 2x15, 3x20, 2x30, 6x60, 4x150, 4x300 and 2x600 s) frames, respectively. All data were normalized and all appropriate corrections were applied for dead time, decay, randoms, scatter and attenuation.

During the therapeutic study, the $[^{15}\text{O}]\text{H}_2\text{O}$ scan was performed after the therapeutic scan with $[^{11}\text{C}]$docetaxel. First, a 50 mAs low-dose CT scan was performed. Then, $[^{11}\text{C}]$docetaxel and docetaxel therapy were administered as an infusion through the same venous catheter. To this end, an injection tube with $[^{11}\text{C}]$docetaxel (median dose, 359 MBq; range, 196-531 MBq; dissolved in a volume of 50 mL saline) and a plastic flexi-bag containing a therapeutic dose of docetaxel (dissolved in a volume of 520-540 mL) were connected with flexible infusion lines to the same venous catheter. To prevent scatter from $[^{11}\text{C}]$docetaxel in the injection tube, a lead castle was built around the infusion pump that contained the tube. Two infusion pumps were used to control the speed of the
infusions of $^{\text{11}}\text{C}$docetaxel and therapeutic docetaxel separately. The administration of $^{\text{11}}\text{C}$docetaxel and therapeutic docetaxel was scheduled in such a way that $^{\text{11}}\text{C}$docetaxel and cold docetaxel entered the venous catheter at identical time points. Simultaneously, a 90 min dynamic scan was started. As patients received their first docetaxel infusion, the infusion was given at a slower rate for the first 15 min ($^{\text{11}}\text{C}$docetaxel at a rate of 0.007 mL·s$^{-1}$ and docetaxel therapy at a rate of 0.075 mL·s$^{-1}$). Thereafter, if patients did not experience any side-effects, infusion rates of $^{\text{11}}\text{C}$docetaxel and docetaxel therapy were increased to 0.014 mL·s$^{-1}$ and 0.150 mL·s$^{-1}$, respectively. At the time when the infusion of therapeutic docetaxel had been completed, $^{\text{11}}\text{C}$docetaxel infusion was discontinued and saline (100 mL at a rate of 600 mL·h$^{-1}$) was infused subsequently. Thereafter, the remaining $^{\text{11}}\text{C}$docetaxel in the tube was measured to calculate the actual injected dose of $^{\text{11}}\text{C}$docetaxel. Immediately after the 90 min $^{\text{11}}\text{C}$docetaxel scan, a 50 mAs low-dose CT scan was performed. This was followed by a 10.5 min dynamic $^{\text{15}}\text{O}$H$_2$O scan. 30 s into this scan, 370 MBq $^{\text{15}}\text{O}$H$_2$O (5 mL at a rate of 0.8 mL·s$^{-1}$) was injected intravenously, followed by a 35 mL saline flush (at a rate of 2 mL·s$^{-1}$). $^{\text{11}}\text{C}$docetaxel and $^{\text{15}}\text{O}$H$_2$O scans from the therapeutic scan were reconstructed into 25 (1x600, 10x60 and 14x300 s) and 27 (1x30 background, 1x10, 8x5, 4x10, 2x15, 3x20, 2x30 and 6x60 s) frames, respectively.

**Blood sampling**

After intravenous injection of $^{\text{11}}\text{C}$docetaxel, sequential 10 mL venous samples were collected in glass tubes containing lithium heparin. During microdosing and therapeutic scans, these samples were taken at 2.5, 5, 10, 15, 20, 30, 40, and 60 min post injection and at 10, 20, 30, 40, 50, 60, 70, and 80 min after start of infusion, respectively. Prior to each sample, 3-5 mL blood was discarded and after each sample the line was flushed with 2 mL saline. Blood samples were analyzed for whole blood and plasma concentrations of $^{\text{11}}\text{C}$docetaxel. In addition, blood samples obtained during the therapeutic scan were analyzed for radiolabeled metabolites of $^{\text{11}}\text{C}$docetaxel (10). Samples obtained from the microdosing scan were not analyzed for radiolabeled metabolites, as these had not been detected in a previous microdosing study (10). Whole blood (0.5 mL) was weighted in duplicate and 0.05 mL 10% Triton X-100 solution was added. After centrifuging the remaining whole blood (5 min; room temperature; 4000 rpm), plasma was harvested and 0.5 mL plasma was weighted in duplicate, again adding 0.05 mL 10% Triton X-100 solution. A well-counter, cross-calibrated against the PET scanner, was used to determine activity concentrations. In addition, plasma samples collected during the therapeutic scan were stored at -80°C until further analysis. Total plasma concentrations of cold docetaxel were determined using liquid chromatography coupled to tandem mass-spectrometric detection as
previously described (13). Trapezoidal integration was used to calculate the area under the plasma curve ($\text{AUC}_{\text{Plasma}}$) of cold docetaxel.

To verify that plasma kinetics of $^{[11]}\text{C}\text{docetaxel}$ and cold docetaxel were identical, radioactivity concentrations of $^{[11]}\text{C}\text{docetaxel}$ in plasma were divided by the specific activity of injected $^{[11]}\text{C}\text{docetaxel}$ during the therapeutic scan and compared to total measured plasma concentrations of docetaxel.

**Input functions**
The ascending aorta in the $^{[15]}\text{O}\text{H}_2\text{O}$ and $^{[11]}\text{C}\text{docetaxel}$ images was used for generating non-invasive image derived input functions, as validated previously (10,14). Volumes of interest (VOI) of 1 cm diameter were drawn over the ascending aorta in approximately 10 consecutive image planes of the frame in which the first pass of the bolus was best visualized. Projection of these VOIs onto all image frames yielded the arterial time-activity curve (TAC) ($C_A(t)$). For the $^{[15]}\text{O}\text{H}_2\text{O}$ images, a similar approach was used for the pulmonary artery in approximately five consecutive planes, thereby providing a TAC for the pulmonary circulation $C_V(t)$ (10). The image derived input function of $^{[11]}\text{C}\text{docetaxel}$ was obtained by multiplying $C_A(t)$ with a sigmoid function describing the measured (manual samples) plasma/whole blood ratios over time in the microdosing scan, and by linear interpolation of measured plasma/whole blood ratios in the therapeutic scan. For the $^{[11]}\text{C}\text{docetaxel}$ microdosing scan, input functions were limited to the first 10 min of data, since the rapid clearance of the tracer precludes reliable input functions at later time points (10).

**Delineation of tumor volumes of interest**
An experienced nuclear medicine physician [E.F.C.] defined all tumors on low-dose CT scans. To this end, low-dose CT images were converted into ECAT-7 format and tumor VOIs were drawn using the CAPP software package (CTI/Siemens, Knoxville, TN, USA). Subsequently, these VOIs were projected onto all dynamic images of the corresponding $^{[15]}\text{O}\text{H}_2\text{O}$ and $^{[11]}\text{C}\text{docetaxel}$ scans, thereby generating tumor TACs for $^{[15]}\text{O}\text{H}_2\text{O}$ and $^{[11]}\text{C}\text{docetaxel}$, respectively.

**Analysis of tumor perfusion**
Kinetic analysis of data was performed using dedicated programs written within the software environment Matlab (The MathWorks Inc., Natick, MA, USA). Tumor TACs derived from the $^{[15]}\text{O}\text{H}_2\text{O}$ images were fitted to a single-tissue compartment model, as described previously (14). To this end, the image derived input function was used as arterial input function, and corrections for spill-over from both arterial and pulmonary circulations were taken into account.
Analysis of $[^{11}\text{C}]$docetaxel kinetics in tumors

First, tumor kinetics of $[^{11}\text{C}]$docetaxel obtained from microdosing and therapeutic studies were analyzed using a two-tissue irreversible compartment model, linearized using the Patlak method (10,15). Previously, it has been shown that this is the method of choice for quantitative analysis of $[^{11}\text{C}]$docetaxel kinetics in tumors (10). Net rate of influx ($K_i$) was determined using the first 10 min of the microdosing scan and the first 60 min of the therapeutic scan. Thereafter, spectral analysis (16) was used to predict tumor kinetics of $[^{11}\text{C}]$docetaxel during the therapeutic scan on the basis of its kinetics during the microdosing scan, according to the method suggested by Rosso et al (17). To that end, impulse response functions (IRFs) were generated. The IRF represents the response of tissue if a true bolus of $[^{11}\text{C}]$docetaxel could be delivered immediately (16). Once the IRF is known, it can be applied to predict tissue response (i.e. tracer concentrations) for any plasma input function by convolution with that plasma input function. For the $[^{11}\text{C}]$docetaxel microdosing scans, tumor IRFs were determined using spectral analysis with fifty basis functions and clearance rate constants ranging from 0 to 1 min$^{-1}$. Next, this IRF was convolved with the $[^{11}\text{C}]$docetaxel plasma input function of the therapeutic scan, resulting in a prediction of tumor TACs during therapy (16). Bootstrap analysis was performed to estimate the confidence intervals of the IRF and the predicted tumor TAC during the therapeutic scan (17). Areas under the predicted and measured tumor time-activity curves (AUC$_{\text{Tumor}}$) for $[^{11}\text{C}]$docetaxel during infusion of the therapeutic dose were compared.

Tumor uptake of cold docetaxel

To further verify whether an $[^{11}\text{C}]$docetaxel $K_i$ from the microdosing scan predicts kinetics of cold docetaxel during therapy, the amount of cold docetaxel in tumor tissue was calculated according to two different methods. First, the accumulated amount of cold docetaxel in tumors was calculated by multiplication of $[^{11}\text{C}]$docetaxel $K_i$ obtained from the microdosing scan with the AUC$_{\text{Plasma}}$ of cold docetaxel during the therapeutic scan. Alternatively, the concentration of cold docetaxel at the last time point of the therapeutic scan was calculated by dividing the radioactivity concentration in tumors at 90 min by the mean specific activity of $[^{11}\text{C}]$docetaxel in plasma. Second, the AUC$_{\text{Tumor}}$ of total docetaxel during the therapeutic scan was calculated by dividing the measured AUC$_{\text{Tumor}}$ of $[^{11}\text{C}]$docetaxel during the same scan by the mean specific activity of $[^{11}\text{C}]$docetaxel in plasma. The schematic diagram in Supplementary figure 1 illustrates the analyses that were carried out to calculate the amount of cold docetaxel in tumor tissue. Finally, the absolute amount of cold docetaxel in tumor was determined by multiplication with the corresponding tumor volume.
**Tumor response to docetaxel treatment**
Response to docetaxel treatment was determined on sequential CT scans. To this end, one-dimensional changes in tumor size (18) were assessed by measuring the diameter of each tumor that could be defined in the field of view of the dynamic PET scan.

**Statistics**
Statistical analysis was performed using SPSS software (SPSS for Windows 16.0, SPSS, Inc., Chicago, IL, USA). The Wilcoxon Signed Ranks test was used to compare variables obtained from microdosing and therapeutic scans. The Spearman’s correlation coefficient was used to explore correlations. A 2-tailed probability value of $P < 0.05$ was considered significant.

**RESULTS**

**Patients and PET scanning**
At the time of the study, all patients were diagnosed with histologically proven non-small cell lung cancer. Retrospectively, however, one patient was diagnosed with thymic carcinoma after surgical resection of the primary tumor. In six patients (four males and two females; median age 68 years; range 46-74 years), both $^{11}$C docetaxel scans were evaluable for analysis. In the remaining patients, the second $^{11}$C docetaxel scan could not be performed because of technical difficulties. All patients underwent a $^{15}$O H$_2$O scan as part of the microdosing scan, whereas four out of six patients underwent an additional $^{15}$O H$_2$O scan as part of the therapeutic scan. The therapeutic dose of docetaxel was administered at 75 mg·m$^{-2}$ with a median dose of 150 mg (range 110–170 mg). Patients did not experience any acute side-effects during infusion of therapeutic docetaxel. The median time of docetaxel infusion was 66 min (range 62-74 min). None of the patients used co-medications consisting of inhibitors or substrates of the efflux transporter ABCB1. Following the $^{11}$C docetaxel PET study, three patients were subsequently treated with a platinum compound.

**Plasma kinetics of $^{11}$C docetaxel correlate with docetaxel kinetics during therapeutic infusion**
Infusion of $^{11}$C docetaxel during the therapeutic scan resulted in different arterial plasma curves of $^{11}$C docetaxel than those obtained during the microdosing scan (Figure 1). Radiolabeled metabolites of $^{11}$C docetaxel could not be detected during the therapeutic scan, confirming previous results for tracer $^{11}$C docetaxel studies (10). During the therapeutic scan, plasma curves of $^{11}$C docetaxel were similar to those of cold docetaxel (Figures 1B and 2A), which were similar to those of historical controls [Figure 2A, (19)].
Median AUC\textsubscript{Plasma} of cold docetaxel was 2.23 µg·mL\textsuperscript{-1}·h (range 1.75-3.42 µg·mL\textsuperscript{-1}·h). When plasma concentrations of cold docetaxel during therapeutic infusion were calculated on the basis of measured radioactivity concentrations in plasma and infused doses of \textsuperscript{[14]C}docetaxel and cold docetaxel, there was a high correlation between measured and calculated plasma concentrations of cold docetaxel (Spearman’s \( p = 0.850; P < 0.001 \); Figure 2B).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Whole blood and plasma concentrations of \textsuperscript{[14]C}docetaxel in the ascending aorta as function of time for (A) a microdosing scan (tracer dose only) and (B) a therapeutic scan (co-infusion with a pharmacological dose of docetaxel).}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Plasma concentrations of cold docetaxel. (A) Plasma concentrations of cold docetaxel during infusion of a therapeutic dose as function of time in the present study (filled circles) and in historical controls (open squares) (19). (B) Measured versus calculated plasma concentrations of cold docetaxel. Calculated concentrations were based on measured \textsuperscript{[14]C}docetaxel plasma concentrations and infused doses of \textsuperscript{[14]C}docetaxel and cold docetaxel. The solid line is a regression line. \( p \), Spearman’s correlation coefficient; \( P \), \( p \)-value.}
\end{figure}
Median AUC$_{\text{Plasma}}$ of cold docetaxel was 2.23 µg·mL$^{-1}$·h (range 1.75-3.42 µg·mL$^{-1}$·h). When plasma concentrations of cold docetaxel during therapeutic infusion were calculated on the basis of measured radioactivity concentrations in plasma and infused doses of $[^{14}\text{C}]$docetaxel and cold docetaxel, there was a high correlation between measured and calculated plasma concentrations of cold docetaxel ($\rho = 0.850; P < 0.001$; Figure 2B).

**$K_i$ values of $[^{14}\text{C}]$docetaxel in tumors are comparable during microdosing and therapeutic scan**

Fourteen tumors with a median size of 3 cm$^3$ (range 1-334 cm$^3$) could be defined on the low-dose CT scans. Figure 3 shows time-activity curves of $[^{14}\text{C}]$docetaxel in tumors during microdosing and therapeutic scans.

![Figure 3](image)

**Figure 3.** Tumor concentrations of $[^{14}\text{C}]$docetaxel as function of time for (A) a microdosing scan (tracer dose only) and (B) a therapeutic scan (co-infusion with a pharmacological dose of docetaxel). The fit in Figure (A) is the best fit for a two-tissue irreversible compartment model.

During the time frame of the therapeutic scan, therapeutic infusion of docetaxel resulted in progressive accumulation of $[^{14}\text{C}]$docetaxel in tumor tissue. During microdosing and therapeutic scans, median $K_i$ values of $[^{14}\text{C}]$docetaxel in tumors were 0.0078 mL·cm$^{-3}$·min$^{-1}$ (range 0.0023-0.0228 mL·cm$^{-3}$·min$^{-1}$) and 0.0077 mL·cm$^{-3}$·min$^{-1}$ (range 0.0023-0.0152 mL·cm$^{-3}$·min$^{-1}$), respectively. These $K_i$ values were highly variable between and within patients. There was a trend towards a correlation between $K_i$ values of $[^{14}\text{C}]$docetaxel obtained from microdosing and therapeutic scans (Spearman’s $\rho = 0.473; P = 0.088$). Although therapeutic infusion changed $K_i$ of $[^{14}\text{C}]$docetaxel in some tumors, overall there was no significant difference (Wilcoxon Signed Ranks test, $P = 0.507$). Both during microdosing and therapeutic scans, $K_i$ of $[^{14}\text{C}]$docetaxel was related to tumor
perfusion (Spearman’s $p = 0.662$ and 0.775; $P = 0.010$ and 0.007, respectively). After therapeutic infusion, tumor perfusion did not change (Wilcoxon Signed Ranks test, $P = 0.131$).

$[^{11}C]$docetaxel microdosing predicts $AUC_{\text{Tumor}}$ of $[^{11}C]$docetaxel during therapeutic infusion

As $K_i$ only represents the net rate of influx of $[^{11}C]$docetaxel in tumor tissue, the $AUC_{\text{Tumor}}$ of $[^{11}C]$docetaxel was used as a measure of $[^{11}C]$docetaxel exposure of tumors during the therapeutic scan. During the 90 min therapeutic scan, the median $AUC_{\text{Tumor}}$ of $[^{11}C]$docetaxel was measured to be 285 kBq-min-mL$^{-1}$ (range 110-634 kBq-min-mL$^{-1}$). In addition, the $AUC_{\text{Tumor}}$ of $[^{11}C]$docetaxel during the therapeutic scan was predicted from the IRF (Figure 4A), obtained using spectral analysis of the microdosing scan, together with the plasma $[^{11}C]$docetaxel curve during the therapeutic scan. Figure 4B shows a representative example of such a predicted $AUC_{\text{Tumor}}$ of $[^{11}C]$docetaxel during a therapeutic scan. There was a high correlation between measured and predicted $AUC_{\text{Tumor}}$ of $[^{11}C]$docetaxel (Spearman’s $p = 0.807$; $P < 0.001$; Figure 5).

**Figure 4.** Spectral analysis of $[^{11}C]$docetaxel uptake in tumor tissue. Confidence intervals were estimated using bootstrap analysis. (A) Impulse response function (IRF) in tumor tissue derived from a microdosing scan. The dashed lines represent the 95% confidence intervals. (B) Tumor time-activity curve for the corresponding therapeutic scan. The solid line represents the curve predicted from the microdosing IRF and the plasma concentration obtained from the therapeutic scan, whereas the dashed lines represent the corresponding 95% confidence intervals. The filled circles represent the measured concentrations.
Figure 5. Measured versus predicted AUC\textsubscript{Tumor} of [\textsuperscript{11}C]docetaxel. The measured AUC\textsubscript{Tumor} was derived from the therapeutic scan, whereas the predicted AUC\textsubscript{Tumor} was based on the impulse response function (IRF) derived from the microdosing scan and the [\textsuperscript{11}C]docetaxel plasma input function from the therapeutic scan. The solid line represents a regression line.

\textit{AUC}, area under curve; \textit{p}, Spearman’s correlation coefficient; \textit{P}, \textit{p}-value.

\textbf{Accumulated amount of cold docetaxel in tumor tissue can be determined from the microdosing scan}

As accumulation of docetaxel in tissue depends on its plasma concentrations, the accumulated amount of docetaxel in tumors was calculated by multiplying the [\textsuperscript{11}C]docetaxel \textit{Ki} from the microdosing scan with the AUC\textsubscript{Plasma} of cold docetaxel during the therapeutic scan. At 90 min after the start of docetaxel infusion, the median accumulated amount of cold docetaxel was 1.56 \textmu g·cm\textsuperscript{-3} (range 0.24-2.90 \textmu g·cm\textsuperscript{-3}), which showed a trend with the concentration of cold docetaxel at the last time point of the therapeutic scan (Spearman’s \textit{p} = 0.516; \textit{P} = 0.059). When the accumulated docetaxel amount was multiplied with the corresponding tumor volume, the median accumulated amount of cold docetaxel in tumors was 5.58 \textmu g (range 1.85-542.66 \textmu g), corresponding with 0.0036% (range 0.0011-0.4933%) of the total infused dose of docetaxel.

[\textsuperscript{11}C]docetaxel \textit{Ki} from the microdosing scan correlates with AUC\textsubscript{Tumor} of cold docetaxel during the therapeutic scan

Based on the measured AUC\textsubscript{Tumor} of [\textsuperscript{11}C]docetaxel during the therapeutic scan, the AUC\textsubscript{Tumor} of cold docetaxel could also be calculated. At 90 min of the therapeutic scan,
the median AUC$_{\text{Tumor}}$ of cold docetaxel was 1.69 μg·mL$^{-1}$·h (range 0.63-2.80 μg·mL$^{-1}$·h), which correlated with the accumulated amount of docetaxel at 90 min (Spearman’s $\rho = 0.675$; $P < 0.001$). In addition, AUC$_{\text{Tumor}}$ of cold docetaxel showed a good correlation with the $[^{11}\text{C}]$docetaxel $K_i$ value and tumor perfusion from the microdosing scan (Spearman’s $\rho = 0.715$ and 0.653; $P = 0.004$ and 0.011, respectively).

**Tumor uptake of $[^{11}\text{C}]$docetaxel is associated with tumor response after docetaxel therapy**

Nine out of fourteen tumors could be evaluated for tumor response. The other five tumors could not be evaluated due to clinical deterioration of one patient, precluding further CT assessment. At evaluation, the median change in the longest tumor diameter was 7% (range -6 to 100%). In this limited number of tumors, there was a negative correlation between the accumulated amount of cold docetaxel in tumors (obtained by multiplication of $K_i$ from the microdosing scan and AUC$_{\text{Plasma}}$ of cold docetaxel; units in μg·cm$^{-3}$) and the percentage change in longest tumor diameter (Spearman’s $\rho = -0.717$; $P = 0.030$). Similarly, $[^{11}\text{C}]$docetaxel $K_i$ and perfusion obtained from the microdosing scan showed a negative correlation with the change in longest diameter (Spearman’s $\rho = -0.800$ and -0.650; $P = 0.010$ and 0.058, respectively). For the purpose of illustration, Figures 6 and 7 show examples of sequential CT images in a patient with relatively high $[^{11}\text{C}]$docetaxel uptake and in another patient with relatively low $[^{11}\text{C}]$docetaxel uptake.

**DISCUSSION**

The concept of PET microdosing may be used to obtain important information on the distribution and kinetics of drugs in humans (20,21). In particular, PET studies using tracer doses (so called microdoses) of anticancer drugs may speed up drug development and could be an essential step in personalized treatment planning in oncology. Based on this concept, numerous anticancer drugs have been radiolabeled (22). Nevertheless, PET microdosing has not been validated appropriately in a clinical setting. The present study evaluated the principle of PET microdosing for $[^{11}\text{C}]$docetaxel in cancer patients. Although microdosing and therapeutic scans showed different TACs of $[^{11}\text{C}]$docetaxel in both plasma and tumor tissue, tumor uptake of cold (therapeutic) docetaxel could be predicted on the basis of the $[^{11}\text{C}]$docetaxel $K_i$ derived from the microdosing scan. In addition, preliminary data showed a relationship between $[^{11}\text{C}]$docetaxel $K_i$ and tumor response to docetaxel therapy.

In line with previous $[^{11}\text{C}]$docetaxel tracer studies (10), no radiolabeled metabolites of $[^{11}\text{C}]$docetaxel were detected during therapeutic infusion. Following a 1 hour infusion of
Figure 6. An example of a patient with non-small cell lung cancer showing relatively high \[^{11}\text{C}]\text{docetaxel} \text{ uptake and high perfusion in a tumor with a volume of 334 cm}^3. \text{ During the therapeutic scan, the patient was treated with 110 mg docetaxel. (A) CT scan showing tumor at baseline (longest diameter, 133 mm). (B) Tumor at three weeks after the first treatment with docetaxel (longest diameter, 128 mm). (C) PET-CT fusion image of a microdosing \[^{11}\text{C}]\text{docetaxel summed image (8-10 min; mean \[^{11}\text{C}]\text{docetaxel } K_i = 0.0089 mL·cm}^{-3}·\text{min}^{-1}). (D) PET-CT fusion image of a therapeutic \[^{11}\text{C}]\text{docetaxel summed image [25-65 min; mean } K_i = 0.0125 mL·cm}^{-3}·\text{min}^{-1}; \text{ total accumulated amount of cold docetaxel at 90 min = 543 } \mu\text{g (at 1.62 } \mu\text{g·cm}^{-3}), \text{ corresponding with 0.49\% of the infused dose docetaxel]. (E) PET-CT fusion image of perfusion during the microdosing scan (mean perfusion = 0.57 mL·cm}^{-3}·\text{min}^{-1}). (F) PET-CT fusion image of perfusion during the therapeutic scan (mean perfusion = 0.58 mL·cm}^{-3}·\text{min}^{-1}).
Figure 7. An example of a patient showing relatively low $[^{11}\text{C}]$docetaxel uptake and low perfusion in a tumor with a volume of 88 cm$^3$. During the therapeutic scan, the patient was treated with 140 mg docetaxel. At the time of the study, the patient was diagnosed with histologically proven non-small lung cancer. After neoadjuvant chemotherapy, the tumor was surgically resected and pathological examination of the resected tumor revealed thymic carcinoma instead of non-small cell lung cancer. (A) CT scan showing tumor at baseline (longest diameter, 54 mm). (B) CT scan showing tumor at three weeks after the first treatment with docetaxel (longest diameter, 59 mm). (C) PET-CT fusion image of a microdosing $[^{11}\text{C}]$docetaxel summed image (8-10 min; mean $[^{11}\text{C}]$docetaxel $K_r = 0.0023$ mL·cm$^{-3}$·min$^{-1}$). (D) PET-CT fusion image of a therapeutic $[^{11}\text{C}]$docetaxel summed image (25-65 min; mean $[^{11}\text{C}]$docetaxel $K_r = 0.0023$ mL·cm$^{-3}$·min$^{-1}$; total accumulated amount of cold docetaxel at 90 min = 21.2 µg (at 0.24 µg·cm$^{-3}$), corresponding with 0.015% of the infused dose docetaxel]. (E) PET-CT fusion image of perfusion during the microdosing scan (mean perfusion = 0.18 mL·cm$^{-3}$·min$^{-1}$). (F) PET-CT fusion image of perfusion during the therapeutic scan (mean perfusion = 0.26 mL·cm$^{-3}$·min$^{-1}$).
100 mg·m⁻² docetaxel, metabolites have been detected previously in about one third of patients at 5 to 30 min (23). In the liver, docetaxel usually is metabolized by the cytochrome P450 enzyme CYP3A4 into four major metabolites (24), which are less cytotoxic than the parent compound (25). This extensive liver metabolism is reflected by high hepatic uptake of the tracer [¹¹C]docetaxel (26,27). The absence of radiolabeled metabolites of [¹¹C]docetaxel indicates that no detectable radiolabeled metabolites of [¹¹C]docetaxel enter plasma, at least not during the time course of a PET study. Consequently, radioactivity measured in tumors can be attributed solely to the presence of [¹¹C]docetaxel.

As expected, plasma TACs of [¹¹C]docetaxel were quite different for microdosing and therapeutic scans due to the differences in administration protocols, i.e. bolus injection versus slow infusion. In line with these differences, tumor TACs were also different between microdosing and therapeutic studies. Although plasma concentrations of [¹¹C]docetaxel decreased rapidly after discontinuation of the infusion, tumor TACs still showed accumulation of [¹¹C]docetaxel. Retention of [¹¹C]docetaxel in tumor tissue at later times could not be confirmed, as the total duration of a PET scan is limited by the short half-life of carbon-11 (20.3 min). Nevertheless, it is reasonable to assume that achieved tumor concentrations of docetaxel persist at later time points, as retention of docetaxel in tumors has been measured for 24 hours in a mouse study (5).

Despite differences in the administration schedule, $K_i$ values of [¹¹C]docetaxel were similar during microdosing and therapeutic studies. During a therapeutic infusion, this [¹¹C]docetaxel $K_i$ is a direct measure of the rate of influx of cold docetaxel in tumor tissue. Using [¹¹C]docetaxel $K_i$, however, the availability of docetaxel in plasma is not taken into account. Therefore, as a measure of overall docetaxel uptake in tumor tissue, the AUC_{Tumor} of [¹¹C]docetaxel was determined during therapeutic infusion of docetaxel. In addition, it was possible to predict this AUC_{Tumor} of [¹¹C]docetaxel using the IRF derived from the corresponding microdosing scan together with the plasma curve of [¹¹C]docetaxel during the therapeutic scan. As the predicted AUC_{Tumor} of [¹¹C]docetaxel showed good correlation with the measured values, further analyses were performed to determine tumor uptake of cold docetaxel.

To the best of our knowledge, the present study is the first in which absolute tumor uptake of chemotherapy is measured non-invasively in patients. As AUC_{Tumor} of [¹¹C]docetaxel could be obtained from the therapeutic scans, it was possible to determine the AUC_{Tumor} of cold docetaxel in absolute values. This AUC_{Tumor} of cold docetaxel correlated well with [¹¹C]docetaxel $K_i$ from the microdosing scan, indicating that the
\[^{11}\text{C}]\text{docetaxel } K_i \text{ from the microdosing scan, which does not account for inter-patient plasma variability of cold docetaxel, is a reliable measure of tumor exposure to cold docetaxel. These findings suggest that the influx into tumor tissue dominates the uptake of docetaxel by tumors in case the administered dose of docetaxel is within the normal therapeutic range, indicating that }^{11}\text{C]}\text{docetaxel PET microdosing studies provide non-invasive means for measuring docetaxel tumor kinetics in patients.}

In the present study, additional analyses revealed that < 1% of the infused therapeutic dose of docetaxel is finally taken up by tumor tissue. This finding underscores the fact that only a small amount of drug accumulates in tumors and indicates that more studies are warranted to investigate how chemotherapy can be delivered more effectively to tumors. In this respect, drug-loaded microbubbles are promising for enhancement of drug delivery to tumor tissue, as these microbubbles can be applied for localized delivery in target tissue by ultrasound triggering (28). In addition, the direct effects of other (anticancer) drugs on metabolism as well as drug delivery to tumors need to be investigated, as other drugs may affect metabolism and drug delivery to tumors. In a recent study, we have shown that the anti-angiogenic drug bevacizumab induces a rapid and significant reduction in delivery of \[^{11}\text{C}]\text{docetaxel to tumors in non-small cell lung cancer patients (29).}

Furthermore, results of the present study and previous studies (10,29) indicate that perfusion is an important determinant for docetaxel delivery to tumors. Although docetaxel has anti-angiogenic effects in tumors (30), the present study did not reveal immediate effects of docetaxel infusion on tumor perfusion. More importantly, the present findings suggest that tumor perfusion is a predictor of docetaxel exposure and consequently tumor response to docetaxel therapy. As tumor perfusion measurements using \[^{15}\text{O}]\text{H}_2\text{O PET are relatively simple (14), it may be worthwhile to conduct further studies on the predictive value of tumor perfusion on response to chemotherapy.}

In conclusion, the current study validated the PET microdosing concept for \[^{11}\text{C}]\text{docetaxel in lung cancer patients. Microdosing data of }^{11}\text{C]}\text{docetaxel PET could be used to reliably predict tumor uptake of cold docetaxel during chemotherapy, which was also associated with tumor response to docetaxel therapy. The present study provides a framework for investigating the PET microdosing concept for other radiolabeled anticancer drugs in patients.}
REFERENCES


SUPPLEMENTARY FIGURE 1

Schematic diagram illustrating the various analyses that were performed to calculate the accumulated amount of cold docetaxel in tumor tissue. Data obtained from the $[^{11}C]$docetaxel microdosing scan are presented at the left, whereas data obtained from the $[^{11}C]$docetaxel therapeutic scan are presented at the right.

$K_i$, net rate of influx; vs., versus; AUC, area under curve

1 calculated using the impulse response function (IRF) derived from the microdosing scan together with the plasma curve of $[^{11}C]$docetaxel during the therapeutic scan

2 calculated by dividing the measured $AUC_{\text{Tumor}}$ of $[^{11}C]$docetaxel during the therapeutic scan by the mean specific activity of $[^{11}C]$docetaxel in plasma

3 calculated by multiplication of $[^{11}C]$docetaxel $K_i$ obtained from the microdosing scan with the $AUC_{\text{Plasma}}$ of cold docetaxel during the therapeutic scan