A pharmacokinetic study of midazolam in prostate cancer patients and patients with solid tumours: *in vivo* phenotyping of CYP3A

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Abstract

Background Pharmacokinetic (PK) studies reported a higher clearance of docetaxel in prostate cancer patients compared to patients with solid tumours. The difference in exposure was 1.8-fold after intravenous (IV) administration and 2.8-fold after oral administration. Docetaxel is metabolized by CYP3A4. Therefore, an altered CYP3A4 activity between patient groups could explain the difference.
Aim The aim was to measure CYP3A activity with *in vivo* phenotyping by administering midazolam as a probe. Midazolam was administered orally and intravenously to differentiate between gastrointestinal (GI) CYP3A activity and hepatic CYP3A activity. CYP3A activity was defined as clearance (Cl).
Methods A prospective, interventional, PK study was executed at the Antoni van Leeuwenhoek hospital/Netherlands Cancer Institute (NCT05518799). 9 patients with prostate cancer and castrate testosterone levels (<50ng/dL) and 9 male patients with solid types of tumours were included. Concomitant use of medication, herbs or food that could influence PK of midazolam was not allowed. After signing written informed consent, patients got administered midazolam on two consecutive days: 2 mg of oral midazolam on the first study day and 1 mg IV midazolam on the second study day. Blood samples were drawn before start, after 15 minutes, 30 minutes, 1 hour, 2 hours, 4 hours and 8 hours. Plasma concentrations were used for PK analysis.
Results 18 male, Caucasian patients were included. Among them, 9 prostate cancer patients with castrate testosterone levels and 9 patients with solid tumour type. Apparent oral clearance was higher in prostate cancer patients (87.22 ± 22.50 L/h vs. 75.67 ± 26.24 L/h). Difference was 16%, however not significant (p=0.14). IV clearance was lower in prostate cancer patients (38.95 ± 8.89 vs. 41.79 ± 13.71). However, difference was very small and not significant (p=0.73).
Conclusion The 1.8-fold difference and 2.8-fold difference in docetaxel PK cannot be explained by difference in CYP3A activity between prostate cancer patients and patients with other types of solid tumours.

1.Introduction

Cancer is still one of the most deadly diseases. Regarding the treatment in oncology, attention towards personalized- and precision medicine is rising [1, 2]. This entails tailoring of medical treatment to the individual characteristics of each patient, with optimal exposure as the goal [3]. Optimal exposure can be defined as a drug concentration in the blood that contributes to both efficacy and safety. This is in particular important for drugs that contain a narrow therapeutic window, such as chemotherapeutic agents [4]. In case exposure levels are not optimal, there is a risk for either too low exposure or toxicity [5,6].

Drug exposure is dependent on the administered dose and pharmacokinetics (PK) of the respective drug. In addition, factors as age, sex, co-medication, renal function and enzyme activity affect PK of a drug in the human body. An example of enzymes that are greatly involved in drug metabolism, are cytochrome P450 (CYP) enzymes. CYP enzymes are expressed throughout the whole body, but mainly in the liver and the gastrointestinal(GI)tract [7]. One of these enzymes, CYP3A, and is responsible for breakdown of approximately 50% of all drugs. Expression of CYP3A, and other enzymes can also be influenced by other factors, such as food, genetics, environmental factors, disease and hormonal status [6, 8-10].

Several studies have reported a difference in docetaxel exposure between patients with solid tumours and prostate cancer patients. Docetaxel is a taxane and used as chemotherapy for amongst others prostate cancer. Taxanes inhibit cell proliferation by binding microtubili and thereby halting the cell proliferating cycle [11]. Breakdown of docetaxel occurs mostly via CYP3A4 [12, 13]. Recently, Schultink *et al*., published a meta-analysis where they investigated docetaxel pharmacokinetics in patients with hormone-sensitive prostate cancer (HSPC) and metastatic castration-resistant prostate cancer (mCRCP) compared to patients with other types of solid tumours [14]. The difference in the mean area under the curve extrapolated to infinity ($AUC_{0\text{-}inf}$) after intravenous (IV) administration of docetaxel was 1.8-fold. Moreover, an oral docetaxel tablet in combination with ritonavir (ModraDoc006/r) caused a difference in $AUC_{0\text{-}inf}$ of 2.8-fold in prostate cancer patients compared to patients with other types of solid tumours [15]. The lower exposure to docetaxel was associated with less neutropenia in prostate cancer patients. The mechanism behind the lower $AUC_{0\text{-}inf}$ remains to be elucidated.

In view of previous findings, our hypothesis was an altered CYP3A activity in castrated prostate cancer patients compared to patients with other types of solid tumours. Therefore, the aim of this study was to determine CYP3A activity in castrated patients with prostate cancer compared to male patients with other types of solid tumours. CYP3A activity was determined trough in vivo phenotyping, with midazolam used as a probe. In addition, we aimed to differentiate between hepatic- and gastrointestinal CYP3A activity by oral and IV midazolam administration.

2. Materials and methods

2.1 Study design

This study was designed as a prospective, interventional pharmacokinetic study. Herein, CYP3A4 activity of patients with prostate cancer and other types of solid tumours were compared with each other. Patients were included from the 22nd of march, 2021 until the 1st of December, 2022. The study was conducted at the Antoni van Leeuwenhoek hospital. The study protocol was approved by the Medical Ethics Committee of the Netherlands Cancer Institute/Antoni van Leeuwenhoek and complied with the principles of the Declaration of Helsinki. The study was registered in clinicaltrials.gov under NCT05518799. All patients signed a written informed consent before taking part in the study.

2.2 Patient eligibility

Two groups of adult patients were included: patients with prostate cancer (group 1) and male patients with other types of solid tumours (group 2) receiving anticancer treatment. Group 1 had to be medically castrated, characterized by a testosterone level less than 50 ng/dL [16]**.** Both hormone-sensitive as castration-resistant prostate cancer patients were included. For both groups, metastatic and non-metastatic patients were eligible. Patients with abnormal hematologic, hepatic and renal profile were excluded. In addition, concomitant use of medication, herbs or food which could influence the pharmacokinetics of midazolam were prohibited 14 days before the start of the study or within five half-lives of the drug (see supplementary 1). In particular, dexamethasone, bicalutamide and enzalutamide, commonly used medication among prostate cancer patients, could not be used regarding its inducing effect on CYP3A [17 - 19]. However, using prednisone in a maximum of 10 mg daily was allowed [20]. Smokers or patients who stopped smoking within 7 days before study allocation were excluded.

2.3 Procedure

The first study day patients received 1 mg of oral midazolam. The following day, which was the second study day, patients received 2 mg of IV midazolam. For both study days, blood samples (4 mL) were drawn at 7 time points in EDTA-containing vials: pre-dose, 15 minutes, 30 minutes, 1 hour, 2 hours, 4 hours and 8 hours after administration. Immediately after collecting, samples were centrifuged for 10 minutes at 2800 rpm at 4 °C. Plasma was collected and stored in Eppendorf tubes at – 80 °C until analysis.

2.4 Bioanalysis

Plasma concentrations of midazolam, 1'-hydroxymidazolam and 4'-hydroxymidazolam were determined with a validated LC-MS/MS method. Liquid-liquid extraction with tert-butylmethylether (TBME) was used as sample pretreatment, using 200 µL plasma aliquots. The samples were mixed (1250 rpm, 10 minutes) and centrifuged (14,000 rpm for 5 minutes). After snapfreezing the sample, the organic layer was collected and evaporated until dryness. Before injection into the HPLC, the residue was reconstituted with 100 µl 20 mM ammonium formate in water (pH 3.5)-MeOH (7:3, v/v). The samples were centrifuged (14,000 rpm for 5 minutes) before transferring the supernatant in vials for analysis. Chromatographic separation was achieved using a gradient of 20 mM ammonium formate in water (pH 3.5; Eluens A) and methanol (Eluens B) and a Acquity BEH C18 column (1.7 um, 2.1 x 50 mm). The analytical runtime took approximately 8 minutes. The triple quadrupole mass spectrometer was operated in positive mode. For quantification, multiple reaction monitoring (MRM) was used. Method validation for all three compounds was conducted over a concentration range of 0.1 ng/mL to 50 ng/mL. Run time was 8 minutes. For the quantification, internal standards labelled with stable isotopes were used. Accuracy and precision for midazolam were respectively ± 7.9% and 5.1%, for 1'-hydroxy midazolam ± 7.2% and 5.7 % and for 4'-hydroxy midazolam ± 8.8% and 3.5%. Lower limit of quantification (LLOQ) was 0.1 ng/mL for all three metabolites.

2.5 Pharmacokinetic analysis

Pharmacokinetic parameters were calculated with a non-compartmental analysis (NCA). The NCA was performed in R with pre-written and validated R scripts (version 4.1.2). Area Under the plasma concentration-time Curve from 0 to 8 ($AUC_{0\text{-}8h}$) was calculated using a log-linear model, computed by the trapezoidal rule. This was extrapolated to infinity ($AUC_{0\text{-}inf}$). Maximum plasma concentration (C_{max}) and time to reach C_{max} (T_{max}) were derived from plasma concentrations. The elimination constant, K_e, was determined by linear regression on log-transformed concentrations of last four time-points (see supplementary 2). When C_{max} was within the four time-points, K_e was determined by last four time-points. Half-life ($T_{1/2}$) (equation 1), apparent clearance (Cl/F) and clearance (Cl) (equation 2), volume of distribution (Vd) (equation 3), bioavailability (F) (equation 4) and metabolite ratio (equation 5) were calculated as following:

$$T_{\frac{1}{2}} = \frac{\ln(2)}{K_e} \tag{1}$$

$$\frac{Cl}{F} = \frac{dose}{AUC_{0-inf}} / \, Cl = \frac{Dose}{AUC_{0-inf}} \tag{2}$$

$$Vd = \frac{Cl}{K_e} \tag{3}$$

$$F = \frac{AUC_{0-inf}(oral)/dose}{AUC_{0-inf}(IV)} \tag{4}$$

$$metabolite\ ratio = \frac{AUC_{0-inf}(1'-hydroxymidazolam)}{AUC_{0-inf}(midazolam)} \tag{5}$$

2.6 Statistical analysis

Difference in demographic data was tested with a student's t-test. A Wilcoxon signed rank test was used to test for differences in PK parameters. All tests were 2-tailed and reached significance when P-value was < 0.05. For exploratory objectives, a linear model was tested to compare GI and hepatic CYP3A4 activity. Predictors of this model were disease and administration. A sample size of 18 patients in total were required to reach 80% power (β) and to find an effect of 20% with significance (α). All calculations were performed in R (version 4.1.2).

3. Results

3.1 Patient characteristics

The patient characteristics are described in table 1. In this study, 18 male, Caucasian patients were included. Group 1 consisted of 9 patients with prostate cancer. Group 2 consisted of 9 patients with other types of solid tumours, among them colon cancer, small cell lung carcinoma (SCLC) and melanoma. The median age of patients in group 1 was somewhat higher compared to patients in group 2, however not statistically significant (69 [58 – 79] vs. 64 [38 – 71]; p=0.07). Patients from group 2 were slightly heavier than patients from group 1, but also not significant (81.8 [65 - 100] vs. 91 [62.5 - 131]; p=0.23). All testosterone levels of prostate cancer patients were under castrate levels, with a median and range of 0.03 [0.02 – 0.05] nmol/L. These levels corresponded with beforehand defined levels in the protocol and were statistically significant from testosterone levels in group 2 (p<0.01). Although monocytes between the groups were significantly different (p=0.04), we consider this difference not to have an influence on midazolam clearance. The remaining baseline characteristics, such as hematology and lab values, were normally distributed. No adverse events related to the study procedure have occurred and all patients completed the study.

3.2 Pharmacokinetics

Midazolam - Oral administration

The mean values with corresponding standard deviations of midazolam PK parameters are reported in table 2. $AUC_{0\text{-}8h}$ (25.17 $\pm$ 5.94 ng/mL*h) and $AUC_{0\text{-}inf}$ (28.74 $\pm$ 8.12 ng/mL*h) were higher in the group with solid tumours, compared to the $AUC_{0\text{-}8h}$ (22.05 $\pm$ 6.98 ng/mL*h) and $AUC_{0\text{-}inf}$ (24.52 $\pm$ 7.38 ng/mL*h) for the prostate group (see figure 1). However, these differences were not significant (p=0.16 and p=0.14, respectively). Looking at the standard deviation and corresponding error bars, the results are highly variable and overlap on most time points.

Mean Cl/F of midazolam in the prostate cancer group was 87.22 $\pm$ 22.50 L/h and in the solid tumour group 75.67 $\pm$ 26.24 L/h (see figure 3). Cl was 16% higher in prostate cancer (p=0.14). Differences in C_{max} and T_{max} were not significant between both groups (p=0.10 and p=0.35, respectively). $T_{1/2}$ was 1.88 ± 0.64 hours for prostate and 2.11 ± 0.71 hours for solid tumours (p=0.39). F was 45% in prostate, 55% in solid tumours.

Midazolam – IV administration

The mean values with corresponding standard deviations of calculated midazolam PK parameters are reported in table 3. $AUC_{0\text{-}8h}$ was higher for the prostate cancer group compared to the group with solid tumours (24.29 $\pm$ 6.60 ng/mL*h vs 22.75 $\pm$ 8.03 ng/mL*h; p=0.34). The same applies to $AUC_{0\text{-}inf}$ (27.01 $\pm$ 6.72 ng/mL*h vs. 26.70 $\pm$ 10.51 ng/mL*h; p=0.73) (see figure 1). Standard deviation shows results are highly variable, and error bars in the graph overlap on all time points.

Mean Cl of midazolam was 38.95 $\pm$ 8.89 L/h for the prostate cancer group and 41.79 $\pm$ 13.71 L/h for the solid tumour group (see figure 3). The difference was approximately 7% (p=0.73). $T_{1/2}$ was 2.60 ± 0.64 hours in the prostate group and 3.14 ± 0.56 hours in the solid tumour group (p=0.22).

1'-hydroxymidazolam – Oral administration

The mean values with corresponding standard deviations of calculated PK parameters of 1'-hydroxymidazolam are reported in table 2. $AUC_{0\text{-}8h}$ and $AUC_{0\text{-}inf}$ were slightly higher in the solid tumour group (5.98 $\pm$ 4.25 ng/mL*h and 6.47 $\pm$ 4.45 ng/mL*h) compared to the prostate cancer group (5.10 $\pm$ 2.44 ng/mL*h and 5.67 $\pm$ 2.48 ng/mL*h) (see figure 2). Although AUC was slightly higher in the prostate group, the differences were not significant (p=0.75 and p=0.65, respectively). The standard deviation shows high variability in the results, error bars in the figure overlap on all time points. $t_{1/2}$ was 2.14 ± 1.07 hours for prostate and 1.98 ± 0.42 hours or solid tumours (p=0.86).

1'hydroxymidazolam – IV administration

The mean values with corresponding standard deviations of calculated PK parameters of 1'hydroxymidazolam are reported in table 3. $AUC_{0\text{-}8h}$ and $AUC_{0\text{-}inf}$ were higher in the solid tumour group (4.05 $\pm$ 4.05 ng/mL*h and 4.71 $\pm$ 4.18 ng/mL*h) compared to the group with prostate cancer patients (3.38 $\pm$ 1.32 ng/mL*h and 3.92 $\pm$ 1.27 ng/mL*h). (see figure 3) Differences were not significant (p=0.05 and p=0.55, respectively). Taking the standard deviation in account, the variability in the results were very high and error bars in the figure overlap on all time points. $t_{1/2}$ was 4.15 ± 0.98 hours for prostate cancer and 3.99 ± 1.18 hours for solid tumours (p=0.19).

Ratio of 1'-hydroxymidazolam and midazolam are reported in table 2. For oral administration, metabolite ratio was higher for the prostate cancer group compared to the other solid tumour group (0.24 $\pm$ 0.08 vs. 0.19 $\pm$ 0.16; p=0.73). After IV administration, metabolite ratio was higher in the prostate cancer group compared to the solid tumour group (0.12 $\pm$ 0.03 vs. 0.09 $\pm$ 0.17) in the solid tumour group and also not significant (p=0.34). Looking at the standard deviation, variability was remarkably lower in the prostate group. For oral administration difference was 2-fold and for IV-administration difference was almost 6-fold.

4'-hydroxymidazolam

Because a large proportion of the measured concentrations of 4'-hydroxymidazolam were below the LLOQ (42,9%, n=108; total was n=252), only few concentration over timepoints were available to plot a graph (see figure 4). Therefore this data was considered as unreliable to execute the NCA.

4. Discussion

In this study, we compared CYP3A activity between prostate cancer patients and patients with other types of solid tumours. Midazolam clearance was not significantly different between patients with prostate cancer and other types of solid tumours. Oral Cl/F was 87.22 ± 22.50 vs. 75.67 ± 26.24; p=0.14 and IV Cl was 38.95 ± 8.89 vs. 41.79 ± 13.71; p=0.73. Consequently, we were not able to distinguish between hepatic an GI clearance. Taken together, the results suggest CYP3A activity is not altered in prostate cancer patients compared to patients with other types of solid tumours.

In another CYP3A phenotyping study, researchers also aimed to investigate CYP3A activity in castrated and non-castrated prostate cancer patients. To determine CYP3A activity, a erythromycin breath test was used. Our results are in line with this study: the researchers did not find a significant difference in CYP3A activity between both groups (p=0.26)[21]. However, erythromycin is not specific for CYP3A, since it is also a substrate for p-glycoproteins (P-gp) [22]. In order to draw a definitive conclusion, we repeated the study with a more specific CYP3A probe. Hence, we executed the CYP3A phenotyping with midazolam as probe [23]. Midazolam is the most studied and widely accepted probe for CYP3A [23-26]. It is very specific, since it is selectively metabolized by CYP3A and not a substrate for P-gp [23, 26, 27]. Another important reason is that the $AUC_{0\text{-}inf}$ of midazolam and metabolization to its major metabolite, 1'-hydroxymidazolam, correlate well with hepatic CYP3A content [27, 28]. Moreover, midazolam PK are highly sensitive to changes in CYP3A [23, 26]. Also, midazolam can be administered both orally and intravenously to investigate gastro-intestinal and hepatic CYP3A function [24, 29, 30]. Lastly, this test is recommended by the FDA and EMA [31]. For these reasons, oral and IV midazolam was used for phenotyping to show CYP3A activity without other influencing factors. The dosages midazolam used in this study are lower than therapeutic dosages. We chose this dosage because we did not want the patients to experience side effects. As reported in table 1, this has not occurred.

In order to investigate whether $T_{1/2}$ and F of midazolam are altered in cancer patients, we compared our data with other literature [32, 33]. Here, midazolam characteristics are determined in healthy humans. According to these studies, oral bioavailability of midazolam is 40-50% due to high first-pass metabolism. Additionally, absorption of is highly variable. Midazolam has an $T_{1/2}$ of 1.5-3 hours. In our study, we found F was 45% in prostate cancer patients and 55% in patients with other types of solid tumours. For prostate cancer and other types of solid tumours, measured $t_{1/2}$ after oral administration was 1.88 ± 0.64 and 2.11 ± 0.71, respectively. Measured $t_{1/2}$ after IV administration was 2.60 ± 0.64 and 3.14 ±0 .56, respectively. Comparing the literature with the values we have found, we conclude they correspond quite well which means F and $t_{1/2}$ of midazolam are not entirely altered in cancer patients.

We did not find a significant difference in CYP3 activity for prostate cancer and other tumours. However, this does not mean that cancer in general does not affect CYP3A activity at all. To investigate whether cancer has an influence on CYP3A activity, we compared our data with midazolam PK in healthy volunteers [34 – 37]. An overview of these studies are displayed in table 4. The last column shows conclusions of the comparison. These conclusions taken together, results are inconsistent. Cancer might be a factor influencing CYP3A activity, but to draw a conclusion, this needs further investigation. This knowledge may be useful when administering drugs, specifically drugs metabolized by CYP3A, to patients suffering from cancer.

Since we found CYP3A activity not to be the main explanation for the difference in docetaxel PK between prostate cancer patients and patients with solid tumours, we searched for an alternative explanation. A possible alternative hypothesis for the difference in docetaxel PK is an upregulation of liver transporters in castrated patients. In the study mentioned earlier by Franke *et. al*. [21], the docetaxel liver and plasma concentration in castrated and non-castrated rats was studied. The

docetaxel AUC in the liver of castrated rats was higher compared to the non-castrated rats (37.0 vs. 18.0 μg * h/g; p=0.01,). Furthermore, this was associated with a reduced peak in the plasma concentration of docetaxel (1.50 vs. 4.07 μg/mL; p=0.04). These results suggest higher uptake of docetaxel in the liver of castrated rats, resulting in lower plasma concentrations. This effect resembles the higher clearance of docetaxel in castrated male patients seen in previous mentioned studies [17, 18, 21]. The researchers also reported a significant greater hepatic expression of the organic anion transporter 2 (OAT2) in castrated rats, wherein docetaxel accumulation was significantly higher than water-injected controls [21]. This suggests that docetaxel is a substrate for the hepatic OAT2 transporter. Building on these findings, Yu *et al*. investigated the influence of the liver receptor homolog 1 (Lhr-1) on OAT2 transporters and docetaxel PK [37]. Lhr-1 is a nuclear receptor, expressed in amongst others the liver. It plays an important role in regulating transporters [38]. Results showed that overexpression of Lhr-1 resulted in 2.2-fold increase of OAT2 mRNA. Consistent with this result, a knockdown of Lhr-1 resulted in decrease of OAT2 mRNA. This suggests a positive regulatory relation between Lhr-1 and OAT2 in hepatic cells. The same effect was demonstrated in mice: a deletion of Lhr-1 downregulated hepatic OAT2. Last, Lhr-1 knockdown led to decreased docetaxel uptake in hepatic cells at a wide range of dosing concentrations. This experiment showed that hepatic deletion of Lhr-1 altered PK of docetaxel in knockout mice. Furthermore, these knockout mice showed increased C_{max} and AUC plasma concentrations, combined with lowered hepatic concentrations. All taken together, the results suggest castrated rodents to have lower docetaxel plasma concentrations, increased hepatic docetaxel AUC and increased expression of OAT2 transporters. Upregulation of Lhr-1 causes increased expression of OAT2 transporters, and herby affecting docetaxel PK by altering hepatic uptake [21, 37]. Because the testicles are removed in castrated rodents, no more testosterone is produced. Therefore, downregulation of Lhr-1 could possibly be a result of the lowered concentration of the sex hormone testosterone. Few studies have been conducted after the influence of testosterone on the Lhr-1 promotor. Strikingly, several studies showed stimulation of Lhr-1 expression under influence of testosterone [39-41], where we would expect an inhibiting effect. Important to mention is that these studies have been executed in granulosa cells originating from female species. Thus, the relation between Lhr-1 and testosterone remains to be elucidated with preclinical research. Furthermore, clinical research is needed to unravel the relation between testosterone, the Lhr-1 receptor and OAT2 transporter in prostate cancer patients.

The study that we conducted has several strengths and limitations. The first limitation in this study was the relatively small sample size. The study was powered to find a two-fold difference in clearance. This lead to inclusion of 18 patients. Perhaps we would have found a significant difference when the study included more patients. However, to explain the difference in docetaxel PK, we needed to find an effect-size of at least two-fold. In this study, we found a difference of 16%. When significant, this still would not explain the difference in docetaxel PK. Furthermore, according to the FDA [42], a weak inducer is a drug that causes 20%-50% decrease in $AUC_{0\text{-}inf}$. Given this fact, a difference in clearance of 16% is considered clinically irrelevant. As a second limitation, difference in age between both groups could have influenced midazolam PK. With aging comes structurally alterations of the human body that can influence PK of certain drugs [43]. However, a review with several study's did not show a significant reduction in midazolam clearance in elderly populations [44]. Although the difference in age was present, it was not significant and relatively small. Considering these conclusions, we do not suspect the difference in age between groups to be of influence on the results. A strength in this study is that we compared the difference in clearance with a group of different types of solid tumours. The conclusion of this study can therefore probably be extrapolated to other solid tumour types. Another strength is that we did intense sampling in de absorption phase. Most PK studies collected the first sample after 30 minutes, while we already took the first sample after 15 minutes. This gives more detailed information of the early absorption phase of midazolam. Last strong point is that we tested on two consecutive study days. For this reason, we expect the intra-individual variability to be minimal.

In conclusion, the results of this pharmacokinetic study suggest the two-fold difference in clearance as seen with docetaxel in prostate cancer patients cannot be explained by CYP3A. To unravel the factor of influence, further research needs to be conducted. Herein, research after the liver transporter OAT2 with its Lrh-1 receptor seems promising.

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Tables

Table 1 Baseline characteristics

	Group 1: prostate cancer (*n*=9)	Group 2: other types of solid tumours (*n*=9)	P-value[c]
Patient characteristics			
• Ethnic origin			
- Caucasian	100% (*n*=9)	100% (*n*=9)	
• Age[a]	69 [58 – 79]	64 [38 – 71]	0.07
• Weight[a]	81.8 [65 - 100]	91 [62.5 - 131]	0.23
• Height[a]	178 [171 - 186]	186 [170 -191]	0.24
• WHO-status			
- 0	100% (*n*=9)	88,9% (*n*=8)	
- 1	0% (*n*=0)	11,1% (*n*=1)	
Tumour information			
• Primary tumor type			
-Prostate	100% (*n*=9)	0% (*n*=0)	
-Colorectal	0% (*n*=0)	44,4% (*n*=4)	
-Melanoma	0% (*n*=0)	44,4% (*n*=4)	
-SCLC	0% (*n*=0)	11,2% (*n*=1)	
• Stage of cancer			
-Local	0% (*n*=0)	22,2% (*n*=2)	
-Locally advanced	11,1% (*n*=1)	0% (*n*=0)	
-Metastatic	88,9% (*n*=8)	77,8% (*n*=7)	
Heamatology[a]			
• Hemoglobin (mmol/L)	8.6 [7.8 – 8.8]	9 [6.7 – 10.4]	0.18
• White-Blood-Count (10^9/L)	7 [3.4 – 9.6]	6.9 [3.4 – 9.1]	0.74
• ANC (10^9/L)	3.8 [1.9 – 6.5]	3.8 [1.7 – 5.8]	0.71
• Platelets (10^9/L)	183 [164 - 257]	221 [4.1 - 326]	0.90
• Lymphocytes (10^9/L)	1.6 [0.6 – 2.4]	1.8 [0.9 – 3.9]	0.51
• Monocytes (10^9/L)	0.5 [0.4 – 0.8]	0.7 [0.5 – 0.9]	0.04
• Eosinophils (10^9/L)	0.1 [0.1 – 0.3]	0.2 [0.1 – 0.4]	0.07
• Basophils (10^9/L)	0.1 [0.1 – 0.1]	0.1 [0.1 - 0.1]	1.00
• Total bilirubin (μmol/L)	9 [6 - 23]	6 [6 - 18]	0.22
• ASAT (U/L)	32 [22- 69]	28 [19 - 42]	0.13
• ALAT (U/L)	22 [17 - 36]	22 [9 - 35]	0.91
• CRP (mg/L)	1 [1 - 56]	2 [1 - 5]	0.46
Lab values[a]			
• Serum creatinine (μmol/L)	72 [61 - 90]	84 [66 - 99]	0.06
• Albumin (g/L)	46 [42 - 49]	44 [39 - 49]	0.25
• Testosterone (nmol/L)	0.03 [0.02 – 0.5]	9 [6.3 - 29]	0.00
• eGFR (mL/min)	90 [71 - 108]	84 [66 – 96]	0.12
Other medical information			
• Prior therapy			
- No	66,7% (*n*=6)	55,6% (*n*=5)	
- Docetaxel	33,3% (*n*=3)	0% (*n*=0)	
- Capecitabine	0% (*n*=0)	11,1% (*n*=1)	
- Pembrolizumab	0% (*n*=0)	11,1% (*n*=1)	
- Nivolumab	0% (*n*=0)	11,1% (*n*=1)	
- Ipilimumab	0% (*n*=0)	11,1% (*n*=1)	
• Prior hormonal therapy			
- No	66,7% (*n*=6)	77,8% (*n*=7)	
- Bevacizumab	0% (*n*=0)	22,2% (*n*=2)	
- Bicalutamide	22,2% (*n*=2)	0% (*n*=0)	
- Cyproteron	11,1% (*n*=1)	0% (*n*=0)	

• Concomitant medication[b]			
- Abiraterone	13,5% (*n*=5)	0% (*n*=0)	
- Antibiotics	0% (*n*=0)	3.8% (*n*=1)	
- Anti-histaminics	2.7 % (*n*=1)	0% (*n*=0)	
- Asthma medication	0% (*n*=0)	3.8% (*n*=1)	
- Cardiovascular medication	21.6% (*n*=8)	15.4% (*n*=4)	
- Calciumregulators	18.9% (*n*=7)	0% (*n*=0)	
- Cetuximab	0% (*n*=0)	3.8% (*n*=1)	
- Corticosteroïds (prednisone 5mg and budesonide 3mg)	0% (*n*=0)	7.7% (*n*=2)	
- Gonadereline-agonists	18.9% (*n*=7)	0% (*n*=0)	
- Immunetherapy	0% (*n*=0)	19.2% (*n*=5)	
- Laxatives	2.7% (*n*=1)	15.4% (*n*=4)	
- Paracetamol	5.4% (*n*=2)	7.7% (*n*=2)	
- PDE-5-inhibitor	2.7% (*n*=1)	0% (*n*=0)	
- PPI	5.4% (*n*=2)	15.4% (*n*=4)	
- Prednisone (5 mg)	8.1% (*n*=3)	0% (*n*=0)	
- Proteinkinaseinhibitor	0% (*n*=0)	3.8% (*n*=1)	
- Thyreomimetics	0% (n=0)	3.8% (*n*=1)	
Symptoms and adverse events			
• Adverse events			
- Yes	0% (*n*=0)	0% (*n*=0)	
- No	100% (*n*=9)	100% (*n*=9)	

SCLC Small Cell Lung Carcinoma, *ANC* absolute neutrophil count, *ASAT* aspartate aminotransferase, *ALAT* alanine aminotransferase, *CRP* C-reactive protein, *eGFR* glomerulair filtration rate, *PDE-5 inhibitor* phosphodiesterase type 5 inhibitor, *n* number of patients

[a] median [range]

[b]Most patients used multiple drug types, therefore total is more than amount of patients

[c]Two-tailed, unpaired student's t-test with α=0.05

Table 2 PK parameters after oral midazolam administration for prostate cancer patients and patients with other types of solid tumours

	Prostate cancer (mean ± SD)	Other types of solid tumours (mean ± SD)	P-value[a]
Midazolam			
Cl/F (L/h)	87.22 ± 22.50	75.67 ± 26.24	0.14
$AUC_{0\text{-}8h}$ (ng/mL*h)	22.05 ± 6.98	25.17 ± 5.94	0.16
$AUC_{0\text{-}inf}$ (ng/mL*h)	24.52 ± 7.38	28.74 ± 8.12	0.14
C_{max}	11.65 ± 3.10	9.28 ± 2.69	0.10
T_{max}	0.49 ± 0.009	0.73 ± 0.37	0.35
$T_{1/2}$ (hours)	1.88 ± 0.64	2.11 ± 0.71	0.39
F	45%	55%	
1'-hydroxymidazolam			
$AUC_{0\text{-}8h}$ (ng/mL*h)	5.10 ± 2.44	5.98 ± 4.25	0.07
$AUC_{0\text{-}inf}$ (ng/mL*h)	5.67 ± 2.48	6.47 ± 4.45	0.65
$T_{1/2}$ (hours)	2.14 ± 1.07	1.98 ± 0.42	0.86
Ratio 1-hydroxymidazolam/midazolam	0.24 ± 0.08	0.19 ± 0.16	0.73

Cl/F Apparent clearance, $AUC_{0\text{-}8}$ Area Under the Curve 0-8 hours, AUC_{inf} Area Under the Curve extrapolated to infinity, C_{max} maximum concentration, T_{max} time on maximum concentration, $T_{1/2}$ half-life, *F* biological availability

[a] Two-tailed, unpaired Wilcoxin test with α=0.05

Table 3 PK parameters after IV midazolam administration for prostate cancer patients and patients with other types of solid tumours

	Prostate cancer (mean ± SD)	Other types of solid tumours (mean ± SD)	P-value[a]
Midazolam			
Cl (L/h)	38.95 ± 8.89	41.79 ± 13.71	0.73
$AUC_{0\text{-}8h}$ (ng/mL*h)	24.29 ± 6.60	22.75 ± 8.03	0.34
$AUC_{0\text{-}inf}$ (ng/mL*h)	27.01 ± 6.72	26.70 ± 10.51	0.73
$T_{1/2}$ (hours)	2.60 ± 0.64	3.14 ± 0.56	0.22
1'-hydroxymidazolam			
$AUC_{0\text{-}8h}$(ng/mL*h)	3.38 ± 1.32	4.05 ± 4.05	0.05
$AUC_{0\text{-}inf}$ (ng/mL*h)	3.92 ± 1.27	4.71 ± 4.18	0.55
$T_{1/2}$ (hours)	4.15 ± 0.98	3.99 ± 1.18	0.19
Ratio 1-hydroxymidazolam/midazolam	0.12 ± 0.03	0.09 ± 0.17	0.34

Cl clearance, $AUC_{0\text{-}8}$ Area Under the Curve 0-8 hours, AUC_{inf} Area Under the Curve extrapolated to infinity, $T_{1/2}$ half-life

[a] Two-tailed, unpaired Wilcoxin test with α=0.05

Table 4 Our data in comparison with midazolam PK in healthy volunteers

Authors	Study population	Midazolam administration	Cl (reported in article)	Converted Cl (mL/min)	Conclusion
C.A. Ribbers, L.T. van der Heijden (2023)	Caucasian prostate cancer patients and patients with solid tumour types	Oral, 2 mg	Prostate: 87.22 ± 22.50 L/h[a] Solid tumour: 75.67 ± 26.24 L/h[a]	1453.67 mL/min[b] 1261.17 mL/min[b]	
		IV, 1 mg	Prostate: 38.95 ± 8.89 L/h[a] Solid tumour: 41.79 ± 13.71 L/h[a]	649.17 mL/min[b] 696.5 mL/min[b]	
Tateishi et al. (2001) [34]	Healthy European American men	Oral, 2 mg	1728 mL/min[b]	1728 mL/min[b]	Higher Cl/F than all cancer patients included in this study
Vanhove et al. (2018) [35]	Healthy male volunteers	Oral, 2 mg	762.7 ± 327.7 mL/min[a]	762.7 mL/min[b]	Lower Cl/F than all cancer patients included in this study
Kharasch et al. (1999) [36]	Healthy male volunteers	IV, 1 mg	Day 1: 6.61 ± 1.99 ml/kg/min[a]	549,7 mL/min for prostate[b] 601,5 m/L min for solid tumours[b]	Cl lower than all cancer patients included in this study
			Day 13: 7.94 ± 2.43[a]	649.5 mL/min for prostate[b] 722,5 mL/min for solid tumours[b]	Cl almost the same as all cancer patients in this study
			Day 21: 7.88 ± 2.52[a]	644.6 mL/min for prostate[b] 717.1 mL/min for solid tumours[b]	Cl almost the same as all cancer patients included in this study

[a] Resported as mean ± standard deviation

[b] Reported as mean

Figures

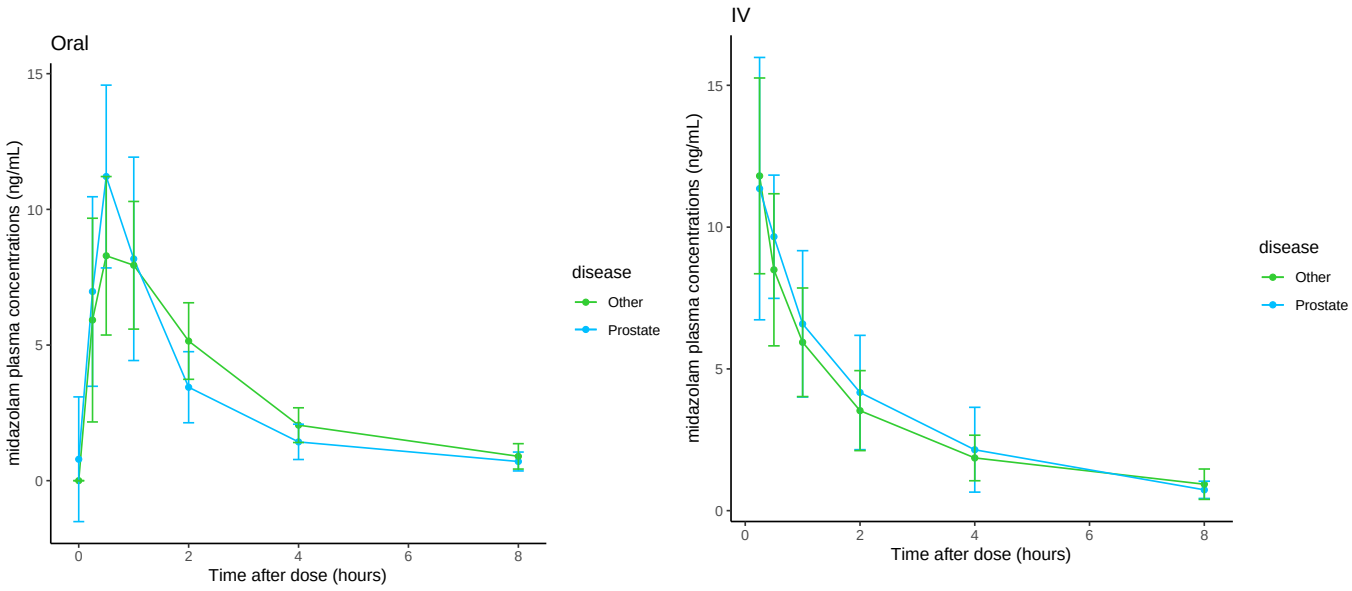


Figure 1 Plasma-over-time concentration curve for midazolam after (A) oral midazolam administration and (B) IV midazolam administration. The blue lines represent the prostate cancer patients. The green lines represent the patients with other solid tumour types.

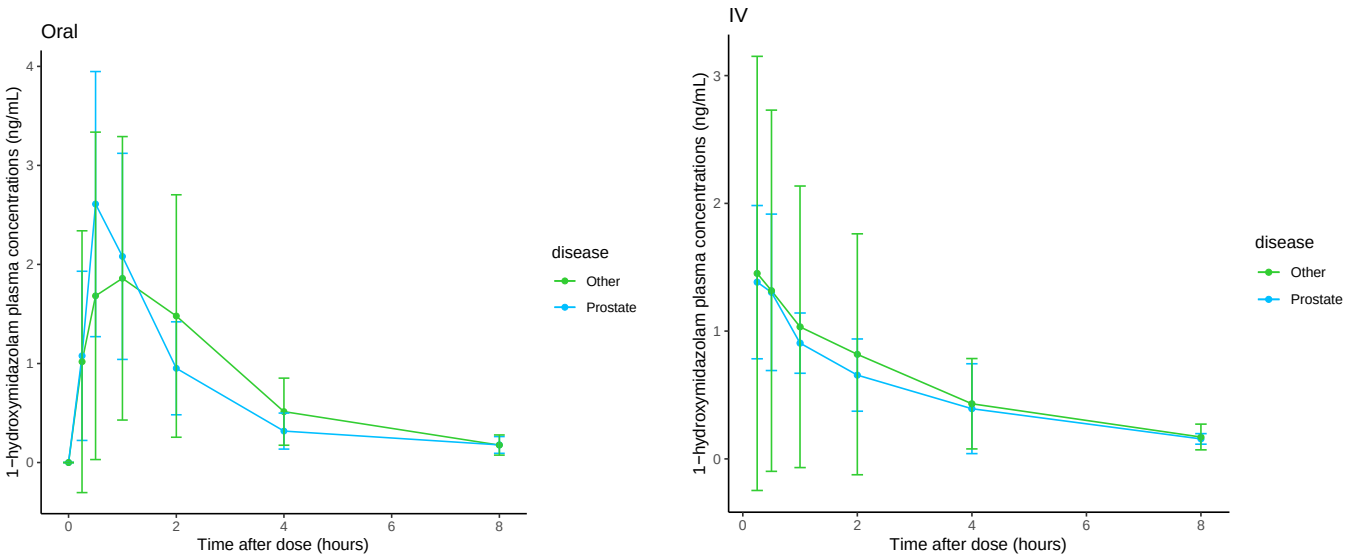


Figure 2 Plasma-over-time concentration curve for 1'hydroxymidazolam after (A) oral midazolam administration and (B) IV midazolam administration. The blue lines represent the prostate cancer patients. The green lines represent the patients with other solid tumour types.

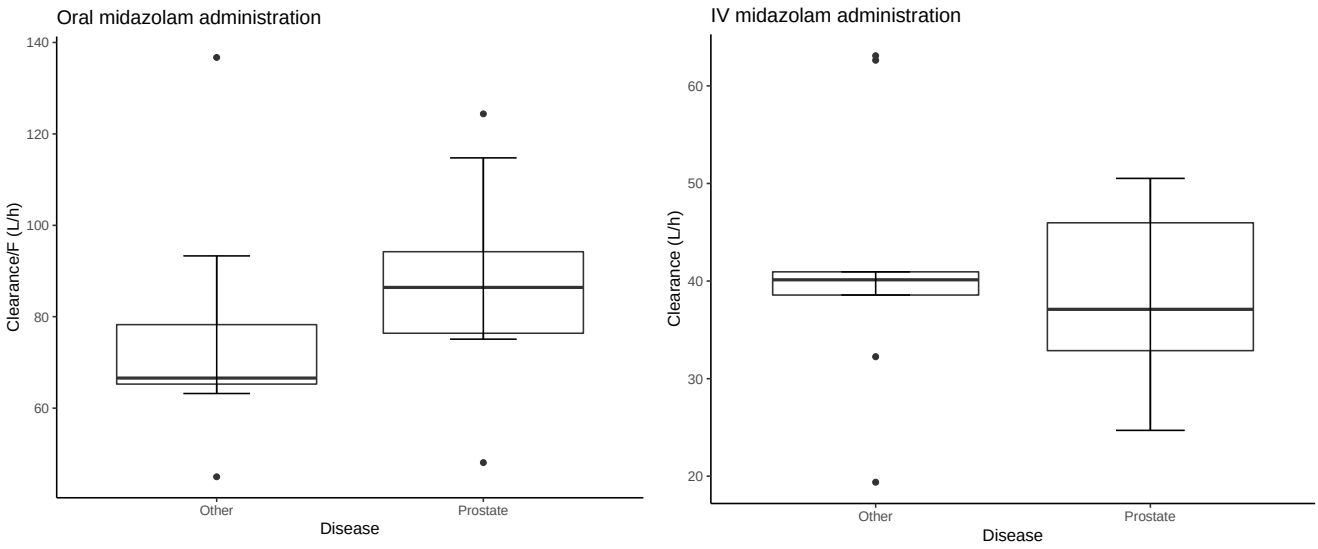


Figure 3 Clearance (L/h) in prostate cancer patients and patients with other solid tumour types (A) after oral midazolam administration and (B) after IV midazolam administration

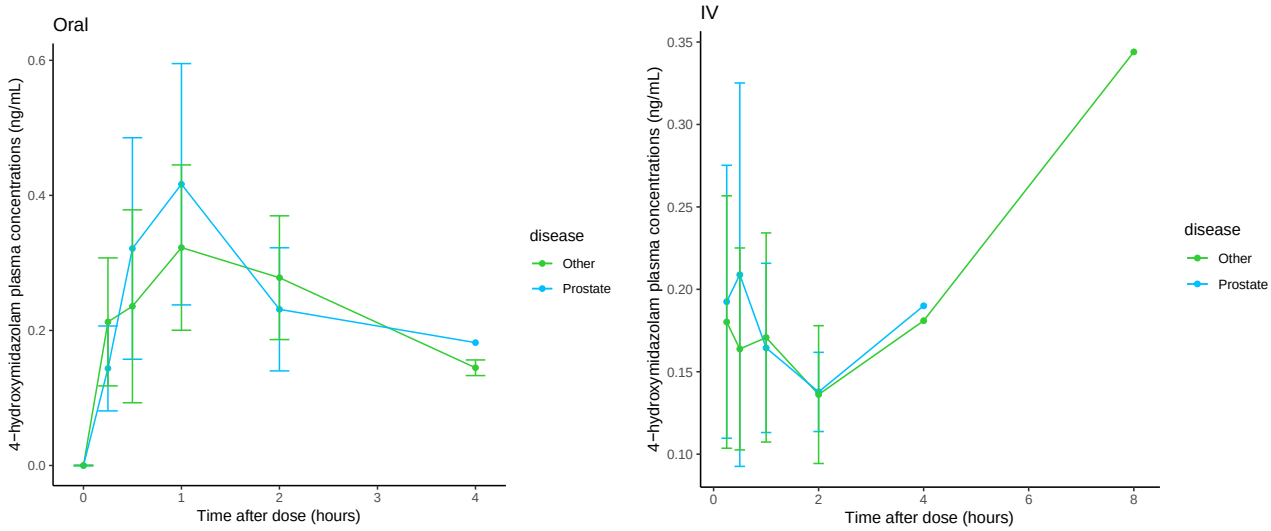


Figure 4 Plasma-over-time concentration curve for 4'-hydroxymidazolam after (A) oral midazolam administration and (B) IV midazolam administration. The blue lines represent the prostate cancer patients. The green lines represent the patients with other solid tumour types.

Supplementary figures

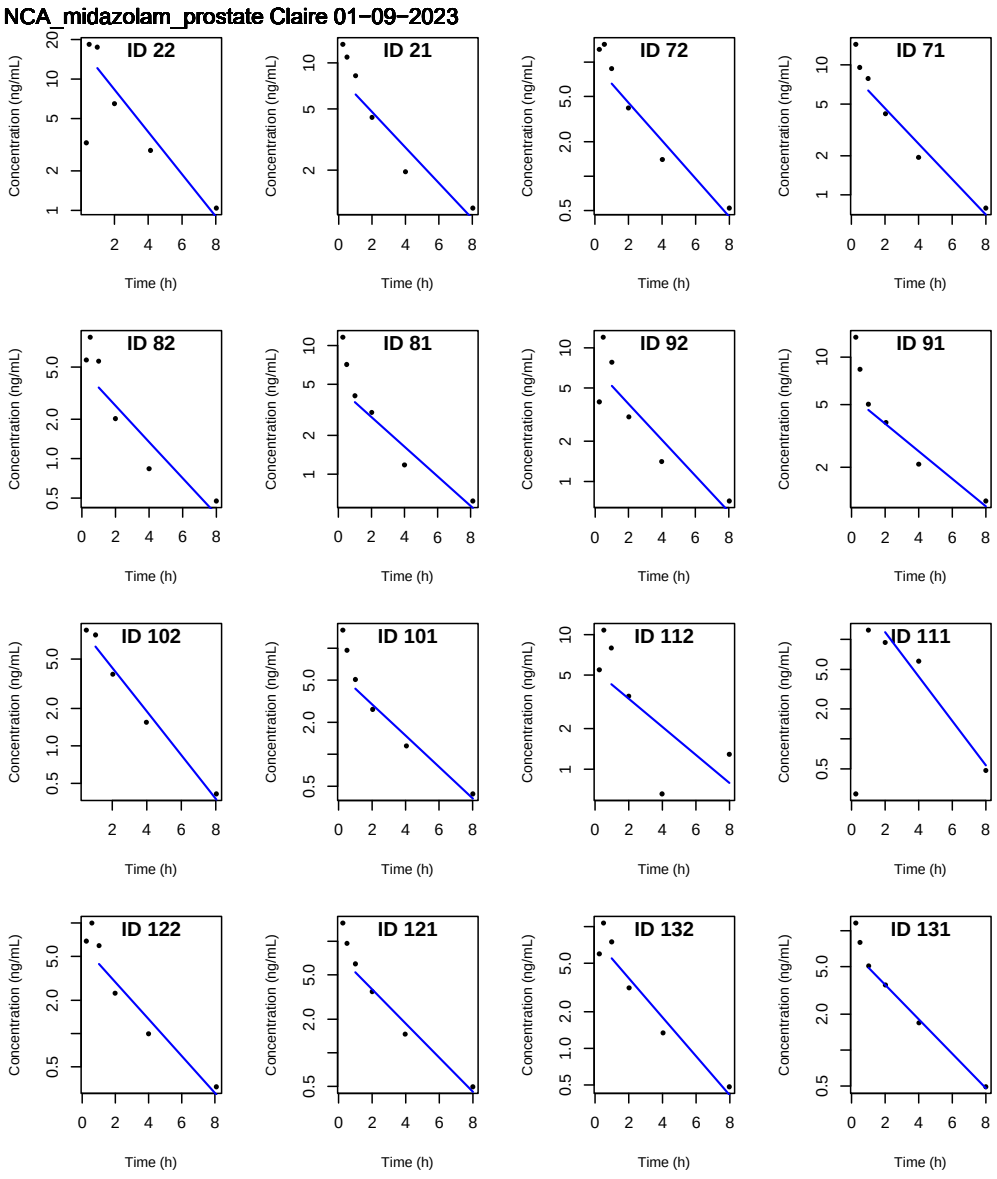
Supplementary I – Forbidden co-medication

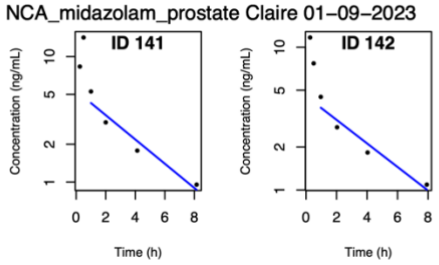
Inhibitors or inducers of CYP3A4 which might alter the pharmacokinetics of midazolam are not allowed to be used in the study, including but not limits to:

These inhibitors of CYP3A4 are not allowed to be used concomitantly in the study	**These inducers of CYP3A4 are not allowed to be used concomitantly in the study**
HIV-antivirals: indinavir, ritonavir, nelfinavir, saquinavir	Enzalutamide (should be discontinued at least 30 days before start of study
Anti-microbial agents: clarithromycin, itraconazole, ketoconazole, nefazodone, telithromycine, fluconazole, chloramphenicol, ciprofloxacin, norfloxacin, voriconazole	Bicalutamide (should be discontinued at least 2 weeks before start of the study)
Cardiac agents: verapamil, diltiazem, cimetidine, amiodarone	Dexamethasone (should be discontinued at least 2 weeks before start of study)
Other agents: fluvoxamine	St. John's Wort
Fruits: star fruit, grapefruit juice	HIV-antivirals: efavirenz, nevirapine
	Other agents: barbiturates, carbamazepine, modafinil, oxcarbazepine, phenobarbital, phenytoin, pioglitazone, rifabutin, rifampicin

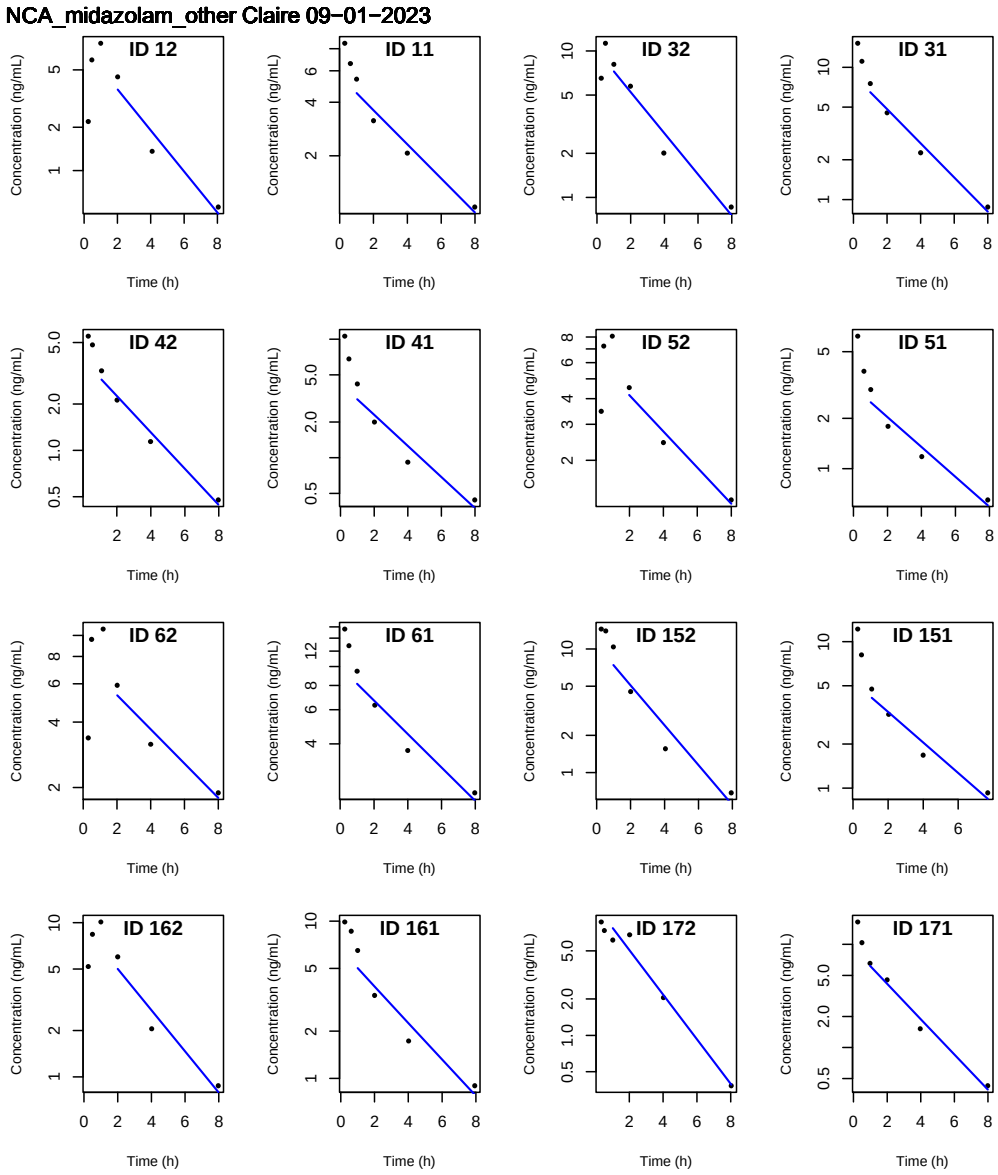
Supplementary II

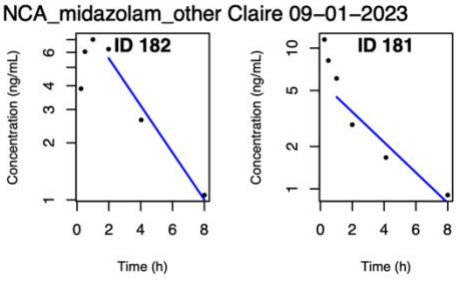
Supplementary 2.1 Midazolam plots for last 4 timepoints per individual – prostate cancer patients



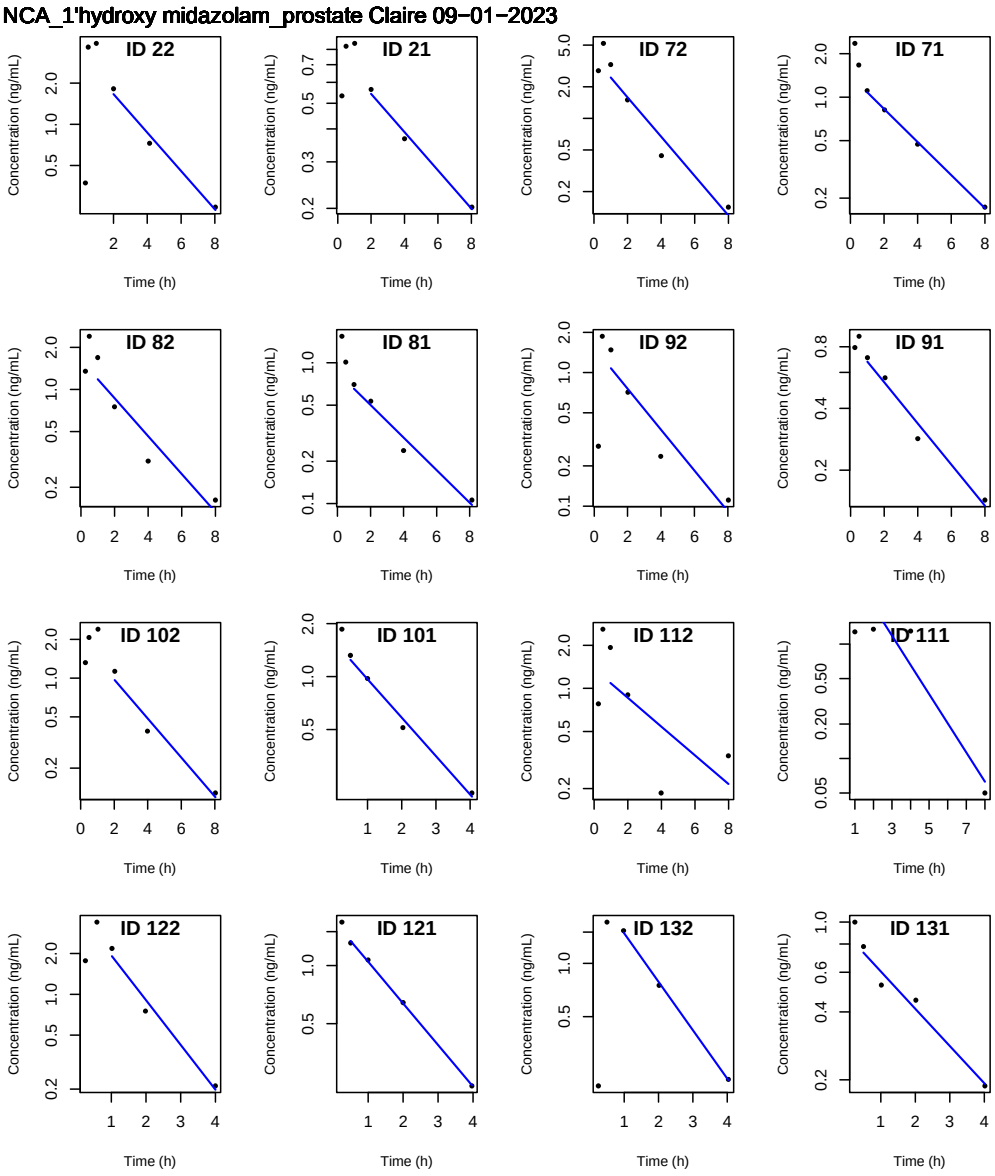


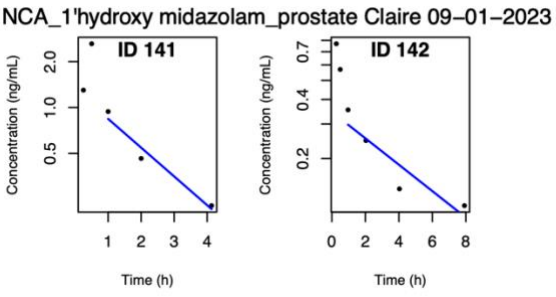
Supplementary 2.2 Midazolam plots for last 4 timepoints per individual – solid tumour patients





Supplementary 2.3 1'-hydroxy midazolam plots for last 4 timepoints per individual – prostate cancer patients





Supplementary 2.4 1'-hydroxy midazolam plots for last 4 timepoints per individual – other tumour patients