Pharmacometric Evaluation of Biomarkers to Improve Treatment in Oncology

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Abstract


Cancer is a family of many different diseases with substantial heterogeneity also within the same cancer type. In the era of personalized medicine, it is desirable to identify an early response to treatment (i.e., a biomarker) that can predict the long-term outcome with respect to both safety and efficacy. It is however not uncommon to categorize continuous data, e.g., using tumor size data to classify patients as responders or non-responders, resulting in loss of valuable information. Pharmacometric modeling offers a way of analyzing longitudinal time-courses of different variables (e.g., biomarker and tumor size), and therefore minimizing information loss.

Neutropenia is the most common dose-limiting toxicity for chemotherapeutic drugs and manifests by a low absolute neutrophil count (ANC). This thesis explored the potential of using model-based predictions together with frequent monitoring of the ANC to identify patients at risk of severe neutropenia and potential dose delay. Neutropenia may develop into febrile neutropenia (FN), a potentially life-threatening condition. Interleukin 6, an immune-related biomarker, was identified as an on-treatment predictor of FN in breast cancer patients treated with adjuvant chemotherapy. C-reactive protein, another immune-related biomarker, rather demonstrated confirmatory value to support FN diagnosis.

Cancer immunotherapy is the most recent advance in anticancer treatment, with immune checkpoint inhibitors, e.g., atezolizumab, leading the breakthrough. In a pharmacometric modeling framework, the area under the curve of atezolizumab was related to tumor size changes in non-small cell lung cancer patients treated with atezolizumab. The relative change from baseline of Interleukin 18 at 21 days after start of treatment added predictive value on top of the drug effect. The tumor size time-course predicted overall survival (OS) in the same population.

Circulating tumor cells (CTCs) are tumor cells that have shed from a tumor and circulate in the blood. CTCs may cause distant metastases, which is related to a poor prognosis. A novel modeling framework was developed in which the relationship between tumor size and CTC count was quantified in patients with metastatic colorectal cancer treated with chemotherapy and targeted therapy. It was also demonstrated that the CTC count was a superior predictor of OS in comparison to tumor size changes.

In summary, IL-6 predicted FN, IL-18 predicted tumor size changes and tumor size changes and CTC counts predicted OS. The results in this thesis were obtained by using pharmacometrics to evaluate biomarkers to improve treatment in oncology.

Keywords: Pharmacometrics, Biomarkers, Oncology, Population PKPD Modeling, NONMEM

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Nog finns det mål och mening i vår färd - men det är vägen, som är mödan värd.

*ur ”I rörelse” av Karin Boye*

_Till mina ♥♥_
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


V **Netterberg I**, Karlsson MO, Terstappen LWMM, Koopman M, Punt CJA, Friberg LE. Circulating tumor cell counts is a better predictor of overall survival than dynamic tumor size changes – a quantitative modeling framework. *Submitted."

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### Contents

1 Introduction ......................................................................................... 11  
1.1 Cancer ............................................................................................. 11  
1.2 Treatment in oncology .................................................................... 12  
1.2.1 Chemotherapy ........................................................................ 13  
1.2.2 Cancer immunotherapy .......................................................... 14  
1.3 Clinical endpoints in oncology ....................................................... 15  
1.4 Biomarkers ...................................................................................... 16  
1.4.1 Managing toxicity .................................................................. 16  
1.4.2 Predicting efficacy ................................................................. 16  
1.5 Pharmacometrics ............................................................................. 18  
1.5.1 General concepts .................................................................... 19  
1.5.2 Myelosuppression model ....................................................... 20  
1.5.3 Tumor size models ................................................................ 21  
1.5.4 Indirect response models ....................................................... 21  
1.5.5 Count models ......................................................................... 22  
1.5.6 Time-to-event models ............................................................ 22  
2 Aims ..................................................................................................... 24  
3 Methods ............................................................................................... 25  
3.1 Patients and data .............................................................................. 25  
3.1.1 Data to manage toxicity ......................................................... 25  
3.1.2 Data to predict efficacy .......................................................... 27  
3.2 Exploratory analysis of atezolizumab biomarkers ......................... 28  
3.3 Applied and developed models ....................................................... 28  
3.3.1 Myelosuppression model – predicting daily ANC ................. 29  
3.3.2 Atezolizumab pharmacokinetic model .................................. 31  
3.3.3 Characterization of biomarker time-courses .......................... 31  
3.4 Time-to-event models ..................................................................... 34  
3.4.1 Febrile neutropenia ................................................................. 34  
3.4.2 Dropout .................................................................................. 35  
3.4.3 Overall survival ...................................................................... 35  
3.5 Data analysis ................................................................................... 37  
4 Results ................................................................................................. 39  
4.1 Description of the analyzed data ..................................................... 39  
4.1.1 CRP, IL-6 and febrile neutropenia ........................................... 39
Abbreviations

AAD All available data
ANC Absolute neutrophil count
AUC Area under the curve
C2YASOT Data censored no later than 2 years after start of treatment for each individual patient
C2YE Data censored no later than at a cut-off date set 2 years earlier than in AAD
C5MALD Data censored a maximum of five months after last dose
CD40 Cluster of differentiation 40
CFB Change from baseline
CI Confidence interval
CIT Cancer immunotherapy
CL Clearance
CRP C-reactive protein
CT Computed tomography
CTC Circulating tumor cells
ctDNA Circulating tumor DNA
DF Degree of freedom
FDA Food and Drug Administration
FEC-Doc 5-fluorouracil (5-FU)-epirubicin-cyclophosphamide and docetaxel
FN Febrile neutropenia
FOCE First-order conditional estimation
FOCEI First-order conditional estimation with interaction
G-CSF Granulocyte colony-stimulating factor
ICAM-1 Intercellular adhesion molecule 1
ICI Immune-checkpoint inhibitor
IDR Indirect response
IIV Inter-individual variability
IL-6 Interleukin 6
IL-8 Interleukin 8
IL-18 Interleukin 18
1 Introduction

There is an unmet medical need in cancer patients despite multiple available treatment options. Accounting for 9.6 million deaths and 18.1 million new cases in 2018, cancer is one of the leading causes of mortality and morbidity worldwide [1]. Consequently, individualization of available treatments as well as development of new therapies are needed. Drug development in oncology is, however, challenging, partly due to the extensive variability in pathogenesis both between and within tumors [2]. One approach to tackle this challenge is to use biomarkers. Biomarkers are characteristics that are objectively measured and evaluated as indicators of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention [3], which can provide valuable predictive properties and therefore be used to predict drug response and consequently to individualize cancer therapy.

Pharmacometric models provide a tool to characterize pharmacokinetic (PK)-pharmacodynamic (PD) (PKPD) relationships between a drug, biomarker, therapeutic efficacy and safety and has been applied both to support drug development and in clinical practice [4–6]. Thus, such models can be used to optimize treatment with anticancer drugs, both with respect to their effect but also the management of toxicity.

1.1 Cancer

Cancer is a family of many different diseases which manifests by uncontrolled cell growth, caused primarily by non-intrinsic factors (e.g., carcinogens, viruses, smoking, nutrient intake, immune and hormone levels) but also (to a lesser extent) by unavoidable spontaneous mutations [7]. Lung cancer is the most common and deadliest (in terms of absolute numbers) cancer type worldwide, across men and women, followed by breast, colorectal and prostate cancer [1]. However, the incidence of different cancer types varies around the world. As an example, the most, second and third most common cancer types in Sweden are prostate, breast and colorectal cancer, respectively, while lung cancer is the fifth most common cancer type [8]. Figure 1 shows a summary of the estimated new cancer cases and deaths in 2018 for the ten most common cancer types in the world and in Sweden [8].
1.2 Treatment in oncology

Historically, the evolution of cancer treatment has been relatively slow. The discovery of anesthesia in the 19th century enabled surgical removal of tumors. Ever since, the surgical methods have been improved and refined, which is why surgery has been, and still is, one of now five pillars of cancer treatment. Radiotherapy was introduced as a second pillar half a century later as Wilhelm Conrad Roentgen presented his discovery of x-rays. Chloromethine (a nitrogen mustard) was the first anti-cancer drug to ever be approved by the Food and Drug Administration (FDA) in the mid-20th century, which was developed as a result of chemical warfare during World War I and additional research in relation to World War II [9]. Nitrogen mustards are non-specific DNA alkylating agents and belong to the third pillar of cancer treatment, i.e., chemotherapy. Additional drug development has resulted in over 100 approved chemotherapeutic drugs. The use of targeted therapy, the fourth pillar, which only act on specific molecules involved in the development of cancer, has increased in the past couple of decades with rituximab as the first FDA approved targeted cancer drug [10]. Cancer immunotherapy (CIT) is the most recent anti-cancer breakthrough, although its first use in patients dates back to the late 19th century when William Coley (“the father of CIT”) injected a mixture of live and inactive bacteria into and around tumors and observed durable remission in several malignancies. Coley was, however, criticized because of the potential and unpredictable toxicity and thus the clinical application of CIT was put on hold [11]. However, CIT experienced a revival in parallel with the finding that cancer can be treated by the inhibition of immune checkpoints (“brakes” of the immune response), a discovery awarded with the 2018 Nobel Prize for Medicine [12]. CIT is now also accepted as the fifth pillar of cancer treatment.
Both tumor (e.g., size, spread and mutational status) and individual (e.g., performance status) characteristics influence the selection of cancer treatment. Commonly, a combination of a main treatment together with additional treatments, given either before (neoadjuvant) or after (adjuvant) the main treatment, is used.

Two of the above mentioned pillars of cancer treatment are in focus in this thesis, i.e., chemotherapy (myelosuppressive) and CIT (with an immune checkpoint inhibitor, ICI).

1.2.1 Chemotherapy

Chemotherapy is an important treatment option which may be used with the intention to cure the disease, control cell growth or improve quality of life. Tumor cells may, however, become resistant to chemotherapy, resulting in an initial decrease in tumor size followed by regrowth. Chemotherapy is unspecific and interferes with key steps in the cell cycle of rapidly dividing cells, such as tumor cells but also healthy cells, e.g., cells of the immune system. Dosing of chemotherapeutic drugs has traditionally relied on the belief that higher drug exposure results in better response, without ever reaching a maximum response. Consequently, the tolerated dose is limited by toxicity. For many chemotherapeutic drugs, the dose-limiting toxicity is bone-marrow toxicity, also referred to as myelosuppression, which is characterized by an abnormally low number of blood cells [13]. The neutrophil is the most abundant white blood cell (i.e., immune cell) in humans [14] and plays an important role in the innate immune system during the acute response to inflammation [15]. Neutropenia is a condition with abnormally low absolute neutrophil count (ANC), commonly as a consequence of chemotherapy, and is graded according to the common terminology criteria for adverse events by the National Cancer Institute [16],

- Grade 1 (mild): $\text{ANC} < 2.0 - 1.5 \cdot 10^9 \text{cells/L}$
- Grade 2 (moderate): $\text{ANC} < 1.5 - 1.0 \cdot 10^9 \text{cells/L}$
- Grade 3 (severe): $\text{ANC} < 1.0 - 0.5 \cdot 10^9 \text{cells/L}$
- Grade 4 (life-threatening): $\text{ANC} < 0.5 \cdot 10^9 \text{cells/L}$

The ANC is monitored in patients receiving chemotherapy to discover severe neutropenia. However, ANC monitoring is often sparse and patients with neutropenia may face life-threatening infections. Approximately 25% of early breast cancer (i.e., the tumor has not yet spread beyond the breast) patients treated with adjuvant 5-fluorouracil (5-FU)-epirubicin-cyclophosphamide and docetaxel (FEC-Doc) experience febrile neutropenia (FN) [17]. FN is severe neutropenia (grade 4 or ANC expected to fall below $0.5 \cdot 10^9 \text{cells/L}$) in combination with fever (oral temperature $> 38.3^\circ\text{C}$ or two consecutive values of oral temperature $> 38^\circ\text{C}$ during 2 h) [18]. An overall mortality of 9.5% in inpatients with FN has been reported, but the rate depends on the cancer type,
demographic factors, the type of infection and comorbidities [19]. Additional consequences of FN include substantial increases in healthcare costs [19] as well as reduced relative dose intensity (i.e., the ratio of delivered to planned chemotherapy dose), which has been related to worse survival [20].

Chemotherapy is often given in cycles of approximately 2-4 weeks in order for the bone-marrow to recover sufficiently, since myelosuppressive chemotherapy exhausts the bone-marrow. However, ANC monitoring is often limited to only just before the next dose is to be given. Potential consequences of the limited ANC sampling include i) delay in identification of patients at high risk of experiencing life-threatening neutropenia, ii) inconvenience for the patient and the clinic if the next cycle needs to be delayed due to low ANC [21, 22] when the patient is scheduled for the next cycle and iii) too cautious choice of dose and thereby suboptimal therapy. Thanks to recent technical advances, it is now possible for patients to monitor their ANC more frequently at home without the need of additional medical assistance [23]. Utilization of frequent ANC measurements, together with model-derived predictions, provide a possibility to improve therapy management by identifying patients that potentially need rescue-medication, e.g., granulocyte colony-stimulating factor (G-CSF) and/or antibiotics, and to predict, several days prior to initiation of the next cycle, if the subsequent dose can be administered on the planned day.

1.2.2 Cancer immunotherapy

The immune system is a complex defense system that protects the host against diseases by detecting unknown structures and eliminate them. Not only does the immune system fight infections, but it also plays an important role in avoiding tumor formation. This part of the immune system has been described as the cancer immunity cycle. The ability to avoid immune destruction by tumor cells, by disrupting the cancer immunity cycle, has been recognized as a hallmark of cancer [24]. The goal of CIT is therefore to sustain and/or reinitiate a functioning cancer immunity cycle without activating autoimmune inflammatory reactions [25].

There are several types of CITs, e.g., cancer vaccines, adoptive cell transfer and oncolytic viruses. This thesis focuses on an ICI. Several ICIs have shown efficacy in a range of cancers during the recent years and continue to attract interest in drug development [26–29], with Ipilimumab being the first ever approved ICI [30]. Another ICI is atezolizumab, an immunoglobulin G1 monoclonal antibody, which inhibits the programmed death-ligand 1 (PD-L1). PD-L1 is one of the so-called immune checkpoints, which suppresses the immune response upon binding to programmed cell death protein 1 (PD-1) or B7-1 expressed on the surface of cytotoxic T cells. Many tumor cells express PD-L1 as a mechanism of evading immune destruction, which is why blockade of PD-L1 results in a sustained immune response and killing of tumor cells by
cytotoxic T cells [31]. The theory behind the atezolizumab mechanism of action is illustrated in Figure 2. As of today, atezolizumab is approved for treatment in bladder, non-small cell lung cancer (NSCLC), triple-negative breast cancer and extensive-stage small-cell lung cancer [32].

1.3 Clinical endpoints in oncology

The Biomarkers Definition Working Group defines a clinical endpoint as “a characteristic or variable that reflects how a patient feels, functions, or survives” [3]. Overall survival (OS) corresponds to the time from randomization (in a clinical study) to death from any cause. Improvement of survival is the most reliable cancer endpoint to provide evidence of clinical benefit and ultimately to support drug approval. However, due to long follow-up times surrogate endpoints may be used to demonstrate clinical efficacy and may result in accelerated approval for new drugs [33]. Several surrogate endpoints rely on assessment of the tumor size, e.g., objective response rate and progression-free survival. Computed tomography (CT) scans are commonly used to measure cancer lesions. The sum of longest diameter (SLD) of all target lesions is computed at baseline, i.e., just before start of treatment, and thereafter regularly during treatment, for example every six or nine weeks. Response Evaluation Criteria in Solid Tumours (RECIST) is used based on these measurements to identify complete response, partial response, progressive disease and stable disease [34].
1.4 Biomarkers

Biomarkers may be used to improve the understanding of the development of a disease and the response to treatment by providing information about a clinical endpoint (safety- and/or efficacy-related) before the clinical endpoint can be observed. Biomarkers are identified by establishing their clinical relevance (i.e., appropriateness to answer relevant clinical questions) and their validity (effectiveness as a biomarker). Validity is, however, a questioned term as it implies that the often complex underlying biology is fully understood. Evaluation is another common term, referring to the ongoing process of studying a biomarker and relating it to a clinical endpoint [35].

1.4.1 Managing toxicity

Primary prophylaxis with G-CSF is recommended by several guidelines when the risk of developing FN, as a consequence of chemotherapy, is $\geq 20\%$ [36–38]. Patients treated with FEC-Doc have a risk of developing FN $\geq 10\%$ [39]. Prophylaxis should, however, be avoided in patients that would not benefit significantly from such therapy, due to potential toxicity and related healthcare costs. It would therefore be of value to improve the prediction of FN before clinical signs of FN become apparent but after administration of chemotherapy. Most of the factors that have been identified as predictors of FN are static, e.g., a baseline value. Alternatively, the dynamics of circulating immune-related biomarkers (other than the ANC) may provide additional predictive or diagnostic value. A pronounced increase in the biomarker concentration could signal that the patient is at high risk of developing FN. In a previous study, the frequently used semi-mechanistic myelosuppression model [40] (illustrated below), was used to describe the time-course of chemotherapy-induced myelosuppression and it was found that a rapid decline in the ANC was related to the probability of developing FN [41].

Interleukin 6 (IL-6) and C-reactive protein (CRP) are two immune-related circulating biomarkers that could potentially be used to predict FN in patients receiving myelosuppressive chemotherapy. IL-6 is produced mainly by macrophages in response to the recognition of pathogen-associated molecular patterns and stimulates the production of CRP by hepatocytes [42]. Both IL-6 and CRP were measured in a previous clinical study [43], however, no pharmacometric analysis has explored the relationship between the longitudinal IL-6 and CRP time-courses and FN.

1.4.2 Predicting efficacy

Biomarkers, or predictors, with respect to efficacy can be separated into three subgroups in this thesis; i) immune-related biomarkers, ii) tumor size-related
markers and iii) circulating tumor cells (CTCs). These are described more in detail below.

1.4.2.1 Immune-related biomarkers
Atezolizumab therapy has demonstrated prolongation of OS, however, not all patients show favorable response to treatment [44]. Consequently, it is important to identify patients that may benefit from atezolizumab treatment. Baseline expression of PD-L1 on tumor and immune cells have been extensively investigated to guide patient selection for treatment with checkpoint inhibitors. Patients with high PD-L1 expression respond better to treatment, although some variability in the response remains unexplained [44–47]. Circulating peripheral immune biomarkers could add predictive value in addition to PD-L1 expression and potentially be used to evaluate treatment efficacy earlier than possible with tumor response or OS.

Both interleukin 18 (IL-18) and interferon-inducible T-cell alpha chemo-attractant (ITAC) are biologically relevant to the mechanism of action of atezolizumab. IL-18 induces interferon gamma, which stimulates and modulates the immune response [48]. ITAC is a chemokine that attracts activated T cells [49]. Dynamic changes in these peripheral markers could potentially reflect treatment response to atezolizumab (i.e., tumor size changes and OS). Additional biomarkers that could be related to the effect of atezolizumab include cluster of differentiation 40 (CD40), CRP, intracellular adhesion molecule 1 (ICAM-1), interleukin 8 (IL-8), vascular cell adhesion molecule 1 (VCAM-1) and proliferating and activated CD8+ T cells expressing human leukocyte antigen – DR isotype and antigen ki67 (TCD8,prolif/activ).

1.4.2.2 Tumor size-related markers
Variables derived from the tumor size may be used to predict OS. Such variables include the tumor size before treatment (i.e., baseline), a ratio between the baseline tumor size and the tumor size (TSR) at a certain week and time to tumor growth (TTG) but also more dynamic metrics derived from PKPD-modeling of tumor size changes, e.g., the tumor growth rate, the current rate of tumor size changes and the tumor size time-course itself. The mentioned variables are illustrated in Figure 3. Modeling frameworks including both tumor size changes and OS have been commonly applied in the area of pharmacometrics [4, 50, 51], although published analyses applied in CIT are limited [52–54].

1.4.2.3 Circulating tumor cells
A relatively new biomarker in oncology is the CTC count. Measuring the CTC count is often referred to as a liquid biopsy, in contrast to the more invasive conventional biopsy where a sample of the tumor is extracted. Liquid biopsies offer a fast and cost-efficient way to follow disease status by a simple blood sample [55]. CTCs may cause development of metastases if they survive their
journey through the vasculature after shedding from the tumor [56] and high CTC counts have been related to worse OS and progression-free survival across tumor types in several studies [57–62].

1.5 Pharmacometrics

Pharmacometrics is commonly described as an interdisciplinary research area where statistics, mathematics, computational science and pharmacology are used to describe, understand and predict PK (absorption, distribution and elimination of a drug by the body) and PD (desired or adverse response related to the given drug) properties of a drug [63]. The relationships between administered dose, drug concentration and effect (exposure-response) can be quantified with pharmacometric models, which are often described by a system of algebraic or differential equations. These models may be developed solely to describe a certain data set (empirical), or by incorporating prior knowledge about physiology, disease and the mechanism of action of a drug (mechanistic). Empirical models can be used to answer questions about PK properties of a drug, e.g., an estimate of the elimination capacity (e.g., clearance, CL) while they are not as useful as mechanistic models when extrapolating to untested scenarios.

Pharmacometrics is used in oncology to evaluate clinical efficacy and safety by characterizing and quantifying PKPD-relationships. It is also used to predict clinical outcome from preclinical and early clinical data, guide dosing selection and assist the design of new clinical trials by testing what-if-scenarios, as well as in the clinical setting to individualize already existing
therapies [4–6, 64, 65]. Below is an overview of general concepts in pharmaco-
metrics, followed by descriptions of already established models utilized in this thesis.

1.5.1 General concepts
PKPD-relationships are most commonly analyzed with nonlinear mixed effects (NLME) models (population models) in pharmaco-
metrics. The term mixed effects relates to the combination of fixed and random effects. Fixed effects describe a typical individual in a population (structural model) and ran-
dom effects describe different levels of unexplained variability from that typical individual (stochastic model) [66]. A NLME model describing a continuous, dependent variable \(y_{ij}\) (e.g., PK, biomarker concentrations, tumor size or ANC) for an individual \(i\) at the observation time \(t_{ij}\) (independent variable) is given in Equation 1;

\[
y_{ij} = f(t_{ij}, \theta, X_i, \eta_i) + \epsilon_{ij}
\]

The fixed effects in this equation are described by the parameter vector \(\theta\). \(X_i\) is a matrix of subject-specific covariates (e.g., drug dose, age or presence of a mutation) which may be used to explain parts of the unexplained variability. The individual random effects (inter-individual variability, IIV) and residual unexplained variability (RUV) are described by the vectors \(\eta_i\) and \(\epsilon_{ij}\), respectively. \(\epsilon_{ij}\) describes the differences between observed data and model predictions (including model misspecification and analytical assay errors). \(\eta_i\) and \(\epsilon_{ij}\) values are assumed to be normally distributed with a mean of 0 and a variance of \(\omega^2\) (defined by the covariance matrix \(\Omega\)) and \(\sigma^2\) (defined by the covariance matrix \(\Sigma\)), respectively. The RUV in Equation 1 is described as an additive term, although other implementations are possible (e.g., proportional and combined proportional and additive).

Maximum likelihood (ML) estimation is a commonly used method to estimate the model parameters that maximize the likelihood of observing the given data. The likelihood for an individual \(i\) is described in Equation 2 [67].

\[
L_i(\theta, \Omega, \Sigma|y_i) = P(y_i|\theta, \Omega, \Sigma) = \int P(y_i|\eta_i, \theta, \Sigma) \cdot P(\eta_i|\Omega) \, d\eta_i
\]

The likelihood for all individuals is the sum of all individual likelihoods (\(L_i\)). An analytical solution to Equation 2 is, however, often missing because of the nonlinearity of \(\eta_i\) and numerical approximations are needed to use ML estimation. A variety of estimation methods are available and the methods used in this thesis are the first-order conditional estimation (FOCE) and the second-order Laplacian method. These are approximations and gradient-based methods which minimize the negative twice log-likelihood, i.e., the objective function value (OFV). The OFV can be used during model development to assess model improvement between hierarchical models. NONMEM [68] is one of
the most commonly used software for pharmacometric analyses and also used in this thesis work.

1.5.2 Myelosuppression model

Friberg et al. published a semi-mechanistic model for the description of myelosuppression as a result of six different chemotherapeutic drugs in 2002 [40]. This model was the first of its kind, incorporating mechanistic aspects of chemotherapy-induced myelosuppression, i.e., i) a self-renewal mechanism, regulating a compartment of proliferative cells, ii) a feedback parameter that increases the production when the number of blood cells is reduced, i.e., mimicking the effect of endogenous G-CSF, iii) a cell maturation process to describe the time-delay between effects on production and changes in circulatory neutrophil counts and iv) a distinct differentiation between system-related and drug-related parameters. A schematic representation of the model is given in Figure 4.

The myelosuppression model by Friberg et al. has been used in a variety of settings since first published, including design and evaluation of clinical trials in drug development [69–72], individualized feedback-adaptive dosing in the clinic [73] and incorporation of a description of endogenous G-CSF [43]. The Friberg myelosuppression model provides an opportunity to improve patient management in the clinics by predicting if/when a patient will develop severe neutropenia and when the ANC has recovered enough for the subsequent dose to be administered.

![Figure 4. Schematic representation of the myelosuppression model by Friberg et al. published in 2002 [40]. Neutrophils proliferate, then mature in a chain of three transit compartments in the bone marrow and are subsequently released to the circulation with the rate constant \( k_{pr} \). Here, a linear \((\text{Slope}_{\text{Drug}}) \) drug effect \( (E_{\text{drug}}) \) inhibits the proliferation rate \( (k_{prol}) \) and a decrease in the circulating pool is later observed. The neutrophils in the circulating pool regulates the proliferation rate through a feedback mechanism described the ANC at baseline \( (ANC_0) \), the ANC time-course \( (ANC(t)) \) and a feedback parameter \( (\gamma) \). Neutrophils are eliminated from the circulating pool with the elimination rate constant \( (k_{circ}) \).](image-url)
1.5.3 Tumor size models

Tumor response to treatment is often characterized by RECIST (see section 1.3 Clinical endpoints in oncology). However, RECIST is not an ideal assessor of treatment response since continuous data is categorized, resulting in loss of information. Furthermore, the total number of metastases and metastatic location may be ignored and for some drugs the observed change in tumor size may not represent the treatment effect well [4]. PKPD-modeling of longitudinal tumor data provides a possibility to retain all continuous data and characterize the time-course of tumor size changes in response to treatment.

Different model types have been suggested to describe tumor time-courses [4, 6, 64, 65]. One such model is the tumor growth inhibition (TGI) model, published by Claret et al. in 2009 [50], which has been applied to several different types of cancers and drugs [74–76]. The TGI model can describe multiple patterns of tumor size changes, including tumor shrinkage and re-growth as the drug effect is washed out. The TGI model is described by the differential equation in Equation 3;

$$\frac{dS(t)}{dt} = k_{grow} \cdot S(t) - k_{kill} \cdot Exposure \cdot e^{-\lambda t} \cdot S(t)$$

$S(t)$ (i.e., the tumor size time-course) is the tumor size at time $t$, $k_{grow}$ is a first-order growth rate constant and $k_{kill}$ is a drug-specific cell kill rate constant relating tumor shrinkage to a drug exposure metric (e.g., dose or daily area under the curve, AUC). The $\lambda$ parameter describes a drug-resistance term. The growth rate may also be logistic or of zero-order, i.e., independent of tumor size, which has previously been observed in lung cancer for example [77, 78].

Pharmacometric models are commonly evaluated with visual predictive checks (VPCs), in which the observed data is compared to the simulated data given the evaluated model. Since dropout in studies of tumor size changes often depends on the observed tumor size (i.e., non-responders dropout and responders remain in the study), dropout needs to be considered in order to evaluate a tumor size model with a VPC without bias.

1.5.4 Indirect response models

Biomarker homeostasis can be described by a turnover model, which is characterized by a zero-order production rate ($R_{in}$) and a first-order elimination rate ($k_{out}$). The biomarker concentration is typically constant in absence of drug (although it is possible to account for disease progression and circadian rythm) and the baseline concentration is derived as $R_{in}/k_{out}$. The indirect response (IDR) model is commonly used to describe the biomarker response to a drug treatment. There are four different indirect PD responses to a drug, i.e., stimulation or inhibition of $R_{in}$ or $k_{out}$ [79]. The drug effect is commonly (but
not always) a function (e.g., linear or E\textsubscript{max} model) of drug exposure (e.g., drug concentration or AUC).

### 1.5.5 Count models

As indicated by the name, count data is a data type that originates from counting and is consequently a non-negative integer. Examples of count data include the number of seizures within a time interval or a cell count. The most common count model is the Poisson model, which describes the probability of observation \( j \) in individual \( i \) (\( Y_{ij} \)) taking the value of \( n=0,1,2,... \) as described in Equation 4;

\[
P(Y_{ij} = n) = \frac{\lambda_{P,i}^n}{n!} \cdot e^{-\lambda_{P,i}}
\]

Only one parameter is estimated in the Poisson model, i.e., the mean count parameter (\( \lambda_{P,i} \)), which is defined as a positive real number. \( ! \) in Equation 4 represents the factorial function [80].

The Poisson model assumes equidispersion, i.e., the mean and the variance of the counts in an individual have the same value. However, if the data violate this assumption by either having a variance that is higher (overdispersion) or lower (underdispersion) than the mean, alternative models of the Poisson family are required, e.g., negative binomial model, generalized Poisson model and Double Poisson model [80].

### 1.5.6 Time-to-event models

Parametric time-to-event (TTE) models can be used to model, for example, OS or dropout from a study. A time is recorded when the studied event, such as death, dropout or development of FN, occurs. TTE data consequently differs in nature from longitudinal continuous tumor size, biomarker or neutrophil data. In TTE analyses, the survival and hazard functions are of central interest. The survival function \( S(t) \), represents the probability that an event occurs sometime beyond time \( t \) and the hazard function, \( h(t) \), describes the instantaneous hazard at which an event occurs, Equation 5;

\[
h(t) = h_0(t) \cdot e^{f(\beta, x_t)}
\]

\( h_0(t) \) is the baseline hazard and is characterized by at least one parameter and can be constant over time (exponential distribution) or time-varying (e.g., Weibull, Gompertz, log-normal or log-logistic distributions) [81]. \( f(\cdot) \) is a function of the estimated coefficient vector \( \beta \) and the vector of patient-specific predictors \( x_t \), explaining parts of the hazard. Potential predictors of the hazard include patient baseline characteristics (e.g., age), model-derived metrics
(e.g., $k_{grow}$) or time-varying metrics (e.g., tumor size or biomarker time-courses) [4].

TTE data may be censored, meaning that the observed variable is only partially known. For example, a patient that survives until at least the end of the study will be right censored at that time and the actual time of death is unknown. In oncology, a patient that progresses on the study treatment will discontinue the treatment but potentially receive another treatment while still remaining in the study for OS follow-up without additional tumor size evaluation. Consequently, the results of a TTE analysis may be influenced by the time of censoring.
The overall aim of this thesis work was to evaluate the value of different biomarkers to improve the management of toxicity in cancer patients and for predicting treatment efficacy. This was done by utilizing established and developing new pharmacometric models.

The specific aims were:

- To investigate how frequent monitoring of the ANC together with model-based predictions could improve therapy management with respect to identifying patients at risk for severe neutropenia and planning the start of the subsequent treatment cycle.

- To characterize the time-courses of IL-6 and CRP in breast cancer patients treated with adjuvant chemotherapy and to explore their value as predictors of FN.

- To characterize the time-course of potential atezolizumab biomarkers and evaluate them, together with additional variables, as potential predictors of tumor size changes and OS in NSCLC patients.

- To quantify the dynamic relationships between CTC count, tumor size changes and OS in patients with metastatic colorectal cancer (mCRC) treated with first-line chemotherapy and targeted therapy.
3 Methods

Pharmacometric models, either already established and published or developed within this work, were used in all five papers of this thesis. Below is a summary of the data and methods that were used in each paper.

3.1 Patients and data

The analyzed data is a combination of simulated data, clinical trial data and data from observational studies. Cancer types vary between breast cancer (Paper II), NSCLC (Papers III and IV) and mCRC (Paper V). Studied drugs include chemotherapeutic drugs (i.e., docetaxel and 5-fluorouracil, epirubicin and cyclophosphamide (FEC), capecitabine and oxaliplatin), targeted therapy (i.e., bevacizumab and cetuximab) and atezolizumab (ICI). A summary of patients, treatment (schedule and dosing information) and analyzed variables in each paper is given in Table 1.

The studies in Papers II-V were approved by relevant ethics committees and all patients provided written informed consent for participation in the studies. Studies were conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. No ethical approval was needed in Paper I since the analysis was a simulation study.

3.1.1 Data to manage toxicity

Evaluation of biomarkers to improve treatment in oncology with respect to safety was performed in Papers I and II. Paper I is a simulation study and the data in Paper II originates from a local clinical study at the Uppsala University hospital.

3.1.1.1 Simulated ANC data

The myelosuppression model by Friberg et al. (described above and illustrated in Figure 4) [40] with parameter estimates (for docetaxel) according to Kloft et al. [82] was used to simulate ANC and obtain “true” individual ANC profiles. To avoid extreme ANC values during recovery of the ANC no variability related to the feedback parameter $\gamma$ was included [83]. The ANC was simulated just before the start of docetaxel treatment and thereafter daily from day 3 to day 21 (first cycle only). No simulated data were generated for days 1 and
### Table 1. Summary of patients, treatment and analyzed variables in each paper

<table>
<thead>
<tr>
<th>Paper</th>
<th>Patients</th>
<th>Treatment</th>
<th>Variables analyzed in this thesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>600 simulated carcinoma, melanoma, and sarcoma patients</td>
<td><strong>Docetaxel</strong> 1-hour IV infusion of 75 or 100 mg/m²</td>
<td>ANC</td>
</tr>
<tr>
<td>II</td>
<td>49 breast cancer patients</td>
<td><strong>Adjuvant treatment</strong> 3 cycles FEC (IV infusions)¹; Epirubicin: 1-hour 75 mg/m² 5-FU: 2-min 600 mg/m² Cyclophosphamide: 15-min 600 mg/m² 3 cycles docetaxel¹; 1-hour IV infusion of 80 mg/m²</td>
<td>IL-6, CRP and FN (in cycles 1 and 4)</td>
</tr>
<tr>
<td>III/IV</td>
<td>88 non-small cell lung cancer patients</td>
<td>**Atezolizumab (every 3 weeks)**² 30-60 min IV infusion of 10, 15 or 20 mg/kg or a fixed dose of 1200 mg</td>
<td>CRP, CD40, ICAM-1, ITAC, IL-18, IL-8, VCAM-1, T&lt;sub&gt;CD8,prolf/activ&lt;/sub&gt;, SLD, dropout and OS</td>
</tr>
<tr>
<td>V</td>
<td>458 metastatic colorectal cancer patients</td>
<td><strong>CB arm (every 3 weeks)</strong> Capecitabine: 1000/1250 mg/m² orally twice daily on days 1-14 in cycles 1-6/subsequent cycles Oxaliplatin: 130 mg/m² IV (max 6 cycles) Bevacizumab: 7.5 mg/kg IV <strong>CBC arm (every 3 weeks)</strong> CB arm Cetuximab: 400/200 mg/m² IV on the first day of the first/subsequent cycles</td>
<td>SLD, dropout, CTC and OS</td>
</tr>
</tbody>
</table>

5-FU, 5-fluorouracil; ANC, absolute neutrophil count; CD40, cluster of differentiation 40; CRP, C-reactive protein; FEC, 5-fluorouracil-epirubicin-cyclophosphamide and docetaxel; FN, febrile neutropenia; ICAM-1, intercellular adhesion molecule 1; IL-6, interleukin 6; IL-8, interleukin 8; IL-18, interleukin 18; ITAC, interferon-inducible T-cell alpha chemoattractant; IV, intravenous; OS, overall survival; SLD, sum of longest diameter; T<sub>CD8,prolf/activ</sub>, activated T cells

¹ Seven patients received the treatment in the opposite order, two patients received six cycles of FEC and trastuzumab/hormonal therapy was added when applicable

² One patient received 16 cycles of 1 mg/kg
2 since glucocorticoids (often administered before and/or early in the cycle to prevent nausea [84]) can cause a short, temporal, increase in circulating neutrophils [85, 86], which is not captured by the applied myelosuppression model.

3.1.1.2 CRP, IL-6 and febrile neutropenia data
The study in Paper II was conducted at the Department of Oncology, Uppsala University hospital, Sweden, between February 2007 and January 2010. A majority of patients received three cycles of FEC followed by three cycles of docetaxel (as described in Table 1) [43]. The data was primarily collected in cycles 1 and 4 and the analysis based on this data consequently included only those two cycles. IL-6 and CRP were collected on seven different days in cycle 1 for the first ten enrolled patients (predose, days 7-9, 9-11, 11-13, 13-15, 15-18 and 20-22 following FEC and predose, days 4-6, 6-8, 8-10, 10-13, 13-15 and 20-22 following docetaxel). For the other 11-49 patients in cycle 1 and 4, as well as for patients 1-10 in cycle 4, IL-6 and CRP were collected on five different days (predose, days 7-12, 12-14, 14-16 and 18-22 following FEC and predose, days 5-8, 8-10, 10-15 and 18-22 following docetaxel). Additional CRP measurements were available in some patients as part of routine care. No drug concentrations were measured. Febrile neutropenia was defined as grade 3 or 4 neutropenia in combination with coexisting fever, regardless of cause.

3.1.2 Data to predict efficacy
Evaluation of biomarkers to improve treatment in oncology, with respect to efficacy was performed in Papers III-V. Below is a description of the data used in these papers.

3.1.2.1 Atezolizumab data
Data from the phase I dose-escalation study PCD4989g were used in Papers III and IV. Patients with locally advanced or metastatic solid tumors or hematologic malignancies were recruited in the PCD4989g study [87], although only patients with NSCLC were analyzed in Papers III and IV. Atezolizumab was administered every three weeks; additional dosing details are available in Table 1. Drug concentrations were measured predose in cycles 1-8, 10, 12, 14-16 and ≥17, 30 min after end of infusion in cycles 1-7, and 1, 3, 7 and 14 days after end of infusion in cycle 1. Biomarker concentrations were collected predose in cycles 1-4 and 7 and 30 min, 1 day and 7 days after end of infusion in cycle 1, except TCD8,prolif/activ which was measured predose in cycles 1-3 and 5. Tumor sizes, i.e., SLD, were assessed by the RECIST version 1.1 [34] and SLD as measured by CT scan was determined every six weeks for 24 weeks and every 12 weeks thereafter until disease progression, death or further systemic cancer therapy. OS data were available.
3.1.2.2 Circulating tumor cell data

*Paper V* was based on data from the multicenter phase III CAIRO2 trial [88, 89]. The study included mCRC patients and CTCs were measured in a subset of 458 patients [62], which were consequently included in the analysis in *Paper V*. Patients were randomized 1:1 to receive treatment with capecitabine, oxaliplatin and bevacizumab (CB group) or the same regimen with the addition of cetuximab (CBC group; further dosing information is given in Table 1). Tumor size, i.e., SLD, was measured by CT scan and evaluated with RECIST 1.0 [90], before initiation of treatment and thereafter every nine weeks. CTC counts were measured in two replicates of 7.5 mL blood using the CellSearch® System (Veridex, LLC, Raritan, NJ), before treatment and after 1-2, 3-5, 6-12 weeks after start of treatment and subsequently every nine weeks at the same time as SLD evaluation for up to a year. No drug concentrations were measured, only the administered doses were available. During model development, all administered doses were normalized to their respective nominal dose and values below and above 1 therefore represented doses lower and higher than the nominal dose, respectively. Each normalized dose was subsequently added to the summary variable total normalized dose (TND). OS was registered as a secondary outcome measure.

3.2 Exploratory analysis of atezolizumab biomarkers

Two biomarkers were selected for PKPD-modeling and further exploration as potential predictors of tumor size changes in response to atezolizumab treatment in *Paper III*. The selection procedure was performed in two steps: The first step was performed outside the work of the current thesis and narrowed down 95 biomarkers to ten biomarkers, which showed statistically significant changes from baseline on treatment (p≤0.05). Eight of these ten biomarkers were prioritized based on biological relevance, i.e., CRP, CD40, ICAM-1, ITAC, IL-18, IL-8, VCAM-1 and TCD8,prolif/activ. An exploratory analysis was performed for these eight biomarkers in the second step of the selection procedure. The selection of two biomarkers was guided by visual inspection of the exploratory analysis and included evaluation of potential exposure-response and tumor size-biomarker relationships, intra- and inter-patient variability and between-biomarker correlations.

3.3 Applied and developed models

The previously published myelosuppression model [40] was used in *Paper I*, both to simulate daily ANC (see section 3.1.1.1 Simulated ANC data) but also to make forecasts of the ANC (described more in detail below). New models
were developed in Papers II-V and the model development in each paper is described below.

3.3.1 Myelosuppression model – predicting daily ANC

Given the daily simulated ANC data and the myelosuppression model as described above, daily ANC, summary variables of the chemotherapy-induced myelosuppression time-course, i.e., time to nadir, \( NADIR_{\text{time}} \), ANC value at nadir, \( NADIR_{\text{ANC}} \) and time to recovery of the baseline ANC, \( RECOVERY-\text{ANC}_0{\text{time}} \) (illustrated in Figure 5) and sensitivity and specificity for classification of severe neutropenia (i.e., Grade 4 and \( \text{ANC} \leq 0.1 \times 10^9 \) cells/L) were predicted in a Bayesian feedback analysis (i.e., no re-estimation of population parameters, only estimation of individual parameters given the model and simulated data).

Predictions were performed for a range of scenarios with varying monitoring durations (baseline and day 3 up to day 21) and monitoring frequencies (every, every other and every third day). Nine different monitoring scenarios, together with the “true” and forecasted ANC profiles are illustrated in Figure 6. Two additional scenarios were explored; i) baseline (only ANC baseline available) and ii) baseline and day 5 (only ANC baseline and day 5 available).

The forecasts of daily ANC were evaluated with an estimated error, Equation 6;

\[
Error_{d,D} = \log(ANC_{\text{ipred,d,D}}) - \log(ANC_{\text{true,D}})
\]

\( ANC_{\text{ipred,d,D}} \) is the individually predicted ANC at day \( D \) given that the ANC was monitored up to day \( d \). \( ANC_{\text{true,D}} \) is the corresponding ANC originating from the “true” profile at day \( D \).

The predictions of the summary variables were evaluated with a root-mean square error (RMSE), Equation 7;

\[
\text{Figure 5. ANC time-course (ANC(t)) for one patient illustrating evaluated summary variables. ANC, absolute neutrophil count; NADIR}_{\text{ANC}}, ANC at nadir; NADIR_{\text{time}}, time for occurrence of NADIR_{\text{ANC}}; RECOVERY-\text{ANC}_0{\text{time}}, time to recovery of the baseline ANC.}
\]
\[ RMSE_d = \sqrt{\frac{\sum_{i=1}^{N} (Value_{\text{pred},d} - Value_{\text{true},i})^2}{N}} \]

\(Value_{\text{pred},d}\) is the individually predicted (forecasted) summary variable given that the ANC was monitored up to day \(d\) and \(Value_{\text{true},i}\) is the corresponding summary variable originating from the “true” profile. \(N\) is the number of patients.

Docetaxel was used as an example drug in this analysis, however, further explorations were performed for three additional hypothetical drugs with: i) less myelotoxicity (by setting the docetaxel toxicity \(Slope\) parameter in the myelosuppression model to half of its value), ii) smaller residual error (approximately 26%, which was the same value as for paclitaxel in the analysis by Kloft et al. [82]) and iii) longer mean transit time (\(MTT\)) (141 hours which was the longest estimated \(MTT\) in the analysis by Kloft et al. [82]).

**Figure 6.** Nine different monitoring scenarios, as described in grey stipes, illustrated for one patient.

ANC, absolute neutrophil count
3.3.2 Atezolizumab pharmacokinetic model

A fit-for-purpose population PK model for atezolizumab was developed outside the scope of this thesis work and was available for further PKPD-modeling in Papers III and IV. The PK model was developed based on 167 patients in the PCD4989g study [87], who received doses of 10, 15 and 20 mg/kg every three weeks. The model used dose-linear elimination and included no time-varying PK components, which is supported by the atezolizumab PK model in metastatic urothelial cancer developed by Stroh et al. [91]. The PK model was used to generate post-hoc PK estimates for the 88 NSCLC patients, which were used to model IL-18 and ITAC and to derive cycle-specific AUCs (\(AUC_{cycle,n}\)) according to Equation 8;

\[
AUC_{cycle,n} = \frac{Dose_n}{CL_i} 
\]

\(Dose_n\) is the dose related to cycle \(n\). The AUC was also calculated based on \(CL_i\) values estimated on PK data from cycle 1 only (\(AUC_{cycle1}\)) to explore potential impact of time-varying PK.

3.3.3 Characterization of biomarker time-courses

The evaluated biomarkers in this thesis can be separated in two groups, circulating biomarkers and tumor size. Additional descriptions of these groups are given below.

3.3.3.1 Circulating biomarkers

The time-courses of IL-6 and CRP, two biomarkers in response to atezolizumab treatment, and CTCs were characterized in Papers II, III and V, respectively. The modeling approaches are described below.

IL-6 and CRP were modeled separately during the initial model development. In a second step, they were modeled simultaneously in order to explore potential correlations. Turnover models were used to describe the homeostasis of these biomarkers. Changes in IL-6 and CRP were not related to drug exposure, but rather to infection and temporal biomarker increases were initially modeled either with an empirical surge function \(g(t)\), Equation 9, or the MTIME-functionality in NONMEM [68]. A time period with a different biomarker production rate than the baseline production rate was estimated with the MTIME approach. The mixture functionality implemented in NONMEM [68] was used to identify subpopulations that had or did not have elevated production, by setting \(g(t)\) to 0 (surge function approach) or not estimating a different production rate (MTIME approach) for the subpopulations that did not have elevated concentrations. The probability for elevated production was assessed by assuming the same probabilities for elevation in cycles 1 and 4 or as separate probability-related parameters.
Three parameters are estimated in Equation 9, the surge amplitude \((SA_{BioM})\), peak time \((PT_{BioM})\) and width \((SW_{BioM})\). \(BioM\) (biomarker) can be replaced with either IL-6 or CRP, as appropriate.

The IL-6 regulation of the CRP production \([42]\) was explored by including covariance between the random effects and/or by letting IL-6 stimulate the CRP production through a linear function or an E\(_{\text{max}}\) model \((E_{IL-6})\). IIV was evaluated on all model parameters and inter-occasion variability (IOV) \([92]\) was tested on the surge related parameters and the parameters related to \(E_{IL-6}\).

IDR models were used to characterize the biomarker response to atezolizumab over time \((BioM)\). By linking a pool compartment (e.g., precursor of the biomarker) to the IDR model a diminishing response over time was evaluated \([93]\). The differential equation system for such a model is given in Equations 10-12;

\[
\frac{dBioM_{\text{pool}}}{dt} = R_{in} - k_{rel} \cdot BioM_{\text{pool}} \cdot (1 + EFF) \tag{10}
\]

\[
\frac{dBioM}{dt} = k_{rel} \cdot BioM_{\text{pool}} \cdot (1 + EFF) - k_{out} \cdot BioM \tag{11}
\]

\[
k_{rel} = k_{out} \cdot \frac{BioM_0}{BioM_{\text{pool},0}} \tag{12}
\]

\(BioM_{\text{pool}}\) is the biomarker concentration in the pool compartment, \(R_{in}\) is the zero-order biomarker production rate constant, \(k_{rel}\) is the first-order rate constant for the release of biomarker from the pool to plasma and \(BioM_0\) and \(BioM_{\text{pool},0}\) is the biomarker concentration in the circulating and pool compartments at baseline, respectively. \(BioM\) can be replaced with the selected biomarker. Linear and saturable \((E_{\text{max}}\) model\) drug effects \((EFF)\) were explored. In addition to the effect delay characterized by the IDR model combination with an effect compartment was also explored \([94]\). The observed tumor size at baseline was evaluated as a predictor of \(R_{in}\).

The time-course of the CTC count in mCRC patients treated with chemotherapy and targeted therapy were described as a mean CTC count in 7.5 mL blood, \(\lambda_{\text{CTC}}(t)\), by applying count models of the Poisson family (i.e., Poisson, zero-inflated Poisson and negative binomial) \([80]\). Relationships between the tumor size time-course (and other tumor size-related metrics) and \(\lambda_{\text{CTC}}(t)\) were explored as well as the inclusion of a drug effect, i.e., categorical (drug or no drug) or continuous relationships. The Kinetic-Pharmacodynamic modeling approach \([95]\) was explored in absence of drug concentration, using the TND,
as well as using TND as a covariate. Drug effects were explored on top of potential tumor size-related relationships. Effect delays were explored through implementation of effect compartments [94].

### 3.3.3.2 Tumor size

Two different tumor size models were developed in this thesis, one based on NSCLC patients treated with atezolizumab (*Paper III*) and one based on mCRC patients treated with chemotherapy and targeted therapy (*Paper V*). Exponential and linear growth rates were evaluated in both tumor size models.

Three different structural models for tumor size changes were explored to characterize the longitudinal tumor time-course in response to treatment with atezolizumab in NSCLC, i.e., the TGI model by Claret et al. in 2009 [50], the model by Simeoni et al. in 2004 [96] and a model developed for another ICI (pembrolizumab) by Chatterjee et al. in 2016 [97]. The tumor shrinkage rate was evaluated to be related to drug exposure (i.e., dose and AUC) and model-derived metrics of the biomarkers. The biomarker metrics included the biomarker concentration over time (absolute and change from baseline, CFB), the predicted (and observed) relative change from baseline on day 21 ($RCFB_{BioM,d21}$) and day 42 ($RCFB_{BioM,d42}$) and cumulative AUC from biomarker baseline ($AUC_{BioM,0-t}$). $RCFB_{BioM,d21}$ and $RCFB_{BioM,d42}$ were predicted for each patient from the biomarker models and set to zero before day 21 and day 42, respectively. A covariate analysis was performed to explore relationships between estimated parameters, i.e., the baseline tumor size ($SLD_0$), tumor growth rate, drug-constant tumor shrinking rate ($k_{Shr,Drug}$) and biomarker-constant tumor shrinking rate ($k_{Shr,BM}$), and baseline covariates, i.e., patient characteristics (e.g., smoking status), laboratory values (e.g., baseline neutrophil/lymphocyte ratio, NLR) and tumor-related variables (e.g., gene expressions, mutations and metastases). The stepwise-covariate model (SCM) building tool was used, where covariates were added to the model in the forward selection and eliminated in the backward step, based on statistical testing. Continuous and categorical covariates were included as power models and as a percentage of a parameter value relative to a reference category, respectively.

A tumor size model for mCRC was developed in order to explore potential relationships between the tumor size and CTC time-courses as well as OS. The primary goal of the mCRC tumor size model was therefore to characterize the individual tumor size time-course well in relation to the observed SLD data. Two different structural models for tumor size changes were explored, i.e., the TGI model suggested by Claret et al. in 2009 [50] and the tumor size model for tumor quiescence and drug-resistance [98, 99]. The mixture functionality in NONMEM, together with skewed and binomial distributions of the random effects, were used to explore subpopulations with different patterns of tumor size changes. The TND was explored as a predictor of tumor size changes. A dropout model, using logistic regression, was developed in
order to evaluate the mCRC tumor size model with VPCs. Variables derived from the observed tumor size were explored as predictors of dropout, including: i) progressive disease (PRD, 20% increase and at least 5 mm increase in tumor size), ii) baseline tumor size, iiiia) absolute and iiib) relative change from baseline (RCFB) tumor size time-course) and iv) time. Predictors were evaluated alone and in combination.

3.4 Time-to-event models
TTE models were developed in Papers II-V. The time-to-febrile neutropenia was characterized in Paper II and a TTE dropout model was developed in Paper III for the purpose of VPC evaluation of the NSCLC tumor size model. OS models, using the TTE approach were developed in Papers IV and V. Additional descriptions of the development of these models are given below.

3.4.1 Febrile neutropenia
Each patient was analyzed as a separate patient in cycle 1 and cycle 4 and only one event (febrile neutropenia) per cycle was allowed (i.e., no repeated TTE). Time constant (exponential distribution) and time-dependent hazards (Weibull distribution) for development of FN were investigated in the initial step. No patient experienced grade 3 neutropenia before 3.5 days and the hazard was consequently set to zero until 3.5 days.

Predictors of FN were explored in three parallel analyses where potential predictors were separated as follows;

i) Available prior-to-chemotherapy (i.e., baseline variables)
ii) Available prior-to-FN (i.e., baseline and IL-6 model-derived variables)
iii) Available when-FN-occurs (i.e., baseline and IL-6 and CRP model-derived variables)

Age, body weight, treatment (FEC or docetaxel), observed ANC, G-CSF, IL-6 and CRP baselines (cycle 1 and cycle-specific), model-estimated ANC, G-CSF, IL-6 and CRP baselines, model-predicted IL-6 and CRP time-courses (absolute, RCFB and normalized to the population baseline and logarithmic) and partial IL-6 and CRP AUCs and model-estimated individual $k_{out,IL-6}$ and $S_{ACRP}$ were explored as predictors in each of the three analyses. The partial AUCs were generated by integrating the change from IL-6 and CRP baselines, respectively, over time from initiation of the cycle until the time of FN. Since the ANC is part of the definition of FN and most patients experienced grade 3 or 4 neutropenia, the ANC time-course was not evaluated as a predictor. Effect compartments were explored, for relevant variables, to describe delayed effects between biomarkers and FN [94].
3.4.2 Dropout
The TTE approach was used to develop a dropout model in Paper III. A dropout event was assumed to occur three weeks after the last tumor observation time. Time constant (exponential distribution) and time dependent hazards (Weibull distribution) for dropout were evaluated. Potential predictors of dropout (alone and in combination), based on the observed tumor size, were explored.

3.4.3 Overall survival
Time constant (exponential distribution of event times) and time-varying (Weibull, Gompertz, log-normal and log-logistic distributions of event times) hazards were explored in the OS models in Papers IV and V. Exploration of predictors of OS were performed in a stepwise manner (forward inclusion and backward elimination) and separated in two steps; i) baseline covariates and ii) model-derived metrics. The variable that provided the best improvement of the model fit was first added to the model and if any additional variable improved the model fit statistically significant on top of the prior included variable it was also added to the model. This procedure was continued until no more predictors could be identified. A final backward elimination step was performed after exploration of both baseline covariates and model-derived metrics. Potential predictors were added exponentially to \( h_0(t) \) and linearly to each other. Continuous baseline covariates were centered around the median value and categorical variables were included as a relative change in a parameter value from a reference category. Continuous and categorical covariates were imputed with the median value and the most common category, respectively, in cases of missing covariate values.

A relative hazard (i.e., to the typical patient with median covariate values, except for the studied covariate, and similar to a hazard ratio) was computed to illustrate the relationship between the observed range of baseline covariates and the hazard. The relative hazard was calculated according to Equations 13 (continuous covariates) and 14 (categorical covariates);

\[
\text{Relative hazard}_{\text{cont}} = e^{\beta_{\text{cont}} \cdot (\text{COV}_{\text{cont}} - \text{COV}_{\text{cont,median}})}
\]

\[
\text{Relative hazard}_{\text{cat}} = e^{\beta_{\text{cat}} \cdot \text{COV}_{\text{cat}}}
\]

where \( \beta_{\text{cont}} \) and \( \beta_{\text{cat}} \) are parameters relating a continuous (\( \text{COV}_{\text{cont}} \)) and categorical (\( \text{COV}_{\text{cat}} \)) covariates, respectively, to the hazard. For a dichotomous covariate, \( \text{COV}_{\text{cat}} \) has a value of 0 for the reference category and 1 for the comparing category.

The impact on the inclusion of predictors of OS and the size of their effects on OS in NSCLC patients treated with atezolizumab, were investigated for four different censoring strategies;
i) AAD All available data
ii) C2YE Data censored no later than at a cut-off date set 2 years earlier than in AAD
iii) C2YASOT Data censored no later than 2 years after start of treatment for each individual patient
iv) C5MALD Data censored a maximum of five months after last dose

Consequently, four different baseline hazards were established prior to the exploration of predictors in NSCLC patients. Several continuous and categorical baseline covariates were explored, including patient characteristics (e.g., smoking status), laboratory variables (e.g., different cell counts, IL-18 and ITAC) and tumor-related variables (e.g., PD-L1 expression and metastases). Model-derived metrics included variables related to the PK model (AUC from baseline to day 21), the IL-18 and ITAC models (accumulated change from baseline AUC from baseline to day 21 and $RCFB_{BioM,d21}$) and the tumor size model (Table 2). The value of longer follow-up (here; AAD) in comparison to the alternative censoring strategies was also explored by predicting AAD given parameter estimates in the final C2YE, C2YASOT and C5MALD models.

Baseline covariates, i.e., tumor size, CTC count, age, treatment arm, normal/increased lactate dehydrogenase, prior chemotherapy and resection of primary tumor, were explored following establishment of a baseline model in mCRC patients treated with chemotherapy and targeted therapy. Subsequently, tumor size- (Table 2) and CTC-related model-derived metrics were explored on top of the baseline covariates. CTC-related model-derived metrics

Table 2. Description of tumor size related model-derived metrics explored as predictors of overall survival

<table>
<thead>
<tr>
<th>Variable</th>
<th>Description</th>
<th>Explored in Paper(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_{Growth}$</td>
<td>Tumor growth rate</td>
<td>IV</td>
</tr>
<tr>
<td>Log($R_{Growth}$)</td>
<td>Log of tumor growth rate</td>
<td>IV</td>
</tr>
<tr>
<td>SLD(t)</td>
<td>Absolute time-course of tumor size changes</td>
<td>V</td>
</tr>
<tr>
<td>RCFB-SLD(t)</td>
<td>Relative change from baseline of the SLD time-course</td>
<td>IV and V</td>
</tr>
<tr>
<td>BN-SLD(t)</td>
<td>Baseline normalized SLD time-course</td>
<td>V</td>
</tr>
<tr>
<td>RCFL-SLD(t)</td>
<td>Relative change from lowest SLD time-course</td>
<td>V</td>
</tr>
<tr>
<td>SLD(t)-slope</td>
<td>Time-course of SLD rate of changes</td>
<td>IV and V</td>
</tr>
<tr>
<td>TSR6/TSR12</td>
<td>Tumor size ratio at week 6/12</td>
<td>IV</td>
</tr>
<tr>
<td>TSR9/TSR18</td>
<td>Tumor size ratio at week 9/18</td>
<td>V</td>
</tr>
<tr>
<td>TTG</td>
<td>Time to tumor growth</td>
<td>IV and V</td>
</tr>
</tbody>
</table>
included absolute and RCFB of $\lambda_{\text{CTC}}(t)$, the absolute and ratio (to baseline) $\dot{\lambda}_{\text{CTC}}(t)$ at weeks 1, 3 and 6 and a categorical effect for $\lambda_{\text{CTC}}(t) \geq 1/2/3$/estimated cut-off.

3.5 Data analysis

The pharmacometric analyses described in this thesis were performed in the previously mentioned software for NLME models, NONMEM 7.2-7.4 [68]; the exact version used in each paper is given in Table 3. The First-order conditional estimation method with interaction (FOCEI) and the Laplacian estimation method were used to estimate model parameters. Perl-speaks-NONMEM (PsN) was used to execute model runs and simulations, process the NONMEM output, produce VPCs, generate relative standard errors (RSEs) with the Sampling Importance Resampling (SIR) approach [100, 101] and perform automated covariate analyses in the SCM program [102]. Pirana was used to facilitate generation of run records [102]. R (www.R-project.org) was used for data management, computation of accuracy, bias and imprecision and for graphical exploration of raw data as well as for graphical evaluation of the NONMEM output, together with the R based programs Xpose [102] and ggplot2 (www.ggplot2.org). Berkeley Madonna was used for simulations performed in Paper III [103].

The OFV provided in the NONMEM output was used to discriminate between models during model development. For nested models (i.e., a full model

<table>
<thead>
<tr>
<th>Paper</th>
<th>p-values</th>
<th>Sequential estimation method</th>
<th>RSE method</th>
<th>NONMEM version</th>
</tr>
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<tbody>
<tr>
<td>I</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>7.2 and 7.3</td>
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<tr>
<td>II</td>
<td>0.05</td>
<td>IPP</td>
<td>SIR</td>
<td>7.3</td>
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<td>III</td>
<td>BM/TS models: 0.01</td>
<td>PPP&amp;D</td>
<td>NONMEM sandwich covariance matrix</td>
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<tr>
<td></td>
<td>DO: 0.05</td>
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<td>Covariate analysis;</td>
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<tr>
<td></td>
<td>Forward: 0.01</td>
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<tr>
<td></td>
<td>Backward: 0.001</td>
<td></td>
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<td>IV</td>
<td>Forward: 0.05</td>
<td>PPP&amp;D</td>
<td>NONMEM R covariance matrix</td>
<td>7.4</td>
</tr>
<tr>
<td></td>
<td>Backward: 0.05</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>TS/CTC/DO: 0.05</td>
<td>IPP (TS model)</td>
<td>SIR</td>
<td>7.4</td>
</tr>
<tr>
<td></td>
<td>OS: 0.01</td>
<td>PPP&amp;D (CTC model)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

BM, biomarker; CTC, circulating tumor cells; DO, dropout; IPP, individual pharmacokinetic (PK) parameters; PPP&D, population PK parameters and data; OS, overall survival; SIR, sampling importance resampling; TS, tumor size
that can collapse to a reduced model), the ΔOFVs are approximately $\chi^2$ distributed and the degrees of freedom (DFs) are the difference in number of parameters. p-values of 0.05, 0.01 and 0.001 correspond to ΔOFVs (OFV_{reduced model} - OFV_{full model}) of 3.84, 6.64 and 10.83 (one DF), respectively. Statistical significance levels used in each paper are given in Table 3. Actual significance levels, in terms of ΔOFV, were generated with the randomization test [102] and used during the evaluation of predictors in the TTE models in Paper II.

IIV was generally included exponentially to a typical parameter ($\theta$), resulting in log-normally distributed individual parameters ($P_i$) as described in Equation 15;

$$P_i = \theta \cdot e^{\eta_i} \eta_i \sim N(0, \omega^2)$$

where $\eta_i$ is a normally distributed variable for individual $i$ with a mean of 0 and variance of $\omega^2$. Other, semi-parametric, distributions of $\eta$ were explored when indicated by graphical evaluation. Proportional (additive on log-scale), additive and combined proportional and additive residual error models were considered. Log-transformed CRP, IL-18 and tumor size (Paper III) data were used.

Modeling frameworks, where one modeled variable influences another (e.g., prediction of tumor size influenced by a biomarker), were developed in Papers II-V. This was achieved either by fitting both variables simultaneously (IL-6 and CRP model in Paper II) or by using a sequential estimation method, i.e., by first fitting one variable and then using the obtained parameter estimates (and potentially observed data) when fitting the other variable. Sequential methods applied in this thesis include the methods similar to the Individual PK Parameter (IPP) and Population PK Parameter and Data (PPP&D) approaches. Individual PK (or biomarker) parameters are added to the data set and used in the model code with the IPP method. Consequently, no re-estimation of any parameters related to the first variable is performed with this method. With the PPP&D method, the population PK (or biomarker) parameters are fixed to the estimated values, while individual PK (or biomarker) parameters are allowed to be re-estimated since the data of the first variable is retained in the data set and may therefore be influenced by the second variable. Details of which sequential estimation method used in each paper are given in Table 3.

Model development was additionally guided by performance of the VPCs/prediction-corrected VPCs (pcVPCs) [104], graphical evaluation of goodness-of-fit plots, reasonability of the parameter estimates and the sizes of the RSEs (generated with SIR or from the NONMEM covariance matrix) [105]. Kaplan-Meier (KM) VPCs were used to evaluate the predictive performance of TTE models and Kaplan-Meier mean covariate (KMMC) VPCs were used to evaluate the appropriateness of included continuous predictors in TTE models [106].
4 Results

The results in this thesis work are given below. The analyzed data is first described which is then followed by a presentation of the results in Papers I-V.

4.1 Description of the analyzed data

Descriptions of the data analyzed in Paper II, Papers III-IV and Paper V are given below in sections 4.1.1 CRP, IL-6 and febrile neutropenia, 4.1.2 Atezolizumab data and 4.1.3 Circulating tumor cell data, respectively.

4.1.1 CRP, IL-6 and febrile neutropenia

The total numbers of IL-6 and CRP measurements were 445 and 482, respectively, which were collected in 49 (cycle 1) and 45 (cycle 4) patients. One patient (who did not develop FN) had a very high IL-6 baseline (i.e., 28 times higher than the median IL-6 baseline) and the following time-course deviated considerably from the pattern in all other patients (i.e., the concentration was high before start of treatment and decreased closely to the end of the cycle). This patient was excluded from all parts of the analysis in Paper II. An additional patient had a high IL-6 baseline (i.e., 17 times higher than the median IL-6 baseline) and that single observation was excluded from the analysis. FN was developed in 11 patients. There were 12 FN events in total, six in cycle 1 and 4 each. Six FN episodes were related to FEC and docetaxel each.

4.1.2 Atezolizumab data

There were 413-458 observations of each of the eight biomarkers (selected for the exploratory analysis in Paper III), except for T_{CD8, prolif/active} which was measured 185 times, in the 88 NSCLC patients. Tumor size was assessed during a range from zero (four patients with a baseline size only) to 153 weeks. The observed median SLD at baseline was 5 cm, which increased modestly during the first two tumor scans (reflection of non-responders) and then decreased to a size below baseline for the remainder of the study (reflection of dropout).

There were 69 (AAD), 56 (C2YE), 54 (C2YASOT) and 28 (C5MALD) deaths in the four data sets exploring different censoring strategies. Follow-up
times ranged from 16 days to 5.2 (AAD), 3.2 (C2YE), 2 (C2YASOT) and 4.7 (C5MALD) years.

4.1.3 Circulating tumor cell data
The median SLD was 87 mm (range: 10-494 mm) prior to start of treatment. SLD evaluation was typically assessed 4 times (range: 1-20) within a patient for a median follow-up of 33 weeks (range: 0-244 weeks). The median CTC count was 0 in 51% (baseline measurements) and 76% (all evaluable replicates) samples. There were typically 5 (range: 1-27) CTC replicates per patient and the median CTC follow-up was 27 weeks (range: 0-104 weeks). The median TND was 3.34 (range: 0.301-23.4). Seven patients and 18 observations in 16 additional patients were identified as outliers, based on visual inspection of the raw data, and consequently omitted from the analysis data set, which therefore included 451 patients. The median OS was 1.7 years and follow-up ranged between 1.5 weeks and 5.1 years.

4.2 Predictions in Paper I
Results based on docetaxel (which was used as an example drug) in Paper I are given and illustrated in this section. The distributions of the accuracy (as defined in Equation 6) of the predictions of daily ANC are illustrated for eight monitoring durations and three monitoring frequencies in Figure 7. The widest distributions were observed around the time for occurrence of nadir, the distribution was however narrowed later on in the cycle as more data became available for the forecasts. As an example, the 2.5th and 97.5th percentiles of the accuracy values were 0.27 and 20.2 for a 4-day forecast with daily data monitored up to day 7 (the fourth orange box in plot e) in Figure 7) and 0.74 and 1.43 when the ANC was monitored daily up to day 15 and predicted at day 21 (sixth orange box in plot g) in Figure 7). The investigated monitoring durations only had a minor impact on the accuracy, although the spread of the error was the (slightly) lowest with daily ANC monitoring.

The RMSE_d (as defined in Equation 7) of the predicted summary variables are illustrated in Figure 8. The lowest RMSE_d was observed with daily ANC monitoring and longer monitoring durations. The imprecision (RMSE_d) of RECOVERY-ANC0time was ±1 day with daily monitoring of the ANC and a monitoring duration of 11 days and no improvements were observed beyond 17 days monitoring duration, which was approximately the true median RECOVERY-ANC0time, left plot in Figure 8. For a monitoring duration roughly one day shorter than the true median NADIRtime and daily ANC monitoring, the RMSE_d was more than ±1 day (NADIRtime) and ±0.2×10^9 cells/L (NADIRANC).
Figure 7. Distribution of the absolute error for scenarios where the absolute neutrophil count (ANC) was monitored until day 3, 4, 5, 6, 7, 10, 15 and 19. $\text{ANC}_{\text{pred},D}$ is the individual predicted ANC at day $D$, given data available up to day $d$ and $\text{ANC}_{\text{true},D}$ is the true ANC at day $D$. Orange, blue and green boxes represent monitoring frequency every, every other and every third day, respectively. The horizontal line represents no prediction error, the blue and red lines illustrate days the ANC was monitored and predicted, respectively. The vertical line inside of each box is the median. Lower and upper hinges of the box and ends of the whiskers represent the 25th and 75th and 2.5th and 97.5th percentiles, respectively. No outliers are presented.
The true rates of grade 4 neutropenia and an ANC≤0.1×10^9 cells/L were 68% and 23%, respectively. The median time to grade 4 neutropenia and an ANC≤0.1×10^9 cells/L were at day 6.0 and at day 7.6, respectively. The sensitivity for Grade 4 neutropenia was overall high, even in the baseline scenario (91.9%), however the specificity was lower with less available data to predict the ANC (48.7% in the baseline scenario). In contrast, for an ANC≤0.1×10^9 cells/L the sensitivity and specificity were low (30.4%) and high (94.8%), respectively, in the baseline scenario. The Grade 4 specificity and the ANC≤0.1×10^9 cells/L sensitivity improved with longer monitoring durations. Sensitivities and specificities for a daily monitoring frequency, and the additional two scenarios, are illustrated in Figure 9.

Similar results, as those described above for docetaxel, were obtained for three additional hypothetical drugs. Overall, compared to docetaxel, the distribution of the accuracy of daily ANC forecasts was narrower around nadir

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**Figure 8.** Root-mean squared error at day \( d \) in the cycle (RMSE\(_d\)) of the ANC at nadir \((\text{NADIR}_{\text{nadir}})\), time to nadir \((\text{NADIR}_{\text{ANC}})\) and time to recover to the ANC baseline value \((\text{RECOVERY-ANC0}_{\text{time}})\). The dots represent the errors, connected by lines. Orange, blue and green colors indicate the daily, every other and every third day monitoring of the ANC, respectively. The empty diamond represents the RMSE\(_d\) of the scenario with data available only at baseline. The shaded grey areas represent 95% (2.5th to 97.5th percentiles) of the true times of nadir and recovery to baseline, respectively.

**Figure 9.** The dots represent the sensitivity (pink) and specificity (blue) for classification of Grade 4 neutropenia (i.e., absolute neutrophil count (ANC)<0.5·10^9 cells/L, left) and an ANC≤0.1·10^9 cells/L (right), based on daily monitoring of the ANC, connected by lines. The diamonds represent the baseline and baseline and day 5 scenarios. The shaded grey areas represent 95% (2.5th to 97.5th percentiles) of the true times for occurrence of Grade 4 neutropenia (left) and an ANC≤0.1·10^9 cells/L (right).
for the less toxic drug, generally better with a longer MTT and differences were negligible for the smaller residual error. For additional details and graphical representation of these results, see Supplementary Material to Paper I.

4.3 Exploratory analysis of atezolizumab biomarkers

The visual inspection of the exploratory analysis of the eight biomarkers resulted in the selection of IL-18 and ITAC for further PKPD-modeling and evaluation as potential predictors of tumor size changes in response to atezolizumab treatment. IL-18 and ITAC showed a possible exposure-response (as assessed in Figure 10). A potential (although difficult to assess due to high variability and dropout) correlation between the biomarker (mainly for ITAC) response at day 21 and the tumor size changes in cycle 6 (Figure 11). IL-18 and ITAC increased during the first cycle and peaked at day 21. While the IL-18 response thereafter slowly returned to baseline, the ITAC response was instead retained (still lower than at day 21) for a longer time.

4.4 Biomarker models

Models of circulating biomarkers were developed in Papers II, III and V and tumor size models were developed in Papers III and V. Three modeling frameworks, all including biomarker models, were developed in total in Paper II (IL-6, CRP and FN), Papers III and IV (atezolizumab PK, IL-18, tumor size and OS) and Paper V (tumor size, CTC and OS). Schematic representations of these modeling frameworks, including TTE variables, are presented in Figures 12-14, respectively (Figure 13 illustrates the extension of OS in Paper IV to the modeling framework developed in Paper III). The details of the different final biomarker models are given below.

4.4.1 Circulating biomarkers

Elevated IL-6 and CRP concentrations were allowed in cycles 1 and/or 4 (or no elevation), resulting in a model with 16 possible subpopulations (as defined by the mixture model). However, only a single probability parameter was estimated for IL-6 and CRP each, which was lower for CRP (44%) than IL-6 (63%). This was however not unexpected since the CRP production was stimulated by a change in IL-6, using a linear function (ΔOFV=61) and the actual number of CRP elevations were higher than for IL-6.

IIV was related to IL-6₀, CRP₀ and kₜₜₜₜₑₜ and IOV was related to SACRP, SWCRP, PTCRP and PTIₚₚ. No parameter correlations could be identified after
Figure 10. Boxplots of the observed relative change from baseline (RCFB) of the eight selected biomarkers versus protocol-specified nominal time after first dose. The plots are grouped by the protocol-specified nominal dose where the light, medium, and dark boxes represent doses of 10, 15 and 20 mg/kg, respectively. (Data observed following dosing at 1 mg/kg are not shown.) Observations related to the fixed dose of 1200 mg are included in the groups most closely related to the corresponding dose in mg/kg; measurements for 5 patients in the 15 mg/kg group and 2 in the 20 mg/kg group. The upper and lower hinges of the boxes represent the 75th and 25th percentiles, respectively. Upper and lower ends of the whiskers correspond to the 75th percentile+1.5·IQR and 25th percentile-1.5·IQR, respectively, where IQR is the interquartile range. No outliers are shown. The dashed line indicates no RCFB.

CD40, cluster of differentiation 40; CRP, C-reactive protein; ICAM-1, intracellular adhesion molecule 1, IL-18, interleukin 18, IL-8, interleukin 8; ITAC, interferon-inducible T-cell alpha chemoattractant; VCAM-1, vascular cell adhesion molecule 1; T_CD8, prolif/activ, activated T cells

Figure 11. The RCFB tumor size (at the end of cycle 6) versus the biomarker RCFB (at day 21) for the eight graphically explored biomarkers. The x-axis is limited in 5 plots to present the majority of the data. The solid black line represents the smoothed line.

CD40, cluster of differentiation 40; CRP, C-reactive protein; ICAM-1, intracellular adhesion molecule 1, IL-18, interleukin 18, IL-8, interleukin 8; ITAC, interferon-inducible T-cell alpha chemoattractant; VCAM-1, vascular cell adhesion molecule 1; T_CD8, prolif/activ, activated T cells
The inclusion of the IL-6 regulation on the CRP production. The \(PT_{IL-6}\) (137 h) was forced to be shorter than \(PT_{CRP}\) (187 h) as the latter was constrained to be the \(PT_{IL-6}\) plus an estimated additional time (\(PT_{CRP+}\)). IL-6 and CRP elevations were predicted in all cycles related to an FN event (except for one patient that had no IL-6 elevation). The peak IL-6 and CRP concentrations were typically predicted 1.3 and 0.4 days prior to FN, respectively.

The pcVPC (Figure 15) of the final model showed an overall good fit of both the IL-6 and CRP data to the model, with some underprediction of the lower percentiles, especially for CRP in cycle 4 (bottom right panel in Figure 15). Parameter estimates and corresponding RSEs for the final model are reported in Table 4.

The IDR model with a linear stimulation by atezolizumab of \(k_{rel}\) (IL-18: \(\Delta OFV=13.8\), ITAC: \(\Delta OFV=67.6\)) together with the inclusion of a pool compartment (IL-18: \(\Delta OFV=66.2\), ITAC: \(\Delta OFV=12.1\)) best described both the IL-18 and ITAC data. Additional effect delay was identified for IL-18 and was characterized through the inclusion of an effect compartment (\(\Delta OFV=27.7\)). The pcVPCs (panel b and c in Figure 16) of the final IL-18 and ITAC models illustrate no major model misspecification. The IL-18 and ITAC parameters were estimated with reasonable precision (Table 5).

The assumption of equidispersion in the Poisson model was violated since the mean of the CTC count data was larger than the variance of the CTC count data in the current data set. Instead, the negative binomial model improved the
The probability of observing a CTC count equal to $n$ in the negative binomial model is described in Equation 16;

$$P(CTC = n) = \frac{\Gamma\left(n + \frac{1}{\sigma}\right)}{\Gamma\left(\frac{1}{\sigma} \cdot n!\right)} \cdot \left(1 + \frac{\lambda_{CTC}(t)}{1 + \lambda_{CTC}(t)}\right)^{\frac{1}{\sigma}} \cdot \left(\frac{\lambda_{CTC}(t)}{1 + \lambda_{CTC}(t)}\right)^n$$

where $\lambda_{CTC}(t)$ is the time-course of the mean CTC count in 7.5 mL blood, $\sigma$ is a parameter describing overdispersion and $\Gamma$ and $n!$ represent the gamma function and factorial function of $n$, respectively.
Since a majority of the CTC measurements did not detect any CTC, the model was evaluated primarily by inspecting the VPC of the proportion of CTC samples equal to 0. The base model did however not characterize the time-course of undetectable samples well (left panel in Figure 17). The OFV dropped additionally when SLD (model described in the next section) was introduced as a predictor of $\lambda_{CTC}(t)$ ($\Delta$OFV=206) and resulted in a better capture of the later re-increase in samples equal to 0 of the VPC (middle panel Figure 17). The initial increase in proportion of CTC samples=0 was captured when a dose-response relationship was included (right panel Figure 17). Addition of TND as a predictor of $\lambda_{CTC}(t)$ also improved the model fit statistically significant ($\Delta$OFV=463). The $\lambda_{CTC}(t)$ can consequently be described as in Equation 17:
**Figure 15.** Prediction-corrected visual predictive check (pcVPC) for the final interleukin 6 (IL-6) and C-reactive protein (CRP) model in *Paper II*. The plot is stratified by biomarker and cycle. The solid and the upper and lower dashed red lines are the median and the 90th and 10th percentiles of the observed data, respectively. The shaded red, upper and lower green areas are the 95% CIs around the median, 90th and 10th percentiles of the simulated data (n=500), respectively. Open circles represent observations.

**Figure 16.** Prediction-corrected visual predictive check (pcVPC) for the final models in *Paper III* for a) tumor size (sum of longest diameter, SLD, with the dropout model included) b) interleukin 18 (IL-18) and c) interferon-inducible T cell alpha chemoattractant (ITAC). The x-axes are limited to 585 (a) and 175 (b and c) days after first dose. The dashed lines represent the observed 10th and 90th percentiles (a) and the 2.5th and 97.5th percentiles (b and c). Solid lines represent median values. The shaded areas are the 95% CIs of the corresponding percentiles computed from the simulated data sets (n=500). The open circles are observed biomarker concentrations and SLD assessments.
Table 4. Parameter estimates and relative standard errors for the final interleukin 6 (IL-6) and C-reactive protein (CRP) and time-to-febrile neutropenia (FN) models in Paper II

<table>
<thead>
<tr>
<th>Parameter (unit)</th>
<th>Typical value (RSE,%)</th>
<th>IIV/IOV, CV% (RSE, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IL-6 and CRP model</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6₀ (pg/mL)</td>
<td>2.50 (9.2)</td>
<td>68.0 (11)</td>
</tr>
<tr>
<td>CRP₀ (mg/L)</td>
<td>1.88 (12)</td>
<td>80.5 (11)</td>
</tr>
<tr>
<td>k_out,IL-6 (h⁻¹)</td>
<td>0.0141 (25)</td>
<td>130 (22)</td>
</tr>
<tr>
<td>k_out,CRP (h⁻¹)</td>
<td>0.0224 (13)</td>
<td>-</td>
</tr>
<tr>
<td>P升降,IL-6 (%)</td>
<td>63.4 (10)</td>
<td>-</td>
</tr>
<tr>
<td>P升降,CRP (%)</td>
<td>44.3 (20)</td>
<td>-</td>
</tr>
<tr>
<td>Slope (RCFBIL-6₀⁻¹)</td>
<td>1.05 (18)</td>
<td>-</td>
</tr>
<tr>
<td>Prop. IL-6 error (%)</td>
<td>54.7 (4.7)</td>
<td>-</td>
</tr>
<tr>
<td>Prop. CRP error (%)</td>
<td>53.0 (4.1)</td>
<td>-</td>
</tr>
<tr>
<td>SₐIL-6</td>
<td>7.99 (16)</td>
<td>-</td>
</tr>
<tr>
<td>SₐCRP</td>
<td>4.40 (21)</td>
<td>61.4 (38)</td>
</tr>
<tr>
<td>SW_IL-6 (h)</td>
<td>32.4 (11)</td>
<td>-</td>
</tr>
<tr>
<td>SW_CRP (h)</td>
<td>53.8 (17)</td>
<td>83.8 (32)</td>
</tr>
<tr>
<td>PT_IL-6 (h)</td>
<td>137 (9.7)</td>
<td>59.7 (14)</td>
</tr>
<tr>
<td>PT_CRP⁺ (h)</td>
<td>50.3 (32)</td>
<td>81.3 (27)</td>
</tr>
<tr>
<td><strong>FN model (prior-to-chemotherapy)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>h₀ (h⁻¹)</td>
<td>5.70x10⁻³ (31)</td>
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</tr>
<tr>
<td>β₁ (years⁻¹)</td>
<td>0.0754 (40)</td>
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<tr>
<td><strong>FN model (prior-to-FN)</strong></td>
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</tr>
<tr>
<td>h₀ (h⁻¹)</td>
<td>3.3x10⁻⁴ (110)</td>
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</tr>
<tr>
<td>kₑ₀ (h⁻¹)</td>
<td>0.491 (40)</td>
<td>-</td>
</tr>
<tr>
<td>β₂ (LN_IL-6₀⁻¹)</td>
<td>3.13 (15)</td>
<td>-</td>
</tr>
<tr>
<td>β₃ (L/10⁹ cells)</td>
<td>-1.07 (33)</td>
<td>-</td>
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<tr>
<td><strong>FN model (when-FN-occurs)</strong></td>
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<td></td>
</tr>
<tr>
<td>h₀ (h⁻¹)</td>
<td>7.61x10⁻⁵ (120)</td>
<td>-</td>
</tr>
<tr>
<td>β₄ (LN CRP₀⁻¹)</td>
<td>2.33 (12)</td>
<td>-</td>
</tr>
</tbody>
</table>

IL-6₀ and CRP₀, baseline IL-6 and CRP concentrations; k_out,IL-6 and k_out,CRP, first-order IL-6 and CRP elimination rate constants; P升降,IL-6 and P升降,CRP, probability for elevated IL-6 and CRP concentrations (regulated by surge functions) in either cycle 1 or 4; Slope, parameter relating the relative change from IL-6 baseline time-course (RCFBIL-6₀⁻¹) to the CRP production; SₐIL-6 and SₐCRP, IL-6 and CRP surge amplitudes; SW_IL-6 and SW_CRP, IL-6 and CRP surge widths; PT_IL-6, IL-6 surge peak time; PT_CRP⁺, time added to PT_IL-6 to get the CRP surge peak time; FN, febrile neutropenia; h₀, the baseline hazard for FN; β₁, β₂, β₃ and β₄, parameter relating age, the effect concentration of the logarithmic and normalized to the population IL-6 baseline time-course (CE LN_IL-6(t))), the model-derived ANC₀ (baseline absolute neutrophil count) and the logarithmic and normalized to the population CRP baseline CRP time-course (LN_CRP(t)) to FN, respectively; kₑ₀, effect compartment rate constant; RSE, relative standard error; IIV, inter-individual variability; IOV, inter-occasion variability; CV%, coefficient of variation.

The RSEs were generated from the SIR procedure.
Table 5. Parameter estimates and relative standard errors for the final interleukin 18 (IL-18), interferon-inducible T cell alpha chemoattractant (ITAC), tumor size and dropout models in Paper III

<table>
<thead>
<tr>
<th>Parameter (unit)</th>
<th>Typical value (RSE,%)</th>
<th>IIV, CV% (RSE, %)</th>
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<tbody>
<tr>
<td><strong>IL-18 model</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( R_{in} ) (pg/mL/day)</td>
<td>23.0 (12)</td>
<td>-</td>
</tr>
<tr>
<td>( k_{out} ) (day(^{-1}))</td>
<td>0.106 (13)</td>
<td>47 (8.7)</td>
</tr>
<tr>
<td>( Pool_0 ) (pg/mL)</td>
<td>499 (18)</td>
<td>78 (18)</td>
</tr>
<tr>
<td>( Slope ) (mL/μg)</td>
<td>0.168 (26)</td>
<td>80 (26)</td>
</tr>
<tr>
<td>( k_{el} ) (day(^{-1}))</td>
<td>4.36·10(^{-3}) (25)</td>
<td>-</td>
</tr>
<tr>
<td><strong>Proportional error (%)</strong></td>
<td>22.3 (2.5)</td>
<td>-</td>
</tr>
<tr>
<td><strong>ITAC model</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( R_{in} ) (pg/mL/day)</td>
<td>3.07 (24)</td>
<td>48 (10)</td>
</tr>
<tr>
<td>( k_{out} ) (day(^{-1}))</td>
<td>7.91·10(^{-3}) (23)</td>
<td>-</td>
</tr>
<tr>
<td>( Pool_0 ) (pg/mL)</td>
<td>271 (20)</td>
<td>115 (15)</td>
</tr>
<tr>
<td>( Slope ) (mL/μg)</td>
<td>0.0599 (35)</td>
<td>-</td>
</tr>
<tr>
<td><strong>Proportional error (%)</strong></td>
<td>28.3 (3.9)</td>
<td>-</td>
</tr>
</tbody>
</table>

| **Tumor size model**      |                       |                   |
| \( SLD_0 \) (cm)         | 4.75 (11)             | 62 (8.3)         |
| \( R_{Growth} \) (cm/week) | 0.0169 (17)           | 93 (17)         |
| \( k_{Shr,Drug} \) (L/mg/day/week) | 2.75·10\(^{-6}\) (23) | 124 (21) |
| \( \lambda_{Drug} \) (week\(^{-1}\)) | 0.0609 (8.7)          | -                |
| \( k_{Shr,IL-18} \) (1/(% week)) | 0.00282 (14)          | 181 (24)        |
| \( \theta_{R-growth-LiverMets.} \) | 8.48 (8.5)            | -                |
| \( \theta_{SLD0-Mets} \) | 0.927 (27)            | -                |
| **Proportional error (%)** | 13.4 (2.1)            | -                |
| **Dropout model**         |                       |                   |
| \( h_0 \) (week\(^{-1}\)) | 0.0306 (1.8)          | -                |
| \( \beta \)              | 1.56 (8.8)            | -                |

\( R_{in} \), zero-order production rate; \( k_{out} \), first-order elimination rate constant; \( Pool_0 \), baseline concentration in the pool compartment; \( Slope \), linear drug effect; \( k_{el} \), first-order effect compartment rate constant; \( SLD_0 \), baseline sum of longest diameter; \( R_{Growth} \), zero-order tumor growth rate; \( k_{Shr,Drug} \), rate constant related to tumor shrinkage by AUC in former or current smokers; \( \lambda_{Drug} \), rate constant for reduced effect; \( k_{Shr,IL-18} \), rate constant related to tumor shrinkage by IL-18; \( \theta_{R-growth-LiverMets.} \), describes the fractional change in \( R_{Growth} \) \((1+\theta)\) for patients with liver metastases at baseline; \( \theta_{SLD0-Mets} \), describes the fractional change in \( SLD_0 \) \((1+\theta)\) for patients with more than two metastases at baseline; \( h_0 \), baseline hazard; \( \beta \), parameter relating the tumor size to dropout RSE, relative standard error; IIV, inter-individual variability; CV\%, coefficient of variation

The RSEs were computed based on the NONMEM variance covariance matrix (S matrix)

\[
\lambda_{CTC}(t) = CTC_0 \cdot e^{\beta_{SLD(t)}(SLD(t)-1)} \cdot \left(1 - \frac{TND}{D50 + TND}\right)
\]

where \( CTC_0 \) is the baseline count for an SLD of 1 mm and in absence of drug, \( \beta_{SLD(t)} \) is a parameter relating \( SLD(t) \) to \( \lambda_{CTC}(t) \) and \( D50 \) is the TND accounting for 50% of the maximum drug-related effect. Parameter estimates and corresponding RSEs are presented in Table 6.
4.4.2 Tumor size models

Two different tumor size models were developed, one in patients with NSCLC (Paper III) and one in patients with mCRC (Paper V). These are further described below.

4.4.2.1 NSCLC tumor size model

The TGI model proposed by Claret et al. [50] described the current tumor size data in NSCLC patients best of the three evaluated structural models (see section 3.3.3.2 Tumor size). A constant growth rate ($R_{\text{growth}}$) described the data better than an exponential rate ($\Delta \text{OFV}=6.4$). When predictors of tumor shrinkage were evaluated in the TGI model, the $\text{AUC}_{\text{cycle,n}}$ provided the best model fit and was added linearly to the model. The $E_{\text{max}}$ model did not provide a statistically significant drop of the OFV. The OFV was approximately eight units higher when $\text{AUC}_{\text{cycle1}}$ was used. When $\lambda_{\text{Drug}}$ (drug-resistance parameter) was added, the OFV dropped 74 units. The estimate of $\lambda_{\text{Drug}}$ was 0.0609 week$^{-1}$, resulting in a loss of the $\text{AUC}_{\text{cycle,n}}$-effect with a half-life of 80 days. None of the investigated IL-18 or ITAC metrics described tumor shrinkage better than $\text{AUC}_{\text{cycle,n}}$, neither when they were evaluated alone nor in combination. However, an improvement of the model fit was observed when they were added on top of the $\text{AUC}_{\text{cycle,n}}$-effect. The model-predicted $\text{RCFB}_{\text{IL-18,d21}}$ provided the largest drop in OFV when added on top of $\text{AUC}_{\text{cycle,n}}$ ($\Delta \text{OFV}=44.2$) followed by $\text{RCFB}_{\text{ITAC,d21}}$ ($\Delta \text{OFV}=38.9$). The IL-18 and ITAC CFB time-courses also provided significant improvement of the model fit.
Table 6. Parameter estimates and relative standard errors for the final, tumor size, circulating tumor cell (CTC) and overall survival models in Paper V

<table>
<thead>
<tr>
<th>Parameter (unit)</th>
<th>Typical value (RSE,%)</th>
<th>IV, CV% (RSE, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tumor size model</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$SLD_0$ (mm)</td>
<td>88.3 (4.2)</td>
<td>^a0.586 (7.6)</td>
</tr>
<tr>
<td>$Box-cox shape$ (IIV-$SLD_0$)</td>
<td>-0.246 (23)</td>
<td></td>
</tr>
<tr>
<td>$FR_{logit}$</td>
<td>0.279 (25)</td>
<td>^a2.30 (12.7)</td>
</tr>
<tr>
<td>$FR$ (%)</td>
<td>56.9</td>
<td></td>
</tr>
<tr>
<td>$R_{Grow, sensitive, slow}$ (mm/week)</td>
<td>8.38∙10^{-2} (11)</td>
<td></td>
</tr>
<tr>
<td>$R_{Grow, sensitive, fast}$ (mm/week)</td>
<td>5.02 (28)</td>
<td></td>
</tr>
<tr>
<td>$k_{Kill}$ (week$^{-1}$)</td>
<td>9.55∙10^{-3} (7.0)</td>
<td>71.4 (7.9)</td>
</tr>
<tr>
<td>$k_{Delay, slow}$ (week$^{-1}$)</td>
<td>4.53∙10^{-3} (0.41)</td>
<td></td>
</tr>
<tr>
<td>$k_{Delay, fast}$ (week$^{-1}$)</td>
<td>66.6∙10^{-3} (0.70)</td>
<td>57.3 (3.0)</td>
</tr>
<tr>
<td>$k_{Grow, resistant}$ (week$^{-1}$)</td>
<td>55.3∙10^{-3} (0.32)</td>
<td>83.2 (7.4)</td>
</tr>
<tr>
<td>$θ_{1Mix,logit}$</td>
<td>-0.103 (230)</td>
<td></td>
</tr>
<tr>
<td>$θ_{2Mix,logit}$</td>
<td>-2.37 (16)</td>
<td></td>
</tr>
<tr>
<td>P(Mix 1) (%)</td>
<td>52.6 ^b(41-64)</td>
<td></td>
</tr>
<tr>
<td>P(Mix 2) (%)</td>
<td>39.7 ^b(26-53)</td>
<td></td>
</tr>
<tr>
<td>P(Mix 3) (%)</td>
<td>7.7 ^b(2.4-22)</td>
<td></td>
</tr>
<tr>
<td>$Error_{proportional}$</td>
<td>0.0889 (3.7)</td>
<td>17.1 (11)</td>
</tr>
<tr>
<td>$Error_{additive}$ (mm)</td>
<td>1.97 (1.45)</td>
<td></td>
</tr>
<tr>
<td><strong>CTC model</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$CTC_0$</td>
<td>0.704 (11)</td>
<td>274 (4.3)</td>
</tr>
<tr>
<td>$O$</td>
<td>1.40 (15)</td>
<td>732 (9.1)</td>
</tr>
<tr>
<td>$β_{SLD0}$ (mm$^{-1}$)</td>
<td>8.11∙10^{-3} (11)</td>
<td></td>
</tr>
<tr>
<td>$D50$</td>
<td>0.503 (11)</td>
<td>176 (11)</td>
</tr>
<tr>
<td><strong>OS model</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$β_0$</td>
<td>9.90 (0.57)</td>
<td></td>
</tr>
<tr>
<td>$γ$</td>
<td>0.589 (4.6)</td>
<td></td>
</tr>
<tr>
<td>$β_{BTS}$ (mm$^{-1}$)</td>
<td>1.75∙10^{-3} ^c(36)</td>
<td></td>
</tr>
<tr>
<td>$β_{Age}$ (years$^{-1}$)</td>
<td>1.73∙10^{-2} ^d(34)</td>
<td></td>
</tr>
<tr>
<td>$θ_{λ_{CTC}}, λ_{CTC} ≥ 3$</td>
<td>1.25 ^c(8.5)</td>
<td></td>
</tr>
</tbody>
</table>

$SLD_0$, baseline SLD; SLD, sum of longest diameter; $FR_{logit}$, fraction drug-resistant tumor of $SLD_0$, estimated on logit scale; $FR$, corresponding actual fraction of $FR_{logit}$; $R_{Grow, sensitive, slow}$, slow growth rate constant of drug-sensitive fraction; $R_{Grow, sensitive, fast}$, fast growth rate constant of drug-sensitive fraction; $k_{Kill}$, tumor kill rate constant; $k_{Delay, slow}$, slow transit compartment delay rate constant from quiescent to tumor to drug-resistant tumor; $k_{Delay, fast}$, fast transit compartment delay rate constant from quiescent tumor to drug-resistant tumor; $k_{Grow, resistant}$, growth rate constant of drug-resistant fraction; $θ_{1Mix,logit}$, mixture parameter 1 estimated on logit scale; $θ_{2Mix,logit}$, mixture parameter 2 estimated on logit scale; $P(Mix 1)$, proportion of patients in mixture 1 (i.e., $k_{Delay, slow} + R_{Grow, sensitive, slow}$); $P(Mix 2)$, proportion of patients in mixture 2 (i.e., $k_{Delay, fast} + R_{Grow, sensitive, slow}$); $P(Mix 3)$, proportion of patients in mixture 3 (i.e., $k_{Delay, slow} + R_{Grow, sensitive, fast}$); $CTC_0$, baseline mean of CTC count per mm SLD in 7.5 mL blood; $O$, overdispersion parameter; $β_{SLD, CTC}$, parameter relating SLD(t) to the mean CTC count; $D50$, total normalized dose accounting for 50% of the maximum drug-related effect; $β_0$, scale parameter in the log-logistic distribution; $γ$, shape parameter in the log-logistic distribution; $β_{BTS}$ and $β_{Age}$ parameters relating baseline tumor size and age to the hazard; $θ_{λ_{CTC}}, λ_{CTC} ≥ 3$, fractional change of the hazard for $λ_{CTC}(t) ≥ 3$ in 7.5 mL blood; CV, coefficient of variation; IV, interindividual variability

^a) IV is reported as a variance

| | ^b) corresponding 95% CI, given the uncertainty in $θ_{1Mix,logit}$ and $θ_{2Mix,logit}$
| | ^c) corresponding 95% CI: 0.52-10^{-1}-3.0∙10^{-1}
| | ^d) corresponding 95% CI: 0.58∙10^{-2}-2.9∙10^{-2}
| | ^e) corresponding 95% CI: 1.0-1.5

52
when added on top of the $AUC_{cycle,n}$-effect ($\Delta OFV=21.1$ and $14.3$, respectively), although the OFV drop was smaller than for $RCFB_{IL-18,d21}$ and $RCFB_{ITAC,d21}$. Resistance to the biomarkers (inclusion of a $\lambda_{Biomarker}$-term) was explored but did not improve the model fit for any investigated model. Since $RCFB_{IL-18,d21}$ provided the best on-top-of-$AUC_{cycle,n}$ improvement, $RCFB_{IL-18,d21}$ was included in the final tumor size model. The pcVPC (panel a in Figure 16) shows an overall good fit of the final model to the data. Parameter estimates and RSEs of the final tumor size model are given in Table 5. The rate of tumor size (i.e., SLD) changes in the final model is described in Equation 18;

$$\frac{d(SLD)}{dt} = R_{Growth} - (k_{Shr, Drug} \cdot AUC_{cycle,n} \cdot e^{-\lambda_{Drug} \cdot t} + k_{Shr, IL-18} \cdot RCFB_{IL-18,d21}) \cdot SLD$$

where $k_{Shr, Drug}$ and $k_{Shr, IL-18}$ are parameters relating $AUC_{cycle,n}$ and $RCFB_{IL-18,d21}$ to tumor shrinkage, respectively.

The estimated baseline SLD ($SLD_0$) for patients with two or less metastases at baseline was 4.8 cm, while it was 9.1 cm for patients with more than two metastases ($\Delta OFV=21.5$ in the covariate analysis). There was almost a 10-fold difference in $R_{growth}$ between patients without (0.017 cm/week) and with (0.16 cm/week) liver metastases at baseline ($\Delta OFV=12.4$ in the covariate analysis). The last identified covariate-parameter relationship was related to smoking and $k_{Shr, Drug}$ ($\Delta OFV=11.2$ in the covariate analysis). $k_{Shr, Drug}$ was not statistically different from zero in patients that never smoked while it was $2.75 \cdot 10^{-6}$ L/mg/day/week in former and current smokers. In summary, three covariate-parameter relationships were included in the NSCLC tumor size model, i.e., number of metastases and $SLD_0$, liver metastases at baseline and $R_{growth}$, and smoking status and $k_{Shr, Drug}$.

The typical tumor time-course for a former/current smoking NSCLC patient with two or less metastases and no liver metastases at baseline was simulated to illustrate the impact of $AUC_{cycle,n}$ and $RCFB_{IL-18,d21}$ on tumor size changes. Time-courses were simulated for the 5th, 50th and 95th percentiles of the individual $AUC_{cycle,n}$ and predicted $RCFB_{IL-18,d21}$ (given a fixed atezolizumab dose of 1200 mg) in the study population. The results are illustrated in Figure 18. $AUC_{cycle,n}$ was more important for tumor shrinkage in the early cycles, whereas the effect by $RCFB_{IL-18,d21}$ was more prominent as the $AUC_{cycle,n}$-effect was washed out, and especially for the 95th percentile of $RCFB_{IL-18,d21}$.

### 4.4.2.2 mCRC tumor size and dropout models

The model of tumor quiescence and drug-resistance performed better than the TGI model with respect to predicting the mCRC tumor size data. Though, patients that progressed immediately after start of treatment were poorly predicted. None of the evaluated skewed or binomial distributions resulted in better prediction of these profiles. However, the addition of a third subpopulation
with a fast growth rate of the drug-sensitive fraction) captured the data from these patients. A linear growth rate of the drug-sensitive fraction improved the model fit in comparison to an exponential growth rate ($\Delta OFV=41.5$). There was no statistically significant relationship between changes in SLD and TND. Overall, the mCRC tumor size model predicted the individual profiles well, illustrated for nine patients (three in each subpopulation) in Figure 19. Parameter estimates and RSEs are presented in Table 6.

**Figure 18.** Simulations of the tumor size, i.e., sum of longest diameter (SLD) time-course for a typical patient (former/current smoker, two or less metastases and no liver metastases at baseline) to illustrate the impact of interleukin 18 (IL-18), i.e., the IL-18 relative change from baseline at day 21 ($RCF_{BIL-18,d21}$) and the cycle-specific atezolizumab area under the curve ($AUC_{cycle,n}$) (right panel) on the tumor dynamics. The value of $AUC_{cycle,n}$ was set to 5600 mg/L•day in the left panel and the value of $RCF_{BIL-18,d21}$ was set to 0.43 in the right panel.

**Figure 19.** Individual tumor size, i.e., sum of longest diameter (SLD) predictions in the final metastatic colorectal cancer tumor size model (lines) and observed tumor sizes (dots) for nine exemplary patients (indicated by the shape of the dots)

Patients in subpopulation 1: slow transit compartment delay rate constant from quiescent to drug-resistant tumor and slow growth rate constant of drug-sensitive fraction

Patients in subpopulation 2: fast transit compartment delay rate constant from quiescent to drug-resistant tumor and slow growth rate constant of drug-sensitive fraction

Patients in subpopulation 3: slow transit compartment delay rate constant from quiescent to drug-resistant tumor and fast growth rate constant of drug-sensitive fraction
Dropout was modeled using logistic regression and the probability of dropping out increased when patients displayed progressive disease ($\theta_{\text{PRD}}=2.05$, 95% CI, 1.61-2.50). The final mCRC tumor size and dropout models were combined to illustrate the predictive performance of the tumor size model. The observed tumor size data agreed well with the simulated data, taking dropout into account (left panel in Figure 20).

![Figure 20](image)

**Figure 20.** Visual predictive check (VPC) of the final metastatic colorectal cancer tumor size model, taking dropout into account (left panel) and Kaplan-Meier (KM) VPC of the final metastatic colorectal cancer overall survival (OS) model (right panel). The solid and dashed lines in the left panel represent the observed median and 5th (lower line) and 95th (upper line) percentiles of the data, respectively. The shaded areas in the left panel are the corresponding 95% CIs of the simulated data, derived from 200 simulations from the final tumor size model. The grey horizontal line represents a sum of longest diameter (SLD) of 10 mm. The right panel illustrates the observed KM curve (black line) in comparison to the 95% CI, generated from 100 simulations (grey shaded area). Black vertical lines indicate censored events.

### 4.5 Models for time-to-events

TTE models were developed in *Papers II-V*. The details of these final models are given below.

#### 4.5.1 Models for febrile neutropenia

The distribution of event times (i.e., for FN) was described by the exponential distribution. A time-varying hazard (Weibull distribution) did not provide a better fit of the FN data. Three final models were developed based on the type of evaluated predictor. KM VPCs of these three models and the base model are given in Figure 21. The observed distribution of FN is within the 95% confidence interval (CI) of percent patients with FN for all models, although the tighter 50% CI indicates some misspecification. The parameter estimates and their RSEs are presented in Table 4. The RSEs related to the baseline
hazard, $h_0$, in these models were high (31-120%). This can, however, be expected when $h_0$ is approaching zero which is the case when included predictors describe a majority of the hazard. The three alternative models are described below and their respective parametrizations of the hazard function, $h(t)$, are given in Equations 19-21. The overall modeling framework is illustrated in Figure 12.

Three of the variables available prior-to-chemotherapy provided statistically significant drops of the OFV, age ($\Delta$OFV=6.06), observed cycle 1 CRP baseline ($\Delta$OFV=4.89) and cycle-specific CRP baseline ($\Delta$OFV=4.28) in the univariate analysis. However, no additional variable improved the model fit after age had been included.

$$h(t) = h_0 \cdot e^{\beta_1 \cdot (AGE-54)}$$

The $\beta_1$ parameter relates age to the risk of developing FN.

A better description of the data was obtained in the analysis with variables available prior-to-FN (total $\Delta$OFV=47.3). Six variables provided statistically significant improvement of the model fit and the predicted IL-6 time-course, logarithmic and normalized to the population IL-6 baseline ($LN_{IL-6}(t)$), resulted in the largest OFV drop ($\Delta$OFV=37.0). An effect compartment was included to describe the delay between IL-6 and FN ($\Delta$OFV=9.47). The model-estimated ANC baseline ($ANC_0$) gave additional descriptive value in the second step, i.e., on top of $LN_{IL-6}(t)$ ($\Delta$OFV=10.3). No additional predictors could be identified.

$$h(t) = h_0 \cdot e^{(\beta_2 \cdot (CE(LN_{IL-6}(t)) + \beta_3 \cdot (ANC_0 - 3.53)))}$$

The $\beta_2$ and $\beta_3$ parameters relate the effect concentration of $LN_{IL-6}(t)$ and $ANC_0$ to the risk of developing FN, respectively.

Figure 21. Kaplan-Meier (KM) visual predictive checks (VPCs) of percent patients with febrile neutropenia (FN) for the base model and the three alternative models with predictors available prior-to-chemotherapy, prior-to-FN and when-FN-occurs. The solid black line represents the observed time-to-FN data in both cycle 1 and 4. The shaded light and dark grey areas are the 95% and 50% CIs based on the simulated data (n=1000).
Larger improvements of the model fit were observed for variables available when FN occurs. Three different parameterizations of the CRP time-course, i.e., logarithmic and normalized to the population CRP baseline (\(\text{LN}_{-}\text{CRP}(t)\)) (\(\Delta\text{OFV}=68.9\)), absolute (\(\Delta\text{OFV}=52.0\)) and relative change from baseline (\(\Delta\text{OFV}=38.8\)) and the partial CRP AUC (\(\Delta\text{OFV}=13.1\)) provided statistically significant drops of the OFV in the univariate analysis. No variable provided additional improvement of the model fit when added on top of \(\text{LN}_{-}\text{CRP}(t)\).

\[
h(t) = h_0 \cdot e^{\beta_4 (\text{LN}_{-}\text{CRP}(t))}
\]

The \(\beta_4\) parameter relates \(\text{LN}_{-}\text{CRP}(t)\) to the risk of developing FN.

4.5.2 Dropout model

A time-varying hazard did not provide a significantly better model fit than the time-constant baseline hazard for description of dropout in Paper III. The model-predicted change from tumor size at baseline was included as a predictor of dropout since it provided the best improvement of the model fit of all investigated variables. The dropout model was used to generate the pcVPC of the final tumor size model (panel a in Figure 16). Estimates and RSEs of parameters in the dropout model are given in Table 5.

4.5.3 Overall survival

Models of OS were developed in Papers IV (NSCLC patients treated with atezolizumab) and V (mCRC patients treated with chemotherapy and targeted therapy). Four final models were developed in Paper IV, for which the influence of censoring strategy on inclusion of predictors and their effect sizes were explored. A summary of all final OS models developed in this thesis is given in Table 7. KM VPCs of the final NSCLC models revealed a good fit of the models to the data with some overprediction between one and two years after start of treatment in the C2YE model (Figure 22). The KM VPC of the final mCRC model revealed no misspecification (right panel in Figure 20). Parameter estimates and corresponding RSEs are presented in Table 8 (NSCLC models) and Table 6 (mCRC model). The RSEs were relatively large in the NSCLC models, with half of all parameters having an RSE above 50%.

The hazard was lower for lower values of alkaline phosphatase (ALP) and higher values of neutrophil/lymphocyte ratio (NLR) in all four models, and all four models except the AAD NSCLC model, respectively. Higher values of lymphocyte count (LYM) predicted a lower hazard in the AAD model. Additionally, patients with higher PD-L1 expression had a higher hazard of dying.
in the C2YE and C2YASOT models. Additional baseline covariates were included in the NSCLC models after the first stepwise procedure (i.e., when only baseline covariates were explored), however these were removed from the

<table>
<thead>
<tr>
<th>Model</th>
<th>Distribution of event times</th>
<th>Included predictor(s)</th>
<th>Model(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSCLC-AAD (Paper IV)</td>
<td>Exponential</td>
<td>LYM, ALP</td>
<td>RCFB-SLD(t) (c-f)</td>
</tr>
<tr>
<td>NSCLC-C2YE (Paper IV)</td>
<td>Exponential</td>
<td>NLR, ALP, 1PD-L1 expression</td>
<td>RCFB-SLD(t) (ext)</td>
</tr>
<tr>
<td>NSCLC-C2YASOT (Paper IV)</td>
<td>Exponential</td>
<td>NLR, ALP, 1PD-L1 expression</td>
<td>RCFB-SLD(t) (ext)</td>
</tr>
<tr>
<td>NSCLC-C5MALD (Paper IV)</td>
<td>Gompertz</td>
<td>NLR, ALP</td>
<td>RCFB-SLD(t) (ext)</td>
</tr>
<tr>
<td>mCRC (Paper V)</td>
<td>Log-logistic</td>
<td>BTS, AGE</td>
<td>( \lambda_{CTC(t)} \geq 3 )</td>
</tr>
</tbody>
</table>

NSCLC, non-small cell lung cancer; AAD, all available data; C2YE, data censored no later than at a cut-off date set 2 years earlier than in AAD; C2YASOT, data censored no later than 2 years after start of treatment for each individual patient; C5MALD, data censored a maximum of five months after last dose; mCRC, metastatic colorectal cancer; LYM, lymphocyte count; ALP, alkaline phosphatase; PD-L1, programmed death-ligand 1; BTS, baseline tumor size; RCFB-SLD(t)(c-f.), relative change from baseline of the SLD time-course carried forward three weeks after last dose; RCFB-SLD(t)(ext.), relative change from baseline of the SLD time-course extrapolated based on the empirical Bayes estimates; \( \lambda_{CTC(t)} \geq 3 \), time-course of the mean CTC count \( \geq 3 \)  
1PD-L1+ immune cells/tumor mass <5% or PD-L1+ tumor cells/tumor mass <50% vs. PD-L1+ immune cells/tumor mass \( \geq 5\% \) or PD-L1+ tumor cells/tumor mass \( \geq 50\% \)

**Figure 22.** Kaplan-Meier (KM) visual predictive checks (VPCs) of the final non-small cell lung cancer (NSCLC) overall survival (OS) models in Paper IV. The four different OS models are based on i) all available data (AAD), ii) data censored no later than at a cut-off date set 2 years earlier than in AAD (C2YE), iii) data censored no later than 2 years after start of treatment for each individual patient (C2YASOT) and iv) data censored a maximum of five months after last dose (C5MALD). The solid black line is the observed KM curve, the grey shaded area is the 95% CI generated from 100 simulations and black vertical lines indicate censored events.
models either in the final backward elimination step or due to numerical difficulties in the estimation. The hazard was lower for lower values of baseline tumor size and age in the final mCRC model. The relative hazard versus the value of the continuous baseline covariate (directions of the relationships and effect sizes) for the baseline predictors are illustrated in Figure 23.

Even though IL-18 and ITAC predicted tumor size changes in Paper III, no statistically significant improvement was observed in any of the NSCLC OS models. Instead, the relative change from baseline of the SLD time-course, $RCFB-SLD(t)$, provided the best improvement in all four NSCLC models. $RCFB-SLD(t)$ was carried forward three weeks after last dose ($c-f$) in the AAD model and extrapolated based on the empirical Bayes estimates ($ext$) in the remaining three models. Only one model-derived variable was allowed in each of the NSCLC models due to model instability and large RSEs following addition of a second model-derived variable. The hazard functions, $h(t)$, of the final AAD, C2YE/C2YASOT and C5MALD models are presented in Equations 22-24, respectively:

$$h(t) = \lambda_{OS,exp} \cdot e^{(\beta_{LYM}(LYM - 1.2) + \beta_{ALP}(ALP - 82) + \beta_{RCFB-SLD(t)}(RCFB-SLD(t)(c-f)))}$$

Table 8. Parameter estimates and corresponding relative standard errors in the final non-small cell lung cancer (NSCLC) overall survival models

<table>
<thead>
<tr>
<th>Value (RSE, %)</th>
<th>AAD</th>
<th>C2YE</th>
<th>C2YASOT</th>
<th>C5MALD</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda_{OS,exp}$ (week$^{-1}$)</td>
<td>9.69$\times 10^{-3}$ (12)</td>
<td>8.12$\times 10^{-3}$ (15)</td>
<td>7.95$\times 10^{-3}$ (48)</td>
<td>-</td>
</tr>
<tr>
<td>$\lambda_{OS,Gomp}$ (week$^{-1}$)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>6.39$\times 10^{-3}$ (56)</td>
</tr>
<tr>
<td>$\gamma_{OS,Gomp}$ (week$^{-1}$)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-17.8$\times 10^{-3}$ (62)</td>
</tr>
<tr>
<td>$\beta_{ALP}$ (L/U)</td>
<td>5.90$\times 10^{-3}$ (22)</td>
<td>4.84$\times 10^{-3}$ (44)</td>
<td>4.67$\times 10^{-3}$ (87)</td>
<td>5.12$\times 10^{-3}$ (8.4)</td>
</tr>
<tr>
<td>$\beta_{LYM}$ (L/10$^9$ cells)</td>
<td>-0.780 (37)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>$\beta_{NLR}$</td>
<td>0.154 (31)</td>
<td>0.159 (29)</td>
<td>0.175 (83)</td>
<td>-</td>
</tr>
<tr>
<td>$\beta_{PD-L1}$</td>
<td>-0.505 (63)</td>
<td>-0.486 (67)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>$\beta_{RCFB-TS(t)} (c-f)$</td>
<td>1.44 (18)</td>
<td>-0.505 (63)</td>
<td>-0.486 (67)</td>
<td>-</td>
</tr>
<tr>
<td>$\beta_{RCFB-TS(t)} (ext)$</td>
<td>-1.26 (55)</td>
<td>1.32 (17)</td>
<td>1.63 (39)</td>
<td>-</td>
</tr>
</tbody>
</table>

$\lambda_{OS,exp}$, exponential scale parameter; $\lambda_{OS,Gomp}$, Gompertz scale parameter; $\gamma_{OS,Gomp}$, Gompertz shape parameter; $\beta_{ALP}$, $\beta_{LYM}$, $\beta_{NLR}$ and $\beta_{PD-L1}$ parameters relating ALP, LYM, NLR and PD-L1 expression to the hazard, respectively; $\beta_{RCFB-TS(t)} (c-f)$, parameter relating the time-course of tumor size RCFB, carried forward three weeks after last dose to the hazard; $\beta_{RCFB-TS(t)} (ext)$, parameter relating the time-course of tumor size RCFB, extrapolated based on empirical Bayes estimates after last observed tumor size measurement to the hazard.

RSE, relative standard error; AAD, all available data; C2YE, data censored no later than at a cut-off date set 2 years earlier than in AAD; C2YASOT, data censored no later than 2 years after start of treatment for each individual patient; C5MALD, data censored a maximum of five months after last dose.

RSEs were computed based on the R covariance matrix in NONMEM.

$^1$PD-L1$^+$ immune cells/tumor mass <5% or PD-L1$^+$ tumor cells/tumor mass <50% vs. PD-L1$^+$ immune cells/tumor mass ≥5% or PD-L1$^+$ tumor cells/tumor mass ≥50%.
Figure 23. Relative (to the median patient) hazard (logarithmic y-axis) versus continuous baseline covariates included in the final overall survival (OS) models. Shaded areas are the 95% CIs, given the standard errors of the parameter estimates, and are colored by the covariate. The relationships are illustrated for the observed range of the covariate value and dashed vertical lines represent the 2.5th and 97.5th percentiles of the observed covariate value. The horizontal grey line indicates no change in relative hazard, i.e., a patient with median values of all covariates and no change from tumor size baseline (non-small cell lung cancer, NSCLC, OS models) or a mean CTC count<3/7.5 mL (metastatic colorectal cancer, mCRC, OS model).

ALP, alkaline phosphatase; LYM, lymphocyte count; NLR, neutrophil/lymphocyte ratio
AAD, all available data; C2YE, data censored no later than at a cut-off date set 2 years earlier than in AAD; C2YASOT, data censored no later than 2 years after start of treatment for each individual patient; C5MALD, data censored a maximum of five months after last dose.
where $\lambda_{OS,exp}$ is the scale parameter of the exponential distribution and $\gamma_{OS,Gomp}$ and $\lambda_{OS,Gomp}$ are the scale and shape parameters of the Gompertz distribution, respectively. $\beta_{LYM}$, $\beta_{ALP}$, $\beta_{RCFB-SLD(t)}$, $\beta_{NLR}$ and $\beta_{PD-L1}$ are parameters relating LYM, ALP, $RCFB-SLD(t)$ NLR and PD-L1 expression to the hazard, respectively. Figure 24 shows the Kaplan-Meier mean covariate (KMMC) VPCs of the base (i.e., without predictors) and final AAD models, which illustrate the improved predictive performance (i.e., better captured trends and tighter CIs) of the mean variable value after inclusion of baseline covariates and $RCFB-SLD(t)$. Similar results were observed for the C2YE, C2YASOT and C5MALD models (see supplementary material to Paper IV for KMMC VPCs of these model).

The AAD was in general well predicted given the final C2YE and C2YASOT models (left and middle panels in Figure 25). However, the

![Graphs showing Kaplan-Meier mean covariate (KMMC) visual predictive checks (VPCs) of the base (top panel) and final (bottom panel) non-small cell lung cancer (NSCLC) overall survival (OS) models, based on all available data (AAD). The solid line is the mean covariate value of patients remaining in the study, the grey area is the 95% CI (generated from 100 simulations). Black vertical lines indicate censored events.](image-url)
C5MALD model failed to predict the AAD (right panel in Figure 25). This was not unexpected partly due to the different distributions of event times, resulting in a lack of long-term survivors.

A majority of the explored model-derived variables improved the model fit in the mCRC model. The biggest drop in OFV was observed for $\lambda_{CTC(t) \geq 3/7.5 \text{mL}}$ ($\Delta$OFV=101), which can be compared to the absolute SLD(t) which resulted in the best improvement of the tumor size-related variables ($\Delta$OFV=66.6). No statistically significant improvement was observed when the CTC cut-off was estimated instead of fixed to 3. The hazard function of the final mCRC model is given in Equation 25;

$$h(t) = \frac{1}{\gamma_{OS,log}} \cdot \frac{1}{t^\gamma_{OS,log}} \cdot e^{(\beta_{BTS} \cdot (BTS-87) + \beta_{Age} \cdot (Age-65) + CTC_{effect})} \cdot \left(1 + \frac{1}{\gamma_{OS,log}} \cdot \frac{1}{t^\gamma_{OS,log}} \right)$$

where $\beta_0$ and $\gamma_{OS,log}$ are the scale and shape parameter of the log-logistic distribution, respectively, and $\beta_{BTS}$ and $\beta_{Age}$ are parameters relating the effect of BTS and age to the hazard. $CTC_{effect}$ is defined in Equation 26;

$$CTC_{effect} = 0 \quad \text{if} \lambda_{CTC(t) < 3/7.5 \text{mL}}$$
$$CTC_{effect} = \theta_{\lambda_{CTC(t) \geq 3}} \quad \text{if} \lambda_{CTC(t) \geq 3/7.5 \text{mL}}$$

where $\theta_{\lambda_{CTC(t) \geq 3}}$ corresponds to a fractional change of the hazard for $\lambda_{CTC(t) \geq 3/7.5 \text{mL}}$. 

\[\text{Figure 25. Kaplan-Meier (KM) visual predictive checks (VPCs) of all available data (AAD) predicted with the final non-small cell lung cancer (NSCLC) overall survival (OS) models based on data censored no later than at a cut-off date set 2 years earlier than in AAD (C2YE), data censored no later than 2 years after start of treatment for each individual patient (C2YASOT) and data censored a maximum of five months after last dose (C5MALD). The solid line is the observed KM curve (AAD) and the shaded area is the 95% CI (generated from 100 simulations). Black vertical lines indicate censored events.}\]
Since $\lambda_{CTC(t)} \geq 3/7.5$ mL was identified as the best predictor of OS and because a dose-response relationship was established between the TND and CTC count, it was reasoned that OS could potentially be improved in patients with a CTC count $\geq 3/7.5$ mL by escalating the dose. Therefore, the CTC modeling framework was used to explore how an increased dose would affect the CTC count. The CTC count was $\geq 3/7.5$ mL in at least one replicate before start of treatment in 144 patients and stayed $\geq 3/7.5$ mL 1-2 weeks after start of treatment in 15 of these patients. A 50% higher TND and doubled and tripled TND reduced $\lambda_{CTC(t)}$ below 3 in 13%, 27% and 53% of these patients, respectively. Similarly, it was also explored if a reduced TND would be acceptable with the aim of retaining the CTC count below 3/7.5 mL after 1-2 weeks after start of treatment. The model predicted 375 patients to have a CTC count $< 3/7.5$ mL 1-2 weeks after start of treatment, given the administered TND. The model predicted a CTC count $\geq 3/7.5$ mL only for a minority of these patients when the TND was reduced by 25% (n=4) and 50% (n=16).
5 Discussion

The discussion of the results in this thesis is separated in two parts (sections 5.1 Improving management of toxicity and 5.2 Relationships between biomarkers and efficacy). These cover the discussion of the results in Papers I and II and Papers III-V, respectively.

5.1 Improving management of toxicity

The potential to improve the management of cancer patients on myelosuppressive chemotherapy was investigated i) by exploring how frequent monitoring of the ANC together with model-based predictions could improve the prediction of neutropenia (Paper I) and ii) by evaluating IL-6 and CRP as predictors of FN (Paper II).

5.1.1 Model-based predictions of neutropenia

Routine clinical practice often allows for only a single baseline (or pre-cycle) measurement of the ANC, or sometimes also a second sample around the expected ANC nadir. Patients may consequently experience severe neutropenia in the middle of the cycle or arrive at the clinic for initiation of the subsequent cycle only to be sent home due to a too low ANC for the next dose to be delivered. This may affect not only the patient (e.g., mentally [107]) but also the clinic (e.g., due to inefficient use of healthcare resources).

The objective of Paper I was to investigate if the myelosuppression model [40, 82] together with frequent monitoring of the ANC could improve the management of cancer patients. As expected, it was shown that model-predictions were more accurate and precise when the forecasts were short and based on more data. However, acceptable levels of accuracy and precision were not available. On the other hand, frequent monitoring always performed better than the baseline scenario in terms of predicting the ANC. However, predictions of NADIR\textsubscript{ANC} and NADIR\textsubscript{time} were more imprecise, indicating difficulties of predicting these variables. However, these results were not unexpected due to the high residual error (42%) [82]. Grade 4 neutropenia was however predicted with high sensitivity, also for the baseline scenario (Figure 9), suggesting that these predictions could potentially be used to make predictions for
patients that would benefit from rescue-medication (e.g., G-CSF and/or antibiotics). Another variable that was predicted with high precision was \textit{RECOVERY-ANC0-time} (i.e., the time for the ANC to recover to the baseline value). It was observed that many patients recovered to their \textit{ANC0} several days before day 21, suggesting that the next dose could be delivered earlier than after the standard 21 days. These predictions could therefore be used to individualize the start of the subsequent cycle. Application of such a system should of course evaluate the effect on survival, safety, healthcare resources and patient convenience since its implementation may be clinically difficult and the activity of the bone-marrow may result in more myelotoxicity than what is usually expected. Similarly, for patients that are predicted to have an ANC so low that the next dose cannot be administered as scheduled, an alternative day for the initiation of the subsequent therapy cycle can be predicted (instead of postponing it for one week, as standard).

5.1.2 Predicting febrile neutropenia

The temporal increases in the biomarkers IL-6 and CRP in breast cancer patients treated with adjuvant chemotherapy were characterized successfully using turnover models together with empirical surge functions and a link between IL-6 and CRP. Estimated baseline levels (IL-6: 2.50 pg/mL and CRP: 1.88 mg/L) were similar to those previously reported in comparable patient populations [108–112]. The half-lives of IL-6 and CRP were derived from their estimates of \( k_{\text{out}} \), corresponding to 49 and 31 hours, respectively. Previous reports of IL-6 and CRP half-lives are variable, ranging from a couple of minutes to nearly one day for IL-6 [113–116] and from 4–62 hours for CRP [115, 117–119]. The differences in the estimates of the half-lives are probably multi-factorial, depending on the study design, study population, bioanalytical assay and method to determine the half-life.

The Multinational Association for Supportive Care in Cancer risk index score [120] and Clinical Index of Stable Febrile Neutropenia [121] can be used to assess the risk of severe complications of a patient with newly diagnosed FN. The risk assessment is further used to guide how the patient should be treated (e.g., in an in- or outpatient setting or with oral or IV antibiotics) [18]. However, these scores cannot be used to predict if a patient will develop FN or not and while guidelines recommends primary G-CSF prophylaxis if the risk of FN is \( \geq 20\% \) (which is often based only on the administered chemotherapy regimen) [18, 37], there is a knowledge gap of how dynamic changes in biomarkers could be used to predict FN. Since IL-6 and CRP increase in response to infection it was hypothesized that these biomarkers could be used to predict FN.

The immune-system in patients with FN may not be functioning as it is in non-neutropenic individuals [122]. Therefore, fever may be the only sign of infection in patients with FN. However, fever in neutropenic patients may
have different origins, such as a clinically or microbiologically documented infection, or it can also be unknown. Also, infections may be bacterial, viral or fungal, and only bacterial infections should be treated with antibiotics [123]. IL-6 and CRP, together with a third biomarker, i.e., procalcitonin, have been studied in a meta-analysis in patients with FN to identify bacterial infections. Procalcitonin was found to be the superior predictor, followed by IL-6 and then CRP. However, the study did not consider dynamic changes of these biomarkers [124]. Since only a low number of episodes of FN was observed in the analysis in Paper II, it was not possible to separate FN of different origins. Instead, three sub-analyses were performed where the evaluated variables were separated into three categories; i) available prior-to-chemotherapy, ii) available prior-to-FN and iii) available when-FN-occurs. It was found that age predicted FN when no IL-6 or CRP variables were included in the analysis (ΔOFV=6.06). The model predicted that a 70-year old patient had a 3.3 times higher risk to develop FN than a 54-year old patient. However, when also the IL-6 model-derived variables were included in the analysis, age did no longer improve the model fit. Instead, the IL-6 time-course (ΔOFV=37.0), together with an effect delay, provided the best model improvement in the univariate step and \( ANC_0 \) contributed to the prediction of FN on top of the IL-6 time-course (ΔOFV=10.3). For a patient with an IL-6 concentration of 10 pg/mL, the risk of developing FN was 8.8 times higher than for a patient with an IL-6 concentration of 5 pg/mL. For an \( ANC_0 \) of 2.5\( \cdot 10^9 \) cells/L, the risk of FN was 5.1 times higher than in patients with the typical \( ANC_0 \) estimate of 3.5\( \cdot 10^9 \) cells/L. However, when also the CRP model-derived variables were allowed in the final analysis, the CRP time-course outstood both age and the IL-6 time-course as a predictor of FN with the clearest drop in OFV (ΔOFV=68.9) and the risk of FN was 5.0 higher in a patient with a CRP concentration of 10 mg/L compared to 5 mg/L. However, peaks in CRP concentration overlapped with the time of FN diagnosis, limiting the value of CRP as a predictor of FN. Consequently, in a clinical setting it would be preferred to monitor IL-6 to predict if a patient will develop FN. Prediction of FN could potentially be facilitated if IL-6 (and potentially CRP) could be monitored similarly to the ANC with a home-based lab.

5.2 Relationships between biomarkers and efficacy

Predictors of tumor size changes and OS, following treatment with atezolizumab in NSCLC patients, were explored in Papers III and IV and quantitative relationships between dynamic tumor size changes and CTC counts and OS in mCRC patients treated with chemotherapy and targeted therapy were evaluated in Paper V. These results are discussed more in detail below.
5.2.1 Atezolizumab biomarkers in NSCLC

An extensive modeling framework, integrating PK, biomarker(s), tumor size and OS was developed in Papers III and IV. So far, there has not been any similar reports for any of the newly developed ICIs, although extensive modeling frameworks have been reported for other therapies [74, 125]. This framework added quantitative knowledge in the area of biomarkers in CIT, as it was illustrated how drug exposure ($AUC_{cycle\ n}$) and IL-18 ($RCFB_{IL-18,d21}$) predicted tumor size changes in NSCLC patients treated with atezolizumab while the tumor time-course was the best predictor of OS. It was possible to establish this framework based on Phase I data, where relationships between changes in tumor size and OS have previously been analyzed primarily on data from later clinical studies [4, 50, 51], demonstrating the value of data from early clinical trials. Since a Phase I study is initiated prior to a Phase III study, data from a Phase I study is naturally more mature (i.e., more events have happened) than data from a Phase III study, using the same date for database lock. Additionally, the Phase I study included multiple dose levels, which is less common in later oncology studies, which may reduce any potential correlation between drug exposure and disease status (selection bias).

Even though CIT presents a novel treatment option for cancer patients to fight cancer, it is not effective in all patients and the response to CIT is highly variable, with interpatient heterogeneity in immune and disease status and the ability of the cancer to evade immune destruction [126]. It is therefore of critical importance to identify patients that will respond to such treatment in order to increase the probability of a successful treatment, preferably with non-invasive and early methods. Most of the biomarker research for ICIs affecting the PD-1/PD-L1 pathway has relied on the expression of PD-L1 on tumor and tumor-infiltrating immune cells. Despite PD-L1 describing parts of the variability in response to ICIs, a non-negligible fraction of patients with negative PD-L1 expression also responds [127]. However, no relationship could be established between PD-L1 expression and tumor size changes in Paper III, while it was included as a predictor of OS in two of four alternative OS analyses in Paper IV (i.e., the C2YE and C2YASOT models). Several parameterizations of PD-L1 expression (both continuous and categorical) were evaluated, although separating PD-L1 expression based on high and low expression on tumor and tumor-infiltrating immune cells consistently improved the model fit most. Interestingly, it was also found that the drug effect (with respect to tumor size changes) was absent in patients that had never smoked, which is in line with previous reports, although the usefulness of smoking history as a biomarker to guide therapy has been questioned [128]. This may be related to a higher mutational burden in smokers or patients that formerly smoked [129], which results in additional neoantigens for the immune system to target.
Based on the exploratory analysis it was observed that the levels of IL-18 increased (according to a dose-response relationship) following start of treatment until day 21 and then slowly returned towards the baseline (Figure 10). It may be that IL-18 is a biomarker of the initial T cell activation in the periphery, while the contribution from the effect site (i.e., the tumor microenvironment) is more modest. Although the drug effect, with respect to tumor size changes, was more pronounced compared to the IL-18 mediated effect, larger values of $RCFB_{IL-18,d21}$ predicted a sustained tumor response, which was not observed for the drug effect (Figure 18). This behavior of sustained tumor response for large values of $RCFB_{IL-18,d21}$ was hypothesized to be related to the previously reported relationship between on-treatment $k_{Grow}$ and OS [52] in NSCLC patients treated with atezolizumab in a Phase II study and a Phase III study. The modeling framework developed in Paper III was therefore extended to include OS in Paper IV.

The growth rate ($R_{Grow}$) predicted OS in Paper IV, similar to the relationship between and $k_{Grow}$ and OS in the analysis by Claret et al. [52], although none of the IL-18- (or ITAC-) related variables predicted OS. Instead, the $RCFB-SLD(t)$ was identified as the best predictor of OS, independently of follow-up time. Since the survival time was allowed to influence predictions of the individual $RCFB-SLD(t)$ (PPP&D applied during estimation), the risk of immortal time bias was reduced. In addition to the relationship between $k_{Grow}$ and OS, these results are comparable also to relationships between $TTG$ and current rate of tumor size changes [54] as well as absolute and percent change in tumor size [53] and OS, established in patients treated with ICIs.

In studies of OS, patients are followed either until they die or until the end of the study (which may include multiple cut-off dates), whichever occurs first. Patients that are alive at the end of the study will be right censored, i.e., their actual time of death is unknown although it is known that the patient survived until at least the date of end of follow-up. Patients that progress while on the study treatment may discontinue further treatment with the study drug, and potentially switch to an alternative treatment, but remain in the study for OS follow-up. Consequently, a switch in therapy may confound the results of an OS analysis, which is why different censoring strategies were applied in Paper IV to explore the impact of follow-up on inclusion of predictors and their effect sizes. It was concluded that the applied censoring strategies only had minor impact on the included predictors (Table 7) and that the parameter estimates relating each predictor to the hazard were similar (Table 8). LYM (AAD model) and NLR (C2YE, C2YASOT and C5MALD models) were the first baseline covariates to be included in the first stepwise procedure. Neutrophils promote tumor activity by triggering inflammation in the tumor microenvironment while lymphocytes suppress cancer activity related to host immunity [130, 131]. Consequently, it is advantageous to have more lymphocytes in relation to neutrophils, which is in line with the identified LYM and
NLR relationships (Figure 23). ALP was included in all four NSCLC OS models. Elevated levels of ALP may be observed in liver disease and could reflect patient status. Based on the final NSCLC OS models, it was found that the relative hazard increased with increasing values of ALP. Finally, the applied censoring strategies had only minor impact on what predictors that were included. Instead, the largest difference between the final models was related to the distribution of the event times. The exponential distribution performed best in the AAD, C2YE and C2YASOT models (i.e., time-constant hazard), whereas the Gompertz distribution described the C5MALD best (with a negative shape parameter corresponding to a hazard that is decreasing monotonically). Therefore, it was not unexpected that the final C5MALD model failed to predict AAD (Figure 25). On the other hand, both the C2YE and C2YASOT models could predict the AAD well, suggesting that it might have been possible to analyze the data with an earlier cut-off date and then extrapolate to later time points. However, the C5MALD is potentially the censoring strategy least influenced by confounding treatments.

5.2.2 SLD and CTC as biomarkers of OS in mCRC

Relationships between the CTC count and OS in mCRC patients treated with chemotherapy (capecitabine and oxaliplatin) and targeted therapy (bevacizumab and cetuximab) in the CAIRO2 trial [89] have been established previously [62]. However, the analysis in Paper V added mechanistic and quantitative understanding of the relationship between changes in tumor size and CTC count as well as a comparison of their predictive value of OS. The model of tumor quiescence and drug-resistance [98, 99], with a slightly modified structure, successfully predicted the variable individual patterns of tumor size changes (Figure 19). The individual predictions of tumor size changes were subsequently related to the probability of observing a CTC count equal to \( n \). Additionally, the TND inhibited the \( \lambda_{\text{CTC}(t)} \) which resulted in a better description of the rapid increase in proportion of samples equal to zero soon after start of treatment. A CTC count \( \geq 3/7.5 \) mL, at any time after start of treatment, predicted OS better than any of the tumor size-related markers, including the tumor size time-course.

The time-course of changes in CTC counts has previously been described with a population PKPD model in patients with metastatic castration-resistant prostate cancer (mCRPC) [132], where Wilbux et al. analyzed relationships between CTC counts, prostate-specific antigen and a latent variable (representing the underlying tumor burden). These variables were, however, not related to OS. However, similar to the CTC model in Paper V, a negative binomial model accounting for overdispersion was also applied in the mCRPC CTC model. The overdispersion parameter was estimated to a value above 1 in both analyses, suggesting that the mean CTC count and the variability in the CTC count is large both in mCRC and mCRPC.
Following the first stepwise procedure of baseline covariates, all explored tumor size-related variables (Table 2) provided statistically significant improvement of the model fit. However, the mean CTC time-course, parameterized as \( \lambda_{CTC}(t) \geq 3/7.5 \text{ mL} \), predicted OS even better (relative hazard: 3.51, 95% CI: 2.85-4.32). When tumor size-related variables were added on top of CTC, no additional model improvement was observed. A threshold of 3/7.5 mL is also consistent with previous findings in mCRC [62, 133–135], while studies in other cancers have identified higher thresholds; breast (\( \geq 5 \text{ CTCs/7.5 mL} \)) [136], prostate (\( \geq 5 \text{ CTCs/7.5 mL} \)) [137], and NSCLC (\( \geq 50 \text{ CTCs/7.5 mL} \)) [138]. The benefits of using the CTC count, rather than the RECIST defined SLD, as a predictor of OS include an earlier response in CTC count and reflection of the total tumor burden (and not only target lesions). However, the utility of using the CTC count to guide clinical decision making has been questioned, i.e., will patient outcome improve based on CTC counts [139, 140].

The developed modeling framework in Paper V was applied to assume that a CTC count \(< 3/7.5 \text{ mL} \) is beneficial with respect to OS. Second, the dose-response relationship was used to explore if dose-escalations could drive the CTC count below 3/7.5 mL in patients with a CTC count \( \geq 3/7.5 \text{ mL} \) both before start of treatment and after 1-2 weeks of treatment. Using a 50% increased TND and doubled and tripled TND, the CTC count was lowered below 3/7.5 mL in 13%, 27% and 53%, respectively. However, the risk of severe toxicity should be considered prior to any dose increase. On the other hand, the model predicted that only a minority of the patients would get a CTC count \( \geq 3/7.5 \text{ mL} \) if the TND was reduced by 25% or 50%. Consequently, extending the framework to also include safety variables could potentially leverage the clinical utility of the proposed modeling framework. Such modeling frameworks have previously been established in patients with gastrointestinal stromal cancer treated with sunitinib [141] and patients with renal cell carcinoma treated with axitinib [74].

Liquid biopsies offer a minimally invasive, simple, fast and cost efficient method to follow disease status [55]. Not only is it possible to measure the CTC count, but also other analytes as potential measures of total tumor burden, e.g., circulating tumor DNA (ctDNA), which is a type of circulating cell-free DNA that could reflect the tumor genome [55, 142]. The information obtained from CTC counts and ctDNA may complement each other [143]. Extending the proposed CTC modeling framework to also include ctDNA (and toxicity) could potentially increase the predictive value of the modeling framework in Paper V.
6 Conclusions

In this thesis, pharmacometric models were developed and applied to evaluate the potential of various biomarkers to improve treatment (with respect to both management of toxicity, Papers I and II, and predicting efficacy, Papers III-V) in oncology. The value of using the myelosuppression model to predict neutropenia was explored in a simulation study. To enhance the understanding of relationships between biomarkers and clinical endpoints, three modeling frameworks were developed. These included different types of models for continuous (i.e., PK, biomarker and tumor size), count (i.e., CTC) and time-to-event (i.e., FN and OS) data, where the continuous/count variables were explored as predictors of the time-to-event outcome. The developed modeling frameworks may be used in i) drug development to study different biomarkers, explore the same biomarkers for a different indication of the investigated drug(s) and investigate what-if-scenarios, e.g., the outcome based on different doses and ii) in clinical practice to early predict life-threatening conditions as well as to individualize dosing.

Specific findings of this thesis include:

- The potential to use the previously developed myelosuppression model [40, 82] together with the novel home-lab for self-monitoring of ANC [23] to predict neutropenia, which may enable earlier treatment with rescue medications and avoidance of severe neutropenia, and to individualize the start of the subsequent dose, was illustrated.

- The development of a modeling framework with successful characterization of the IL-6 and CRP time-courses (including description of the underlying relationship between IL-6 and CRP) and the development of FN suggested that IL-6 may potentially be used to predict FN, while CRP seems more suitable for confirmation of FN.

- The time-courses of IL-18 and ITAC were successful characterized in NSCLC patients treated with atezolizumab. Both IL-18 and ITAC predicted tumor size changes, with IL-18 being the better predictor, which contributed to the model fit on top of a drug effect mediated through the atezolizumab AUC. The drug effect was, however, absent in patients that had never smoked, which may be related to a lower tumor mutational burden in these patients. Furthermore, large values
of the IL-18 RCFB at day 21 predicted a sustained tumor response in NSCLC patients treated with atezolizumab.

- A relationship between the tumor time-course and OS was identified based on Phase I data in NSCLC patients treated with atezolizumab, which was independent from follow-up.

- The relationship between changes in tumor size and CTC count in mCRC patients treated with chemotherapy and targeted therapy was identified and quantified. It was also shown that a CTC count $\geq 3/7.5$ mL was a better predictor of OS than changes in tumor size.

6.1 Future perspectives

The results that have been presented in this thesis may serve as a foundation for future projects related to this thesis, including:

- Extension of the modeling frameworks by including variables related to both safety and efficacy.

- Challenge of the findings based on simulated ANC by analyzing real ANC data measured with the home-lab and use of the model-based predictions to guide clinical decisions.

- Further evaluation of using IL-6 as a predictor of FN, potentially by performing a clinical study (similar to the G-CSF study [43]) designed to primarily study IL-6 as a predictor of FN and including a larger patient population to possibly also allow for analysis of different types of origin of FN. Such a study could also include procalcitonin as a study variable.

- Application of the developed frameworks to other indications and for designing new clinical trials.

- Extension of the CTC modeling framework to also include ctDNA, which may complement CTC as a predictor of OS.

- Exploration of if CTC counts also are predictive of OS in patients treated with CIT.
7 Populärvetenskaplig sammanfattning


Den vanligast dosbegränsande toxiciteten för cellgifter är neutropeni, vilket innebär ett lågt absolut neutrofilantal (ANC). Potentialen av att använda modellbaserade prediktioner tillsammans med frekventa mätningar av ANC, för att förutsäga vilka patienter som riskerar allvarlig neutropeni samt uppskjutning av nästa dos, utforskades i Artikel I. Neutropeni kan utvecklas till febril neutropeni (FN), vilket kan utgöra en livsfara för den som drabbas. Interleukin 6, en immunrelaterad biomarkör, identifierades som en prediktor av FN i bröstcancerpatienter som behandlats med adjuvanta cellgifter i Artikel II. C-reaktivt protein (en annan immunrelaterad biomarkör, ofta kallad snabb-sänka), visade inget prediktivt värde utan kunde istället användas för att konfirmera en FN diagnos.

Immunologisk cancerbehandling är det senaste genombrötten inom cancerbehandling, och då framför allt med T-cellsaktiverande antikroppar (dvs., immune checkpoint inhibitors), t.ex., atezolizumab. I Artikel III identifierades ett samband mellan exponeringen av atezolizumab och förändringar i tumörstorlek hos patienter med icke-småcellig lungcancer som behandlats med atezolizumab. Den relativa förändringen från baslinjen av biomarkören Interleukin 18 21 dagar efter behandlingsstart förbättrade prediktionen av tumörstorleksförändringar. Tidsförloppet av tumörstorleksförändringar predikterade överlevnadstiden i samma population i Artikel IV.

Cirkulerande tumörceller (CTCs) är tumörceller som spredits från en tumör och cirkulerar i blodet. Dessa kan ge upphov till metastaser (dvs. tumörer i andra organ än det ursprungliga), vilka är relaterade till en sämre prognos. I Artikel V utvecklades flera modeller för tumörstorlek, CTC antal samt överlevnadstid, som vidare kopplades samman för att undersöka samband dem emellan i patienter med metastaserande kolorektalcancer som behandlats med cellgifter och målinriktade läkemedel. CTC antal visade sig vara en bättre prediktor av total överlevnad än förändringar i tumörstorlek.

Sammanfattningsvis har resultaten i denna avhandling erhållits genom farmakometrisk utvärdering av biomarkörer för att förbättra cancerbehandling.
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