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Computational modeling of pharmacokinetics and tumor dynamics to guide anti-cancer treatment

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Chapter 8

General discussion



Introduction

Although anti-cancer treatments have significantly advanced over the past decades, obstacles to accomplishing successful treatment still exist. The occurrence of treatment resistance is one of the major factors that limit the long-lasting efficacy of anti-cancer therapies [1, 2]. Evolutionary mechanisms are increasingly acknowledged as key factors that contribute to the occurrence of treatment resistance [2-5]. A better characterization and understanding of evolutionary tumor progression, and subsequent use of this knowledge to design new treatment regimens would increase the chance to suppress the development of cancer treatment resistance. Another important factor that challenges successful treatment is the substantial variability in pharmacokinetics (PK) / pharmacodynamics (PD) of anti-cancer drugs, which is especially frequently observed in real-world patients. This can result in suboptimal treatment outcomes for part of the patients especially when the therapeutic window is narrow [6, 7]. Moreover, the typically applied maximum tolerated dose (MTD) paradigm in cancer treatment may not be optimal for real-world patients due to high risk of toxicity [8]. These factors highlight the need to gain more insight into the PK/PD profiles and variability of anti-cancer drugs in real-world patients, and to further develop optimized and individualized treatment regimens.

Quantitative modeling with mixed-effect models is widely applied in pharmaceutical research which enables quantitative characterization and prediction of the PK and PD of therapeutic agents. It also allows quantifying inter- and intra-individual variability and identify covariates that explain the variability [9, 10]. With a Bayesian framework, individual parameters can be obtained based on prior knowledge from the model and patient characteristics and data, which can be used to capture and predict individual PK/PD characteristics [7]. In oncology research, the model-based approach is a helpful tool to make use of longitudinal data, such as drug concentrations, tumor burden, and other PD biomarkers, to gain knowledge about the interaction between drug treatment and the human body, as well as cancer progression. This knowledge and developed models can subsequently support the identification of optimal therapeutic regimens and guide individualized treatment rationally (model-informed precision dosing, MIPD) [7, 11, 12].

The studies presented in this thesis applied quantitative modeling approaches to characterize the evolutionary tumor progression and PK/PD of anti-cancer drugs. The developed models were subsequently applied to evaluate and develop optimal and individualized regimens for oncology patients.

Better understanding of evolutionary tumor progression

Intra-tumor heterogeneity

Intra-tumor heterogeneity, which suggests distinct cells exist in the same tumor, is considered to be one of the main factors that drive the evolving adaptation of cancer to treatment. Capturing intra-tumor heterogeneity is therefore of importance for a better understanding of evolutionary treatment resistance. As summarized in **chapter 2**, various kinds of quantitative models have been applied to describe and predict tumor dynamics and resistance evolution in cancer patients. Among the reported tumor dynamics models, intra-tumor heterogeneity has been considered when describing tumor regrowth by separating the tumor into components consisting of cells that are sensitive or resistant to therapy. The interaction between sensitive and resistant cells is also the cornerstone for the models that characterize the evolutionary development of drug resistance.

In the studies in **section I**, intra-tumor heterogeneity has served as a key element in the applied models to support the understanding of evolving tumor progression. The presence of pre-existing resistant components (primary resistance) and/or acquired resistance and their interaction have also been frequently discussed. In **chapter 3**, a model that accounted for various clonal populations was developed and it well captured the tumor sizes and mutant *KRAS* levels in circulating tumor DNA (ctDNA) versus time curves from patients with metastatic colorectal cancer (mCRC). In addition to the clonal populations that are sensitive or resistant to the original treatment, a hypothetical third clonal population was also introduced in the model to describe tumor response to multiple treatments. The same structure was also applied to characterize the dynamics of tumor sizes and ctDNA measurements in non-small cell lung cancer (NSCLC) patients. The inclusion of primary or acquired resistance in this study was supported by the detected mutation in ctDNA, which was suggested to be a mediator of acquired resistance [13, 14]. The model therefore included acquired resistance, and primary resistance was only considered for patients with detectable *KRAS* mutation pre-treatment. The developed model allowed us to capture not only the dynamics of total tumor size but also that of sub-clones in the tumor, which reflects the evolutionary progression of the tumor.

The study presented in **chapter 4** further characterized the tumor dynamics in NSCLC patients treated with erlotinib while considering tumor heterogeneity. In this study, we explored models with or without primary resistance while including an acquired resistance for both. The results indicated that the model assuming no primary resistance could adequately fit the obtained data, and estimating primary resistance did not improve the model fit. This might indicate that for NSCLC patients with an activating *EGFR* mutation,

it is mainly the acquired resistance, which was due to the acquisition of *EGFR* p.T790M mutation or other mechanisms, that limits the treatment response. Among previously reported model-based studies on tumor size dynamics in NSCLC patients treated with erlotinib, one study also considered tumor heterogeneity [15]. Their results also showed that the models with or without primary resistance could describe the data equally well even though erlotinib was used as a second-line treatment in their study [15].

In fact, studies on the probability of having resistance at the start of treatment have been performed. They demonstrated that such probability increased as tumor burden increased and it could reach up to > 90% [16, 17]. The study that provided the original data for **chapter 3** also suggested that drug resistance is likely to be present prior to the initiation of anti-cancer drug treatment [13]. Yet, the estimated baseline size of the resistance clonal population only accounted for a small part of the total tumor cell population [13]. In **chapter 4**, the estimated baseline size of primary resistance accounted for a small proportion (5.9%) of the baseline tumor size. Therefore, although resistance may be present prior to the treatment, considering the small proportion and the complexity of the model, the primary resistance has been omitted in the models used in our studies. In addition, the data of genetic biomarkers is believed to be viable evidence to support the differentiation of heterogeneous components in the tumor when modeling tumor dynamics considering tumor-heterogeneity [18].

Interaction among clonal populations and treatment

In addition to intra-tumor heterogeneity, capturing the interaction among clonal populations in the tumor and anti-cancer drug treatment is also a cornerstone when describing evolving development of resistance in tumor. We have addressed such interaction by accounting for the differences in proliferation rates of tumor cells, the response of tumor cells to the therapy, and the transition between sensitive and resistant tumor cells in response to treatment.

In order to obtain resistance to treatment, tumor may give up some proliferation capability, which is represented by a fitness cost [19]. Due to this fitness cost, the proliferation rate of the resistant clonal population can be lower than that of the sensitive clonal population [19, 20]. In **chapter 3**, we adopted this concept and assumed that the growth rate of resistant cells was 70% of the sensitive cells. In **chapter 4**, we have also estimated separate growth rates for different cell populations during model development. The estimated growth rate of sensitive cells was 2.19 fold higher than that of the resistant cells. However, the high relative standard error (RES) (104%) indicated a high uncertainty in the estimation.

Therefore, the growth rates of treatment sensitive and resistant clonal populations were eventually set to be the same in this study. This lack of identifiability of separate growth rates is considered to be caused by the limited amount of data.

The response of tumor cells to the therapy has been mainly addressed by adding a regression term on drug susceptible tumor cells. In **chapter 3**, we have included treatment effect with a drug-dependent regression term. This is due to the lack of data on drug exposure or dose in this study. In the meantime, the trough concentrations of the used monoclonal antibody therapy have shown to be able to reach above 90% of the saturation levels at standard treatment regimens, suggesting almost a maximum effect in all patients [21]. However, for other molecules the exposure of which correlates to response, such as tyrosine kinase inhibitors (TKIs), drug levels are important to be included in the analysis. This would be beneficial for the understanding the exposure-response relationship and how drug exposure is driving the evolutionary progression of tumor. Therefore, we explored a model that incorporated exposure-dependent treatment effect in **chapter 4**. However, we did not identify a clear exposure-tumor inhibition relationship within the studied concentration range (the median predicted drug concentrations at the tumor size monitoring time points was 992 ng/ml (range of 284–1554 ng/mL)). A dose-tumor inhibition relationship was also not identified. This lack of relationship between erlotinib exposure and responses, which may be because of the saturated treatment effect, is in line with previous findings [6, 22–24]. Although the influence of drug exposure on the evolving tumor progression could not be investigated in this case, the results may suggest a potential option to decrease the dose of erlotinib to target a lower concentration that still ensures sufficient efficacy but can be better tolerated, especially since a significant proportion of erlotinib-treated patients can have severe toxicity [25].

Because of the selection pressure of anti-cancer drug treatment, our studies in **chapter 3** and **4** assumed that mutations were able to be acquired which resulted in a transition from sensitive to resistant cell population. A back transfer process from drug resistant to sensitive clonal population was also introduced in **chapter 3** during the treatment interruption periods. This assumption allowed capturing the recovery of sensitivity to the treatment upon withdrawal of treatment, which was supported by in vitro observations [26]. This process could also describe the phenomenon that in the absence of the drug, susceptible tumor cells have the benefit of growing back again at the expense of resistant tumor cells. When the back transfer process was removed, the simulation outcomes of evaluated regimens were only slightly affected but the decline of ctDNA upon withdrawal of treatment, which has been observed in mCRC patients [26, 27], could not be captured anymore. It was also observed that under this circumstance, the remaining susceptible

tumor cells had no growth advantage over the resistant tumor cells during the withdrawal of treatment, hence the tumor would not regain susceptibility. Therefore, the introduction of a transition between clonal populations in this study allowed the description of the dynamics of and the competition among different clonal populations based on current available data. More data under intermittent therapy would be valuable to better characterize this dynamic process, and to better estimate parameters.

Insight provided by ctDNA

Clinically available genetic biomarkers such as ctDNA have been shown to be able to provide insight into tumor heterogeneity and evolution of resistance, and also correlate with tumor burden [18]. Studies have already utilized the available ctDNA data to support the estimation of parameters that are required in the tumor evolution model or to evaluate the simulation results of the models [13, 28, 29]. Thus, we see opportunities to incorporate the ctDNA measurements in model-based tumor dynamics studies to enable better understanding and prediction on the tumor progression and dynamics of tumor sub-clones. Such models would be of help in investigating treatment regimens that increase the chance of overcoming treatment resistance. The model developed in **chapter 3** enabled the characterization of the time-curves of both tumor sizes and ctDNA measurements in patients with mCRC. The link between the generation of genetic variants in ctDNA and tumor burden was accounted by a sub-clonal tumor-size dependent shedding rate which was expressed with Hill equations with tumor size as the independent variable. This model allowed us to describe the delayed emergence of genetic variants in ctDNA indicating treatment resistance as well as the earlier emergence of detectable mutation than disease progression, which was observed in the original studies [13, 30]. The ctDNA measurements also informed the inclusion of primary or acquired resistance.

The study in **chapter 4** demonstrated that in NSCLC patients treated with erlotinib, the baseline ctDNA measurements on variant allele frequency (VAF) of mutant *EGFR* and the presence of a *TP53* mutation have a potential correlation with the estimated parameters related to tumor dynamics (mainly the growth rate constant k_g and mutation rate constant k_m), especially that higher baseline *EGFR* VAF was significantly correlated with increased growth rate constant k_g . This indicates that patients with higher *EGFR* VAF at baseline may have a worse response to the treatment, which is in line with the clinical findings from an *EGFR* cohort in the START-TKI study, i.e. patients without detectable ctDNA at baseline had a lower rate of radiological progression [25]. An explanation could be the association between ctDNA levels and tumor burden [18, 31]. Our result also supports previous findings suggesting that baseline concomitant *TP53* mutations may relate to

worse clinical outcomes in patients with NSCLC [25]. After incorporating baseline ctDNA measurements, the developed tumor dynamics model could better predict the tumor size dynamics in response to erlotinib treatment in NSCLC patients. This finding also demonstrates the potential to use ctDNA as an early biomarker to support decision making for the treatment of NSCLC patients [32].

Design treatment to overcome resistance

Designing treatment with gained knowledge on treatment resistance evolution and applying personalized treatment would increase the chance of overcoming cancer treatment resistance [2, 33]. Based on this concept, adaptive treatments where drug selection is guided by the mutation detected in ctDNA, and intermittent treatment which utilizes the fitness advantage of sensitive cells during the withdrawal of treatment to regain sensitivity to treatment have been suggested for better treating cancer patients [18, 33, 34]. This also brings forward opportunities to treat cancer as a chronic disease and has been increasingly studied in the oncology field. Traditional approaches of anti-cancer therapy have not exploited these theoretical advantages. Current protocols typically apply treatment agents at the MTD until evidence of progression [33].

The study presented in **chapter 3** evaluated different designs of adaptive and intermittent treatment regimens with simulations based on the developed model. These regimens aim to prolong the duration of suppressing treatment resistance and thereby overcoming treatment resistance. The adaptive schedules also enabled the personalized design of therapy since the switch of drugs was guided by individual ctDNA measurements. The results of this study showed that the adaptive and intermittent treatment regimens, with appropriate designs, outperformed the conventional continuous treatment. The simulated intermittent regimen which consisted of an 8-week treatment and a 4-week suspension prolonged median progression-free survival (PFS) of the simulated population from 36 weeks to 44 weeks. The simulated adaptive regimens were shown to further prolong median PFS to 56–64 weeks.

Our results are in line with the evolutionary principle, and evidence that supports the feasibility of suggested regimens is present. An example of the adaptive therapy can be seen from the treatments of NSCLC patients. Acquisition of T790M mutation is the main mechanism of acquired resistance upon treatment of erlotinib/gefitinib in NSCLC patients, and osimertinib can be selected for T790M-positive patients [35]. In the study, we introduced a second hypothetical treatment targeting the resistant population that harbors *KRAS*

mutation. Lately, the U.S. Food and Drug Administration (FDA) also granted accelerated approval to the first KRAS-blocking drug [36]. This indicates the potential feasibility of successfully implementing the suggested adaptive treatment.

As for the intermittent treatment, the advantage has been seen from some clinical observations. A study has shown that adaptive intermittent treatment of abiraterone based on prostate-specific antigen (PSA) levels resulted in a better clinical outcome than the typical continuous treatment [34], although the study design may need to be refined [37]. Another retrospective analysis demonstrated that intermittent use of enzalutamide in metastatic castration-resistant prostate cancer patients prolonged the time to PSA failure and improved overall survival [38]. In patients with colorectal cancer, a re-challenge of EGFR blockade has shown to be efficient again [26]. Yet, several clinical studies failed to show improved outcomes in patients undergoing intermittent therapy and the underlined mechanism remains unclear [39-44]. We believe that, in this case, a model-based approach may be helpful for understanding these conflicting results and support identification of the optimal designs. For example, a previous *in silico* study indicated that an intermittent abiraterone followed by a lead-in period was not beneficial for prostate cancer patients, while the adaptive intermittent treatment guided by PSA was the best option [34]. Moreover, the results derived from our study also raised attention to the length of the treatment holiday if improved treatment outcome is desired, as extending the treatment holiday can result in inferior results.

Model-informed precision dosing (MIPD)

Quantify variabilities and identify covariates

Our studies in **section II** demonstrated that with the population modeling approach, the variabilities in PK/PD of a therapeutic agent as well as the influence of relevant covariates can be quantified. This would be of great importance to guide dose tailoring for an individual patient prior to the start of treatment to achieve personalized therapy. In **chapter 6**, we have developed a two-compartment population PK model which well described the PK of mitotane in patients with ACC. The covariates that significantly correlate with mitotane PK have been identified, which explained 35.8% and 30.7% of random inter-individual variabilities (IIV) on apparent clearance (CL/F) and central distribution volume (V_c/F), respectively. In this study, we were able to investigate separate effects of lean body weight (LBW) and fat amount (total body weight – LBW) on mitotane distribution volumes, as they are more physiologically plausible covariates [45, 46]. Furthermore, the

inter-occasion variability (IOV) on CL/F was also incorporated to capture the intra-subject variability. The estimates of IOV indicate an overall increasing clearance during the first 500 days followed by a decrease thereafter. This dynamic indicates that a self-induction in mitotane clearance, which has been suggested previously [45], may exist temporarily. This study also for the first time explored and quantified the potential effect of pharmacogenetic variation on mitotane clearance. Eventually, three SNPs, i.e. *CYP2C19**2 (rs4244285), *SLCO1B3* 699A>G (rs7311358), and *SLCO1B1* 571T>C (rs4149057), were included in the final model. The model estimated that carrying 'A' variant in *CYP2C19**2 reduced the mitotane CL/F by 44.9%. This is in line with the fact that the 'A' variant of *CYP2C19**2 is a nonfunctioning variant and has been demonstrated to decrease the activity of CYP2C19 [47, 48]. The power of pharmacogenetic analysis may be influenced by the small number of included patients and the exploratory characteristic of this analysis. However, as the dataset enabled differentiation between IIV and IOV, the certainty of the possible genotype effect on clearance, which is more likely to be covered by IIV, was increased. Our result suggests that enzyme CYP2C19 and transporters SLCO1B3 and SLCO1B1 for drug uptake in the liver might be involved in mitotane PK pathways, and their polymorphisms should be considered for mitotane dose selection, but further validation is required to translate the findings into an implementable clinical recommendation.

The study in **chapter 7** performed a population PK analysis for high-dose methotrexate (HD-MTX) in patients with central nervous system (CNS) lymphoma based on data from 3 medical centers. In addition to the impact of patients' demographics and physiological condition on HD-MTX PK, the study also enabled an investigation on the variation among patients from different medical centers receiving different treatment regimens. The results show that the identified covariates on clearance (CL) of MTX are in accordance with the known PK characteristics of MTX [49, 50]. Moreover, the CL of MTX also showed to vary among treatment regimens, and the difference in CL was able to be quantified. This might suggest a need to alter the dose when targeting to the same level of exposure. The possible factors that contributed to this result could be the differences in infusion duration / rate of HD-MTX, patients' status, and the combined medications among these treatment groups. However, the impact of those factors cannot be distinguished as they highly overlapped with each other. The included covariates in the final model explained 46.9% of the variability on CL between and within patients. Additionally, body weight was identified as a significant covariate on distribution volume of central compartment which reduced random IIV significantly. Currently, HD-MTX is dosed per body surface area (BSA) in CNS lymphoma patients. However, our study demonstrated that the influence of BSA on MTX PK is less significant, although BSA has been identified as a covariate in

previous PK studies [51, 52]. A few previous studies have also pointed out that BSA is not the most predictive factor to MTX PK, and BSA-guided dosing should be reconsidered especially for overweight patients [53-55]. In our study population, an increasing trend of the estimated MTX area under the concentration-time curve (AUC) from 24 hours after drug administration to infinity ($AUC_{24-\infty}$) and MTX concentration at 24 hours (C_{24h}) over BSA has also been observed. Additionally, a dose reduction for HD-MTX has already been suggested for patients with reduced renal function [56, 57]. Taking these facts into account, a potential to dose HD-MTX with a model-based approach that involves multiple covariates including renal function is implied. This is considered to be more rational and accurate than BSA-guided dosing, and can help to further reduce PK variability.

Better prediction of toxicity

Toxicity can cause unfavorable outcomes in the treatment of cancer patients. Because of this, studies on risk factors and thresholds that predict high toxicity are of great importance. In **chapter 7**, the baseline predictors as well as exposure thresholds that predict a high risk of renal and hepatotoxicity in patients with CNS lymphoma treated with HD-MTX were identified with the model-based approach. Based on the modeling and simulation results, we recommended a baseline eGFR target of $> 66.6 \text{ mL/min/1.73 m}^2$ for patients with CNS lymphoma to use HD-MTX in order to lower the probability of renal toxicity. This is in accordance with a previous review which indicated that renal function is a key prognostic factor for the tolerance of HD-MTX [57]. Additionally, a higher risk for hepatotoxicity in CNS lymphoma patients is foreseeable if the administrated dose of HD-MTX is higher than 3500 mg/m^2 . The study also identified correlations between MTX exposure metrics and renal toxicity. In addition to the AUC of MTX, C_{24h} was also investigated as an exposure metric, as a threshold on C_{24h} is valuable for early identification of patients at risk and early application of rescue treatment. The modeling results provided potential exposure thresholds that correlate with a high risk of renal toxicity in patients with CNS lymphoma ($> 60\%$). The threshold of C_{24h} ($8.66 \text{ } \mu\text{mol/L}$) is also in line with what was found in a previous study ($10 \text{ } \mu\text{mol/L}$) [56]. For patients with a higher risk of toxicity that still need HD-MTX treatment, they should be carefully monitored and rescue therapy with high dose folate or, in severe cases, glucarpidase could be considered [58-60]. In addition, due to the feature of mixed-effect modeling, once patients' toxicity results of the first cycle are known, the model can also be applied to provide individual threshold that predicts high toxicity. In this circumstance, we believe our study holds great potential for further individualizing HD-MTX dosage and preventing acute organ toxicity, which can improve HD-MTX therapy in CNS lymphoma patients.

Guide individualized treatment

Based on the identified covariates and pre-defined therapeutic targets, coupled with Bayesian forecasting, MIPD can be applied to guide optimal initial dose selection and dose adaptation for cancer patients. The optimal therapeutic drug monitoring (TDM) strategies can also be explored. The study presented in **chapter 6** designed and evaluated several mitotane dosing strategies, given that TDM was performed, by simulating with the final population PK model. The results indicated that determining the starting dose with the developed model considering included covariates is most beneficial in terms of shortening the time to reach the therapeutic target, compared with starting with the fixed dose for all patients. This design can also limit the risk of toxicity to a relatively low level, together with the designed TDM strategies. Under the setting of individualized starting dose, the regimens with stepwise increasing dose at the start required less time to reach the therapeutic target, while the one with constant starting dose demonstrated the lowest risk of having toxicity. However, due to the fact that a shorter time to reach the therapeutic target is normally paired with a higher probability of toxicity, it is suggested to consider patients' condition on whether the increased risk of having toxicity can be tolerated in order to gain the benefit of reaching the therapeutic target quicker when selecting a dosing regimen. A regimen with a loading dose followed by a maintenance dose would also be desired to allow a fast target attainment. However, we didn't consider this regimen in our study as it requires a high dosage which is not tolerable for most patients. When one (or more) TDM result becomes available, individual parameters could be estimated with the population PK model. The dose amount for subsequent drug administrations can then be determined according. This approach is also demonstrated to be a promising strategy which was predicted to further decrease the risk of toxicity while providing a satisfactory target reaching time. Only that patients' tolerance to the high level of dose increase needs to be considered when applying this strategy. Potentially, with the individual PK parameters, an adequate dose for maintaining a steady drug concentration level after reaching the therapeutic window can be estimated so that the frequency of dose adaptation can be decreased. In **chapter 7**, our findings imply that dosing HD-MTX with a model-based approach would potentially be more rational for further reducing PK variability. In addition, on the basis of our results on toxicity analysis, further investigation on the exposure-response relationship of MTX would be of interest for establishing a therapeutic range for HD-MTX for future model-based personalized dosing.

Challenges and future perspectives

Addressing treatment resistance considering evolutionary resistance development and applying precision treatment would be beneficial to improve the treatment outcome for oncology patients. The results presented in this thesis show that with the quantitative models, the evolutionary tumor progression and PK/PD of anti-cancer drugs can be characterized and predicted, thereby optimal treatment strategies can be designed and evaluated for oncology patients. However, beyond what has been demonstrated and discussed, challenges still remain regarding data availability, model development, and validation and implementation of the results. Further research and collaborations are needed to overcome the challenges and facilitate better implementation of the findings in the clinic.

Section I

Knowledge and data availability

In order to make use of genetic biomarkers to understand the dynamics of tumor sub-clones, previous knowledge of the genetic variants that reflect treatment sensitivity is required. Available data is also essential for developing models to characterize the correlation between anti-cancer treatment responses and biomarkers, and to support decision making. As for ctDNA, although its value in oncology treatment has now been increasingly acknowledged, ctDNA monitoring has not yet been widely applied in routine clinical practice and the availability and collection of longitudinal ctDNA data are limited [31, 32, 61]. Whether patients had metastatic disease and the available sequencing assay and gene panel can also impact the availability of ctDNA data. In **chapter 3**, detectable mutant *KRAS* concentrations were only available from 9 patients out of 25 mCRC patients. In **chapter 4**, detectable mutant *EGFR* VAFs were available in 13 out of 18 NSCLC patients. The limited capability to develop a ctDNA dynamics model and adequately estimate all parameters. The missing data, such as the missing baseline ctDNA measurements in **chapter 4**, may also affect the interpretation of the results. Therefore, more and more detailed data is desired to validate our findings. Since ctDNA is being increasingly studied and the analysis method is improving, together with active collaborations, we see opportunities in the future to gain sufficient knowledge and data on longitudinal ctDNA measurements. This will better support the development of models capturing ctDNA dynamics and the incorporation of ctDNA time curves in the tumor dynamics model, which would benefit the in-depth study on evolutionary resistance development. In addition, once an adequate model is developed, sparsely sampled data can also be well utilized and missing data can be imputed rationally. Currently, effort is being made to establish standards and best practices to better systematize the evaluation

of ctDNA kinetics [31]. Moreover, if sequencing data of multiple variants are available, efforts need to be made to handle these data in a quantitative manner and a selection of variants to be included in the analysis may be required.

Model development for evolutionary tumor progression

When modeling tumor dynamics in our studies, the sum of the longest diameters (SLD) of all target lesions has been the observation of interest. Nevertheless, the dynamics of each separate lesion would also be suitable for supporting the investigation of the progression of heterogeneous tumors, especially when differences can be observed between primary and metastatic lesions. Thus, further investigation on the dynamics of separate lesions and comparing the findings with what is presented in this thesis can be of interest for future studies.

In addition to what are proposed in this thesis, other modeling strategies that characterize evolutionary tumor dynamics are also available, which can be applied in studies having different focuses. One example would be game theory models which have a stronger focus on the interaction and payoff matrix among different cell populations. The changes in the fitness of cells (fitness cost or benefit) when interacting with therapy and other types of cells are accounted for in game theory models [19, 34]. Another commonly applied modeling strategy is stochastic models which allow describing the stochastic process of proliferation, death, and mutation of tumor cells in the tumor, although the expected outcome can be comparable to those that are derived from ordinary differential equations [62]. In addition, the studies presented in this thesis assumed tumor cells accumulate one mutation that leads to resistance to one drug each time. The possibility of acquiring multiple mutations at a time which leads to multi-drug resistance has not been included in the analysis. This can also be a point of consideration for future studies.

In terms of modelling the time-curves of ctDNA measurements, our study presented in **chapter 3** proposed a concept model for capturing ctDNA dynamics which consists of a sub-clonal tumor-size dependent generation and a first-order elimination. The model considered the correlation between tumor size and ctDNA amount and well characterized the data from mCRC and NSCLC patients. We have also seen recent studies applying models that are classically used to capture tumor size to describe ctDNA time course dynamics. One study characterized the time-curves of mutant *EGFR* in ctDNA in NSCLC patients with a model with zero-order increase, first-order decay, and time-dependent regrowth, and tumor size dynamic was not incorporated [63]. Another study successfully modeled the ctDNA time course using a bi-exponential model (first-order increase and first-order decay) [64]. The correlation between tumor shrinkage and ctDNA drop has been observed

and described by linking the decay rates of tumor sizes and ctDNA data [64]. These studies provide more simple model options with fewer parameters for future pharmacometric studies. However, the underlying biology and tumor heterogeneity were not considered [64]. Moreover, in addition to characterizing the observed data, the prediction of newly acquired mutation which has not yet occurred in the data would also be interesting to be further explored.

Validation and extrapolation of the proposed model and treatment design

The studies in **section I** illustrated how quantitative models can support the study on evolving tumor progression and treatment optimization so that anti-cancer resistance can be better overcome. However, due to the characteristics of being based on limited data, further validation with external datasets is required to confirm the performance of the model and the added value of the suggested schedules. In addition, prospective clinical studies are warranted before the application of the suggested treatment designs. The validation should concern not only the predictability on the observed time-curves of data, but also on the treatment outcome such as PFS. Regarding clinical trials, several clinical studies on intermittent therapies have been reported, which however failed to show improved outcomes and the underlined mechanism remains unclear [39-44]. The need for clinical trials on adaptive therapy guided by ctDNA is however not met yet [31]. Currently, our group is carrying out a clinical study on intermittent enzalutamide therapy in prostate cancer patients (NCT05393791). The findings would be of great value to evaluate the concept proved in our study.

In addition, our studies were mainly performed in mCRC and NSCLC patients treated with anti-EGFR therapies, and focused explicitly on the use of tumor size measurements and ctDNA data. It would be of interest for future studies to extrapolate the concept models and findings to other targeted treatments and cancer types. Moreover, other oncologic biomarkers would in principle also be valuable to provide insight into the evolutionary dynamics of tumor and guide treatment. A previous study has demonstrated the value of PSA in guiding the intermittent treatment of prostate cancer patients [34].

Furthermore, to support further research and enable the achievement of the ultimate goal of optimizing and personalizing anti-cancer treatment, a multidisciplinary collaboration is essential. This is due to the requirement of in-depth knowledge about tumor and clonal dynamics as well as skills needed for complex modeling and simulation.

Implementation of proposed treatment design

Challenges also remain to apply the proposed novel treatment strategies in **chapter 3** that could better overcome resistance in clinical practice. First of all, our study indicated that intermittent therapy may only work for the responders to certain targeted treatment. Thus, for patients who had detectable resistance mutation pre-treatment, a better option would be to choose another treatment from start. Moreover, despite that the intermittent regimens were predicted to provide better treatment outcome than the continuous regimen in a population level, opposite results can be seen when looking at simulated subjects individually, same as when comparing adaptive and intermittent regimens. This indicates that variability between individuals can affect the choice of regimen. Thus, the idea of individual intermittent treatment, the concept of which has been proposed in the treatment of prostate cancer patients [34], could be further investigated.

Furthermore, in order to apply adaptive treatment guided by ctDNA measurements, the mutations indicating sensitivity to treatment need to be acknowledged beforehand. If multiple mutations have been reported, a selection may be required based on the strength of evidence and capability of the quantification technique, such as the gene panel in the assay and the number of mutations that can be detected simultaneously. To strengthen clinical implementation of ctDNA in the future, the turnaround times of the sequencing assays should also be short. In **chapter 3**, the study focused on the most representative mutation that is associated with resistance. However, not all patients developed detectable *KRAS* mutation during the course of treatment. This indicates that in order to better implement adaptive treatment, multiple relevant mutations may need to be considered. In addition, our study demonstrated that the frequency of monitoring ctDNA and the thresholds of adjusting treatment also matters when implementing adaptive treatment to improve treatment outcome. We have evaluated frequencies of once every 4–12 weeks which has been shown to be feasible [13, 65], but there is no clear validated optimal time point for ctDNA analysis [31]. The sampling frequency can also depend on the disease, therapy, sequencing assays, financial burden, and burden on the patients. After validation, the proposed computational model can be of help to inform the best practice on monitoring ctDNA and guide optimized treatment accordingly [31].

Section II

Implementation of MIPD

As discussed in **chapter 5**, to facilitate the implementation of MIPD in clinical practice, efforts are still required to overcome several challenges, such as to evaluate the model and to translate the research findings into user-friendly MIPD software [7]. Currently, multiple programs have been developed and are already in use for model-informed TDM [7, 66]. In **chapter 6**, we have also developed a Shiny app to elucidate how precision dosing advice of mitotane for ACC patients can be informed by the developed population PK model. We have implemented the final PK model and an optimized individualized dosing regimen into this app. With this program, based on the input of the characteristics of a certain patient, an individualized starting dose can be determined by the model and be visualized together with the predicted mitotane concentration-time curves for this patient. Currently, the build-in algorithm only allows the determination of the starting dose according to the input information corresponding to the included covariates. As a R package that supports empirical Bayesian estimation is now available [67], we see a potential to implement the regimen where a more precise dose amount can be determined according to individual parameters estimated based on available TDM results. Nevertheless, this app is currently intended for research purpose only. Validation in hospital settings is still needed for its application in clinic or transferring the model to a commercial platform.

Moreover, given that programs are available for model-informed TDM, the developed models in our studies are believed to be able to be further applied to support model-based TDM of mitotane and high-dose MTX.

Further PK/PD analysis for precision dosing

In addition to PK, variabilities in PD should also be taken into consideration when implementing precision treatment. FDA recently proposed the Project Optimus which encourages improving dose selection and optimization for oncology drugs by accounting for both efficacy and tolerability rather than automatically selecting the MTD [8, 68]. In **chapter 7**, we have developed a toxicity model which allows quantifying the probability of having renal or hepatotoxicity in patients with CNS lymphoma treated with HD-MTX given the value of risk factors. The identified exposure thresholds on C_{24h} can also be applied to guide the early use of rescue therapy. Nevertheless, in order to better guide personalized treatment, further PK/PD analyses are still warranted. Firstly, in addition to already investigated factors, the impact of pharmacogenetic polymorphisms on the PK and toxicity probability in patients with CNS lymphoma treated with HD-MTX would

be of interest to future studies. Previous studies have demonstrated the influence of ABCC2 polymorphisms on the PK of HD-MTX in patients with lymphoid malignancy [69, 70]. Gene *MTHFR*, *SLC19A1*, and *ABCB1* were reported to potentially associate with an increased risk for hepatic toxicity [71]. Exploring the impact of pharmacogenetic polymorphisms has the potential to better explain inter-patient variability. Additionally, studies on the penetration of MTX to the CNS would also be of interest as CNS is the target site of MTX and neurotoxicity is also a major problem for patients receiving HD-MTX treatment. This goal can be achieved by applying physiologically based pharmacokinetic modelling (PBPK) approach [72]. Furthermore, although high drug exposure can result in toxicity, sufficient exposure is still essential to guarantee the efficacy. In our study, an exposure-efficacy relationship was not investigated. A previous study suggested that $AUC_{0-\infty} > 1100 \mu\text{mol/L}\cdot\text{h}$ is associated with a favorable treatment outcome [73]. Due to an identified correlation of $AUC_{0-\infty}$ with C_{24h} , the same group recommended a C_{24h} target of 4–5 $\mu\text{mol/L}$ [74]. Nonetheless, the direct relationship between C_{24h} and the efficacy has not been reported. Therefore, further investigation is warranted to explore the possibility of establishing a therapeutic range for HD-MTX, which could better facilitate future personalized dosing.

Conclusion

Addressing treatment resistance considering evolutionary resistance development and applying personalized drug treatment would be beneficial to improve the treatment outcome for oncology patients. This thesis has applied the quantitative modeling approach to characterize the evolutionary tumor dynamics and ctDNA dynamics and quantify PK/PD variabilities for anti-cancer drugs. The developed model can facilitate the identification of optimal treatment designs and guide individualized treatment rationally, although challenges remain for the results implementation and further research and more data is warranted to validate the findings and support better practice of personalized treatment.

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