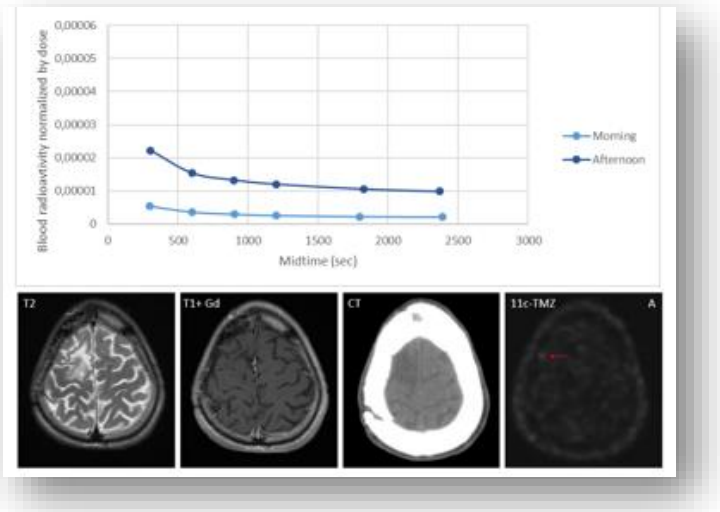
The use of PET/CT imaging data for a mechanistic PK-model of radiolabeled temozolomide

FA-MA203 ONDERZOEKSPROJECT



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ABSTRACT (Dutch)

Achtergrond

Temozolomide is een cytostaticum geïndiceerd voor de behandeling van maligne gliomen. Het wordt gedoseerd op basis van de BSA (body surface area). Ondanks de effectiviteit van het middel moeten tot op heden veel patiënten de behandeling met temozolomide vroegtijdig staken door het optreden van tumorprogressie of hematologische toxiciteit. Het is onduidelijk welke factoren bijdragen aan de effectiviteit en toxiciteit van temozolomide maar mogelijk speelt verschil in tumorpenetratie een rol. Om de behandeling te optimaliseren is het essentieel om de farmacokinetiek (PK) van temozolomide, inclusief de tumorpenetratie, in kaart te brengen. Een vorige microdose 11C-temozolomide PET(CT) studie maakte het mogelijk om tumor opname te visualiseren in twee glioblastoom patiënten. Het doel van het huidige onderzoek was om aan de hand van de beschikbare data de haalbaarheid van een pilot PET(CT)-PK studie met temozolomide te bepalen om uiteindelijk de behandeling met temozolomide te optimaliseren bij glioblastoom patiënten.

Methode

Om te beginnen is er een uitgebreide literatuurstudie uitgevoerd om te voorspellen of de PK van temozolomide lineair is in de range van microdose naar therapeutische dosering. Het PK profiel van temozolomide en de metabolieten zijn onderzocht om een semi-mechanistisch PK modellering plan te maken. Vervolgens is met de opgedane kennis gekeken naar de bruikbaarheid van de beschikbare 11C-temozolomide PET(CT) data om PET(CT) te gebruiken voor het kwantificeren van de tumorpenetratie. Tot slot is een plan opgesteld om aan voldoende data te komen voor het onderzoek van de PK van temozolomide.

Resultaten

Temozolomide is substraat voor meerdere drug transporters en ABP's (albumine binding proteins) en vertoont hierdoor waarschijnlijk geen lineair PK in de te onderzoeken dosering range. Temozolomide is een prodrug dat spontaan degradeert naar het actieve methyldiazonium-ion. De actieve stof methyleert vervolgens DNA. Het ontwikkelde model om de PK semi-mechanistisch te beschrijven bevat een perifeer, centraal en breincompartiment waarin temozolomide ongebonden, gebonden of geconverteerd naar een DNA adduct kan voorkomen.

De huidige 11C-temozolomide PET en bloedsample (verkregen uit SPE) data kon gekwantificeerd worden. Door de spontane degradatie van de stof kon er echter uit de bloedsample data, dat via standaard procedures verkregen was, niet het onderscheid gemaakt worden tussen temozolomide, metabolieten en DNA adducten. Hierdoor was de data niet geschikt om de PK van temozolomide te onderzoeken.

Het opgestelde onderzoeksplan heeft een innovatieve aanpak door een therapeutische dosering temozolomide toe te dienen voorafgaand aan de microdose 11C-temozolomide en door PET(CT) te combineren met LC-MS analyse om het onderscheid te kunnen maken tussen temozolomide, metabolieten en DNA-adducten in bloed en tumorweefsel. De bevindingen over de PK van temozolomide zullen gebruikt worden om in een grote groep patiënten individuele PK te voorspellen om vervolgens een exposure-respons relatie te beschrijven in een multicenter studie. Uiteindelijk zou dit kunnen leiden tot een geïndividualiseerde vorm van doseren met mogelijk een betere effectiviteit en lagere toxiciteit tot gevolg.

Conclusie

Deze studie laat zien dat klinische farmacologie, farmaceutisch en PET tracer kennis gecombineerd dient te worden om tracer studies met complexe moleculen te ontwikkelen. Innovatieve methoden zoals het combineren van LC-MS met PET(CT) maken het haalbaar. Het voorgestelde onderzoeksplan kan mogelijk de behandeling van glioblastoom patiënten verbeteren en nieuwe inzichten opleveren om PET(CT) studies met andere small molecules te verbeteren.

ABSTRACT (Dutch) 1
ABBREVIATIONS 3
INTRODUCTION/BACKGROUND 4
Temozolomide efficacy and toxicity 4
Properties of temozolomide 5
PET(CT)-PK 6
METHODS 7
RESULTS 8
PK of temozolomide 8
Absorption, distribution, metabolism and excretion 8
Efflux transporters 10
TMDD/protein binding 10
Lysosomal accumulation 10
PK model 13
Current data 15
Research plan to obtain the necessary data 16
PET(CT)-PK study 16
PK study 17
PK-sample analyses 18
DISCUSSION/CONCLUSION 19
REFERENCES 20
APPENDIX 24
Appendix I: Use of erlotinib, afatinib and osimertinib data in PET(CT)-PK 24
Validating the quantification method 24
PK of erlotinib, afatinib and osimertinib 26
Appendix II: Abstract for the Dutch Hospital pharmacy days 2021 33
Appendix III: Cancer Center Amsterdam fund application 34
Appendix IV: Abstract of KWF kankerbestrijding fund application 36

ABBREVIATIONS

AAG	alpha-1-acid glycoprotein
ABP's	albumin-binding proteins
AIC	4-amino-5-imidazole-carboxamide
AUC	area under the curve
BBB	blood-brain-barrier
BCRP	breast cancer resistance protein
BSA	body surface area
CCA	cancer center Amsterdam
CNS	cerebrospinal fluid
CT	computed tomography
DNA	deoxyribonucleic acid
EGFR	epidermal growth factor receptor
GI tract	gastrointestinal tract
HPLC	high pressure liquid chromatography
HSA	human serum albumin
IV	intravenous
LC-MS	liquid chromatography–mass spectrometry
MRP	multidrug resistance protein
MTIC	5-(3-methyltriazen-1-yl)imidazole-4-carboxam-ide
NONMEM	non-linear mixed effects modeling
P-gp	p-glycoprotein
PBPK model	physiological based pharmacokinetic model
PD	pharmacodynamics
PET	positron emission
PK	pharmacokinetics
PK model	pharmacokinetics model
ROI	region of interest
SPE	solid phase extraction
TKI	tyrosine kinase inhibitor
TMA	temozolomide acid
TMDD	target mediated drug disposition
UMC	university medical centers

INTRODUCTION/BACKGROUND

Temozolomide efficacy and toxicity

Temozolomide is a non-cell cycle specific chemotherapeutic drug belonging to the group of alkylating agents. It is currently dosed according to the body surface area (BSA). Temozolomide is used for the treatment of newly diagnosed glioblastoma multiforme or malignant glioma showing recurrence or progression after standard therapy.(1) The prognosis of glioblastoma patients is not good although in a recent meta-analysis the 2-year and 3-year survival has doubled to 18% and 11% in the last 10 years.(2,3) Forty-seven percent of patients fails to complete treatment due to tumor progression (43%) or severe hematological toxicity (17%).(4) Patients who complete chemoradiation have a much better prognosis (13.5 months) than patients that stop treatment for any reason (1.9 months).(3,5–7) These data suggest that temozolomide efficacy can be improved and that the occurrence of hematological toxicity hampers a sustainable tumor maintenance. The addition of temozolomide to radiation contributes to the risk of several subtypes of hematological toxicities – the relative risk on thrombocytopenia increases 29 times, leukocytopenia 18 times, neutropenia 10 times, anemia 7 time and lymphocytopenia 3 times.(6,8) During adjuvant therapy, a higher dose of temozolomide increases the risk of all severe toxicities compared to lower dose treatment schedules.(9–11) Therefore dose reduction is useful in preventing severe toxicity. However, at present we don't know how reduction in temozolomide affects treatments efficacy and consequently survival.(9,12) An additional problem is the fact that for this group of patients there are no alternative drugs which makes temozolomide their last resort.

Preliminary data from the Amsterdam UMC confirms that there is a possibility to increase temozolomide efficiency and reduce toxicity in some patients. Figure 1 shows a clear correlation between cumulative dose and decrease of thrombocytes. Figure 2 on the other hand shows patients with a high cumulative dose have a better survival. These figures support the idea that individual dosing may improve temozolomide efficacy and help prevent toxicity in glioma patients. At present however it is unclear which patients should get a low cumulative dose and which patients should get a high dose for optimal treatment. A better understanding of temozolomide pharmacokinetics (PK) and its correlation with pharmacodynamics (PD) is essential for achieving optimal individual dosing.

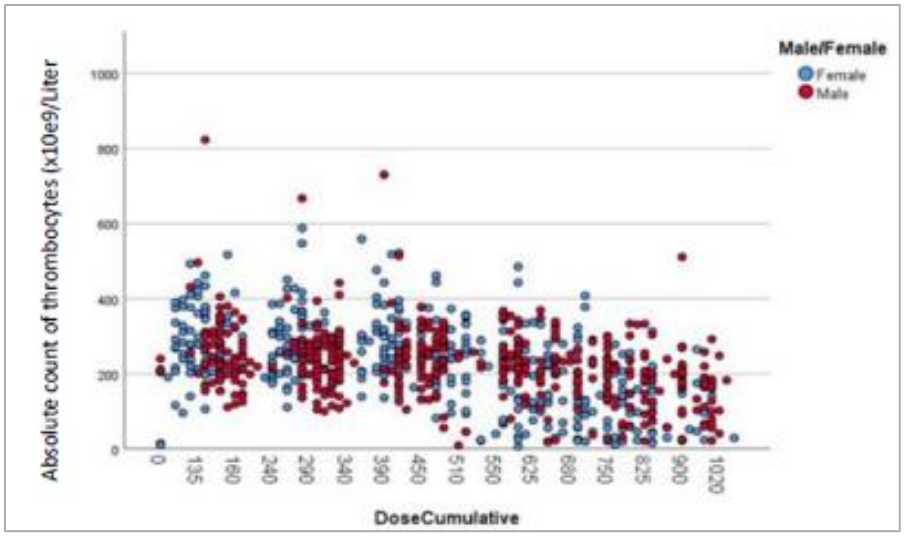

Figure 1: Absolute count of thrombocytes in relation to the cumulative dose of temozolomide during chemoradiation.

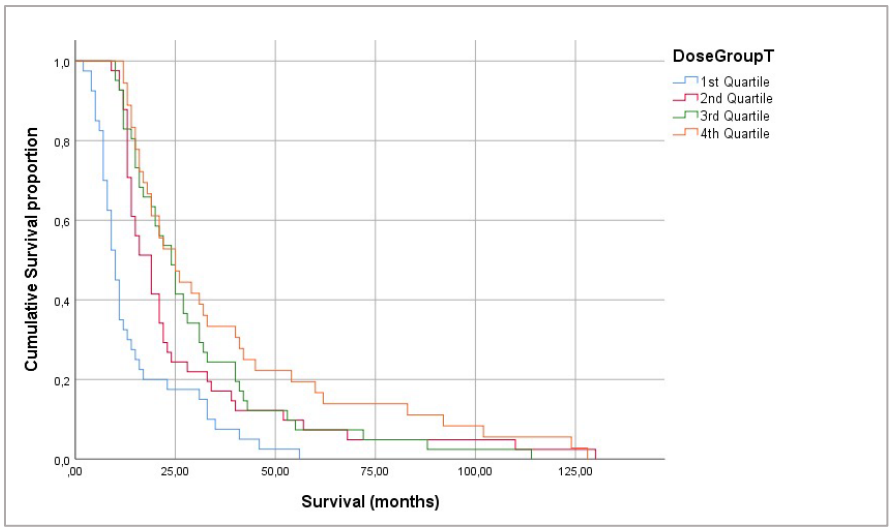

Figure 2: Survival of patients according to the administered dose. Patients in the 1st quartile received the lowest cumulative dose of temozolomide followed by the 2nd, 3rd and 4th.

There have been studies in the past which aimed to clarify the PK of temozolomide at the therapeutic dose levels to improve the dose regimen. Prior studies quantified the plasma PK and cerebrospinal fluid penetration (CNS).(9,12) The obtained population PK can aid to quantify temozolomide exposure in individual patients but are, at present, not validated. Moreover, in these studies exposure in plasma and CNS did not correlate with toxicity or efficacy. Therefore, a better way to dose temozolomide was not found and continuing dosing according to BSA was proposed.(9,12) A next step in trying to improve the dose regimen so that eventually the efficacy and safety of temozolomide improves is to visualize and predict variability in drug penetration at the tumor site in glioblastoma patients using data obtained from microdose radiolabeled 11C-temozolomide PET imaging studies and studies with therapeutic dose levels of temozolomide.

Properties of temozolomide

Temozolomide is absorbed rapidly and completely after oral administration. It's a prodrug and the cytostatic effect of the active compound is achieved due to methylation of DNA. The methylation can occur on several places but happens mainly at the N7 and O6 positions of guanine (figure 3).(13) The molecule structure of temozolomide (figure 4) is highly polar (Log P: -1.1).(1,14)

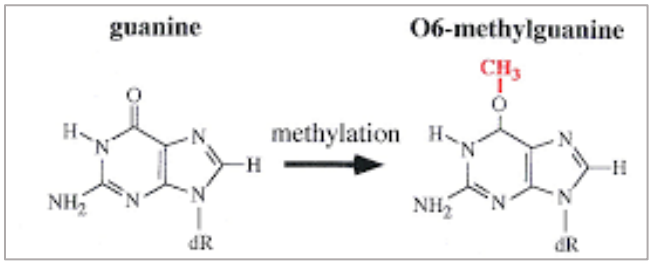

Figure 3: Formation of O6-methylguanine out of guanine after methylation.

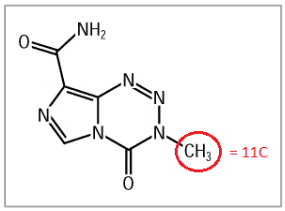

Figure 4: Structure of [3-N-11C-methyl]-temozolomide.

Amsterdam UMC previously developed an efficient - good manufacturing practice compliant - method to synthesize 11C-temozolomide for use with clinical PET. The 11C-carbon atom is located on the 3-N-methyl position which is indicated by a red circle in figure 4 and has a half-life of 20.4 min.(15)

PET(CT)-PK

When conducting PET(CT)-PK research Computed Tomography (CT) is used to image the human body (and localize the tumor) while Positron Emission (PET) can visualize a tracer or radiolabeled substance. With PET it is possible to receive information like the amount of radioactivity in a 3D region of interest (ROI), like a tumor. By quantifying the radioactivity with the use of calculations it is possible to measure drug amounts in the ROI.

Combining PET(CT) data with blood sample analysis (using techniques like solid phase extraction (SPE) and high pressure liquid chromatography-ultraviolet (HPLC-UV)) allows studying the PK of substances. SPE is suitable to separate substances based on differences in polarity by applying the sample on a non-polar column and washing the column with a polar liquid.(16) HPLC also separates substances based on polarity differences and UV is used to detect every compound immediately after leaving the HPLC column.(17) In the Amsterdam UMC the golden standard of sample analysis is to first use SPE in an attempt to separate the intact drug from metabolites/adducts. Subsequently HPLC will be used to further distinguish intact drug from other metabolites/adducts. The separation with HPLC however can't always be used since some radiolabeled substances will lose their radioactivity before a proper separation can been achieved. An ISO-qualified lab is available in the Amsterdam UMC for the sample analysis. This process is schematically shown in figure 5. An example of the use of PET(CT) to examine PK with TKI's can be found in appendix 1.

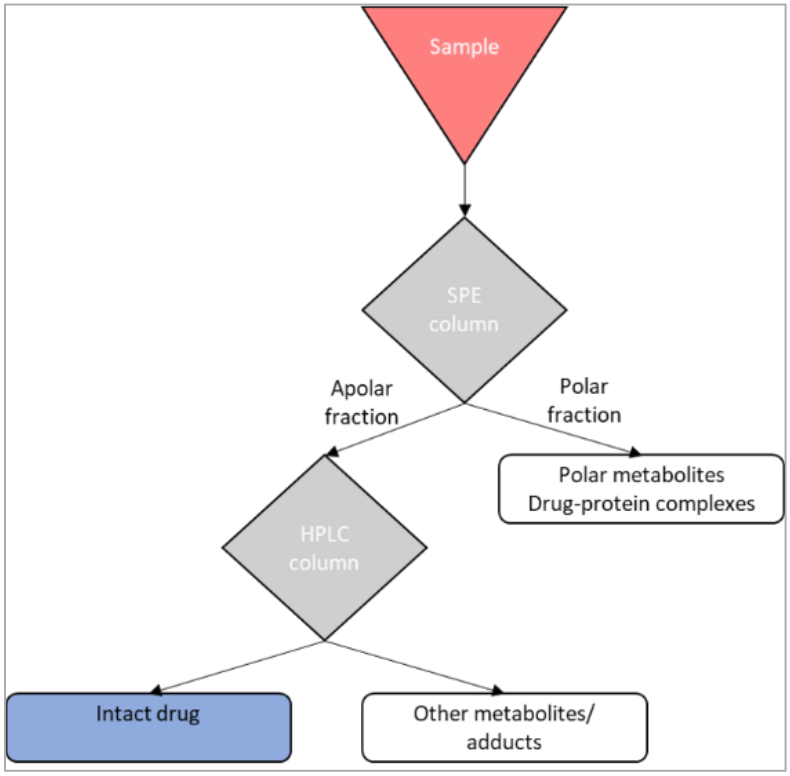

Figure 5: Schematic representation of the golden standard blood sample analysis. SPE and HPLC are used to separate the intact drug from any metabolites, complexes, adducts, etc. UV spectrometry is used to detect the intact drug after HPLC.

In PET(CT)-PK studies it's common to use a microdose of radiolabeled substance to minimize the patients exposure to radioactivity as well as to be able to trace the substance throughout the body without the substance actually having an effect on the body. Tissue-uptake of microdosed radiolabeled drug may differ from uptake at the therapeutic dose level. Enzymes involved in binding and metabolism, drug-specific transport and target-binding may become saturated at the therapeutic dose level, but not at the microdose level.(18) This possible PK-difference poses a major challenge when using PET/CT probes for prediction of drug penetration at the site of disease at the therapeutic dose level, especially for high affinity/high protein bound drugs.

In the recent past a PET(CT) temozolomide PK study was performed in Amsterdam UMC using a microdose of 11C-temozolomide in glioblastoma multiforme patients (study: the effect of dexamethasone on the biodistribution of [3-N-11C-methyl]temozolomide in glioblastoma multiforme patients; a pilot study). Due to stringent inclusion criteria the researchers were not able to include enough patients in the study. Furthermore, due to tumor excision prior to the PET(CT) study, quantification of drug penetration into the tumor was not feasible. For these reasons the study was discontinued after inclusion of only two patients.

Building on these preliminary data, we now aimed to assess whether a pilot PET(CT)-PK study combined with a larger PK study with temozolomide is feasible to examine the PK of temozolomide and quantify tumor penetration in glioblastoma patients. We aimed to combine the microdose radiolabeled 11C-temozolomide PET imaging study data and previously published studies with therapeutic dose levels of temozolomide to develop a PK strategy and clinical study protocol for evaluation of temozolomide. This PK protocol was included in two fund applications (KWF Kankerbestrijding & Cancer Center Amsterdam). In future, a better understanding of the PK-PD of temozolomide may lead to dose individualization with as a result a better outcome by promoting efficacy and preventing toxicity.

METHODS

First a literature study was conducted to fully understand the PK of temozolomide as it is known at present. Our main focus was evaluating linearity amongst microdose and therapeutic dose levels. The results were visualized by building a physiological based pharmacokinetic model (PBPK model). The findings were subsequently used to build a PK model.

The next step in examining the possibilities to conduct a temozolomide PET(CT) study was validating the quantification method. As mentioned earlier, when using PET(CT) to clarify the PK of radiolabeled substances, whether the substance is temozolomide or not, the acquired radioactivity data should to be quantified to obtain drug amounts. Currently, sample analyses in blood and in biopsies are the golden standard for drug quantification, but biopsy samples are difficult to obtain and to interpret due to spatial and temporal heterogeneity in drug uptake into the tumor.(19,20) To evaluate the quantification method the acquired knowledge from the literature search about the PK of temozolomide was used to analyze the previously obtained PET(CT) temozolomide data of two patients. The available data were analyzed blood samples (SPE derived) data and PET images. The data was examined to predict whether quantification of the data was feasible.

After analyzing this data a research plan was developed on how to approach a future study to acquire all the (quantifiable) data needed for a proper examination of the PK of temozolomide including drug penetration at the tumor site in glioblastoma patients.

For this study R and R studio (version 4.0.3) were used to process data. For visualization of data R studio and Excel were used.

RESULTS

PK of temozolomide

The focus of this literature search was understanding what kind of substance temozolomide really is and combining these findings with possible mechanisms to take account of when evaluating linearity in PK amongst microdose vs therapeutic dose levels. A summary of all the findings is visualized in the PBPK model of figure 9 and figure 10.

Absorption, distribution, metabolism and excretion

Temozolomide is absorbed fully and quickly after oral administration with a Tmax of approximately 0,5-1,5 hour.(13,21) Radiolabeled microdoses of temozolomide are however administered IV.

The distribution volume of temozolomide is low, namely 0,4 L/kg. About 10-20% of the temozolomide is bound to plasma proteins. It has been shown that temozolomide can cross the blood-brain-barrier (BBB) for about 30%.(1,13)

Temozolomide is a very interesting substance because it behaves different than most drugs. It metabolism isn't real metabolism but rather a spontaneously occurring degradation process that is mostly influenced by pH (figure 10b). When taken orally temozolomide stays stable in the acidic environment of de GI tract but starts degrading when reaching the physiological pH of blood. Practically no interference of enzymes occur in the degradation process.(1,21,22) After administration of a therapeutic dose of temozolomide about 5-10% will eventually be eliminated as intact drugs, approximately 2% will be converted to temozolomide acid (TMA) and the rest will be converted to 5-(3-methyltriazen-1-yl)imidazole-4-carboxam-ide (MTIC).(1,13,21,23) MTIC quickly breaks down to 4-amino-5-imidazole-carboxamide (AIC) and a methyl-diazonium ion. AIC is a known intermediate in purine and nucleic acid biosynthesis and has no pharmacological effect. The highly reactive methyl-diazonium ion on the other hand is the active methylating species which causes the cytostatic effect. The clearance is 5,5 L/hour/m^2. Temozolomide has a half-life of 1.8 hour, while MTIC has a half-life of only 2,5 min. The half-life of the methyl-diazonium ion is extreme short due to the highly reactive nature of the substance.(1,13,21–24)

The first mechanism to be examined that could possibly give non-linear PK was metabolism. It was hypothesized that when administrating a microdose the metabolism rate of the drugs could be much higher than when a therapeutic dose was given. As a result the clearance of the drugs should be higher and the half-life shorter after administration of a microdose. This mechanism has been described before and is allocated to the saturation of metabolism pathways.(18,25–28) Figure 6 shows schematically how saturation of a pathway can cause non-linear pharmacokinetics. Since the metabolism of temozolomide is a spontaneous nonenzymatic, chemical degradation process, there isn't something in the process which can get saturated. For this reason temozolomide metabolism can't cause non-linear PK as displayed in figure 6b.

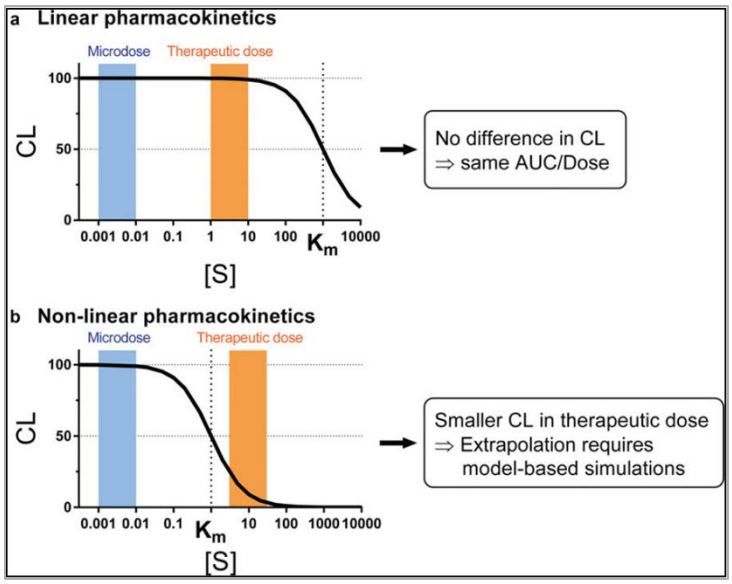

Figure 6: In 6a the metabolism pathway isn't saturated whether a microdose or a therapeutic dose is given. Therefore the PK is linear amongst these dose levels. In 6b the pathway gets saturated resulting in a slower metabolism and smaller clearance at therapeutic dose. S = substrate, CL = clearance, AUC = area under the curve, Km = Michaelis-Menten constant.(18)

The spontaneous degradation of temozolomide makes a proper sample analysis challenging and requires an adequate sample preparation. Another challenging property of temozolomide is the fact that both temozolomide and all the metabolites are polar(13,23,29,30) making separation with the method displayed in figure 5 difficult or perhaps even impossible. When using 11C-temozolomide the 11C atom will be part of MTIC and methyl-diazonium ion during the degradation steps as is displayed in figure 7.(15)

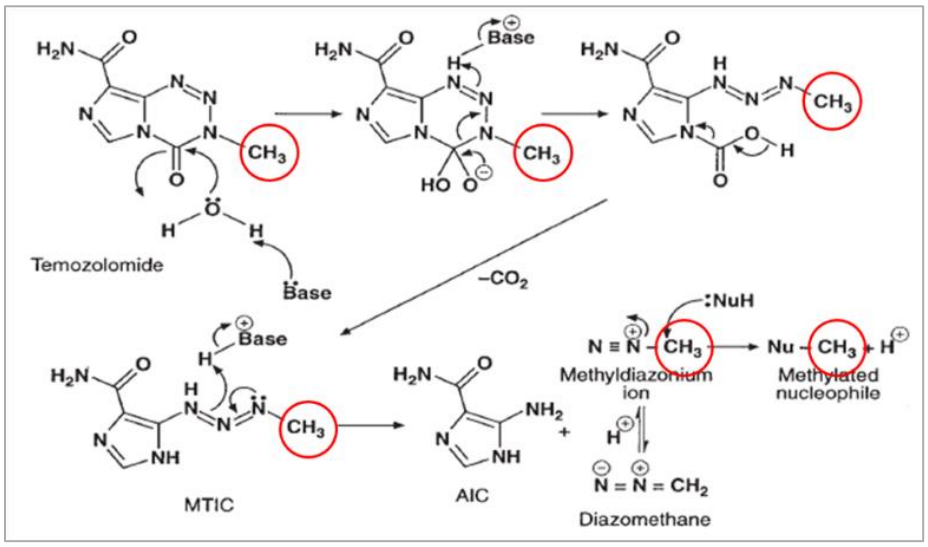

Figure 7: Formation of AIC and methyl-diazonium out of temozolomide. 11C atom is marked by the red circle.(15,31)

Excretion of temozolomide occurs mainly by renal clearance as intact drug or metabolites (figure 9). Only a very small fraction (<1%) is excreted the feces. An interesting fact is that not all of the temozolomide is recovered shortly after administration in the urine or feces. This is because of the incorporation of AIC and methyl-diazonium ion in DNA making it stay in the body for a long time.(1,13,21)

Efflux transporters

Another mechanism that can give substances a different PK after microdose administration compared to therapeutic dose is the effect of efflux transporters.(18,27,28) The most important efflux transporters are P-glycoprotein (P-gp), Breast Cancer Resistance Protein (BCRP) and Multidrug Resistance Proteins (MRP's). These transporters occur on different places throughout the body and actively pump substances out of certain tissues.(32) The transporters in the gastrointestinal (GI) tract for instance can actively counter the absorption of certain drugs after oral administration including.(28,33,34) When given a microdose the effect of these transporters can be relatively large compared to administration of a therapeutic dose due to less saturation.(28)

Temozolomide is substrate for the transport proteins P-gp and BCRP (figure 9 & 10a) which can be present at the BBB, tumor cells, GI tract and kidneys.(32,35–37) The transporters in the GI tract however are irrelevant for this examination because temozolomide is absorbed rapidly and completely.(13,21) It has been shown that P-gp and BCRP activity limit the brain uptake of temozolomide and that upregulation of these transporters can cause temozolomide resistance in mice. Additionally it was shown that higher temozolomide concentrations relatively reduced the efflux activity of the transporters, meaning some sort of saturation is possible.(36,37) This is an indication that these efflux transporters could cause non-linear PK in the microdose, therapeutic dose range. Perhaps more temozolomide is pumped out of the brain when a microdose is given instead of a therapeutic dose. (38,39) Whether and how P-gp and BCRP elsewhere in the body effect the PK of temozolomide hasn't been examined yet.

TMDD/protein binding

The phenomenon of drugs binding to plasma proteins or showing Target Mediated Drug Disposition (TMDD) by binding to high affinity, low capacity targets is another mechanism that can cause non-linear PK.(18,27) The idea is that when given a microdose a relatively large fraction of the drugs will quickly bind to the proteins or targets and therefore the clearance will be high in the first instance while giving a therapeutic dose will cause the proteins or target getting saturated quickly with a slower clearance and non-linear PK as a result. This mechanism is mostly known to occur with large molecules (biologicals) but can also affect the PK of small molecules, especially with really low dose (microdose) studies.(27,40,41)

As mentioned before temozolomide is bound to plasma proteins for approximately 10-20%.(1,13) HSA is practically the only plasma protein temozolomide is bound to under physiological conditions (figure 9). The nature of the binding is non-covalent and reversible and it protects temozolomide against degradation.(24,42) Since binding to plasma proteins only plays a minor role in temozolomide PK, it is not expected to cause a major difference in PK when a microdose is administered. There is however an interesting effect of protein binding on temozolomide reported. Albumin-binding proteins (ABP's) like SPARC and gp60 are apparently overexpressed on tumor cells and tumor vessel endothelium to increase the uptake of nutrients necessary for growth. As a result, HAS can act as a molecular carrier to transport temozolomide to the tumor tissue.(42)

It is expected that TMDD does not occur with temozolomide. TMDD is a phenomenon that occurs with substances which have a high affinity, low capacity target.(40,41) Temozolomide is a unstable molecule which for the most part degrades and the active metabolite has very much molecules or targets to react with.(21,24) For this reason TMDD is unlikely.

Lysosomal accumulation

The last mechanism that is examined is lysosomal accumulation or also known as cellular uptake or ion-trapping. Lysosomal accumulation occurs when substances have basic functional groups which

can get ionized in the low pH environment of lysosomes. The idea is that some substances occur mostly unionized in the neutral pH (7,4) of the cytosol but become ionized in the lysosome due to the lower pH (5,0). The now ionized molecules can't easily cross the lysosome membrane anymore and thus are ion-trapped.(43–45) Figure 8 displays this process schematically.

Temozolomide has no relevant basic functional groups for the occurrence of lysosomal accumulation and thus has this phenomenon no role in the PK of temozolomide.(13,29,30,43)

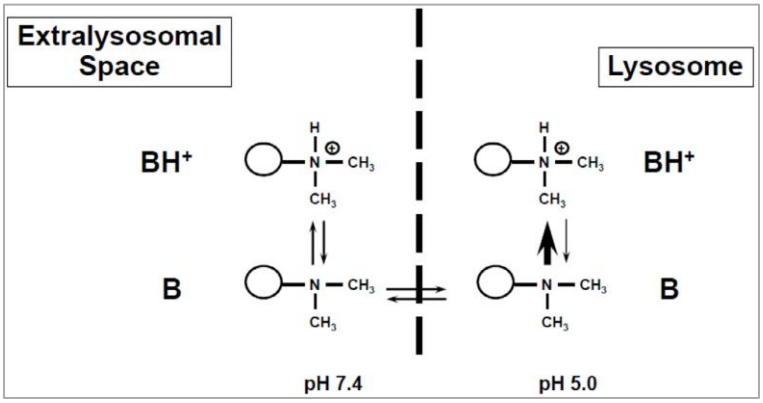

Figure 8: Schematic display of the concept of io-trapping. Basic compounds get more ionized in the low pH environment of the lysosome and therefore get trapped.(43)

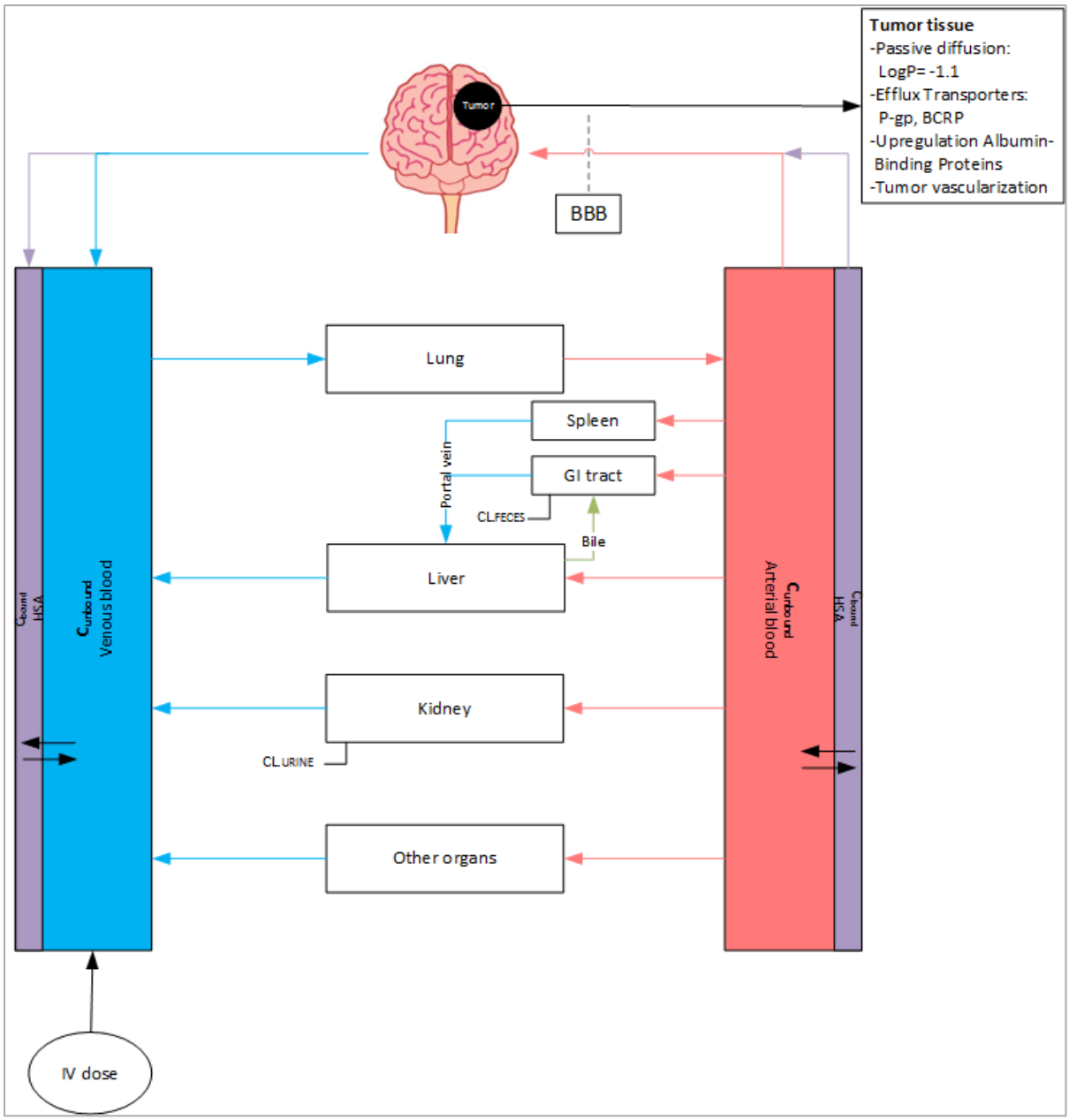

Figure 9: PBPK model of an IV microdose temozolomide administration. Temozolomide can bind reversible to HSA in blood and metabolism can occur practically everywhere under physiological pH. Excretion occurs predominantly through urine and only a little through the feces. Not relevant organs are assembled as 'other organs.' Temozolomide can penetrate the BBB and The 'tumor tissue' compartiment shows processes which might influence the behavior of temozolomide at the tumor site like passive diffusion, effect of transporters, upregulation of ABP's and tumor vascularization. Figure 10 zooms in on some processes of this PBPK model.

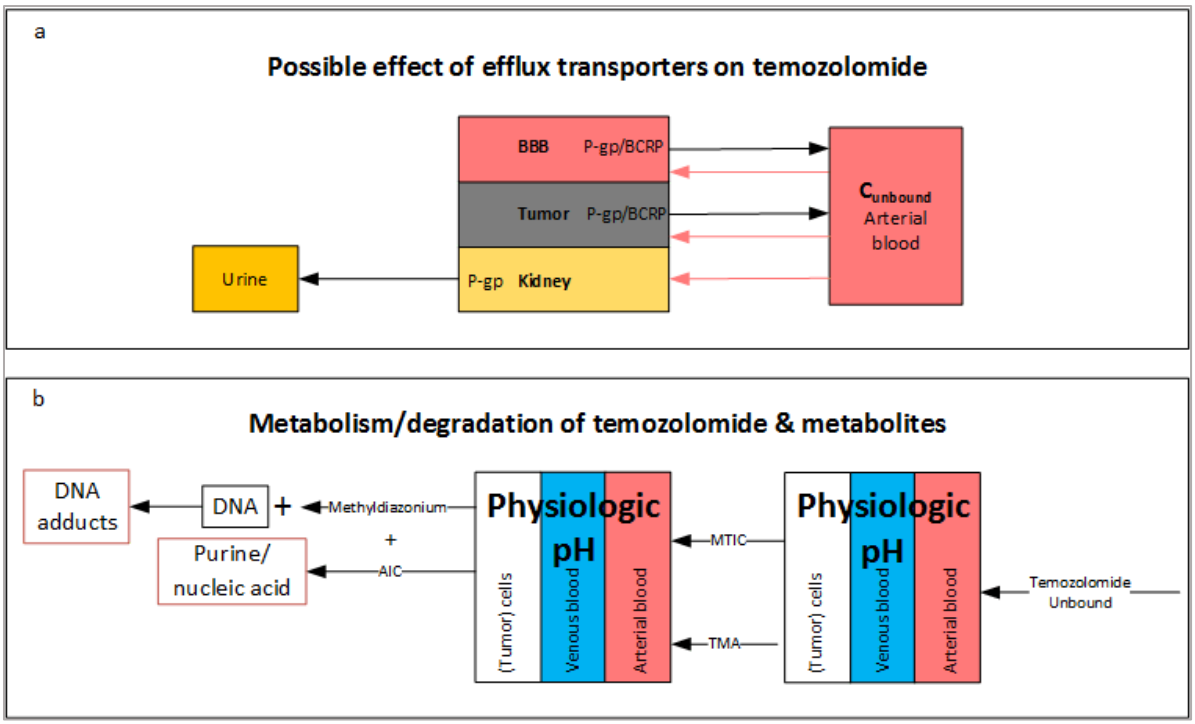

Figure 10: a) Schematic display of the possible effect of P-gp and BCRP on temozolomide in the BBB, tumor and kidney. b) Schematic display of the metabolism of temozolomide in vivo.

PK model

All the previously described findings about the PK of temozolomide was used as a basis to build a semi-mechanistic PK model that can be used for PK modelling (figure 11). The physiological compartments were exchanged for fictious compartments to obtain a model that can be used in a more data driven approach. Some processes from the PBPK model were therefore simplified. The PK model for instance has only three main compartments: peripheral, central and braintumor. ''Peripheral'' and ''central'' have quotation marks because they are fictious compartments to enable modelling, while braintumor is less fictious. The braintumor has a tumorcell compartment to indicate the growth and shrinkage of the tumor. The DNA adducts are cleared by incorporation into DNA of tumor cells or erythrocytes. In these compartments temozolomide can occur bound, unbound or become a DNA adduct. The metabolism of temozolomide as displayed in figure 10b is shortened to temozolomide becoming a DNA adduct in just one step. This has been done because MTIC and methyl-diazonium ion have a very short half-life and so data of these two compounds will not be used when modelling. Another difference between the PBPK and the PK model is the absence of efflux transporters in de latter one. This is done with the assumption that the effect of the transporters will be relatively constant between patients and to simplify this already quite complex model.

The parameters for this PK model will mostly be obtained from the literature. Missing parameters will either be determined by experiments or estimated. The found parameters are listed in table 1 and 2.

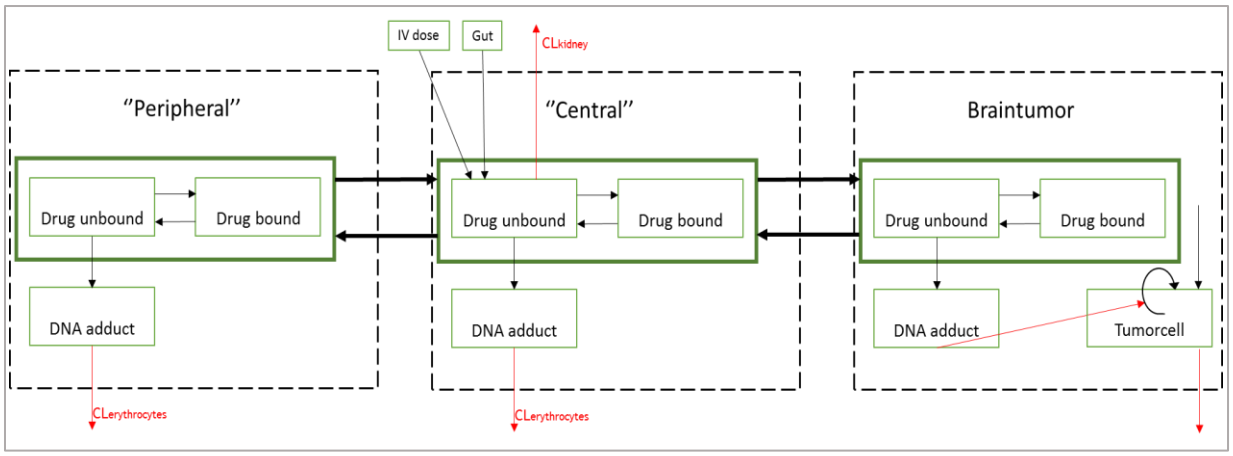

Figure 11: Semi-mechanistic PK model of temozolomide. Both oral and IV administration are taken into account. Black arrows are transfer rates, red arrows are clearance mechanisms. The circular arrow describes the tumor cell growth characteristics and it inhibition by temozolomide.

Table 2: relevant PK parameters of temozolomide and DNA adducts for the PK model. The missing parameters need to be determined or estimated.(9,12)

PK parameter	TMZ	DNA adducts
AUC (ng*hr/ml)	17	
Cmax (ng/ml)	5,2	
Ctrough		
Absorption constant (h^{-1})	3/ 2,66/ 5,8	N/A
Lag-time (h)	0,52	N/A
BBB penetration (%)	20-30%	
Transfer rate plasma to CSF ($K_{plasma \rightarrow CSF}$) ($h^{-1}$)	$7,2 \times 10^{-4}$	
Backwards rate ($K_{CSF \rightarrow plasma}$) ($h^{-1}$)	0,76	
Protein-binding HSA (%)	10-20%	N/A
Kd HSA (mM)	0,25	N/A
Volume of distribution (L/Kg)	0,4	

Table 1: Parameters regarding to the tumorcell growth inhibition (PD) in the temozolomide PK model.(73)

Parameter	Estimate
MTT	11,7
Gamma	0,146
IC50 temozolomide	

Half-life (min)	108	129,600
Clearance total ($L/h/m^2$)	5,5	N/A
CL metabolism	90-95% 4,8	N/A
CL kidney	5-10% 0,42	N/A
CL faeces	<1% -	N/A

Current data

To be able to use the PK model data is needed. First of all the previously obtained 11C-temozolomide microdose data of two patients was examined. This data was available after a prior study with 11C-temozolomide was conducted but discontinued prematurely. In the prior study the blood/plasma sample were analyzed using the gold standard method displayed in figure 5. Temozolomide however is a very complex and challenging compound to work with due to things like spontaneous degradation and the polarity of temozolomide and all its metabolites. Because of the polarity, the used standard analyzing method wasn't suitable to distinguish temozolomide from metabolites/adducts. Furthermore the samples weren't properly prepared to inhibit the spontaneous degradation. For these reasons the available data could not be used to examine the PK of temozolomide.

The data however did give some useful information. By measuring the total radioactivity in the samples it showed that 11C did give a signal (figure 12 graphs). This demonstrates that administration of a microdose of 11C-temozolimde and subsequently taking blood samples can give measurable PK information. Furthermore it is seen that 11C-temozolomide is visible in the brain scan images (figure 12 brain scans). These findings confirm that 11C-temozolomide can be used as a tracer in PET(CT)-PK studies.

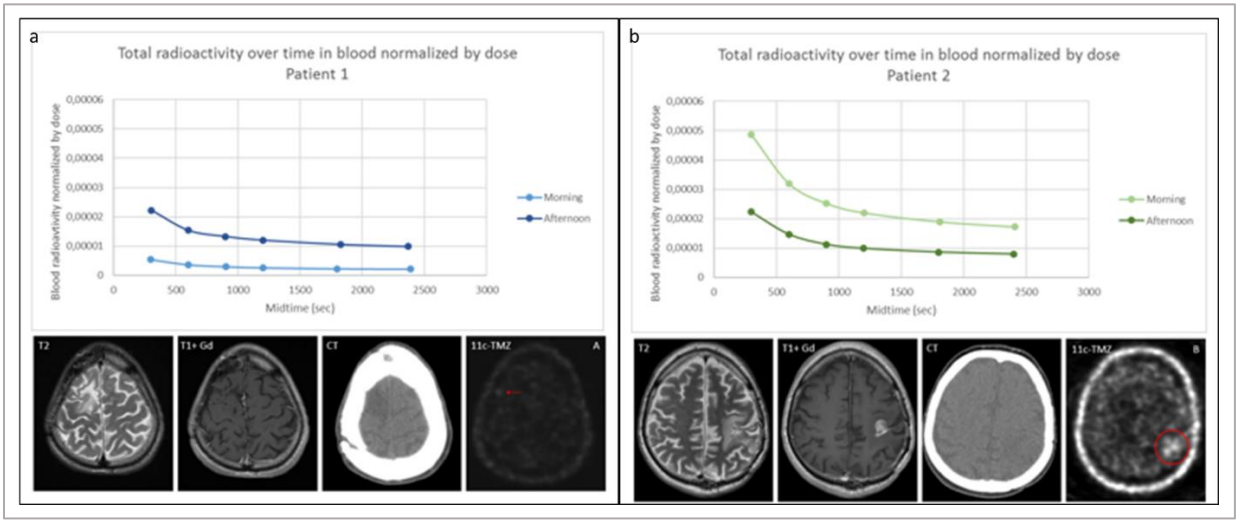

Figure 12: Graphs for two patients (a and b) with total radioactivity measured in blood samples and normalized by administered dose of 11C-temozolomide over time. Both patients got screened in the morning and the afternoon of the same day. Several brain scan images are added for each patient with the 11C-temoolomide marked by red dash.

Research plan to obtain the necessary data

To actually obtain all the data necessary to study the PK and PD of temozolomide a research plan was developed. The plan consist of substudies which will be discussed next.

PET(CT)-PK study

First of all a PET(CT)-PK study (n=10) will be discussed. In this study blood samples of patients will be examined and PET scans will be taken. The relatively small amount of patients is due the difficulty of including patients. The incidence of gliomas isn't very high but more importantly these patients are very sick and usually don't have much time to live so patients tend to say no when asked to be screened a whole day for a study that doesn't gain them anything. Therefore this study will be a feasibility study with a small amount of patients.

Since it is a PET(CT) study patients will receive a microdose 11C-temozolomide IV. A major difference with the previous 11C-temozolomide is the method displayed in figure 5 will not be used for the sample analysis. Rather first the total radioactivity of the samples will be measured followed by the use of liquid chromatography–mass spectrometry (LC-MS). LC-MS should be able to identify the substances in the samples and therefore be able to distinguish temozolomide from metabolites and DNA-adducts.(46) The PET(CT) screening will be performed as usual. The blood samples will be taken during the PET(CT) scan on 5, 10, 15, 20, 30, 40 and 60 min.

To make LC-MS possible and reliable it is important to have enough substance to measure. Therefore instead of just using a microdose 11C-temozolomide, patients will receive an additional 'cold' therapeutic dose (150 mg/m^2) orally. This additional 'cold' dose should also prevent the effect of efflux transporters being more than expected with less brain penetration as a result.

Another main difference is to use more adequate sample storage in this study to prevent spontaneous degradation of temozolomide in this study. Storing the samples at a low pH (pH 4) and a low temperature (4 °C) should keep the substances stable. The decrease in pH will be achieved by the use of phosphoric acid.(47)

To achieve additional information about tumor penetration of temozolomide this study might be expanded with taking biopsies of more than 10 patient. In this way the quantification of the PET(CT) data will me more reliable because this data can be compared to the biopsy data. Furthermore the variability can be examined better if data of more than 10 patients is included.

To quantify the data of this study the quantification formulas displayed in figure 13 will be used. The LC-MS data will be used to examine the fraction parent compound, metabolites and DNA-adducts.

Eq. 1: $$dose\ (\mu g) = \left(\frac{dose\ injected\ \ (MBq)}{specific\ \ activity\ \left(\frac{GBq}{\mu mol}\right) * 1000}\right) * Mw$$

Eq. 2: $$PET\text{-}Imaging\ deived\ Conc.\left(\frac{\mu g}{L}\right) = \left(\frac{mean\ \ acitivity\ \left(\frac{Bq}{ml}\right)}{specific\ \ activity\ \left(\frac{GBq}{\mu mol}\right) * 10^6}\right) * Mw * \left(\frac{cold\ \ dose\ (\mu g)}{tracer\ \ dose\ (\mu g)}\right)$$

Eq. 3: $$Blood\text{-}Conc.\left(\frac{\mu g}{L}\right) = \left(\frac{plasma\ \ concent\ ration\ \left(\frac{MBq}{g}\right) * \rho}{specific\ \ activity\ \left(\frac{GBq}{\mu mol}\right)}\right) * Mw * \left(\frac{cold\ \ dose\ (\mu g)}{tracer\ \ dose\ (\mu g)}\right)$$

Figure 13: Formulas to quantify PET(CT) radioactivity data in the temozolomide study. Equation 1 can be used to calculate the adminestered dose, equation 2 to calculate the PET deriverd concentration and equation 3 to calculate the blood concentration.

PK study

The next substudy (n=80) is quite similar to the previous one but is mainly focused on gathering additional PK information without studying tumor penetration. Since PET won't be used, the use of 11C-temozolomide is not necessary. Instead patients will only receive the oral 'cold' therapeutic dose (150 mg/m^2). Once again LC-MS is the technique used to analyze the plasma samples.(46) The samples time were determined based on the PK profile of temozolomide (figure 14) and will be taken at the following fixed time points: predose (if applicable); 0,5; 1,5; 3; 4 and 6 hours after administration. A limited sampling method is used in which 3-6 samples are taken of each patients at the previous described fixed time points. Furthermore at the first and last sample point of each patient an additional whole blood/PBMC sample will be taken. The sample storage will be the same as described in the previous substudy.(47)

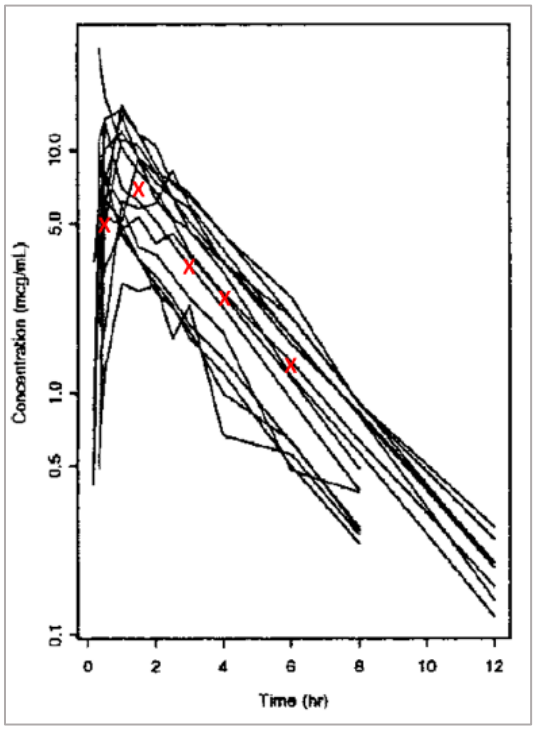

Figure 14: Concentration time curve of temozolomide with the fixed sampling points displayed by red crosses.(9)

To achieve dose optimalization of temozolomide the findings of the previous two substudies will be used in a large PK-PD study (n=1750). In this study general data like demographics and temozolomide dosing of every patients will be collected. Subsequently the validated population PK-model obtained in the previous two substudies will be used to predict individual PK. By doing this the exposure-response relation of temozolomide will be examined to achieve dose individualization for promoting efficacy and prevent toxicity.

Figure 15 schematically displays the structure of the three studies.

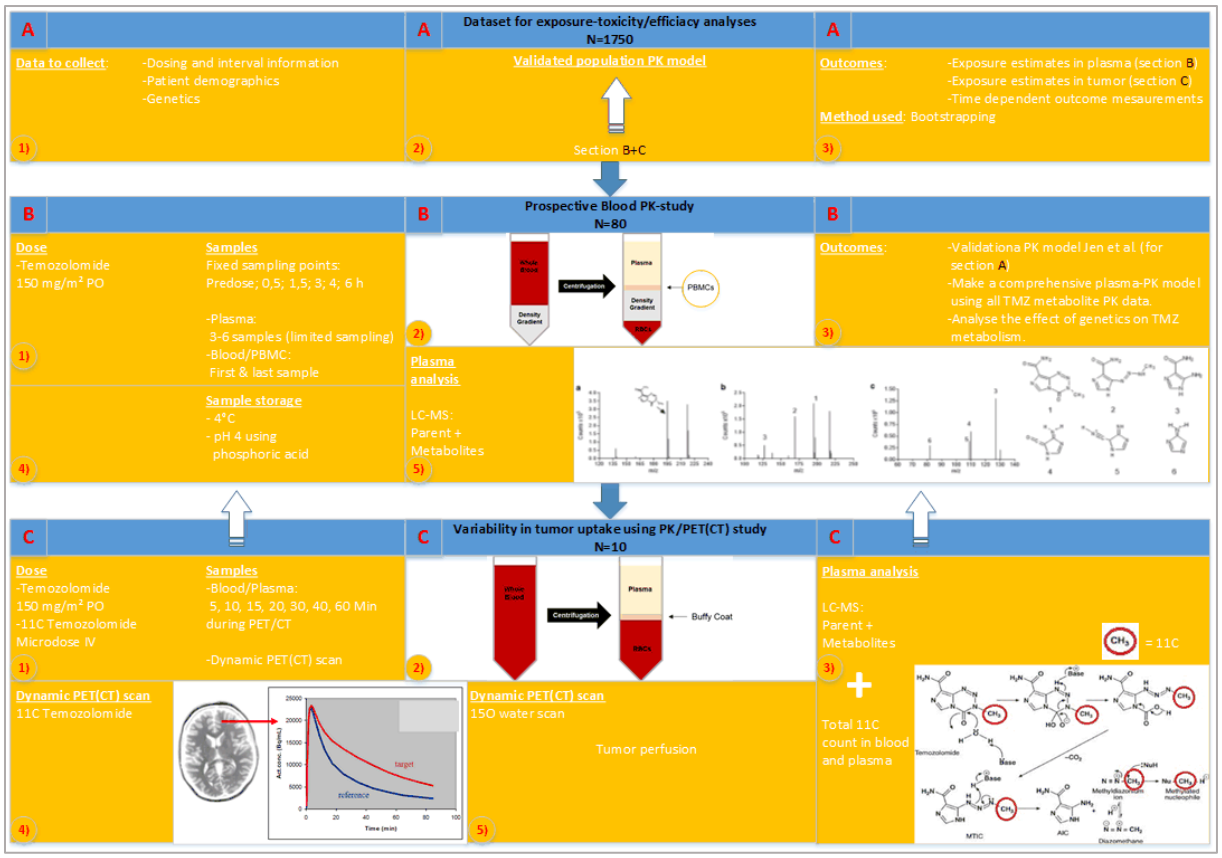

Figure 15: Overview of the PK-PD study (section A), PK study (section B) and PET(CT)-PK study (section C).

PK-sample analyses

Based on the PK characteristics and metabolic profile (figure 10b), we suggest to use LC-MS to determine the PK of temozolomide in plasma, whole blood and the DNA adducts in PBMC. As mentioned before data of the metabolites MTIC and methyl-diazonium will not be gathered and the PK will not be examined. The reason is the very short half-life of these metabolites making determination of the PK nearly impossible. The metabolites TMA and AIC will also not be analyzed because they give little information about the efficiency of temozolomide. So LC-MS will be used to distinguish temozolomide from it metabolites but not for determination of the metabolites PK. DNA adducts PK on the other hand will be determined since it gives essential information about the pharmacology of temozolomide and the half-life of the DNA adducts is longer.

We suggest to perform the following steps for sample preparation before analyses. The first step is stopping the temozolomide degradation by storing the samples in a low temperature, low pH environment as previously described. In case of 11C-temozolomide use the total radioactivity has to be measured and then waited until there is no radioactivity left. Than the samples have to be centrifuged to obtain the right sample fractions (figure 15b2 and 15c2).

DISCUSSION/CONCLUSION

Although the available temozolomide data gave some useful information as regards to the use of 11C-temozolomide as a tracer, the data primarily confirmed temozolomide being a complex and challenging compound to work with and quantification of the data was not feasible. For this reason the research plan that was developed had to be all new and innovative. The resulting plan shows a different approach for the blood sample analysis with combining the microdose 11C-temozolomide with a 'cold' therapeutic dose and using LC-MS for detecting the different compounds. Because of the innovative nature this project can be classified as 'high risk'. The developed method first needs validation. Nevertheless we think that with this method a pilot PET(CT)-PK study with temozolomide is feasible and can be a huge step forward in a better understanding of the PK-PD of temozolomide and ultimately can lead to dose individualization. For this reason we applied for funding at KWF Kankerbestrijding and CCA. The (abstract of the) applications can be found in appendix 3 and 4. If eventually the project succeeds the obtained knowledge about combining a 'hot' microdose with a 'cold' therapeutic dose and the use of LC-MS can be used in PET(CT)-PK studies with other compounds.

The next steps are (if funded) validating the analysis method, gathering data, validating the PK model including defining the parameters of table 1 and eventually examine the PK-PD of temozolomide. Furthermore, this work with temozolomide can be extended to other small molecule tumor uptake studies. We provide an extension of the PK model for TKI's in appendix 1.

Temozolomide is the most potent chemotherapy for glioblastoma patients, but many patients stop prematurely due to severe toxicity. When inter-patient variability and exposure-response relationships are quantified, the predictive model can be used to individualize the temozolomide dosing scheme leading to optimal exposure to promote efficacy and prevent hematological toxicity. This proposal is innovative in combining PET imaging and LCMS during clinical treatment to quantify this unstable prodrug to gain knowledge that can be used in future PET imaging studies. Describing the pharmacokinetics and pharmacodynamics of temozolomide and DNA adducts may give us insight on how drug and on-target exposure can influence the efficacy and toxicity of (oncolytic) drugs.

REFERENCES

1. The Royal Dutch Pharmacists Association (KNMP) kennisbank. Informatorium Medicamentorum, Temozolomide. 2021.

2. Poon MTC, Sudlow CLM, Figueroa JD, Brennan PM. Longer-term (≥ 2 years) survival in patients with glioblastoma in population-based studies pre- and post-2005: a systematic review and meta-analysis. Scientific Reports. 2020 Dec 1;10(1).

3. Zreik J, Moinuddin FM, Yolcu YU, Alvi MA, Chaichana KL, Quinones-Hinojosa A, et al. Improved 3-year survival rates for glioblastoma multiforme are associated with trends in treatment: analysis of the national cancer database from 2004 to 2013. Journal of Neuro-Oncology. 2020 May 1;148(1):69–79.

4. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJB, et al. Radiotherapy plus Concomitant and Adjuvant Temozolomide for Glioblastoma. 2005; Available from: www.nejm.org

5. Burton E, Yusuf M, Gilbert MR, Gaskins J, Woo S. Failure to complete standard radiation therapy in glioblastoma patients: Patterns from a national database with implications for survival and therapeutic decision making in older glioblastoma patients. Journal of Geriatric Oncology. 2020 May 1;11(4):680–7.

6. Hart MG, Garside R, Rogers G, Stein K, Grant R. Temozolomide for high grade glioma. Vol. 2013, Cochrane Database of Systematic Reviews. John Wiley and Sons Ltd; 2013.

7. Wang Y, Zhao W, Xiao Z, Guan G, Liu X, Zhuang M. A risk signature with four autophagy-related genes for predicting survival of glioblastoma multiforme. Journal of Cellular and Molecular Medicine. 2020 Apr 1;24(7):3807–21.

8. Hanna C, Lawrie TA, Rogozińska E, Kernohan A, Jefferies S, Bulbeck H, et al. Treatment of newly diagnosed glioblastoma in the elderly: a network meta-analysis. Vol. 2020, Cochrane Database of Systematic Reviews. John Wiley and Sons Ltd; 2020.

9. Jen JF, Cutler DL, Pai SM, Batra VK, Affrime MB, Zambas DN, et al. Population Pharmacokinetics of Temozolomide in Cancer Patients. 2000.

10. Riccardi A, Mazzarella G, Cefalo G, Garrè ML, Massimino M, Barone C, et al. Pharmacokinetics of temozolomide given three times a day in pediatric and adult patients. Cancer Chemotherapy and Pharmacology. 2003 Dec;52(6):459–64.

11. Rudek MA, Donehower RC, Statkevich P, Batra VK, Cutler DL, Baker SD. Temozolomide in Patients with Advanced Cancer: Phase I and Pharmacokinetic Study. Pharmacotherapy. 2004 Jan;24(1):16–25.

12. Ostermann S, Csajka C, Buclin T, Leyvraz S, Lejeune F, Decosterd LA, et al. Plasma and Cerebrospinal Fluid Population Pharmacokinetics of Temozolomide in Malignant Glioma Patients. 2004.

13. EMA. Temomedac EPAR public assessment report [Internet]. 2010. Available from: http://www.ema.europa.eu

14. National Center for Biotechnology Information. PubChem Compound Summary for CID 5394, Temozolomide. PubChem . 2021.

15. Eriksson J, van Kooij R, Schuit RC, Froklage FE, Reijneveld JC, Hendrikse NH, et al. Synthesis of [3-N-11C-methyl]temozolomide via in situ activation of 3-N-hydroxymethyl temozolomide and alkylation with [11C]methyl iodide. Journal of Labelled Compounds and Radiopharmaceuticals. 2015;58(3):122–6.

16. Ötles S, Kartal C. Solid-Phase Extraction (SPE): Principles and Applications in Food Samples. Acta Scientiarum Polonorum Technologia Alimentaria. 2016 Mar 30;15(1).

17. Blum F. High performance liquid chromatography. British Journal of Hospital Medicine. 2014 Feb;75(Sup2).

18. Burt T, Yoshida K, Lappin G, Vuong L, John C, de Wildt S, et al. Microdosing and Other Phase 0 Clinical Trials: Facilitating Translation in Drug Development. Vol. 9, Clinical and Translational Science. Blackwell Publishing Ltd; 2016. p. 74–88.

19. Achour B, Al-Majdoub ZM, Grybos-Gajniak A, Lea K, Kilford P, Zhang M, et al. Liquid Biopsy Enables Quantification of the Abundance and Interindividual Variability of Hepatic Enzymes and Transporters. Clinical Pharmacology and Therapeutics. 2021 Jan 1;109(1):222–32.

20. Labots M, Pham T v., Honeywell RJ, Knol JC, Beekhof R, de Goeij-De Haas R, et al. Kinase inhibitor treatment of patients with advanced cancer results in high tumor drug concentrations and in specific alterations of the tumor phosphoproteome. Cancers. 2020 Feb 1;12(2).

21. Baker SD, Wirth M, Statkevich P, Reidenberg P, Alton K, Sartorius SE, et al. Absorption, Metabolism, and Excretion of 14 C-Temozolomide following Oral Administration to Patients with Advanced Cancer 1. 1999.

22. EMA. Temozolomide teva EPAR product information NL. 2010.

23. Reid JM, Stevens DC, Rubin J, Ames MM. Pharmacokinetics of 3-Methyl-(triazen-1-yl)imidazole-4-carboximide following Administration of Temozolomide to Patients with Advanced Cancer'. Vol. 3. 1997.

24. Friedman HS, Kerby T, Calvert H. Temozolomide and Treatment of Malignant Glioma 1. 2000.

25. Zhou XJ, Garner RC, Nicholson S, Kissling CJ, Mayers D. Microdose pharmacokinetics of IDX899 and IDX989, candidate HIV-1 non-nucleoside reverse transcriptase inhibitors, following oral and intravenous administration in healthy male subjects. Journal of Clinical Pharmacology. 2009 Dec;49(12):1408–16.

26. Ordonez AA, Wang H, Magombedze G, Ruiz-Bedoya CA, Srivastava S, Chen A, et al. Dynamic imaging in patients with tuberculosis reveals heterogeneous drug exposures in pulmonary lesions. Nature Medicine. 2020 Apr 1;26(4):529–34.

27. Lappin G, Kuhnz W, Jochemsen R, Kneer J, Chaudhary A, Oosterhuis B, et al. Use of microdosing to predict pharmacokinetics at the therapeutic dose: Experience with 5 drugs. Clinical Pharmacology and Therapeutics. 2006 Sep;80(3):203–15.

28. Bosgra S, Vlaming MLH, Vaes WHJ. To Apply Microdosing or Not? Recommendations to Single Out Compounds with Non-Linear Pharmacokinetics. Clinical Pharmacokinetics. 2016 Jan 1;55(1):1–15.

29. Drugbank. Temozolomide. 2021.

30. Drugbank. Metabolite 5-(3-methyltriazen-1-yl) imidazole-4-carboxamide (MTIC). 2021.

31. Koukourakis G v., Kouloulias V, Zacharias G, Papadimitriou C, Pantelakos P, Maravelis G, et al. Temozolomide with radiation therapy in high grade brain gliomas: Pharmaceuticals considerations and efficacy; a review article. Vol. 14, Molecules. 2009. p. 1561–77.

32. Schinkel AH, Jonker JW. M ammalian drug efflux transporters of the ATP binding cassette (ABC) family: an overview [Internet]. Vol. 55, Advanced Drug Delivery Reviews. 2003. Available from: www.elsevier.com/locate/drugdeliv

33. Rudin CM, Liu W, Desai A, Karrison T, Jiang X, Janisch L, et al. Pharmacogenomic and pharmacokinetic determinants of erlotinib toxicity. Journal of Clinical Oncology. 2008 Mar 1;26(7):1119–27.

34. Marchetti S, de Vries NA, Buckle T, Bolijn MJ, van Eijndhoven MAJ, Beijnen JH, et al. Effect of the ATP-binding cassette drug transporters ABCB1, ABCG2, and ABCC2 on erlotinib hydrochloride (Tarceva) disposition in in vitro and in vivo pharmacokinetic studies employing Bcrp1-/-/Mdr1a/1b-/- (triple-knockout) and wild-type mice. Molecular Cancer Therapeutics. 2008;7(8):2280–7.

35. Lin JH, Yamazaki M. Role of P-Glycoprotein in Pharmacokinetics Clinical Implications.

36. Munoz JL, Walker ND, Scotto KW, Rameshwar P. Temozolomide competes for P-glycoprotein and contributes to chemoresistance in glioblastoma cells. Cancer Letters. 2015 Oct 10;367(1):69–75.

37. de Gooijer MC, de Vries NA, Buckle T, Buil LCM, Beijnen JH, Boogerd W, et al. Improved Brain Penetration and Antitumor Efficacy of Temozolomide by Inhibition of ABCB1 and ABCG2. Neoplasia (United States). 2018 Jul 1;20(7):710–20.

38. Burt T, Yoshida K, Lappin G, Vuong L, John C, de Wildt S, et al. Microdosing and Other Phase 0 Clinical Trials: Facilitating Translation in Drug Development. Vol. 9, Clinical and Translational Science. Blackwell Publishing Ltd; 2016. p. 74–88.

39. Bosgra S, Vlaming MLH, Vaes WHJ. To Apply Microdosing or Not? Recommendations to Single Out Compounds with Non-Linear Pharmacokinetics. Clinical Pharmacokinetics. 2016 Jan 1;55(1):1–15.

40. An G. Concept of Pharmacologic Target-Mediated Drug Disposition in Large-Molecule and Small-Molecule Compounds. Vol. 60, Journal of Clinical Pharmacology. Blackwell Publishing Inc.; 2020. p. 149–63.

41. An G. Small-Molecule Compounds Exhibiting Target-Mediated Drug Disposition (TMDD): A Minireview. Vol. 57, Journal of Clinical Pharmacology. Blackwell Publishing Inc.; 2017. p. 137–50.

42. Rubio-Camacho M, Encinar JA, Martínez-Tomé MJ, Esquembre R, Mateo CR. The interaction of temozolomide with blood components suggests the potential use of human serum albumin as a biomimetic carrier for the drug. Biomolecules. 2020 Jul 1;10(7):1–22.

43. Derendorf H. Excessive lysosomal ion-trapping of hydroxychloroquine and azithromycin. International Journal of Antimicrobial Agents. 2020 Jun 1;55(6).

44. de Klerk DJ, Honeywell RJ, Jansen G, Peters GJ. Transporter and lysosomal mediated (Multi)drug resistance to tyrosine kinase inhibitors and potential strategies to overcome resistance. Vol. 10, Cancers. MDPI AG; 2018.

45. Zheng S, Matzneller P, Zeitlinger M, Schmidt S. Development of a population pharmacokinetic model characterizing the tissue distribution of azithromycin in healthy subjects. Antimicrobial Agents and Chemotherapy. 2014 Nov 1;58(11):6675–84.

46. Peer CJ, Ronner L, Rodgers L, McCully CML, Warren KE, Figg WD. Quantification of temozolomide in nonhuman primate fluids by isocratic ultra-high performance liquid chromatography-tandem mass spectrometry to study brain tissue penetration following intranasal or intravenous delivery. Separations. 2016 Mar 1;3(1).

47. Kim H, Likhari P, Parker D, Statkevich P, Marco A, Lin C-C, et al. High-performance liquid chromatographic analysis and stability of anti-tumor agent temozolomide in human plasma [Internet]. Vol. 24, Journal of Pharmaceutical and Biomedical Analysis. 2001. Available from: www.elsevier.com/locate/jpba

48. Bahce I, Smit EF, Lubberink M, van der Veldt AAM, Yaqub M, Windhorst AD, et al. Development of [11C]erlotinib positron emission tomography for in vivo evaluation of EGF receptor mutational status. Clinical Cancer Research. 2013 Jan 1;19(1):183–93.

49. Bahce I, Yaqub M, Errami H, Schuit RC, Schober P, Thunnissen E, et al. Effects of erlotinib therapy on [11C]erlotinib uptake in EGFR mutated, advanced NSCLC. EJNMMI Research. 2016 Dec 1;6(1):1–13.

50. Abdelgalil AA, Al-Kahtani HM, Al-Jenoobi FI. Erlotinib. Profiles of Drug Substances, Excipients and Related Methodology. 2020 Jan 1;45:93–117.

51. van Erp NP, Gelderblom H, Guchelaar HJ. Clinical pharmacokinetics of tyrosine kinase inhibitors. Vol. 35, Cancer Treatment Reviews. 2009. p. 692–706.

52. Rood JJM, Toraño JS, Somovilla VJ, Beijnen JH, Sparidans RW. Bioanalysis of erlotinib, its O-demethylated metabolites OSI-413 and OSI-420, and other metabolites by liquid chromatography-tandem mass spectrometry with additional ion mobility identification. Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences. 2021 Mar 1;1166.

53. Hendrikse NH, Windhorst AD, El-Khouly F, Klok RP. Investigator brochure [11C]Erlotinib. 2017 Jul.

54. The Royal Dutch Pharmacists Association (KNMP) kennisbank. Informatorium Medicamentorum, Osimertinib. 2021.

55. Vishwanathan K, So K, Thomas K, Bramley A, English S, Collier J. Absolute Bioavailability of Osimertinib in Healthy Adults. Clinical Pharmacology in Drug Development. 2019 Feb 1;8(2):198–207.

56. Dickinson PA, Cantarini M v., Collier J, Frewer P, Martin S, Pickup K, et al. Metabolic disposition of osimertinib in rats, dogs, and humans: Insights into a drug designed to bind covalently to a cysteine residue of epidermal growth factor receptor. Drug Metabolism and Disposition. 2016 Aug 1;44(8):1201–12.

57. AstraZeneca. Investigator brochure Osimertinib. 2017 May.

58. The Royal Dutch Pharmacists Association (KNMP) kennisbank. Informatorium Medicamentorum, afatinib. 2021.

59. Wind S, Schnell D, Ebner T, Freiwald M, Stopfer P. Clinical Pharmacokinetics and Pharmacodynamics of Afatinib. Vol. 56, Clinical Pharmacokinetics. Springer International Publishing; 2017. p. 235–50.

60. The Royal Dutch Pharmacists Association (KNMP) kennisbank. Informatorium Medicamentorum, Midazolam. 2021.

61. Niwa T, Murayama N, Emoto C, Yamazaki H. Comparison of Kinetic Parameters for Drug Oxidation Rates and Substrate Inhibi-tion Potential Mediated by Cytochrome P450 3A4 and 3A5. Vol. 9, Current Drug Metabolism. 2008.

62. Katsuya Y, Fujiwara Y, Sunami K, Utsumi H, Goto Y, Kanda S, et al. Comparison of the pharmacokinetics of erlotinib administered in complete fasting and 2 h after a meal in patients with lung cancer. Cancer Chemotherapy and Pharmacology. 2015 Jul 2;76(1):125–32.

63. Brown K, Comisar C, Witjes H, Maringwa J, de Greef R, Vishwanathan K, et al. Population pharmacokinetics and exposure-response of osimertinib in patients with non-small cell lung cancer. British Journal of Clinical Pharmacology. 2017;83(6):1216–26.

64. Zhang XY, Zhang YK, Wang YJ, Gupta P, Zeng L, Xu M, et al. Osimertinib (AZD9291), a mutant-selective EGFR inhibitor, reverses ABCB1-mediated drug resistance in cancer cells. Molecules. 2016 Sep 1;21(9).

65. Dömötör O, Pelivan K, Borics A, Keppler BK, Kowol CR, Enyedy ÉA, et al. Comparative studies on the human serum albumin binding of the clinically approved EGFR inhibitors gefitinib, erlotinib, afatinib, osimertinib and the investigational inhibitor KP2187. 2018.

66. Dickinson PA, Cantarini M v., Collier J, Frewer P, Martin S, Pickup K, et al. Metabolic disposition of osimertinib in rats, dogs, and humans: Insights into a drug designed to bind covalently to a cysteine residue of epidermal growth factor receptor. Drug Metabolism and Disposition. 2016 Aug 1;44(8):1201–12.

67. Yang L, Zhu M, Kang Y, Yang T, Ma W. Investigation of the binding characteristics between ligands and epidermal growth factor receptor by cell membrane chromatography. Journal of Molecular Recognition. 2018 Jun 1;31(6).

68. EMA. SUMMARY OF PRODUCT CHARACTERISTICS, giotrif. 2021.

69. Leeuwerik A. The predictive value of low dose radiolabeled tyrosine kinase inhibitor PET/CT-imaging kinetics for precision medicine: a feasibility study. Amsterdam; 2021.

70. Joly-Tonetti N, Ondet T, Monshouwer M, Stamatas GN. EGFR inhibitors switch keratinocytes from a proliferative to a differentiative phenotype affecting epidermal development and barrier function. BMC Cancer. 2021 Dec 1;21(1).

71. Trapp S, Rosania GR, Horobin RW, Kornhuber J. Quantitative modeling of selective lysosomal targeting for drug design. European Biophysics Journal. 2008 Oct;37(8):1317–28.

72. Honeywell RJ, Hitzerd SM, Kathmann GAM, Peters GJ. Subcellular localization of several structurally different tyrosine kinase inhibitors. ADMET and DMPK. 2018;6(3):258–66.

73. Schmitt A, Gladieff L, Laffont CM, Evrard A, Boyer J-C, Lansiaux A, et al. Factors for Hematopoietic Toxicity of Carboplatin: Refining the Targeting of Carboplatin Systemic Exposure. Journal of Clinical Oncology. 2010 Oct 20;28(30).

APPENDIX

Appendix I: Use of erlotinib, afatinib and osimertinib data in PET(CT)-PK

To further validate the quantification method available tyrosine kinase inhibitors (TKI's) data was analyzed. As mentioned earlier, when using PET(CT) to clarify the PK of radiolabeled substances, whether the substance is temozolomide or not, the acquired radioactivity data should be quantified to obtain drug amounts. Previously published data from former PET(CT)-PK studies with microdoses of radiolabeled erlotinib was available in the Amsterdam UMC to be used to evaluate the quantification method.(20,48,49)

The evaluation was executed by comparing the PET(CT) data with blood sample analysis (SPE derived) data of erlotinib. A literature search was performed to interpret the PET(CT) vs SPE data comparison and clarify possible mechanisms. A main point of interest was the possible difference in PK when administering a microdose or a therapeutic dose making the PK non-linear amongst these dose levels. To further explore the quantification method the evaluation was extended by examining similar not yet published osimertinib and afatinib data. For these two drugs the blood sample data was derived after analysis with not only SPE but also HPLC.

Validating the quantification method

The developed method of quantification was calculating drug amounts out of radioactivity data using the formulas displayed in figure 16. The discussion about the quantification method arose about the '% parent compound' part of the formulas. To be able to validate these formulas it had to be clarified what the fraction of intact erlotinib or 'parent compound' was. After all the only way to properly quantify data is by knowing as well as possible which fraction is what. In an attempt to understand what the parent fraction was and what other fractions there might have been, previously published erlotinib data was re-analyzed.

Eq. 1: $$dose\ (\mu g) = \left(\frac{dose\ injected\ \ (MBq)}{specific\ \ activity\ \left(\frac{GBq}{\mu mol}\right)*1000}\right) * Mw$$

Eq. 2: $$PET\text{-}Imaging\ derived\ Conc.\left(\frac{\mu g}{L}\right) = \left(\frac{mean\ \ acitivity\ \left(\frac{Bq}{ml}\right)}{specific\ \ activity\ \left(\frac{GBq}{\mu mol}\right)*10^6}\right) * Mw * \%\ parent\ compound$$

Eq. 3: $$Blood\text{-}Conc.\left(\frac{\mu g}{L}\right) = \left(\frac{plasma\ \ concentration\ \left(\frac{MBq}{g}\right)*\rho}{specific\ \ activity\ \left(\frac{GBq}{\mu mol}\right)}\right) * Mw * \%\ parent\ compound$$

Figure 16: Formulas used to quantify PET(CT) radioactivity data. Equation 1 can be used to calculate the adminestered dose, equation 2 to calculate the PET deriverd concentration and equation 3 to calculate the blood concentration. The point of discussion in these formulas is the meaning of '% parent compound.'

The previously published microdose erlotinib data showed something notable.(49) Figure 17 shows an patient example with the SPE and PET/CT data quantified and displayed against time. In these graphs the total radioactivity of the sample is measured and the radioactivity is measured after processing with SPE (blue dots is total, blue crosses is after SPE). The measured radioactivity after SPE is allocated to the parent compound (based on Log P of erlotinib) which means the difference between total (blue dots) and parent (blue crosses) is caused by another radiolabeled substance like a metabolite or adduct of erlotinib. To be able to truly understand what the parent fraction is and what '% parent compound' in the formulas should be, it is essential to understand what this 'other radiolabeled substance' is which causes the seen difference in total and parent compound.

What makes these results interesting is that the half-life of erlotinib at a therapeutic dose is 36 hours(50) but seems a lot shorter in this (microdose) experiment. Moreover it is shown that the difference between total and parent is much smaller in the right graph which displays the same data but with the patient using an additional therapeutic dose of erlotinib. This indicates that the process that's responsible for the difference between total and parent gets saturated when given a therapeutic dose of erlotinib. Figure 18 confirms these findings by showing a clear drop within an hour of the parent fraction in patients without erlotinib therapy while the parent fraction only slightly decreases in patients with erlotinib therapy.(49)

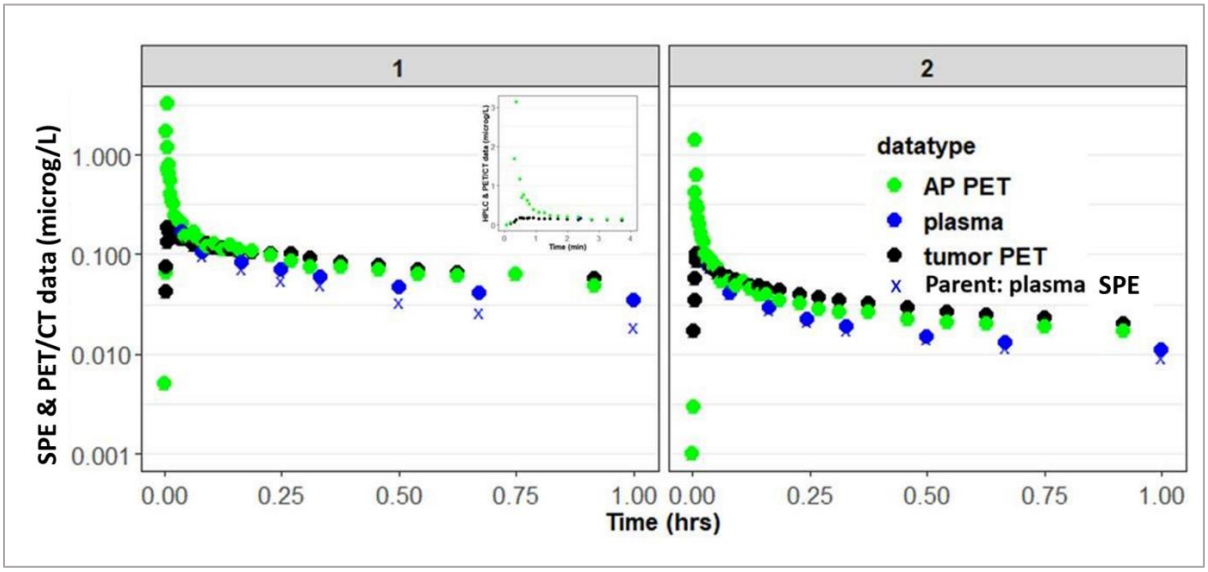

Figure 17: Patient example plot with SPE and PET/CT data displayed against time after administration of a microdose radiolabeled erlotinib. The radioactivity data is quantified to obtain microg/L on the y-axis. The green dots show show the PET data measured on the aorta, the black dots show the PET data measured on the tumor, the blue dots show the total activity of the plasma sample and the blue crosses show the activity of the plasma samples after SPE processing. Graph 1 (left) shows the results with the patient without having erlotinib therapy and graph 2 (right) shows the patient while on erlotinib therapy.

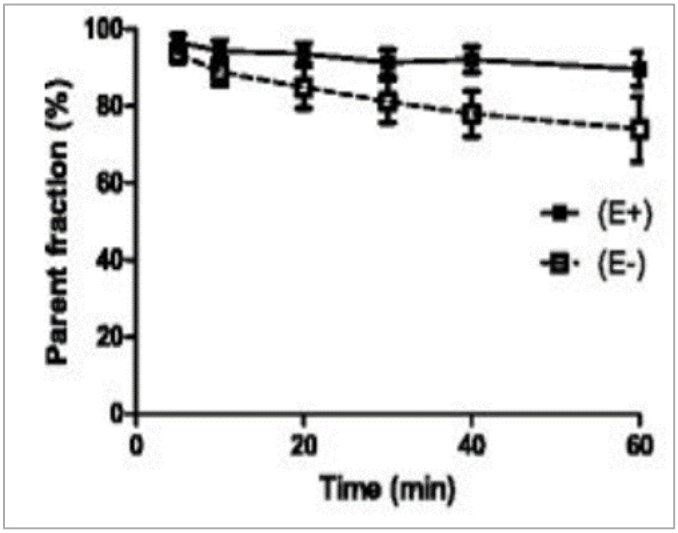

Figure 18: Change in parent fraction compared to total radioactivity in plasma samples over time. E- patients had no erlotinib therapy and E+ patients were on erlotinib therapy.(49)

The available afatinib and osimertinib microdose data showed an even larger decline in parent fraction (figure 19b & 19c). This data however, especially osimertinib, was of lower quality. Another

main difference with the erlotinib data is that the afatinib and osimertinib samples were processed using SPE and as an addition HPLC which obviously causes more separation of parent compound from other compounds with as a result a lower parent fraction. Even though the data of these three drugs can't fully be compared, it is still interesting to see that afatinib and osimertinib also show a drop in parent compound after administration of a microdose. Once again the half-life as known in the literature (with a therapeutic dose) is longer than what this data reflects.

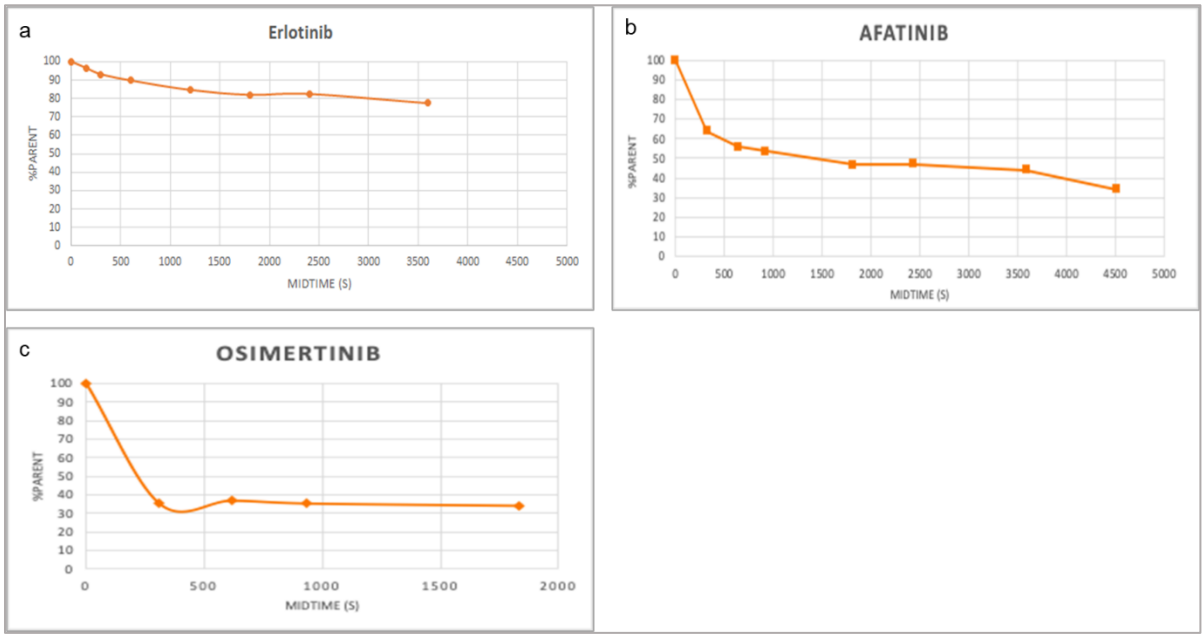

Figure 19: a) Erlotinib parent fraction in blood samples over time after microdose administration, average of 15 patients. Parent fraction determined by SPE. b) Afatinib parent fraction in blood samples over time after microdose administration, average of 6 patients. Parent fraction determined by SPE & HPLC. c) Osimertinib parent fraction in blood samples over time after microdose administration, average of 3 patients. Parent fraction determined by SPE & HPLC.

PK of erlotinib, afatinib and osimertinib

To understand what causes the quick drop in parent compound after administration of a microdose of these TKI's a literature search was performed to fully examine the PK of these drugs. The literature search could be used to make hypothesis on what might happened to these compounds after administration and to get an idea of which mechanisms have to be taken into account when given a microdose. A summary of all the findings is visualized in the PBPK model of figure 22 and figure 23.

Metabolism

The first mechanism that was examined to explain the drop of parent fraction was metabolism. It was hypothesized that when administrating a microdose the metabolism rate of the drugs could be much higher than when a therapeutic dose was given. As a result the clearance of the drugs should be higher and the half-life shorter after administration of a microdose. This mechanism has been described before and is allocated to the saturation of metabolism pathways.(18,25–28) Figure 20 shows schematically how saturation of a pathway can cause non-linear pharmacokinetics. If metabolism indeed caused the drop in parent fraction, this would mean the ''not parent'' radioactivity measured in the samples are metabolites containing 11C.

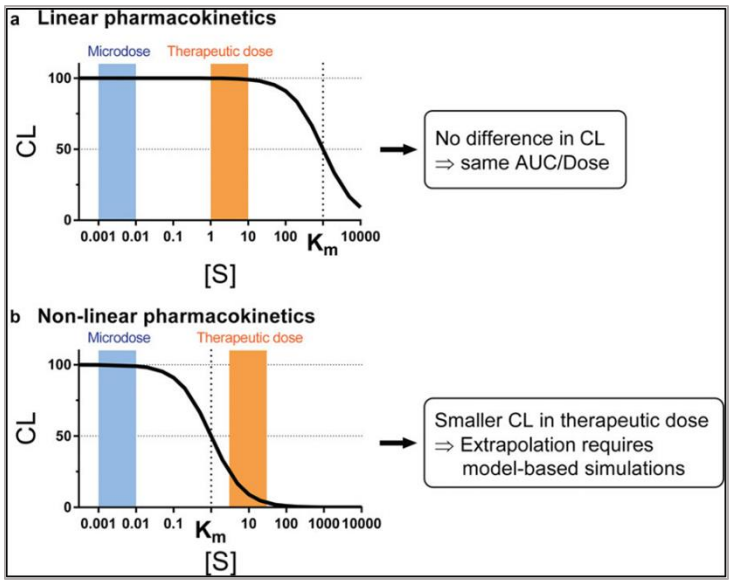

Figure 20: In 10a the metabolism pathway isn't saturated whether a microdose or a therapeutic dose is given. Therefore the PK is linear amongst these dose levels. In 10b the pathway gets saturated resulting in a slower metabolism and smaller clearance at therapeutic dose. S = substrate, CL = clearance, AUC = area under the curve, Km = Michaelis-Menten constant.(18)

Erlotinib is predominantly metabolized in the liver by the action of CYP3A4 with a minor role for CYP1A1 and CYP1A2 and has a half-life of 36 hours.(50,51) The active metabolites OSI-420 and OSI-413 are the most abundant metabolites (10% AUC erlotinib).(33,50–52) Erlotinib has many more metabolic pathways with other enzymes being involved than the ones mentioned (figure 21a). These other pathways however only play a minor role when given a therapeutic dose and are not well examined at present.(51,52) Of the active metabolites only OSI-413 contains the 11C atom when administering 11C-erlotinib. It is now known whether the other metabolites of 11C-erlotinib also contain 11C or not.(53)

Osimertinib has quite a similar metabolic profile but with CYP3A4 and CYP3A5 as the main enzymes in the liver responsible for the metabolism and a half-life of 44 hours.(54,55) Osimertinib also has two active metabolites, namely AZ7550 and AZ5104 with the latter being more active than the parent compound. These are the most abundant metabolites of osimertinib with about 10% representation of the osimertinib AUC.(54,55) As figure 21b displays, osimertinib also has many more metabolic pathways but these pathways and there metabolites only play a minor role and are not well studied.(55,56) AZ7550 contains the 11C atom after administration of 11C-osimertinib and it is unknown which of the less examined metabolites might also contain 11C.(57)

Afatinib is barely eliminated through enzymatic metabolism. It gets predominantly excreted as intact drug and has a half-life of 37 hours.(58,59)

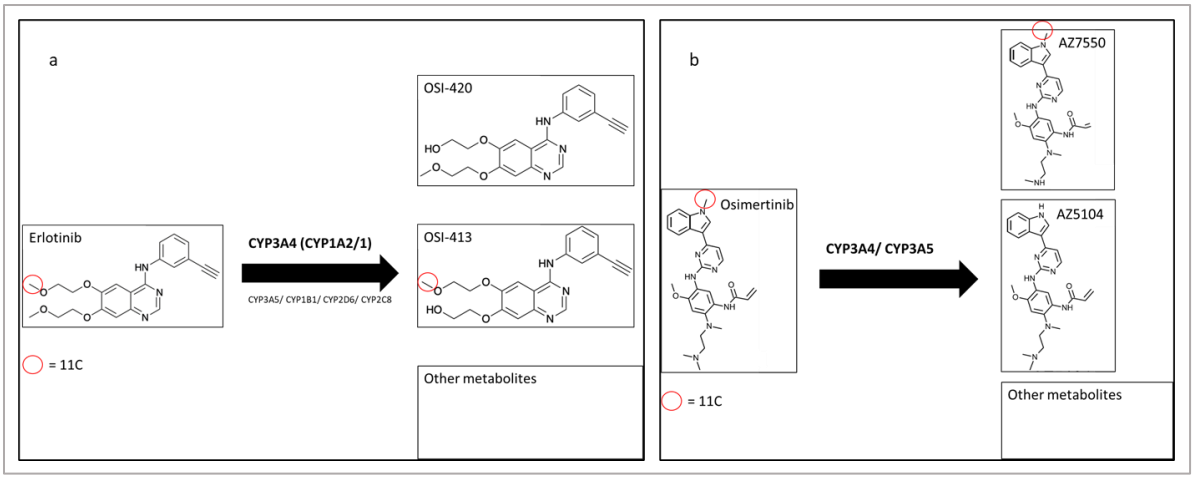

Figure 21: a) Formation of OSI-420, OSI-413 and other less known metabolites from erlotinib under influence of CYP enzymes. b) Formation of AZ7550, AZ1504 and other less known metabolites from osimertinib unther influence of CYP enzymes. The 11C atom is indicated by the red cirkel in both images.

To assess whether the metabolism of the mentioned TKI's could cause non-linear PK a drug is examined that has some similarities as regards to metabolism and is studied at a microdose, namely midazolam. Midazolam shows a large first pass effect through and the effect is caused by metabolism through CYP3A4 and CYP3A5. Almost all the administered midazolam is metabolized by these two CYP enzymes.(27,60) What makes things interesting is the fact that midazolam shows linear PK within the microdose-therapeutic dose range (figure 20a).(27) This is an indication that erlotinib and osimertinib, which get mainly metabolized by the same enzymes and show a smaller first pass effect (50,54), should also have linear PK.

Although midazolam showed an indication of erlotinib and osimertinib having linear PK, there were still too many uncertainties to assess whether the PK is actually linear. Too minimalize these uncertainties the Michaelis constant (Km) of the substances were searched for in the literature. Midazolam had a Km of 5 and 14 µM for the main chemical reaction with CYP3A4 and CYP3A5 respectively.(28,61) The Cmax (at therapeutic dose) of midazolam is 0,016 µM and it has been described that the Cmax,liver of a substance is usually comparable or a bit higher than the Cmax in plasma.(28) Since the Km is higher that the Cmax (at therapeutic dose) these findings describe a situation as displayed in figure 20a and confirm the previous described property of midazolam showing linear PK. Erlotinib had a Km of 1,74 µM for the main conversion reaction with CYP3A4 and a Cmax of 4,6 µM.(61,62) These findings demonstrate a situation as displayed in figure 20b and therefore propose non-linear PK for erlotinib. The literature does not describe a Km for osimertinib so comparing the Cmax with the Km of osimertinib was not possible. Afatinib barely has any enzymatic metabolism so finding a Km was irrelevant. Table 3 displays the so far discussed metabolism information of these compounds.

Table 3: Properties relevant for the metabolism of erlotinib, afatinib, osimertinib and midazolam.(27,28,50,51,54,55,58–63)

	Erlotinib	Afatinib	Osimertinib	Midazolam
Metabolism enzymes	CYP3A4 + (CYP1A2/1)	Hardly any metabolism	CYP3A4 + CYP3A5	CYP3A4 + CYP3A5
T1/2 Therapeutic dose (Hour)	36	37	44	1,5-2,5
Km (µM)	1,74	N/A	?	5 + 14
Cmax (µM)	4,6	0,101	0,501	0,016

In conclusion it can be said that the major metabolism routes through the liver (figure 23a) of these three TKI's can't (completely) explain the quick drop in parent compound. Erlotinib was the only one which might have non-linear PK, osimertinib was uncertain, but most importantly afatinib barely has any enzymatic metabolism at therapeutic dose and a not existing mechanism can't be saturated. Furthermore only one of the two active metabolites of erlotinib and osimertinib contain 11C and could therefore cause decrease of parent fraction. There is however another way in which metabolism could cause the quick drop in parent fraction. As mentioned before there are many more metabolism routes for erlotinib and osimertinib which aren't properly examined at present because they only play a minor role at therapeutic dose.(51,52,55,56) Afatinib might also have multiple metabolism routes or degradation pathways which aren't properly examined because they only play a minor role at therapeutic dose. These other 'less known' pathways could theoretically play a more prominent role at microdose (figure 23b) and therefore might cause the drop in parent fraction.

Efflux transporters

Another mechanism that can give substances a different PK after microdose administration compared to therapeutic dose is the effect of efflux transporters.(18,27,28) The most important efflux transporters are P-glycoprotein (P-gp), Breast Cancer Resistance Protein (BCRP) and Multidrug Resistance Proteins (MRP's). These transporters occur on different places throughout the body and actively pump substances out of certain tissues.(32) The transporters in the gastrointestinal (GI) tract for instance can actively counter the absorption of certain drugs after oral administration including TKI's.(28,33,34) When given a microdose the effect of these transporters can be relatively large compared to administration of a therapeutic dose due to less saturation.(28)

The three discussed TKI's are all substrate for the same two transporters, namely P-gp and BCRP.(51,59,64) Since all three radiolabeled TKI's where administered intravenous (IV), GI tract transporters could not have affected the PK of these drugs. Efflux transporters in the tumor on the other hand could have influenced the PK (figure 22 and 23e).(32) But even though these transporters could have an effect on the PK of erlotinib, afatinib and osimertinib after microdose administration, they could not explain an decrease in parent fraction in the plasma samples.

TMDD/protein binding

The phenomenon of drugs binding to plasma proteins or showing Target Mediated Drug Disposition (TMDD) by binding to high affinity, low capacity targets is another mechanism that can cause non-linear PK.(18,27) The idea is that when given a microdose a relatively large fraction of the drugs will quickly bind to the proteins or targets and therefore the clearance will be high in the first instance while giving a therapeutic dose will cause the proteins or target getting saturated quickly with a slower clearance and non-linear PK as a result. This mechanism is mostly known to occur with large

molecules (biologicals) but can also affect the PK of small molecules, especially with really low dose (microdose) studies.(27,40,41)

Erlotinib, afatinib and osimertinib all bind to the plasma proteins Human Serum Albumin (HSA), Alpha-1-Acid Glycoprotein (AAG) and there target is Epidermal Growth Factor Receptor (EGFR) making (soluble) EGFR a potential TMDD causing substance (figure 22, 23d and 23e).(65–67) The binding nature however do differ with erlotinib binding non-covalent and thus reversible to HSA and AAG, afatinib can bind both covalent and non-covalent and osimertinib binds covalent and thus irreversible. At therapeutic dose the unbound fraction of these drugs is 5%, 5% and 1,32% respectively.(65,66,68) Although theoretically protein binding could give non-linear PK it can't explain all our findings. Erlotinib for instance binds non-covalent which means after sample processing with SPE the non-covalent bound should dissolve with erlotinib becoming free intact parent compound again.(69)

The binding nature of the TKI's with EFGR also differs with erlotinib binding reversible, afatinib irreversible and osimertinib also irreversible.(67,70) Once again it is theoretically possible that soluble EGFR causes TMDD in at least one of these compounds, except for erlotinib since that binding is reversible and would probably dissolve during SPE sample processing. One could say that the differences in the decrease of parent fraction between these TKI's (figure 19) could be caused by the occurrence of TMDD in afatinib or osimertinib and not in erlotinib. However as mentioned before the data of the three substances can't be fully compared due to differences in analyzing method (only SPE or SPE + HPLC).(69)

Lysosomal accumulation

The last mechanism that is examined is lysosomal accumulation or also known as cellular uptake or ion-trapping. Lysosomal accumulation occurs when substances have basic functional groups which can get ionized in the low pH environment of lysosomes. The idea is that some substances occur mostly unionized in the neutral pH (7,4) of the cytosol but become ionized in the lysosome due to the lower pH (5,0). The now ionized molecules can't easily cross the lysosome membrane anymore and thus are ion-trapped.(43–45) Figure 23c displays this process schematically.

When looking at the TKI's it shows that erlotinib has one basic functional group while afatinib and osimertinib have two. Based on these properties it is expected for erlotinib to only become ionized for approximately 60% while afatinib and osimertinib would be ionized for >99% in lysosomes.(44,71,72) Since macrophages relatively have much lysosomes, ion-trapping can occur relatively much in these kind of cells. Macrophages can be abundant around tumor tissue and can therefor trap TKI's and lead them to the spleen (figure 22).(44,72) Because of processes like this lysosomal accumulation can have a major impact on the PK of substances, especially when working with microdoses. However the TKI samples analyzed with SPE and HPLC are plasma samples and plasma contains no cells so lysosomal accumulation can't influence these results.(69)

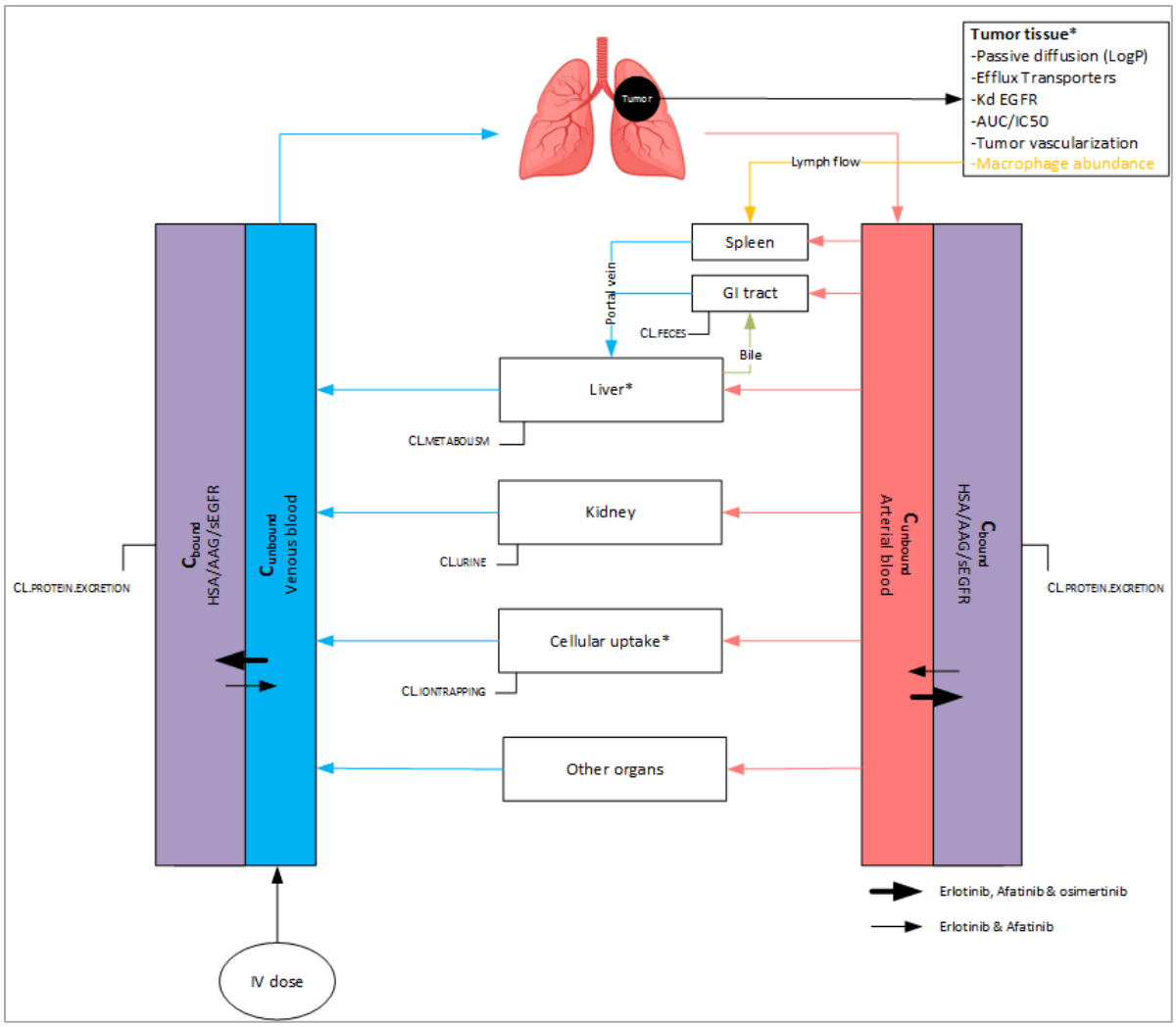

*Figure 22: Combined PBPK model for erlotinib, osimertinib and afatinib. The microdose TKI's are administered IV and can bind to HSA, AAG or soluble EGFR whether the're in venous or arterial blood. Clearance can occur due to cullular uptake, kidney function, metabolism, through feces and by protein excretion. Not relevant organs are assembled as 'other organs' and 'cellular uptake' is visualized as a compartiment for simplicity while in reality cellular uptake can occur in basically all cells. The 'tumor tissue' compartiment shows processes which might influence the behavior of TKI's at the tumor site like passive diffusion, effect of transporters, the Kd of the TKI for EGFR, the AUC/IC50, tumor vascularization and the occurance of machrophages. Figure 23 zooms in on some processes of this PBPK model, visible by *.*

Although this literature search didn't give an absolute answer on which mechanism causes the quick drop in parent fraction, it did give an idea of mechanism that should be taken in account when conducting microdose studies. The obtained knowledge was used to subsequently perform a similar literature search for temozolomide.

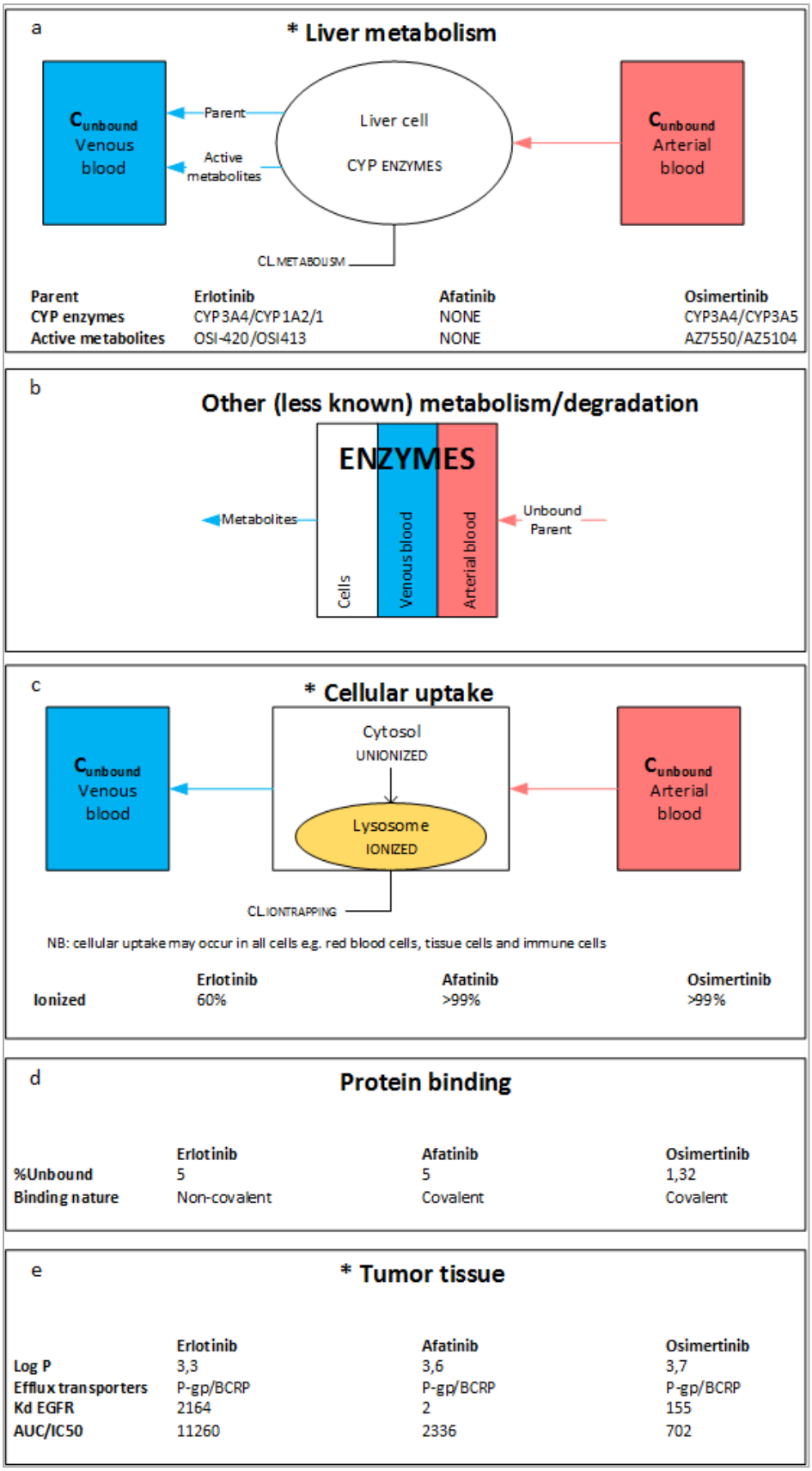

Figure 23: a) Schematic representation of the major metabolism routes of erlotinib, afatinib and osimertinib. b) Schematic display of how less known metabolism/degradation routes can influence the PK of the TKI's. c) Schematic display of how cellular uptake can take place. d) Protein binding information of the TKI's. e) Information about the processes which can influence the TKIs behavior around the tumor.

Appendix II: Abstract for the Dutch Hospital pharmacy days 2021

The use of PET/CT imaging data for a mechanistic PK-model of radiolabeled temozolomide

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2. Amsterdam UMC, location VUmc, Department of Neurology, The Netherlands
3. Amsterdam UMC, location VUmc, Department of Medical Oncology, Cancer Center Amsterdam, The Netherlands.
4. Amsterdam UMC, location VUmc, Department of Radiology and Nuclear Medicine, Cancer Center Amsterdam, The Netherlands

Background
Temozolomide is a chemotherapeutic drug that is currently dosed according to the body surface area (BSA) in malignant gliomas. Although effective, many patients stop prematurely due to early tumor progression or treatment-related hematological toxicity. Factors that contribute to treatment efficacy and toxicity in individual patients are unclear, but variable tumor penetration has been proposed. To optimize treatment, describing the pharmacokinetics (PK) of temozolomide, including tumor tissue penetration, is essential. A prior microdosed ^{11}C-temozolomide PET(CT) allowed us to visualize tumor uptake in two glioblastoma patients. Building on these preliminary data, we now aimed to assess whether a pilot PET(CT)-PK study, combined with a larger PK study with temozolomide is feasible in order to optimize therapeutic responsess of temozolomide in patients with glioblastoma.

Methods
First, we performed a literature search to predict linearity among microdose and therapeutic dose levels. We studied the PK-profile of temozolomide and its' metabolites to form a semi-mechanistic PK modeling plan. Then, the preliminary ^{11}C-temozolomide PET/CT patient data were analyzed, aiming to assess feasibility of using PET/CT to quantify tumor penetration. Finally, a clinical study protocol was developed for evaluation of temozolomide PK.

Results
As substrate for multiple drug transporters and albumin binding proteins, temozolomide is unlikely to have linear PK among dose levels in all tissues. Temozolomide is a prodrug that degrades spontaneously, forming a methyl diazonium ion. This active compound methylates DNA. The proposed model to assess PK semi-mechanistically consists of a peripheral, central and brain compartment, in which temozolomide can occur unbound, bound or converted to a DNA adduct.

Preliminary ^{11}C-temozolomide PET and blood samples (SPE derived) data-analyses enabled quantification. However, due to its' spontaneous degradation, the blood samples - obtained using standard PET-procedures - were not suitable to distinguish temozolomide from metabolites and active DNA adducts.

The developed study protocol has an innovative approach, in which one therapeutic dose is administered prior to the micro-dosed tracer and PET/CT is combined with LC-MS sample analyses to distinguish temozolomide, metabolites and DNA adducts in blood and tumor tissues. PK-findings of this study will be used to predict PK in a large population to assess the exposure-response association in a multicenter study. This could ultimately lead to dose individualization to promote efficacy and prevent toxicity.

Conclusions
This study demonstrates that clinical pharmacology, pharmaceutic and PET tracer knowledge should be combined when developing tracer studies with complex molecules. Innovative methods, combining LC-MS with PET imaging, are feasible. The proposed study may improve glioblastoma treatment and provide new insights to optimize small molecule PET(CT) studies.

Keywords
Temozolomide, pharmacokinetics, PET(CT).

Contact person: i.bartelink@amsterdamumc.nl

Appendix III: Cancer Center Amsterdam fund application

Amsterdam UMC
Universitair Medische Centra

Pre-proposal form CCA grant, round 2021

1	**Title project:** The predictive value of low dose radiolabeled temozolomide PET/CT imaging kinetics for precision medicine: a feasibility study
2	**Proposal for** - Duration 1 or 2 years: 2 years - Kind of personnel: Postdoc/pharmacometrician & research technician (0.1 FTE each) - Additional Budget: Biobanking (neuro-onco biobank) € 2000 & materials € 20.000.
3	**Project fits in CCA research theme (select only 1):** 2.1. Imaging
4	**Principal Investigator and department** 1. dr. Imke Bartelink, Clinical Pharmacology and Pharmacy (VUmc CCA) Supporting PIs (max. 3) 1. prof. dr. Harry Hendrikse, Radiology and Nuclear medicine (VUmc CCA) 2. dr. Mathilde Kouwenhoven, Neurology (VUmc CCA) 3. dr. Richard Honeywell, Clinical Pharmacology and Pharmacy (VUmc CCA) **First applicant: date (month and year) of obtaining the PhD degree:** April 2012
5	**Is funding for this project also requested elsewhere? If so, where?** The method development was not requested, but the results of the current proposal will be used to execute clinical PKPD analyses in 1750 patients in; KWF proposal "A rational approach towards efficacy and risk on toxicity assessment of glioblastoma treatment (KWF 2021-2 No. 13743)"
6	**Aim of the project:** Microdosed ^{11}C-temozolomide PET/CT allowed us to visualize tumor uptake in glioblastoma patients. To extend this work, we now hypothesize that temozolomide PET/CT results reflect therapeutic tumor uptake in individual patients. There are major challenges to individual tumor drug uptake predictions, making pharmacokinetic studies of temozolomide essential for future dose individualizations. The aim of the project is to develop methods to map the pharmacokinetics (PK) of temozolomide, its metabolites in plasma and DNA-adducts in leucocytes and cerebral tumors. First, we aim to identify essential drug-specific characteristics determining temozolomide whole body biodistribution (AIM1). To fulfill this aim, we will use previously obtained LCMS, PET/CT data and preclinical PK parameters, to construct a mechanistic PK-model to predict temozolomide concentrations in blood, leucocytes and cerebral tumor. This model will be used to study the power to predict the dose for optimal cerebral exposure - AUC above preclinical IC_{50} - in a new prospective clinical PK-study. Then, we aim to develop methods to quantify temozolomide, its metabolites and DNA-adducts (AIM 2). Positron emission tomography (PET) imaging with radiolabeled ^{11}C-temozolomide will be used to quantify temozolomide uptake in blood & tumor tissue. LCMS will be used to distinguish between temozolomide, its metabolites and DNA-adducts in blood and PBMCs. AIM 3 is to perform prospective clinical PET/CT and blood pharmacokinetic studies of temozolomide in plasma, PBMCs and tumor tissue, based on power calculations and quantification methods developed in AIM1/2.
7	***Relevance of the project, discuss the following aspects:*** Temozolomide is the most potent chemotherapy available for the treatment of glioblastoma – an aggressive primary brain tumor. Patients with glioblastoma have a median survival of 14.6 months even after aggressive treatment. More than half of the patients cannot complete the 10-month palliative treatment due to early tumor progression or treatment-related hematological toxicity. Factors that contribute to treatment efficacy or risk on severe toxicity in the individual patient are unclear. Even whether temozolomide sufficiently penetrates the tumor is unclear. Temozolomide is dosed according to a one-fits-all strategy at the maximal tolerated dose obtained from phase 1/2 studies. To optimize treatment, describing the pharmacokinetics of temozolomide is essential. If we can predict tissue penetration, we can use the model to predict the optimal temozolomide exposure necessary to promote efficacy and prevent hematological toxicity in glioblastoma patients. This can help objectify the chance of efficacy and risk on toxicity. This project is highly innovative due to the use of ^{11}C-temozolomide and PET combined with LCMS to quantify tumor uptake and quantify temozolomide whole body bio-distribution which fits well within the infrastructure of the Brain Tumor Center Amsterdam, Clinical Pharmacy & Nuclear Medicine. Because of the instability and complexity of temozolomide, describing the pharmacokinetics is a high risk/high benefit

	project. If funded, this project will provide a solid base to examine the pharmacokinetics and dynamics of temozolomide and its metabolites. If successful, we will extend our knowledge of metabolite research to other drugs, including pomalidomide, for which a PK-study in Amsterdam UMC is currently being planned. PET/CT imaging is an innovative technique in understanding drug behaviour. When combined with mechanistic PK-modelling, this can help predict optimal drug exposure necessary to promote efficacy and prevent toxicity for small molecule-oncolytic drugs. When the prospective clinical PET/CT and PK-studies of temozolomide are finalized, we will extend the PK-model to derive a general mathematical framework of small-molecule uptake in tumors as a function of individual PET/CT and therapeutic plasma data and drug-specific variables and include multiple ongoing PET/CT studies using tyrosine kinase inhibitors. This framework may therefore largely contribute in understanding and improving the way we use dose-individualize small molecules in cancer.
8	***Feasibility of the project, discuss the following aspects:*** We visualized ^{11}C-temozolomide within the brain and in blood in glioblastoma patients. We quantified the blood and tumor using the PET/CT data. Since there is currently no method to distinguish temozolomide from its metabolites in the blood samples of the previously screened patients, the total radioactivity of the samples was measured over time. Within this project, we will optimize these measurements to allow quantification of all metabolites and adducts in plasma, whole blood and PBMCs using LCMS and in cerebral tumors using PET/CT. First the total radioactivity of every sample will be measured. Then the samples go through the LCMS to distinguish temozolomide, metabolites and DNA adducts. The use of LCMS will be made possible by giving the participants a ''cold'' therapeutic dose of temozolomide along with the radiolabeled microdose. With the obtained data the temozolomide, metabolites and DNA adducts within the samples and images will be quantified. Temozolomide and its metabolites degrade within minutes in human plasma at 37°C and neutral pH. Therefore, measurement of its concentration in body tissues requires specific pharmacological expertise and validated methods. Prior research shows that stable samples can be obtained through storage at low temperatures (4°C; t1/2 >30min) and plasma acidification (pH=4; at least 30 days at 20°C). In the current proposal we plan to perform sample method development, stability tests, optimize the PET/CT dose and optimize the sampling scheme in clinical trial protocols. There is extensive experience with PET-imaging studies, PK-samples analyses and PK modelling within the Amsterdam UMC. This expertise is essential since temozolomide and its metabolites are highly unstable and it's downstream pharmacology is complex, making description of the pharmacokinetics challenging. All the needed facilities are available including PET scanners, ISO-compliant LCMS and GMP-compliant ^{11}C-temozolomide. Patients will be identified at the Brain Tumor Board/Triage panel that takes place 3 times a week. At the Brain Tumor Center Amsterdam 120 new patients with a high grade glioma are treated every year.
9	**Scientific Quality, discuss the following aspects:** We hypothesize that sample analysis with LCMS can give us insight in the way temozolomide and its metabolites behave in the human body. Furthermore we expect to be able to quantify tumor tissue exposure to temozolomide using PET-imaging. If successful, we can construct a complete PK-model for temozolomide. Unique to this proposal is that we plan to use a rational mechanistic PKPD model to predict response and toxicity. Temozolomide is the most potent chemotherapy for glioblastoma patients, but many patients stop prematurely due to severe toxicity. When inter-patient variability and exposure-response relationships are quantified, the predictive model can be used to individualize the temozolomide dosing scheme leading to optimal exposure to promote efficacy and prevent hematological toxicity. This proposal is innovative in combining PET imaging and LCMS during clinical treatment to quantify this unstable prodrug to gain knowledge that can be used in future PET imaging studies. Describing the pharmacokinetics and pharmacodynamics of temozolomide and its metabolites may give us insight on how drug and on-target exposure can influence the efficacy and toxicity of (oncolytic) drugs.
10	***Key references (max.5):*** - Jen, J.F.*et al.* Population PK of temozolomide in cancer patients. Pharm Res 2000;17(10):1284-9. - Adema, AD *et al*. Cell cycle effects … in cell lines. J Chemother 2009;21(3):338-46. - Eriksson, J., *et al.* Synthesis .. [(11) C]methyl iodide. J Labelled Comp Radiopharm 2015;58(3):122-6 - Brown, T.J., et al., Association.. Meta-analysis. JAMA Oncol, 2016. 2(11):1460-1469. - Grun N *et al*. Trombocytopenie bij patiënten met een glioblastoom behandeld met bestraling en temozolomide. Wetenschappelijke dag Landelijke Werkgroep Neuro-Oncologie (LWNO) april 2021 (award winning presentation)

Appendix IV: Abstract of KWF kankerbestrijding fund application

The Dutch glioblastoma cohort: A rational approach towards efficacy and risk on toxicity assessment of glioblastoma treatment.

Problem description: Temozolomide is currently the most potent drug for the treatment of glioblastoma – an aggressive primary brain tumor with a median survival of only 14.6 months. Since 2005, the standard treatment of patients with glioblastoma consists of maximal safe resection followed by chemoradiation and adjuvant treatment with temozolomide. More than half of the patients cannot complete the 10-month palliative treatment due to early tumor progression (43%) or severe hematological toxicity (17%). The clinical characteristics of patients that relate to efficacy and toxicity of temozolomide are undetermined; whether dose-reductions that patients receive due to severe toxicity affect treatment efficacy is unclear. We hypothesize that dosing temozolomide according to the current one-fits-all contributes to the suboptimal efficacy and to the significant hematological toxicity of temozolomide. We will construct a more rational approach to predict treatment efficacy and treatment-related toxicity based on patient, pharmacological and pharmacogenetic factors.

Research direction: At present we lack sufficient information on clinical and pharmacological factors to predict treatment efficacy and severe hematological toxicity. We will identify clinical factors associated with treatment efficacy (survival S12 months) and a high risk on severe hematological toxicity in a large cohort of patients with a histologically confirmed glioblastoma who underwent standard treatment. We will determine the relation between the proportion of temozolomide that reaches the tumor and the concentrations of temozolomide in blood over time to give a comprehensive description of the pharmacokinetic (PK) profile of temozolomide. Temozolomide causes DNA damage and eventually cell death through methylation of guanine residues at the O ^{6}-position (pharmacodynamics, PD). These temozolomide-induced methyl-groups can be visualized at the tumor site with ^{11}C-labeled temozolomide and PET-imaging and will serve as a surrogate marker for exposure- efficacy . We will incorporate previous knowledge on patient specific prognosticators and drug-specific pharmacological (PKPD) factors to optimize the prospective population-based pharmacokinetic model of Jen et al. [1] for patients with 'de novo' glioblastoma. We will use this model to establish the exposure-efficacy relation for temozolomide.

If temozolomide generates DNA-adducts on the DNA of hematopoietic cells, these cells consequently undergo cell death, which results in hematological toxicity. We will measure the proportion of methyl groups bound to the DNA of peripheral blood mononuclear cells (PBMCs) and will use this as a read-out for treatment-related toxicity. Not all patients are equally vulnerable to the toxic effects of temozolomide. Genetic predisposition to the toxic effects of temozolomide has been described in scarce reports. At present, the reported DNA-variations have not been validated in a sufficiently large cohort and cannot be used in daily practice. We will construct a pharmacokinetic-pharmacodynamic (PKPD) model that can predict if patients that harbor certain genetic polymorphisms have a higher risk of severe toxicity.

Patients who experience severe toxicity or have early progression visit the hospital more often. Surprisingly, the impact of extra visits and time spend at the hospital is slacking in the registration studies of temozolomide for patients with glioblastoma. We will collect this information to inform patients and physicians on the 'burden of treatment'.

Plan of investigation: We identified a cohort of 1750 patients with histologically confirmed glioblastoma treated with standard treatment from the brain tumor databases of three large brain tumor centers (UMCU, ErasmusMC, AUMC) in the Netherlands. Beside clinical characteristics, we will extract for each patient the administered temozolomide doses and types and grades of hematological toxicities. This cohort serves as the backbone of our clinical efficacy and toxicity models.
The instability of temozolomide encompasses a major obstacle to measure temozolomide concentrations in blood and at the tumor site. We circumvent this problem by quantifying temozolomide- labeled at the ^{11}C position, the carbon that will form the methyl-adduct on the tumor DNA- with PET-imaging. This technique has been established at the Amsterdam UMC.
To measure the concentrations of temozolomide and its metabolites
in blood, we will acidify and cool the blood samples. This procedure will stabilize temozolomide and its metabolites [2]. With the gained knowledge on temozolomide concentrations in the tumor and in blood, we can construct a solid pharmacokinetic model for treatment efficacy. We will use this model to validate and optimize the population-based PKPD model of Jen et al. [1]. The optimized model will allow us to calculate temozolomide exposure for individual patients with glioblastoma and to correlate temozolomide exposure to treatment outcome.
We can measure temozolomide-induced DNA-adducts in cell lines [3] and will optimize this assay to measure DNA adducts in PBMNCs. Through PKPD modelling, we can predict if patients that harbor certain genetic polymorphisms implicated in detoxification of temozolomide have a higher risk on severe toxicity. We already collected a large number of normal DNAs to validate the proposed markers and to explore new genetic vulnerabilities for hematological toxicity such as genes involved in DNA repair and temozolomide detoxification.
We collect information on extra hospital visits, emergency room visits, and hospitalization to quantify the effects of unsuccessful treatment and toxicities for the patients. For glioblastoma patients that all have a limited prognosis, it is important to have access to such data to make personalized treatment decisions.

Aims: In this project, we will:

1. Determine the concentration-time profile of temozolomide and its active metabolites in blood and at the tumor site (WP1);
2. Identify clinical and pharmacological factors that predict treatment efficacy in the individual patient (WP2);
3. Investigate clinical and pharmacological risk factors for severe toxicity (WP3) and
4. Explore the effects of early disease progression and severe toxicity on the treatment-burden for patients (WP3).

Expected outcome: The knowledge we gain in this project should lead to identification of patients who: 1) are unlikely to benefit from standard treatment or 2) have high risk on severe hematological toxicity and early treatment abrogation. Patients with low estimated efficacy of standard treatment could benefit from direct start with alternate treatment in a clinical study – for example gemcitabine [3]showed enhanced efficacy in combination with temozolomide or predicted promising combination therapies [4]. Patients with high risk on severe toxicity could receive more conservative temozolomide dosing if predicted treatment-efficacy is not compromised.