Thesis for the Degree of Doctor of Philosophy

Modeling of combination therapy to support drug discovery in oncology

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Svensk populärvetenskaplig sammanfattning

Kombinationsterapi innebär att flera läkemedel ges samtidigt, vilket görs bland annat för uppnå bättre effekt och för att undvika resistens. Kombinationsterapier blir ofta komplicerade vad gäller att välja bra läkemedel att kombinera samt när och vilka doser som ska ges. Istället för att enbart genomföra experimentella studier, kan man använda matematiska modeller för att försöka förutspå utfall. Detta görs genom att man kalibrerar en modell till en begränsad mängd experimentella data och låter modellen prediktera utfallet av t.ex. en högre eller lägre dos, eller om man doserar tre gånger i veckan istället för varje dag.

Den här avhandlingen betraktar flera matematiska modeller för kombinationsterapi av kemiska substanser, eller kombinationer bestående utav en kemisk substans och strålning. Ett genomgående koncept är Tumor Static Exposure (TSE), vilket är den exponering utav läkemedel som gör att tumören varken växer eller krymper. Ger man ett läkemedel är TSE den lägsta exponeiring av läkemedlet som man behöver uppnå för att tumören ska sluta växa, medan om man ger två läkemedel samtidigt, blir TSE en kurva där alla punkter längs kurvan har denna egenskap.

Biologiska processer är väldigt komplicerade, varför man också behöver ta hänsyn till variabilitet mellan individer samt annat osäkerhet. Exempelvis kan man ta fram TSE som predikterar den exponering som krävs för att tumörerna börjar krympa för 90% av populationen.

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Abstract

Mathematical models based on ordinary differential equations, with impulses, are used to describe tumor growth after different treatment combinations, including chemicals as well as radiation. The models are calibrated, using a nonlinear mixed-effects framework, based on time series data of tumor volume from animal experiments. Important features incorporated into the models include natural cell death, and short-term as well as long-term response to radiation treatment, with or without co-treatment with a radiosensitizing compound. Tumor Static Exposure, defined as the treatment combinations that yield stability of the trivial solution to the system model, is introduced as a prediction tool that can also be used to compare and optimize combination therapies. The Tumor Static Exposure concept is illustrated practically, using calibrated models and data from animal experiments, as well as theoretically, using a linear cell cycle model to describe cancer growth subject to treatment with an arbitrary number of anticancer compounds.

Keywords: Combination Therapy, Model-Based Drug Development, Nonlinear Mixed-Effects Models, Oncology, Pharmacokinetics/Pharmacodynamics

List of Appended Papers

The following papers are included in this thesis:

I T. Cardilin et al. Tumor Static Concentration curves in Combination Therapy, *AAPS J.*,19(2):456-467, 2017

II T. Cardilin et al. Model-based evaluation of radiation and radiosensitizing agents in oncology. *CPT: Pharmacometrics Syst. Pharmacol.* 7(1):51-58, 2018

III T. Cardilin et al. Modeling long-term tumor growth and kill after combinations of radiation and radiosensitizing agents. *Cancer Chemother. Pharmacol.* 83(6):1159-1173, 2019

IV T. Cardilin et al. A modeling approach to the selection and ranking of anticancer combinations. Manuscript, 2020

V T. Cardilin et al. Optimization of combination therapy in oncology for a general linear cycle model. Submitted, 2020

Additionally, a review paper was published based on the contents of Paper I and Paper II, see T. Cardilin et al. Evaluation and Translation of Combination Therapies in Oncology, *Eur. J. Pharmacol.*, 834:327-336, 2018. Early results from Papers I, II, and III, were originally presented in the form of posters at the PAGE meetings in 2015, 2016, and 2018, respectively. A summary of the work these three papers comprise was also presented at the PAGE meeting in 2019.

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Tim Cardilin
Göteborg, May 2020

Contents

1 Prologue

Cancer is one of the leading causes of death worldwide. Cancer is not a single disease, but more of a collective term for a condition where cells discontinue their normal responsibilities and start to divide uncontrollably and irrevocably. In most types of cancers this leads to the formation of lumps, or tumors, consisting of many cancers cells that no longer act symbiotically with the rest of the body, and only care about their own proliferation. As the tumor grows large, it puts considerable strain on the human body, and to make matters worse clusters of cancer cells eventually migrate to other parts of the body, in search of nutrients and additional spaces to grow. These clusters traverse by means of the blood stream or lymphatic system and eventually find themselves occupying critical organs such as the lungs and liver, and as these organs start to fail, loss of life is inevitable.

The exact causes of cancer are not completely understood, but many genetic as well as environmental factors have been established. Cancer is closely linked to aging, not only because older people have had more time to develop cancerous mutations in their cells, but also because they have been exposed to environmental and life-style based risk factors for a longer period of time. Aging is also well-known to weaken the immune system, which increases the likelihood that the body fails to discover and kill cells that have undergone potentially dangerous mutations that can lead to cancer.

Cancers are generally treated using one or multiple of the trifecta consisting of surgery, chemicals, and radiation. The purpose of surgery is to cut out most, if not all, of the tumor or tumors, and can be preceded (what is known as neoadjuvant treatment) as well as superseded (what is known as adjuvant treatment) by chemical intervention and/or radiation. Neoadjuvant treatment usually has the purpose of temporarily shrinking the tumor to make it easier to cut out, whereas adjuvant treatment is intended to catch and kill any remnants of cancerous tissues, and to discourage and prevent recurrences.

Radiation therapy involves bombarding the cancer with high-energy electromagnetic radiation such as x-rays and gamma rays, or particles such as protons, hoping to cause enough damage to the cancerous cells that the are eventually destroyed. Radiation can be given by means of external beam, where one tries to focus as much of the exposure on the tumor and minimizing the damage causes to the surrounding healthy tissues, or by internal radiation (what is known as brachytherapy) where a radioactive device is placed inside the body, close to the cancerous tissue. Approximately fifty percent of all cancer patients receive radiation treatment at some point, and sometimes it is the only treatment that is needed.

Last of the trifecta are chemotherapy and various targeted treatments. Chemotherapy is often described as particularly chosen poison, given in the hopes that the cancer is killed or harmed worse before the rest of the body. Targeted treatments are molecules that bind to a target in a specific gene, protein, or part of the cancer environment in order to destabilize cancer growth. In contrast to standard chemotherapy, targeted treatment do not affect all cells, but instead only a certain subset that is vulnerable.

Combination therapy means that two or more cancer treatments are given simultaneously, such as a targeted therapy plus chemotherapy or radiation. One important reason for combination therapies becoming more popular is to combat treatment resistances that can occur during treatment. Some cells carry a mutation that make them impervious to a particular kind of treatment, so that these cells become dominant over time. However, a multi-pronged attack carried out by different treatments is less likely to encounter these types of obstacles that would mandate multi-resistant cancer cells. Another reason for combination therapy is drug synergies, by which is meant that the effect is greater than some predefined baseline referred to as the 'additive' effect. What constitutes additivity is somewhat situational, and is wanting universal agreement.

In this thesis, mathematical models have been constructed based on data from animal experiments on mice, using a chemotherapeutic known as cisplatin, radiation therapy in the form of x-rays, a targeted therapy called cetuximab that prevents cell proliferation, and an early discovery compound that interferes with the repair of DNA damage, in this case caused by radiation. These data are generated from so-called xenograft studies, whereupon cancer cells from a patient or cell line are implanted under skin of the neck or leg of a mouse with a compromised immune system and is allowed to grow. Tumor size is then measured by caliper which allows for estimation of tumor volume over time. An experimental study includes a number of animals divided into different treatment groups. In a combination therapy experiment with two drugs, there are usually at least four treatment groups: a control, or vehicle group that does not receive any treatment, a treatment group that receives only one of the drugs, a treatment group that receives only the other drug, and a treatment group that receives both drugs.

Mathematical models of tumor growth contain unknown parameters, such as the doubling time of cancerous cells, and the potency, or efficiency of cell killing, of a particular chemotherapeutic drug. Plausible values for some of these parameters may be found in the literature or through other sources, but some need to be estimated from data. Parameter estimation can be done using a variety of statistical techniques, the main point being to find the parameter values that minimizes the discrepancy between model prediction and experimental data. The modeling in this thesis has invariably made use of so-called mixed-effects modeling, which is a statistical framework that takes into consideration the fact that data comes from a population of individuals, and that some of the parameters may vary between individuals in the population. For instance, by looking only at the control group of a xenograft experiment, one finds that tumor growth is approximately exponential. However, the growth rate, and therefore also the tumor doubling time, is typically different for different animals.

Mathematical models in pharmacology are not merely used to describe data. Three main areas of application should be mentioned. The first is to make predictions by simulating models to consider various scenarios using different administration schedules, or drug combinations. These are typically performed in order to help guide new experiments to save time and resources. It is also ethically warranted to min-

imize the number of animals that are sacrificed. The second application is to make particular kinds of predictions across species, referred to as translations. One can often find relationships between how certain parameters or responses vary between for example mouse and man, by considering factors such as heart rate and weight. Since the mouse has a significantly higher heart rate than the human, physiological and chemical process occur much faster, and since the mouse is much smaller than the human, much lower quantities of substances are required to achieve various effects. The third and last application that we mention is to gain biological insights. Exploration of a model through mathematical analysis and computer simulation can trigger questions and hypotheses about the underlying mechanisms and biology that may then be explored and tested experimentally. For instance, the mechanisms through which a particular drug operates may not be fully understood, but by creating mathematical models that are challenged by experimental data, information about which of several plausible scenarios is most likely can be obtained.

This thesis also discusses the concept of Tumor Static Exposure (TSE). TSE is the exposure of an anti-cancer drug that leads to stable disease, i.e., when the tumor is neither growing nor shrinking. Exposure above TSE leads to tumor shrinkage, whereas exposure below TSE leads to tumor growth. The concept can be naturally extended to combination therapy with two or more drugs. TSE for a combination of two drugs consists of all combinations of simultaneous exposures that lead to stable disease. This is illustrated in Figure 1, taken from paper I, where the horizontal axis represents the concentration of drug A, and the vertical axis represents the concentration of drug B. The blue curve is the TSE curve of all concentration pairs leading to stable disease. It divides the plot into two regions: a red region of exposure pairs that leads to tumor growth, and a green region of exposure pairs that leads to tumor shrinkage. These kinds of predictions have been analyzed for each of the models featured in this thesis.

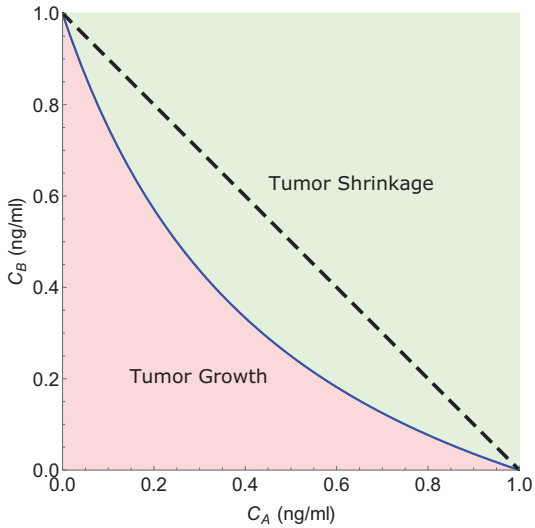

Figure 1: *Tumor Static Exposure (TSE) curve for combinations of two drugs A and B. The TSE curve, shown in blue, separates the concentration plane into regions corresponding to tumor growth (red) and tumor shrinkage (green).*

2 Background

Preclinical drug discovery involves experiments performed *in vitro*, i.e., removed from their normal biological context, as well as *in vivo*, i.e., using animal models. In oncology, a typical *in vivo* experiment is the so-called xenograft study, where cancer cells are placed under the skin of laboratory mice, which are subsequently the recipients of one or multiple test compounds [35]. By repeated measurements, time series of tumor volume can be generated with animals divided into treatment groups with different drugs and administration schedules.

In order to support design and evaluation of such and other experimental studies, mathematical modeling can be used to predict the outcome in an alternative setting, e.g., using a different dose, cell line, or drug combination [26, 5]. In fact, modeling can be particularly helpful in a combination therapy setting owing to the combinatorial explosion of combining different drugs as well as doses. Recent decades have also seen an increased interest in developing combination treatments, as a way to combat drug resistance and to benefit from drug synergy, i.e., when drugs, in some sense, act in tandem to produce a greater-than-expected result [63]. A considerable number of models have been developed and are frequently used to support xenograft studies, c.f. the review [57]. Nonetheless, with an increasing number of treatment candidates and modalities, e.g., combinations involving targeted therapy, standard chemotherapy, or different types of radiation, modeling tools and techniques need to be developed that can be readily reused for different treatments and cancers [52]. Currently, there is a

a lack of generic model structures for combination therapies that can be reused for different combinations, especially combining chemical treatment with e.g. radiation, and a lack of tools and predictive techniques to select the best combinations. Even when a good model exists, it is not always clear which predictions are the most appropriate and how the model can and should be used to compare and rank combinations, as well as optimize a given treatment combination.

Dynamics of tumor volume are commonly described by systems of ordinary differential equations, with the states constituting a heterogeneous cell population. For such models, the most important aspects to cover are cell growth due to uncontrolled mitosis, and the impact of various anti-cancer drugs on the tumor. The models are calibrated to experimental data using inference techniques ranging from ordinary least squares to more sophistical frameworks such as nonlinear mixed-effects modeling, the latter being well suited to describe variability within and across populations, and is described in more detailed in Section 8.

The conclusion is then, that in order to produce good predictions, the following three properties are desirable. First, experiments must be performed that yield informative data, i.e., with low levels of noise and showing a range of outcomes. Second, an appropriate model structure is required, preferably with a connection to current biological understanding, and with a reasonable number of unknown parameters. Third, an appropriate statistical framework is necessary to calibrate the model and to capture different aspects such as average behavior, variability, and uncertainty. Ideally, a positive feedback loop is created, where experimental data is produced, then analyzed, then new experiments are suggested, informed by modeling, which leads to a refinement of the model, and so on. In the next section, three research questions are formulated, each of which is connected to one of the three desirable properties that have been listed here.

3 Research questions

The research described in this thesis has been focused on addressing the following three questions, in the context of preclinical drug discovery and development in oncology

- Q1: How can current mathematical models of combination treatments be refined, and what changes should be made to accommodate additional treatments and treatment modalities?
- Q2: Given a model, what predictions do we use to choose the most promising combinations for further study and to guide additional study designs?
- Q3: How do we best utilize the statistical framework used for model calibration, in order to enhance strengths and mitigate weaknesses, with respect to average behavior, variability, and uncertainty?

3.1 Limitations and scope

This research is on mathematical modeling of combination therapy in oncology, with an eye towards the preclinical stages of the development cycle. As such, priority has been given to issues in the intersection of combination therapy *and* oncology. Generalization about combination therapy in general, or to other disease areas, have only received peripheral treatment. Moreover, issues that are particular to the clinical setting have not been considered, and the important, but difficult, translational step, from animal to human has not been a primary focus.

The focus has been on describing tumor volume over time, using time series data from xenograft experiments, and not e.g. models that include spatial tumor growth. For this reason, and for reasons of accessibility and integrability into existing drug development processes, the models encountered here are ordinary differential equations and not partial differential equations. Lastly, the focus has been to model efficacy, i.e., tumor growth or shrinkage, and not toxicity, although the latter receives some treatment in the shape of minimization of drug exposure.

4 Main contributions

In this section we highlight the three main contributions from this thesis, in increasing order of importance and generality.

4.1 Natural cell death

Paper I introduces natural cell death to a typical tumor growth model with a chain of damage compartments. This provides a more biologically feasible description of the tumor where, even in the absence of drug provocation, only a portion of the tumor cells are actively proliferating. The appropriate initial conditions for such as model were also a novelty introduced in paper I.

4.2 Modeling of radiation treatment

The second main contribution is the radiation model introduced in paper II, and subsequently modified in paper III. The idea, to allow lethally irradiated cells additional cell divisions before dying, is not novel, although it is true for the particular implementation of the idea. Moreover, to the best of our knowledge, these were the first models to describe xenograft data after radiation therapy in a combination therapy context, as well as using mixed-effects modeling.

4.3 Tumor Static Exposure

The third contribution, which is developed throughout all five papers contained in this thesis, is the concept of Tumor Static Exposure (TSE). TSE is defined as the set of all exposure combinations that result in stable disease, and relates to stability of the corresponding dynamical system. Aspects considered in this thesis include practical applications and implementation, generalizations to an arbitrary number of compounds and to different treatment modalities, convexity and its relationship with synergy, and treatment optimization via minimization of a weighted total exposure.

4.4 My contributions to the manuscripts

This section is meant to clarify the contributions that I, Tim Cardilin, have made to the five manuscripts that are part of this thesis. Firstly, I should mention that I did not take any part in the design, execution, or data collection for any of the experiments described in the manuscripts, rather data were delivered to me in the form of excel spreadsheets. I used a Mathematica package developed by Joachim Almquist, Jacob Leander, and Mats Jirstrand to perform parameter estimation. I was not part in the development and did not write any of the code, although I did find and correct several bugs. I frequently consulted the authors, particularly Joachim Almquist, regarding technical aspects of nonlinear mixed-effects modeling and the Mathematica implementation. Below follows a summary of my contributions to each manuscript.

Paper I: I performed data exploration, modeling fitting, and subsequent analyzes. I took a leading role in the intellectual effort regarding model construction and related analysis supported by discussions with Mats Jirstrand, Johan Gabrielsson, and Joachim Almquist. Discussions were also had with co-authors from Merck KGaA. I drafted the manuscript, produced all figures, and was responsible for the submission process through to publication.

Paper II: I performed data exploration, modeling fitting, and subsequent analyzes. I took a leading role in the intellectual effort regarding model construction and related analysis supported by discussions with Mats Jirstrand, Johan Gabrielsson, and Joachim Almquist. Discussions were also had with co-authors from Merck KGaA. I drafted the manuscript, with the exception of parts related to experimental details which where partly written by my co-author Astrid Zimmermann, produced all figures, and was responsible for the submission process through to publication.

Paper III: I performed data exploration, modeling fitting, and subsequent analyzes. I took a leading role in the intellectual effort regarding model construction and related analysis supported by discussions with Mats Jirstrand, Johan Gabrielsson, and Joachim Almquist. Discussions were also had with co-authors from Merck KGaA. I also drafted the manuscript, produced all figures, and revised it into its current form.

Manuscript IV: I performed data exploration, modeling fitting, and subsequent analyzes. I took a leading role in the intellectual effort regarding model construction and related analysis supported by discussions with Mats Jirstrand, Johan Gabrielsson, and Joachim Almquist. Discussions were also had with co-authors from Merck KGaA. I drafted the manuscript, with the exception of parts related to experimental details which where partly written by my co-author Astrid Zimmermann, produced all figures, and was responsible for the submission process through to publication.

Manuscript V: I choose/found the subject to study. I constructed all proofs, examples, and mathematical derivations in the manuscript. I also drafted the manuscript and produced all figures. Discussions and feedback was provided continuously by Mats Jirstrand and Torbjörn Lundh.

5 Mathematical Pharmacology

Mathematical pharmacology is defined by the use of advanced mathematical techniques to improve our understanding of pharmacological processes, and should be regarded as a subdiscipline to mathematical biology. One of the first papers that mentioned the term was published by van der Graaf, Benson, and Peletier, in 2016, and discusses, through two examples, how mathematical modeling and analysis can help improve our understanding of complex biological systems and dynamics [27]. The topic is relatively new and emerging, and is rapidly increasing in popularity, as can be seen from that fact that it was recently chosen as the subject of a special issue in the Journal of Pharmacokinetics and Pharmacodynamics, a leading journal about the modeling of pharmacological processes [39]. The purpose of this section is illustrate the range of mathematical techniques that have been used to support pharmacology and drug discovery.

Firstly, we should mention standard pharmacokinetic and pharmacodynamic modeling, which describe what happens to the drug and body, respectively, that typically utilizes systems of ordinary differential equations as well as standard statistical techniques used to estimate model parameters, ascertain differences between groups, correlation and covariate analyzes, etc [22, 9]. An important framework for such analyzes is mixed-effects modeling, which is used to quantify differences between individuals in a population, see e.g., [17] for a classical reference. Modeling and parameter estimation has also been considered using stochastic differential equations [41], which are appropriate when describing dynamics that are inherently non-deterministic, but which can also be used to correct for model mis-specification. Identifiability analysis is another area that is used to established whether the parameters in a model can be estimated given observations about certain quantities, or whether model reduction may be necessary [32].

Adjacent to identifiability is observability and optimal control theory, which are used to concoct an optimal treatment regimen, given dynamics described by a mathematical model of the disease, various constraints, and an objective function related to the toxicity and other adverse treatment effects [61, 51].

Partial differential equations have also been successfully used to describe diseases and treatments, e.g., radiotherapy treatment of brain tumors, supported by medical imaging techniques (which are also mathematical) to better understand spatial aspects of cancer growth and migration to different tissues [58].

Other examples include models of target-mediated drug disposition (TMDD), which describe the binding of a drug molecule to a target, and can be used e.g., to understand the translation from in vitro (a closed system) to in vivo (an open system) [24]. These models can be complicated, with many parameters that can be difficult to properly estimate. However, under certain assumptions that certain processes occur on different time scales, so-called quasi-equilibrium approximations of the full TMDD models have also become popular ways to describe the kinetics [44].

6 Oncology: An Overview

This section gives an overview of the field of oncology, i.e., cancer biology, (diagnosis), and treatment, with a focus on aspects that are relevant for this thesis. First, we characterize what is meant by cancer, and follow this up by a brief description of the cell cycle and some basic facts about tumors. Thereafter, we describe different cancer treatments, including surgery, chemotherapy, and radiation, which is followed up by an overview of combination theraphies and related concepts such as drug synergy.

6.1 Hallmarks of cancer

In simple terms, cancer is the name for a class of diseases characterized by uncontrolled cell division and invasion to nearby tissues. A more detailed description was famously proposed by Hanahan and Weingberg in 2000, who defined *six hallmarks of cancer*, and in a subsequent update from 2011, included two additional hallmarks and two enabling traits [29, 30].

The first hallmark, *self-sufficiency in growth signals*, reflects that cancer cells are able to go from a resting state to a proliferating state without having been told to do so through exogenous growth signals. This happens because tumor cells have developed the ability to generate their own growth signals, creating a positive feedback loop that reduces the dependence on exogenous factors. An example of this is epidermal growth factor (EGF), whose receptors (EGFR) are overexpressed on the surface of many tumor cells. An inhibitor of EGFR, cetuximab, is featured is paper 1, where it is combined with the standard-of-care treatment cisplatin.

The second hallmark, *insensitivity to anti-growth signals*, means that anti-growth signals, which can either force a cell into a resting state, or remove its proliferating capabilities altogether, are disrupted in many cancer cases.

The third hallmark is *tissue invasion and metastasis*. It is characterized by the tendency for clusters of cancer cells to migrate from the primary cancer site to other parts of the body. Metastases allow the cancer to obtain additional nutrient supplies and space to grow without restriction. Metastatic clusters often travel through the blood stream or the lymphatic system, which is why swollen lymph nodes close to the primary site are among the first signs that the disease is starting to spread. It is for similar reasons that the most common organs for metastases are the lungs and liver [1].

The forth hallmark concerns the *limitless replicative potential* of cancer cells. A non-cancerous cell can divide approximately 60 to 70 times, and each division carries a loss of telomeres, the ends of the chromosomes, which eventually becomes unsustainable. Cancer cells, in contrast, have up-regulated telomerase enzyme, and can activate a mechanism, called alternative lengthening of telomeres (ALT), that allow telometric information exchange to maintain chromosomal integrity. In this respect, cancer cells are *immortalized* and contain limitless replicative potential.

The fifth hallmark describes *sustained angiogenesis*. Angiogenesis is the process by which new blood vessels are formed, and is an essential component for any tissue to grow. In order for the tumor to receive a sufficient supply of oxygen and nutrients,

angiogenic growth signals are up-regulated compared to normal cells, and similarly, anti-angiogenic growth signals are disrupted. One example is vascular endothelial growth factor (VEGF), which is overexpressed in many tumors [13].

The last of the original six hallmarks, *evading apoptosis*, relates to the various strategies which cancers have developed to avoid apoptosis, the process of programmed cell death that is executed when mutations or DNA damage is detected in a cell. The cancer must have developed a resistance to apoptosis, either by avoiding detection, or by disabling the successful executing of the apoptotic process. One example is the mutation of the p53 tumor suppressor gene, which is responsible for the activation of apoptosis [55].

The two additional hallmarks, *evading immune response* and *reprogramming energy metabolism*, reflect the fact that cancer cells must set up a protective barrier, or a cloak of invisibility, not to be destroyed by the marcophages of the immune system, and up-regulate energy metabolism to maintain its growth rate. The latter has been shown to depend greatly on the tumor micro-environment [30].

The two enabling traits, *genome instability* and *tumor-promoting inflammation* help explain how many of the hallmarks listed above come about. Certain mutations, inherited from a parent cell, can give the daughter cells a selective advantage over normal cells that allow them to dominate the local environment. Moreover, inflammation from the presence of immune cells have been shown to have pro-tumorigenic properties, such as providing additional supply of growth factors and enzymes to facilitate angiogenesis, and releasing chemicals with mutagenic properties.

6.2 Tumor composition and the cell cycle

A cancer tumor is composed of cells that have abdicated their normal responsibilities in favor or uncontrolled cell division. Tumors are heterogeneous constructions, made up of different types of cells that serve different functions. The direction of growth, and consequently the tumor geometry, depends on environmental factors such as oxygen and nutrient supply. Depending on the homogeneity of the surrounding tissue, the tumor may be more or less well-approximated by a sphere or an ellipsoid. Many tumors also contain a necrotic core due to, among other things, hypoxia, i.e., the inability to supply sufficient amounts of oxygen to the innermost cells [12]. More generally, the ability to proliferate depends on the location of a cell in the tumor, with the surface, the outermost layer, having the best opportunity to grow.

The process of mitosis, or the cell cycle, is a complicated process through which one cell divides into two daughter cells [64]. A simplified description of the process includes four stages, G1, S, G2, and M, as well as an additional resting stage G0. Essentially, G1 and G2 represent gaps, or growth phases, between the S and M phases during which, respectively, DNA synthesis (duplication) and mitosis occur. G1 and G2 each contain a 'checkpoint' to verify cell integrity and that the cell is ready for the next phase [33].

6.3 Cancer treatment

Cancer is difficult to treat and is one of the leading cause of dead worldwide, and has recently overtaken heart disease as the number one killer in the Unites States [1]. There are three principal modes to treat cancer: surgery, chemicals, and radiation.

Surgery aims to cut out as much of, or all, cancerous tissue. It can be preceded by radiation or chemical intervention in order to shrink the tumor and make it easier to remove, or superseded by such treatment for prophylactic purposes. Surgery is the most effective when the disease is localized and has not infiltrated much of the surrounding tissue, at which point a complete removal can be curative. In contrast, surgery is much less promising when the cancer has metastasized, since even though the primary tumor has been cut out, the disease is still present in other parts of the body. Surgery may still be performed on metastatic cancer patients for palliative reasons, extending life or improving quality-of-life, or with the hope that the remaining cancer can be treated by other methods.

Chemical intervention can be classified as either chemotherapy, targeted therapy, hormone therapy, and immunotherapy. Chemotherapy includes compounds that kill cancer cells as well as normal cells. Due to their accelerated growth rate, cancer cells are more vulnerable to chemotherapy than many normal cells. However, quickly proliferating normal cells such as those in the mouth, hair, and intestines are also particularly sensitive. As such, chemotherapy is poison that aims to kill the cancer before it kills the patient. Paper I provides an example of a chemotheraputic drug in cisplatin, a platinum-based compound which interferes with DNA replication. Cisplatin is used to treat various cancers, including lung cancer and lymphomas. It has been particularly effective versus testicular cancer, increasing the cure rate from 10% to 85% [18].

Targeted therapies are molecules that bind to a particular target on the cancer cell. It works e.g., by inhibiting proliferation or triggering apoptosis in the cell. Targeted therapies are selective and do not typically affect non-cancerous cells. However, it can sometimes be too selective, affecting only the subset of cancer cells that carry a certain mutation. Examples of targeted therapies are cetuximab, an EGFR inhibitor, from paper I, and the early discovery compounds from papers II-IV that interfere with the repair of DNA damage.

Hormone therapy is the administration or manipulation of hormones to, e.g., inhibit the production of a hormone that stimulates cell growth. It can be used to treat cancers in hormonally sensitive tissues such as the breast, prostate, and adrenal cortex [20].

Immunotherapy aims to activate or amplify the body's own immune system to thwart cancer growth or spread. Recently, a lot of attention has been put on cancer immunotherapies, as clinical trials have indicated greatly improved survival for several different compounds [38].

Finally, radiation treatment involves subjecting the tumor to ionizing radiation, which can be photons (x-rays or γ-rays), or charged particles such as protons or electrons [15]. By definition, ionizing radiation creates ions by removing electrons from atoms and molecules in tissues that are hit by the radiation. This causes damage

to the DNA of proliferating cells in the form of single or double-strand breaks [65]. In contrast, cells that are in the resting stage, G0, are less sensitive to radiation. Because of this, and to give healthy tissue time to heal, radiation is often fractionated into lower doses of e.g. 2 Gray (Gy), where 1 Gy corresponds to 1 Joule per kilogram of exposed tissue. The most suitable type of radiation depends on the type of cancer, with photon radiation being the most commonly used treatment and is the only type of radiation featured in this thesis.

The linear-quadratic model is an important empirical model used clinically. It describes the average number of lethal lesions inflicted after radiation dose D as

$$L = \alpha D + \beta D^2, \tag{1}$$

where α and β are two parameters that depend on the type of cancer, radiation, tumor composition etc. The standard assumption is that lethal lesions are Poisson distributed among the cell population [11] and so the probability for a given cell to have incurred zero lethal lesions, and therefore surviving, is given by $\exp(-\alpha D - \beta D^2)$ so that, by independence, the surviving fraction of cells is given by

$$SF(D) = \exp(-\alpha D - \beta D^2). \tag{2}$$

While there is no definitive derivation of the linear-quadratic model that has been confirmed experimentally, a number of derivations have been proposed, e.g. relating the linear term to lethal lesions inflicted by a single photon, whereas the quadratic term corresponds to lethal lesions inflicted by two different photons [11, 59].

6.4 Combination therapy

Different drugs or treatments can be combined to create a combination therapy. This can include a mixture of chemicals and/or radiation. Most combinations include two different treatments, but triple combinations have also been studied in the clinic [54]. The main reasons why combination treatment could be advantageous compared to single-agent treatments are to avoid and overcome resistances, reduce toxicity, and obtain synergistic effects [63].

Drug resistance is a common in oncology, as well as modern medicine in general, that occurs when a subset of cancer cells carry a mutation that makes them less sensitive, or impervious, to a particular treatment. As other tumor cells are destroyed by the treatment, such cells begin to dominate the local tumor environment, which eventually leads to treatment failure. Combination therapy reduces the risk of such scenarios, since it would require cells that are resistant to multiple compounds.

There is a possibility to reduce overall toxicity by employing a combination treatment regimen with lower doses. This can be achieved only if the combined toxic effects of the drugs are tolerable, and the compounds do not produce any undesirable toxic interactions. Any treatment is a trade-off between positive and negative effects, and combination therapy presents a setting with additional degrees of freedom on either side, which carries danger as well as opportunity. It therefore becomes particularly important to have a solid understanding of the individual components

and their interplay, and to optimize treatment to maximize effects while minimizing side-effects.

A synergistic effect is one that is greater than some baseline, referred to as the additive effect. In contrast, a sub-additive effect is referred to as antagonistic. This reduces the problem to that of defining what it means for a combined drug effect to be additive. The answer depends on the measured effect, and many different definitions have been proposed, the two classical definitions being Loewe additivity and Bliss independence [43, 8]. Loewe additivity, also called dose additivity, applies to drugs with the same target, in which any convex combination of doses (or concentrations) with the same effect, should also have the same effect. On the other hand, Bliss independence applies to drugs with independent target in which case the combined effect is multiplicative. More complicated models that define synergy have also been proposed, c.f. [28]. However, one way to look at synergy is that it is an effect that is greater than expected, but what is expected is conditioned on our understanding of the drugs and their mechanisms. Since our understanding changes over time, measures of synergy can therefore be thought of more as a tool to guide and improve said understanding, than as a therapeutic goal in itself. Therefore, in this thesis, we do not focus on synergy, but on modeling and quantifying combination effects to aid the effort to select the best drugs and the best doses or concentrations.

7 Mathematical modeling of tumor growth

This section gives an overview of the typical mathematical models used in preclinical drug development, particularly those that are relevant to the manuscripts included in this thesis. We start with the most general models: systems of impulsive differential equations, and compartment models, which are ubiquitous in life science. Then, we move on to a brief overview of the most common pharmacokinetic and pharmacodynamic models, before focusing on tumor growth models, starting with the most common single state models and moving towards multi-compartment models that describe drug interventions with chemicals as well as radiation.

7.1 Impulsive differential equations

Models in pharmacotherapy are frequently described by so-called impulsive differential equations, i.e., equations of the form

$$\dot{x}(t) = f(t, x(t)),\ x(t) \in \Omega \subset \mathbb{R}^n,\ t \geq 0,\ t \neq \tau_i, \tag{3}$$

$$x(\tau_i^+) = x(\tau_i^-) + g_i(x(\tau_i^-)),\ i = 1, 2, ..., \tag{4}$$

$$x(0^+) = x_0, \tag{5}$$

where $x = (x_1, ...x_n)$ is an n-dimensional vector of system state variables, t is the independent variable, invariably used to represent time, $f = (f_1, ..., f_n)$ is a smooth vector field, and $g_i = (g_{i,1}, ..., g_{i,n}),\ i = 1, 2, ...$ are smooth functions representing jumps that occur at times τ_i, and x_0 is the initial state vector. Here, the limit notation is used for the initial condition in case $\tau_i = 0$ for some i.

Given that the application is to describe cancer treatment, we need only consider the case with fixed impulse times, i.e., when τ_i do not depend on the system state x, and we can also assume that $\tau_{i+1} > \tau_i$ and that $\tau_i \to +\infty$, as $t \to +\infty$. This avoids problems with an infinite number of jumps over a finite time interval, and is fully sufficient for the studied area of application. For equations that satisfy these assumptions, the standard theory regarding existence and uniqueness of solutions for ordinary differential equations remains intact, i.e., if f is Lipschitz continuous there exists a unique solution $x(t)$ to (3) - (5), defined on some maximal interval $[0, t^+]$, and if also for any interval $[0, t^+]$, there exists a compact set $K \subset \Omega$ such that $x(t) \in K$, for all $t \in [t_0, t^+]$, then there exists a unique solution to (3) - (5) on $[0, \infty)$. For convenience, we also assume that $\tau_i \neq 0$ for all i and drop the limit notation for the initial condition. Note that by choosing $g_i = 0$ for all but finitely many i we obtain an impulsive system with finitely many jumps, and if all g_i are chosen to be zero, we obtain a standard system of ordinary differential equations.

A solution $x(t)$ to (3) - (5) is a piecewise continuous function with potential discontinuities of the first kind, i.e., jumps, at times τ_i. The most common type of jump in pharmacotherapy models is state-independent, due to a bolus dose of a drug. However, in this thesis we also consider state-dependent jumps due to radiotherapy, where a fraction of the mass in one state is instantaneously transferred to another

state. In such cases, for physical reasons, it is important to preserve non-negativity of the state vector x , i.e., if $g_i = -kx$ then $k \leq 1$ must hold.

Linear impulsive systems can be written in the form

$$\dot{x}(t) = A(t)\, x(t),\ t \neq \tau_i, \tag{6}$$
$$x(\tau_i^+) = x(\tau_i^-) + B_i\, x(\tau_i^-),\ i = 1, 2, ..., \tag{7}$$
$$x(0) = x_0, \tag{8}$$

where A and B_i are square matrices. The entries of A may be time-dependent, e.g., due to a time-varying drug concentration. For such equations, with fixed impulse times that satisfy the assumptions listed above, a unique solution always exists on $[0, \infty]$ [3].

Impulsive differential equations can also be written using the Dirac delta distribution, as was done in paper II. Unfortunately, the notation used in paper II is erroneous, and we therefore now present the correct formulation. Consider the scalar equation

$$\dot{x}(t) = f(t, x(t)) + g(x(t))\delta(t - \tau), \quad x(0) = x_0 \tag{9}$$

where f and g are globally Lipschitz continuous functions, and τ denotes the time of the jump. For this equation, Nedeljkov and Oberguggenberger [53] proved that if the primitive $G(x) := \int \frac{dx}{g(x)}$ is invertible, then

$$x(\tau^+) = x(\tau^-)G^{-1}(G(x(\tau^-)) + 1). \tag{10}$$

In this thesis, we are primarily interested in the case $g(x) = -ax$. Then $G(x) = -\frac{1}{a} \ln x$ and the solution after the jump is given by

$$x(\tau^+) = x(\tau^-)e^{-a}. \tag{11}$$

Thus, if $a > 0$ then e^{-a} is the remaining fraction of x after the jump and the removed fraction is given by $(1 - e^{-a})$. In the next section we introduce compartment models, a special class of differential equations that are ubiquitous in life sciences.

7.2 Compartment models

This section gives brief description of (linear) compartment models. For a standard reference, see [31]. Compartment models are systems of differential equations consisting of a number of states x_i, referred to as compartments, each representing some sort of well-mixed materia. The compartments are allowed to interact by exchanging materia with one another. A general model with n compartments can be written

$$\dot{x}_i = f_{0i} + \sum_{j=1}^{n} f_{ji} - \sum_{j=1}^{n} f_{ij} - f_{i0},\ i = 1, 2, ..., n, \tag{12}$$

where x_i is the amount of materia in compartment i, $f_{ij} \geq 0$ is the flow of materia from compartment i to compartment j, $f_{i0} \geq 0$ is the flow of materia from compartment i to the environment, and $f_{0i} \geq 0$ represents the flow of materia from the environment into compartment i. The flow rates $f_{ij} = f_{ij}(x, t)$ may in general depend on the compartment vector $x = (x_1, ..., x_n)$ as well as time t.

Linear compartment models occur when the flow rates are assumed to be proportional to the amount in outflow compartment, i.e., $f_{ij} = q_{ij}x_i$, for $i = 1, ..., n,\ j = 0, 1, ..., n$, where $q_{ij} \geq 0$, and $f_{0i} = q_{ii}x_i$ where $q_{ii} \geq 0$ in order to allow to for the possibility that i reacts with itself. The system then becomes linear and homogeneous and can be described by equation $\dot{x} = Ax,\ \ x(0) = x_0$, where the diagonal entries of the matrix A are given by

$$A_{ii} = q_{ii} - \sum_{j=1}^{n} q_{ij}, \tag{13}$$

and the off-diagonal entries are given by

$$A_{ij} = q_{ij} \geq 0,\ i \neq j. \tag{14}$$

In subsequent sections we present multiple examples of compartment models in order to describe pharmacokinetics, i.e., what the body does to a drug, as well as pharmacodynamics, i.e., what the drug does to the body. The contents of these sections can be found in any standard reference on the subject, e.g. [22] or [9].

7.3 Pharmacokinetic models

Drug effect is often modeled in two steps: first, by a pharmacokinetic model that connects drug administration to the concentration of drug in the body, and second, by a pharmacodynamic model that connects drug concentration to an effect of interest, e.g., tumor growth. Sometimes, when the drug concentration is difficult to measure, or, as in this thesis, when describing radiation treatment, it is necessary to link drug administration, i.e., dose, directly to effect.

The pharmacokinetics of a drug can be described by a, usually linear, compartment model where each compartment represents the amount or concentration of the drug in a different type of tissue. The simplest pharmacokinetic model consists of a single compartment x representing, e.g., the concentration of a drug in plasma decaying exponentially over time. The differential equation for this process is

$$\dot{x} = -k_e x,\ x(0) = \frac{D}{V}, \tag{15}$$

where k_e is the elimination rate, D is the drug dose, and V is the distribution volume of x converting dose into concentration. Here, we have assumed that the drug is administered intravenously such that all of the drug is available in plasma at time zero. Any type of drug administration that is not intravenous is called extravascular, e.g., drugs given orally, or intraperitoneally (into the stomach cavity). Extravascular administration is typically described by adding an absorption compartment from

which the drug is absorbed into plasma. As a second example, we consider the two compartment model described by a system of two differential equations

$$\dot{x}_1 = -(k_e + k_{12})x_1 + k_{21}x_2,\ x_1(0) = \frac{D}{V}, \tag{16}$$

$$\dot{x}_2 = k_{12}x_1 - k_{21}x_2, \qquad x_2(0) = 0, \tag{17}$$

where k_{12} and k_{21} are the transfer rate coefficients from x_1 to x_2 and vice versa, and k_e, D and V are the same as before. In this model, x_1 is referred to as the central compartment, usually representing plasma or serum, and x_2 is called the peripheral compartment, representing other tissues.

Nonlinear pharmacokinetic models also exist, such as models with saturable elimination, i.e., if x denotes the drug concentration in plasma, then instead of $\dot{x} = -k_e x$ one has

$$\dot{x} = -\frac{V_{max}x}{k_m + x}, \tag{18}$$

where V_{max} is the maximum elimination rate, and k_m is the Michaelis-Menten constant, corresponding to the concentration at which elimination is at half of its maximum rate [49]. Another example of nonlinear pharmacokinetics occurs in so-called target-mediated drug disposition models, where a drug molecule binds to a target, forming a drug-target complex [42]. This leads to nonlinear elimination whether one is considering the concentration of free, bound, or total drug.

7.4 Pharmacodynamic models

Pharmacodynamic models link the exposure of a drug, i.e., dose or concentration, to a, usually desirable, effect of interest. Before considering models of tumor growth specifically in the next section, we should mention two general models that are frequently used in pharmacodynamics. The first is the so-called Emax-model, providing a direct relationship between drug exposure C and effect E

$$E(C) = \frac{E_{max}C^n}{EC_{50}^n + C^n}, \tag{19}$$

where C is the drug concentration (sometimes dose is used instead), E_{max} is the maximum drug effect, EC_{50} is the concentration at which half of the maximum effect is achieved, and n is the so-called Hill coefficient. In order to describe an inhibitory drug effect one can instead consider $K - E(C)$ where $K \geq E_{max}$ is a baseline constant. The other classical example is the indirect response or turnover model for a quantity R, described by a single differential equation

$$\dot{R} = k_{in} - k_{out}R, \tag{20}$$

where k_{in} is a production, or input rate, and k_{out} is an elimination rate, and R denotes some response variable of interest. A drug effect is then introduced as inhibition or

stimulation of either k_{in} or k_{out}, e.g., $k_{in} \mapsto k_{in}(1 + E(C))$ when production is increased in the presence of a drug. Typically response is assumed to start in steady-state, i.e., $R(t_0) = k_{in}/k_{out}$.

7.5 Tumor growth models

We first consider models described by a single differential equation, and then proceed to consider delays using chains of damage compartments, examples from the papers included in this thesis, including how to incorporate radiation therapy. Finally, we mention a cell cycle model used to analyze combination therapy in paper V.

7.5.1 Single-compartment models

We first consider tumor models consisting of a single state, where V denotes the tumor volume, mass, or number of cells. These three quantities can be used interchangeably by assuming that the tumor is homogeneous with constant density $\rho = 1$. The simplest model describes exponential growth with rate α, i.e., $\dot{V} = \alpha V$, but more generally we may consider a modified exponential growth given by

$$\dot{V} = G(V)V,\ V(t_0) = V_0, \tag{21}$$

where V_0 denotes the tumor volume at time t_0. Popular choices for the function G include $G(V) = \alpha(1 - V/K)$, the logistic model, and $G(V) = \alpha \ln(K/V)$, the so-called Gompertz model, where in both cases the carrying capacity K represents the maximum tumor volume [6]. These are both special cases of the generalized logistic model given by

$$\dot{V} = \alpha\nu \left(1 - \left(\frac{V}{K}\right)^{1/\nu}\right) V,\ V(0) = V_0, \tag{22}$$

where the logistic model is obtained for $\nu = 1$ and the Gompertz model is obtained in the limit as $\nu \to +\infty$. Another example is the Von Bertalanffy model [7] given by

$$V = \alpha V^{2/3} - \beta V,\ V(0) = V_0, \tag{23}$$

where $\beta > 0$ is the death rate of tumor cells. The exponent $2/3$ in the growth term is commonly explained as follows. Tumor growth depends on the nutritional intake, which comes from the surface of the tumor. Given a spherical approximation of the tumor it follows that the surface $S \propto V^{2/3}$. However, cell death can occur everywhere in the tumor and the death term is therefore proportional to volume. A comparison of typical tumor trajectories for the exponential, Gompertz, logistic, and van Bertalanffy models is shown in Figure 2.

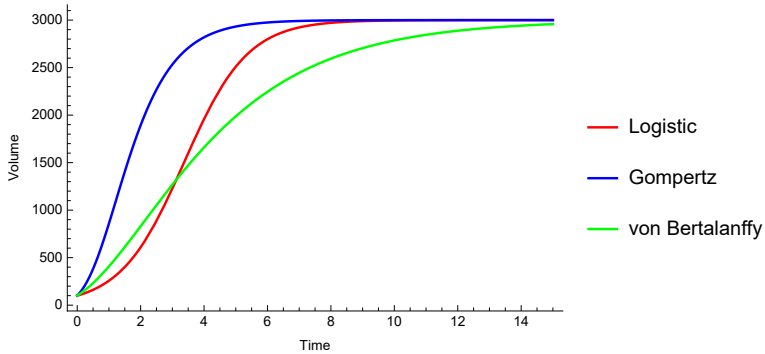

Figure 2: *Illustration of the logistic ($\alpha = 1, K = 3000$), Gompertz ($\alpha = 1, K = 3000$), and van Bertalanffy ($\alpha = 14.42, \beta = 1$) models to describe saturable growth of tumor volume.*

7.5.2 Models with damage compartments

A common way to go from the single-compartment models of the previous section to a multi-compartment model is to introduce a chain of damage compartment, representing dying cells in an increasingly degraded state [36, 25, 34, 66, 21, 47, 48, 50]. Such a chain has been used in papers I-IV. A typical model consists of a main compartment x_1 of proliferating cells and $n-1$ damage compartment $x_2, ...x_n$, and is described by the equations

$$\dot{x}_1 = G(x; u), \tag{24}$$

$$\dot{x}_i = g_{i-1}(u)x_{i-1} - g_i(u)x_i, \ i = 2, ..., n, \tag{25}$$

where G is the net growth function, which in general depends on the state vector x as well as the drug concentration vector u, and g_i is the transfer rate from compartment x_i to compartment x_{i+1}, that may depend on the drug concentration u. The total tumor volume is given by the sum over all compartments $V = \sum x_i$. In the absence of drug provocation, a typical choice for G and g_i is given by $G(x) = k_g x_1$, corresponding to exponential growth, and $g_i = k_k$. Such sets of damage, or delay, compartments can be interpreted as the lifespan of cells having a certain distribution, that is affected by the introduction of anticancer agents [40]. A sequence of n damage compartments with rate parameter k_k is therefore described by a gamma distributed lifespan with rate parameter k_k and shape parameter n. Delay compartments are also commonly used to describe delay in an input signal, i.e., to describe a process when it takes time for an administered drug to properly enter the system [60].

In particular paper I features a four-compartment linear model, $\dot{x} = Ax$, where the system matrix A is given by

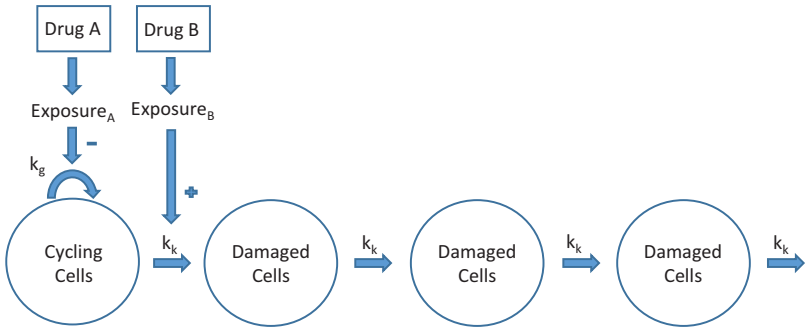

Figure 3: *Compartment model used to describe tumor growth after intervention with cetuximab and cisplatin combinations. The model consists of a main compartment V_1 of proliferating cells and three damage compartments V_2, V_3, and V_4 that cells go through before dying. Drug action of cetuximab is described as inhibition of the growth rate k_g using an Emax function, whereas drug action of cisplatin is described by a linear stimulation of the natural kill rate k_k.*

$$A = \begin{bmatrix} k_g I(C_1) - k_k S(C_2) & 0 & 0 & 0 \\ k_k S(C_2) & -k_k & 0 & 0 \\ 0 & k_k & -k_k & 0 \\ 0 & 0 & k_k & -k_k \end{bmatrix}, \tag{26}$$

where k_g is the growth rate, k_k is the natural death rate, I is an inhibitory Emax function depending on the concentration of the drug cetuximab denoted by C_1, and S is a linear stimulatory function depending on the concentration of the drug cisplatin denoted by C_2. Here

$$I(C_1) = 1 - \frac{I_{max} C_1}{IC_{50} + C_1}, \quad S(C_2) = 1 + bC_2, \tag{27}$$

where I_{max} is the maximum achievable inhibition due to treatment with cetuximab, IC_{50} is the concentration at which 50% of maximum inhibition is achieved, and b is a pharmacodynamic potency parameter of cisplatin. An illustration of the model is provided in figure 3.

Long-term tumor growth without drug intervention is given by the largest eigenvalue of A, i.e., $\lambda_1 = k_g - k_k$. One finds that an eigenvector corresponding to λ_1 is given by

$$v_{\lambda_1} = \left(1, \frac{k_k}{k_g}, \left(\frac{k_k}{k_g}\right)^2, \left(\frac{k_k}{k_g}\right)^3\right). \tag{28}$$

Initial conditions for the system were therefore chosen to be proportional to long-term growth. Thus, if V_0 denotes the initial volume in the first compartment, x_1, then the initial distribution is given by $x(0) = V_0 v_{\lambda_1}$.

7.5.3 Models of radiation therapy

Papers II-IV expand on this model by incorporating the effects of radiation treatment. The most general of these is presented in Paper III, which is a linear impulsive model (see equation (7)) with six states: $x = (V_1, U_1, U_2, V_2, V_3, V_4)$, and where the system matrix A is given by

$$A = \begin{bmatrix} k_g I(D_{acc}, C) - k_k & 0 & 0 & 0 & 0 & 0 \\ 0 & -k_g - k_k & 0 & 0 & 0 & 0 \\ 0 & 2k_g & -k_k & 0 & 0 & 0 \\ 0 & k_k & k_k & -k_k & 0 & 0 \\ 0 & 0 & 0 & k_k & -k_k & 0 \\ 0 & 0 & 0 & 0 & k_k & -k_k \end{bmatrix}, \tag{29}$$

and the jump matrices $B_i = B$ are given by

$$B = \begin{bmatrix} SF(D, C) - 1 & 0 & 0 & 0 & 0 & 0 \\ 1 - SF(D, C) & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \end{bmatrix}, \tag{30}$$

where $I(D_{acc}, C)$ is a long-term inhibition of tumor growth depending on the accumulated radiation dose D_{acc} and the concentration of a radiosensitizing drug, C, at the time of irradiation and is given by

$$I(D_{acc}, C) = \exp\left(-(1 + aC)\gamma D_{acc}\right). \tag{31}$$

Here, a is a pharmacodynamic potency parameter of the radiosensitizer, and γ is a potency parameter associated with long-term radiation exposure. The function $SF(D, C)$ is the surviving fraction of cells given irradiation with dose D and simultaneous radiosensitizer concentration C and is given by

$$SF(D, C) = \exp\left(-(1 + bC)(\alpha D + \beta D^2)\right), \tag{32}$$

where b is a pharmacodynamic potency parameter of the radiosensitizer, and α and β are the linear and quadratic constants from the linear-quadratic model of radiobiology, see section 6.3. The model is illustrated in Figure 4. The idea of long-term inhibition of growth is similar to a model by Querdani et al., used to describe anti-angiogenetic treatment [56].

Similar models have been proposed for radiation therapy alone, and excluding the long-term inhibition of growth, e.g., in the paper by Watanabe et al. where the authors describe the response to a single dose of radiation [62]. A more detailed

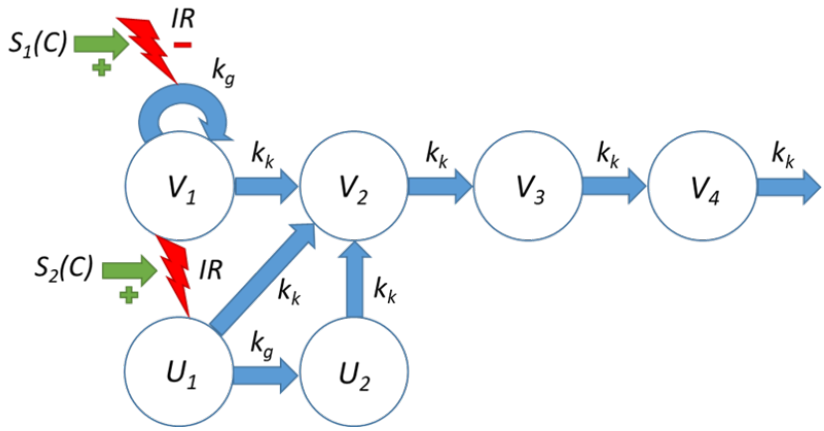

Figure 4: *Tumor model for treatment with radiation and radiosensitizer combinations. Upon irradiation, a fraction of cells are transferred from V_1, the proliferating compartment, to U_1, where they are allowed up to one more cell division (and in the process transferring the daughter cells to U_2), before dying through a series of damage compartment, V_2, V_3, and V_4. The fraction of proliferating cells that is transferred depends on the radiation dose according to the linear-quadratic model, stimulated by the presence of radiosensitizer. The model also includes a permanent inhibition of the growth rate k_g depending on the accumulated radiation dose, and the concentration of radiosensitizer at the time of irradiation.*

systems pharmacology model, supported by various in vitro and in vivo data, was published by Checkley for combinations of radiation and a radiosensitizer [14]. That model combines a cell cycle model, with a tumor model including an inner core and an outer shell (see [19]), where also cells in the outer shell are sensitive to radiation.

7.5.4 Models of the cell cycle

Lastly, tumor growth is modeled as a linear cell cycle model in paper V. The model consists of n states, $x = (x_1, ..., x_n)$, and is described by the following system of differential equations

$$\dot{x}_1 = 2k_n x_n - k_1 x_1, \tag{33}$$
$$\dot{x}_i = k_{i-1} x_{i-1} - k_i x_i, \; i = 2, ..., n, \tag{34}$$

where k_i is the transfer rate from state x_i to x_{i+1}. After the last state, x_n, mitosis occurs, and the two daughter cells are transferred to x_1. This model is illustrated in Figure 5, taken from manuscript V. For this model we consider combination therapy with m cytotoxic drugs, with corresponding concentration vector $u = (u_1, ..., u_m)$, that act linearly on x. The model subject to drug provocation can be written

$$\dot{x} = \left(A - \sum_{i=1}^{m} D_i u_i \right) x, \tag{35}$$

where A is the system matrix for the system (34), given by

$$A = \begin{pmatrix} -k_1 & 0 & \dots & 0 & 2k_n \\ k_1 & -k_2 & 0 & \dots & 0 \\ 0 & k_2 & \ddots & & \vdots \\ \vdots & 0 & \ddots & \ddots & 0 \\ 0 & \dots & 0 & k_{n-1} & -k_n \end{pmatrix}, \tag{36}$$

and D_i are non-negative diagonal matrices whose entries $D_{i,jj}$ correspond to the drug action of drug i on state x_j. We can also collect the diagonal matrices D_i as columns of a full matrix B so that $B_{ij} = D_{i,jj}$. Then, the system may be written

$$\dot{x} = (A + \mathrm{diag}(Bu))\, x. \tag{37}$$

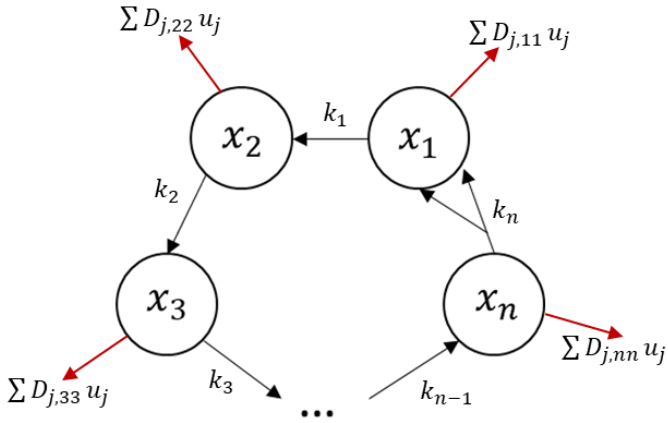

Figure 5: *Illustration of a linear cell cycle model with n states, $x_1, ..., x_n$, with transfer rates $k_1, ..., k_n$. During transfer between x_n and x_1 mitosis occurs and the cell splits into two daughter cells. Cell death induced by a combination of m drugs that may act differently on cells in different stages of the cell cycle, is indicated with red arrows.*

8 Mixed-effects modeling

In this section we consider the problem of model fitting and parameter estimation for a model described by a system of ordinary differential equations and time-series data from a population of individuals, e.g., from a xenograft experiment as described earlier. We consider what is arguably the most appropriate and sophisticated framework for population data, namely the mixed-effects modeling framework. The term 'mixed-effects' refers to the fact that effects, typically parameters in the model, are composed of a fixed as well as a random component. E.g., we may want to describe the tumor growth rate, denoted k_g, with a lognormal distribution with parameters θ and σ. Then we write $k_g = \theta e^{\eta}$, where η is a normally distributed random variable with zero mean and variance σ^2

8.1 The mixed-effects framework

In a general setting, we consider population model described as a system of impulsive differential equations

$$\dot{x}(t) = f(x(t), u(t), \theta, \eta, t), \ t \geq 0, t \neq \tau_i, \tag{38}$$

$$x(\tau_i^+) = x(\tau_i^-) + g_i(x(\tau_i^-), u(\tau_i^-), \theta, \eta), \tag{39}$$

$$x(0) = x_0(u(t), \theta, \eta), \tag{40}$$

where x is the vector of dependent variables, or states, u is an input, or control vector, t is the dependent variable representing time, $\{\tau_i\}_{i=1}^{\infty}$ is a strictly increasing sequence of fixed impulse times, not including the origin, such that $\tau_i \to +\infty$ as $i \to +\infty$, θ is a parameter vector that is common to the entire population, and η is a multivariate normally distributed random vector with zero mean and covariance matrix $\Omega = \Omega(\theta)$. The matrix Ω is symmetric and positive definite, and if it contains any unknown parameters, these are included in the vector θ. When it is feasible, one may assume that Ω is a diagonal matrix, since this greatly reduces the number of parameters. In this case, η is a vector of independent normally distributed random variables. For the tumor models considered in this thesis, x is a vector of states that tumor cells can be inhabit, e.g., amounts of cells different stages of the cell cycle, or simply proliferating or non-proliferating.

Let y be an observation vector modeled as

$$y = h(x, u, \theta, \eta, t) + e, \tag{41}$$

where h is a vector-valued (smooth) function and e is an error term, assumed to be multivariate normally distributed with zero mean and covariance matrix $R = R(x, u, \theta, \eta, t)$. Once again, if R contains any unknown parameters that need to be estimated, these are included as part of the vector θ. For a xenograft study, only the total tumor volume is measured, i.e., the sum of all states

$$y = \sum_i x_i + e, \tag{42}$$

where we have used an additive error, $R = \sigma^2$, in Paper I, a multiplicative error, $R = \left(\sum_i x_i\right)^2 \sigma^2$ in paper II, and a combination of both $R = \sigma_1^2 + \left(\sum_i x_i\right)^2 \sigma_2^2$ in Paper III.

The parameter vector θ can be estimated using the maximum likelihood method. Let d_{ij} denote an observation of individual i at time t_{j_i}, and define the residual

$$\epsilon_{ij} = d_{ij} - E[y_{ij}]. \tag{43}$$

where E denotes expectation. The likelihood function for individual i, assuming η_i are known, is given by

$$L_i(\theta) := p_1(d_i|\theta, \eta_i), \tag{44}$$

where p_1 is the probability density for the observations given θ and η_i, and is given by

$$p_1(d_i|\theta, \eta_i) = \prod_j \frac{1}{\sqrt{2\pi \det R}} \exp\left(-\frac{1}{2}\epsilon_{ij}^T R_{ij}^{-1} \epsilon_{ij}\right), \tag{45}$$

where the product is taken over all observation times for individual i. Considering η_i as a random vector, we can obtain the likelihood function for θ by marginalizing the joint likelihood with respect to η_i using the law of total probability to obtain

$$L_i(\theta) := \int p_1(d_i|\theta, \eta_i)\, p_2(\eta_i|\theta)\, d\eta_i, \tag{46}$$

where p_2 is the probability density of η_i given θ and is given by

$$p_2(\eta_i|\theta) = \frac{1}{\sqrt{2\pi \det \Omega}} \exp\left(-\frac{1}{2}\eta_i^T \Omega^{-1} \eta_i\right). \tag{47}$$

The population likelihood function L is given as the product of the individual likelihoods

$$L(\theta) = \prod_i L_i(\theta). \tag{48}$$

Given the functional form of each individual likelihood, the population likelihood can also be written in terms of the individual log-likelihood functions l_i according to

$$L(\theta) = \prod_i \int \exp(l_i)\, d\eta_i, \tag{49}$$

where l_i are given by

$$l_i = -\frac{1}{2}\sum_j \left(\epsilon_{ij}^T R_{ij}^{-1} \epsilon_{ij} + \ln\det(2\pi R_{ij})\right) \tag{50}$$

$$-\frac{1}{2}\eta_i^T \Omega_{ij}^{-1} \eta_i - \frac{1}{2}\ln\det(2\pi\Omega). \tag{51}$$

Once θ has been estimated, we may obtain the optimal values for η_i, which we denote by η_i^* and refer to as the empirical Bayes estimates (EBEs) by maximizing the individual likelihood for each individual in the population.

Several algorithms have been developed in order to maximize $L(\theta)$ including the First Order Conditional Estimation (FOCE) method and the Stochastic Approximation Expectation Maximization (SAEM) algorithm. We give a brief overview of the FOCE method, an implementation of which has been developed inhouse at the Fraunhofer-Chalmers Centre using Wolfram Mathematica. For details, we refer to the paper by Almquist et al. [4].

Since L does not have a closed-form solution for any reasonable practical problem, we approximate the individual log-likelihood l_i using a second order Taylor expansion in η_i around η_i^* to give

$$l(\theta) \approx l_F(\theta) := \sum_i \left(l_i(\eta_i^*) - \frac{1}{2}\ln\det\frac{-H_i(\eta_i^*)}{2\pi}\right), \tag{52}$$

where H_i is the Hessian matrix whose elements are given by $[H_i]_{jk} = \partial_{\eta_k}\partial_{\eta_j} l_i$. The hessian matrices include terms involving first and second order partial derivatives of ϵ_{ij} and R_{ij}. This makes them computationally expensive and an approximation is therefore made by ignoring all second order terms. An argument in favor of this

exclusion is that the expected value of these terms can be shown to be zero. It should be noted that the residuals ϵ_{ij} as well as the matrices R_{ij}, and their partial derivatives, generally depend on the state vector x and the state sensitivities, meaning that an ODE system has to be solved when these are quantities are to be evaluated.

The optimization problem can be solved using a Quasi-Newton method such as the Broyden-Fletcher-Goldfarb-Shanno (BFGS) algorithm [10], where, in order to evaluate the population likelihood for a given θ, an inner optimization problem is solved to find the corresponding η^* . Derivatives can be approximated using finite-difference, or be exactly computed using so-called sensitivity equations, derivatives of the state vector x with respect to η and θ. We consider the technical details regarding this particular implementation of the FOCE method using sensitivity equations to be outside the scope of this thesis, and instead refer to the paper by Almquist et al. [4].

8.2 Model evaluation

It is standard procedure to consider the precision of the parameter estimates θ obtained from maximum likelihood estimation. This can be done in a variety of ways, e.g., by bootstrapping, where data is re-sampled, with replacement, a large number of times, N, yielding a sequence of estimates $(\hat{\theta}_1, ..., \hat{\theta}_N)$ of $(\theta_1, ..., \theta_N)$, from which the sample variance for each component of θ can be estimated. This procedure is computationally expensive, and we have therefore made use of the Fisher information matrix $I(\theta)$ to estimate the relative standard error (RSE) of each parameter estimate. The Fisher information is the Hessian of the (population) log-likelihood function

$$I(\theta)_{jk} := \frac{\partial^2 l(\theta)}{\partial\theta_j \partial\theta_k}. \tag{53}$$

We remark that, as before, the partial derivatives can be computed approximately using finite differences, or exactly using sensitivity equations [4]. The RSE for θ_i, which we denote by $RSE(\theta_i)$, is then approximated by

$$RSE(\theta_i) \approx \frac{100}{|\theta_i|} \times \sqrt{[I(\theta)^{-1}]_{ii}}. \tag{54}$$

A calibrated model can be evaluated by inspecting of the residuals and EBEs (η^*) for trends. A typical indication that the model is not good enough could be that EBEs are clustered with respect to the treatment groups.

Models can also be compared using the Akaike information criterion (AIC), which is defined as

$$AIC = 2n - 2l(\theta^*), \tag{55}$$

where n is the total number of parameters in the model, and $l(\theta^*)$ is the maximum log-likelihood value. AIC is a measure of the information lost when using a given model to describe data, and weighs goodness-of-fit against model complexity [2].

Thus, a model with a lower AIC value should be preferred over one with a higher AIC value. Note that if two models have the same number of parameters, comparing AIC values is equivalent to comparing likelihood values.

Finally, it is typical for a model to be evaluated using a technique known as Visual Predictive Check (VPC). A VPC involves using the calibrated model to simulate a large number of individuals (or identical studies) so that different percentiles in the simulated data can be compared with the observations in the original data. By simulating many different synthetic data sets with the same covariates as the original data, it is also possible to construct confidence bands for different percentiles (particularly the median) of the observed population. An example of a set of VPCs, taken from paper III, is shown in Figure 6.

8.3 Naive pooled vs. Two-stage vs. Mixed-effects

Parameter estimation can be approached in different ways, and in this section we compare mixed-effects modeling to two alternative techniques, the naive pooled approach and the two-stage approach for population modeling. We consider, in particular, the situation with data from a combination therapy (xenograft) experiment with treatment groups including: a vehicle group, monotherapy groups with the different compounds, and combination treatments. For a thorough discussion of these approaches see, e.g., [9].

The naive pooled approach treats all data from individuals in a treatment group as though it came from the same individual. This leads to a simultaneous nonlinear regression problem for N models, where N is the number of treatment groups, with a common parameter vector θ to be estimated using e.g. maximum likelihood or the least squares method. This approach does not quantify between-subject variability, and is therefore more appropriate when there is little such variability, or to get a rough estimate of the typical behavior. In contrast, when there is significant variability between individuals, which is the case for the animals treated with radiation therapy that are described in this thesis, a naive pooled approach is less effective, and moreover, quantifying and understanding the variability is important to avoid future treatment failure.

The two-stage method is an approach for quantifying between-subject variability that is simpler than mixed-effects modeling. The approach treats each subject individually, and statistics, such as the sample mean and standard deviation, are computed afterwards for the model parameters. This works well if there is a lot of data from each individual, so that the parameter estimates have decent precision, but does not work very well if for some individuals there is only one or a few measurements. This can be somewhat remedied by only estimating a subset of the parameters individually, and letting the rest be the same for the entire population. However, this would require simultaneous fitting of all individuals, which is not significantly easier than using mixed-effects modeling.

In conclusion, in this thesis we have used mixed-effects modeling since it is the most sophisticated framework for treating between-subject variability, and it makes use of the information in all data in the estimation of all model parameters. In

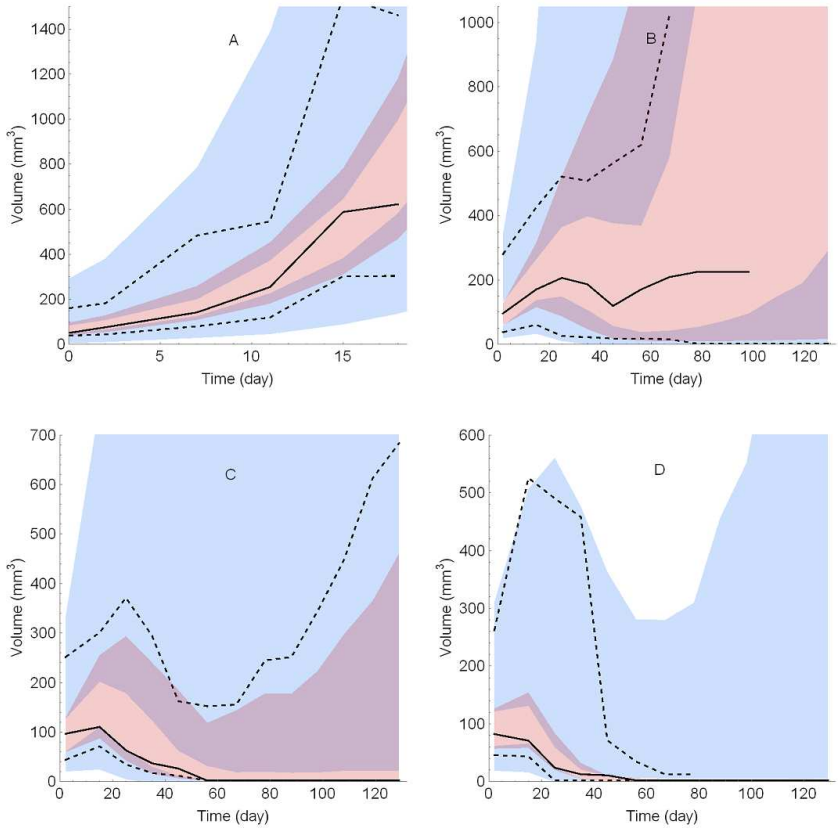

Figure 6: *Examples of visual predictive checks (VPCs) taken from paper III. The figure shows simulated confidence regions for the 10th, 50th, and 90th percentiles obtained by simulating a large number of data sets, for the treatment groups vehicle (A), radiation only (B), radiation and low dose of radiosensitizer (C), and radiation and high dose of radiosenzitier (D).*

contrast, a naive pooled approach would not have been adequate due to significant variability, and the two-stage approach would have been similar to using a uniform distribution compared to a lognormal distribution, the latter of which has the desirable properties of ensuring positivity and penalizing outliers.

9 Tumor Static Exposure

In the absence of treatment cancerous tumor grow larger over time. Intervention with an anti-cancer compound can slow down the cancerous growth, and in good cases even reverse it. Starting with low exposures of the compound and gradually increasing it, one may find a point when the tumor stops growing and starts shrinking instead. We call such an exposure level the Tumor Static Exposure (TSE) of the given compound and denote it by u^*. By this definition all exposures u less than u^* will yield tumor growth, and it is reasonable to assume that any u greater than u^* results in tumor shrinkage. Thus, u^* divides all possible exposures, represented by the positive real line $[0, +\infty)$ into two intervals, $\mathcal{G} := [0, u^*]$ and $\mathcal{S} := (u^*, +\infty)$ that yield different qualitative tumor evolutions.

The construction of TSE and the growth and shrinkage sets, $\mathcal{G}$ and $\mathcal{S}$, can be naturally extended to interventions with multiple anti-cancer agents. For two compounds, with exposures u_1 and u_2, the TSE set is a curve consisting of pairs (u_1, u_2) that separates the first quadrant of the exposure plane into a region of tumor growth $\mathcal{G}$ and one of tumor shrinkage $\mathcal{S}$, whereas for three or more compounds the TSE set will be a (hyper-)surface in the first orthant. The case with two compounds is illustrated in Figure 7, which is taken from Paper I.

It is clear that knowledge of TSE for a given combination is of considerable therapeutic interest, since it would allow for treatment that results in a desirable effect, i.e., tumor shrinkage, while avoiding toxic effects associated with overdosing. As shall be discussed more later in this section, TSE can also be used optimize a given combination and to compare and rank combinations against each other.

TSE can be difficult obtain experimentally, not only due to variability and uncertainty in measurements, but also because it would require testing many different exposure levels to sufficiently map out and identify the sets $\mathcal{G}$ and $\mathcal{S}$, which becomes even more arduous for combination therapies. Therefore, it is convenient to use modeling to make a prediction of TSE based on a smaller set of experiments.

Tumor Static Exposure and similar concepts are considered in all five papers included in this thesis. These concepts have also been featured in a number of other publications, see e.g., [48, 23, 34, 36, 37, 45]

9.1 Definition of Tumor Static Exposure

Consider a compartment model of a tumor described by an impulsive system of autonomous differential equations as in equation (3) - (5), with fixed impulsive times, a state vector $x = (x_1, ..., x_n)$, and an input or exposure vector $u = (u_1, ..., u_m)$ that is assumed to be constant. The components, u_i, can e.g. be the (average) plasma concentration of a particular compound, or the daily dose of radiation. Moreover, we assume that $x_u = 0$ is a solution to the system for any u. Define the shrinkage set

$$\mathcal{S} := \{u \in \mathbb{R}^m_+ : x_u = 0 \text{ is a (locally) stable solution}\}. \tag{56}$$

Recall that a solution solution $\varphi(t)$ to a system of (impulsive) differential equations is stable if any other solution $\varphi_\epsilon(t)$ that starts near $\varphi(t)$, remains close to $\varphi(t)$ for

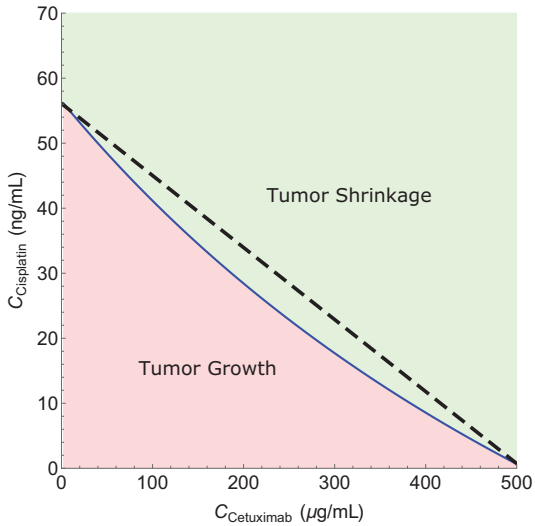

Figure 7: *Tumor Static Exposure curve for the model given by the system matrix* (26)*, for combinations of the compounds cetuximab and cisplatin, using the parameter estimates from fitting the model to xenograft data. The shrinkage region S is marked in green. The intersections of the TSE curve with the coordinate axes correspond to the concentration resulting in tumor shrinkage when the corresponding compound is administered as monotherapy.*

all t. More precisely, if for any $\epsilon > 0$ and $t > 0$ there exists a δ such that if $||\varphi(t_0) - \varphi_\epsilon(t_0)|| < \delta$ then $||\varphi(t) - \varphi_\epsilon(t)|| < \epsilon$.

If the system does not contain any jumps, or at least the jumps do not depend on the exposure u, stability can be established by linearization and the Hartman-Grobman theorem. Let A_u be the linearization of the system dynamics f about the origin. Then the origin is a stable equilibrium solution if all eigenvalues of A_u have negative real parts. This can be used to identify the shrinkage set $\mathcal{S}$ for the models in papers I and V, which are already linear and do not feature radiation impulses. For the radiation models in papers II-IV, however, one could use general definition of stability above, which for a general impulsive system can be quite difficult. However, the radiation models featured in this thesis have a main compartment V_1 such that if $V_1 \to 0$, as $t \to +\infty$, then the volumes of all other compartment also vanish. Hence, the stability problem can be reduced to a single differential equation, and the stability problem reduces to ensuring that the outflow from the main compartment is greater than the inflow due to cell proliferation.

We can define the TSE set as the boundary of shrinkage set, i.e., TSE:= $\partial\mathcal{S}$, and the growth set $\mathcal{G}$ as the complement to $\mathcal{S}$, i.e., $\mathcal{G} := \mathcal{S}^c \cap \mathbb{R}^m_+$. Thus, TSE divides the positive orthant into the regions $\mathcal{G}$ and $\mathcal{S}$. Exposure combinations u on the TSE surface leads to stable, bounded disease, either at steady state, or exhibiting oscillatory behavior.

To illustrate, we consider the set $\mathcal{S}$ for the model in paper I, is of the following form

$$\mathcal{S} = \{(u_1, u_2) \in \mathbb{R}^2_0 : k_g I(u_1) - k_k S(u_2) < 0\}, \tag{57}$$

for some functions I and S. If I is a convex function and S is concave, it follows immediately that S is a convex set. In particular, this is true for linear functions, or E_{max}-type functions with Hill-coefficient $n \leq 1$. The case $I(u_1) = 1 - \frac{I_{max} u_1^{n_1}}{IC_{50}^{n_1} + u_1^{n_1}}$ and $1 + \frac{S_{max} u_2^{n_2}}{SC_{50}^{n_2} + u_2^{n_2}}$ is illustrated in Figure 8 for different values of n_1 and n_2.

Paper V provides a generalization of when the shrinkage set is convex for more general compartment models. Assume that the linearization (with respect to x) of a non-impulsive system can be written

$$\dot{x} = \left(A - \sum_{i=1}^{m} H_i(u_i) \right) x, \quad x(0) = x^0, \tag{58}$$

where A is a matrix with non-negative offdiagonal entries (a so-called Meltzer matrix) and H_i are diagonal matrices, whose components are convex functions of u_i. It then follows, as a corollary to a theorem first proved by Cohen (see [16]) regarding convexity of the spectral radius for non-negative matrices, that the corresponding shrinkage set

$$\mathcal{S} := \{u = (u_1, ..., u_m) \in \mathbb{R}^m_+ : x_u = 0 \text{ is a locally stable solution}\}, \tag{59}$$

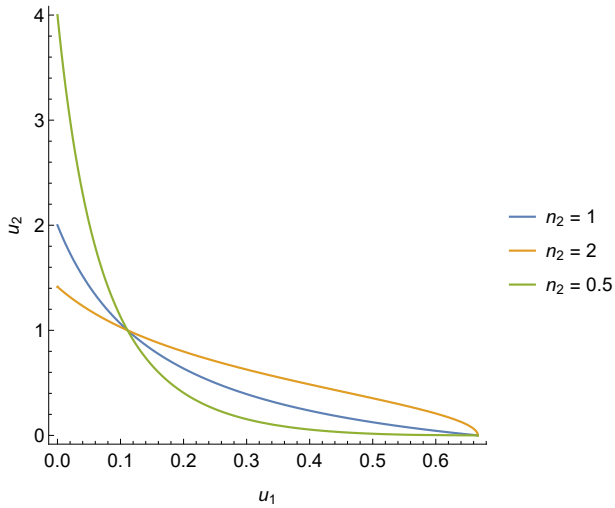

Figure 8: *Tumor Static Exposure curves for different values of the sigmoidicity parameter n_2. The other parameters were chosen as $k_g = 0.5, k_k = 0.3, I_{max} = S_{max} = IC_{50} = SC_{50} = n_1 = 1$. The figure illustrated that the TSE curves are convex for $n_2 < 1$, but not necessarily for $n_2 > 1$.*

is a convex set. Convexity is a nice property not only for optimization purposes, but also it results from synergistic effects between treatment, by which we in this context mean nonlinearities that reward combining compounds.

9.2 Uncertainty and Variability

Above, TSE was introduced based on a deterministic model with known parameters. However, for practical applicability, parameters need to be estimated, which includes a level of uncertainty in the estimates. Moreover, if a mixed-effects approach was followed for model calibration based time series data, then between-subject variability has also been quantified. In the context of TSE, it is desirable to incorporate such variability and uncertainty, in order to understand how the outcome varies in a population. In particular, we may want to predict tumor shrinkage, i.e., the set $\mathcal{S}$, not only for the typical individual, but for a larger percentage, say 95%, of the population.

If the set $\mathcal{S}$ permits an analytic expression of the form

$$\mathcal{S} = \{u \in \mathbb{R}_+^m : h(u, \theta, \eta) < 0\},$$

for some function h, where θ is a vector of population parameters (or, more precisely, their estimates), and η is a random vector with a known distribution, then $H = h(u, \theta, \eta)$ defines a new random variable. In special cases, the distribution of H

can be determined exactly, e.g., using the fact that the product (quotient) of two independent log-normally distributed random variables is again log-normally distributed. In general, Monte Carlo simulations can be used to compute the set $\mathcal{S}_\alpha$ defined by

$$\mathcal{S}_\alpha := \{u \in \mathbb{R}_+^m : \mathbb{P}_\eta(h(u, \theta, \eta) < 0) = \alpha\}, \tag{60}$$

for some $\alpha \in (0, 1)$. Alternatively, $\mathcal{S}_\alpha$ may be approximately obtained by taking the α-percentile among the TSE curves for the individuals in the observed population, which can be computed using η_i^* for each individual that were obtained by maximizing the individual log-likelihood given the final parameter estimates for θ. An example, taken from paper II, is shown in Figure 9. Moreover, the estimated uncertainty in the parameter vector θ can be used to construct a confidence region for $\mathcal{S}_\alpha$, either by bootstrapping, or using the asymptotic normal distribution and the Fischer information matrix $I(\theta)$.

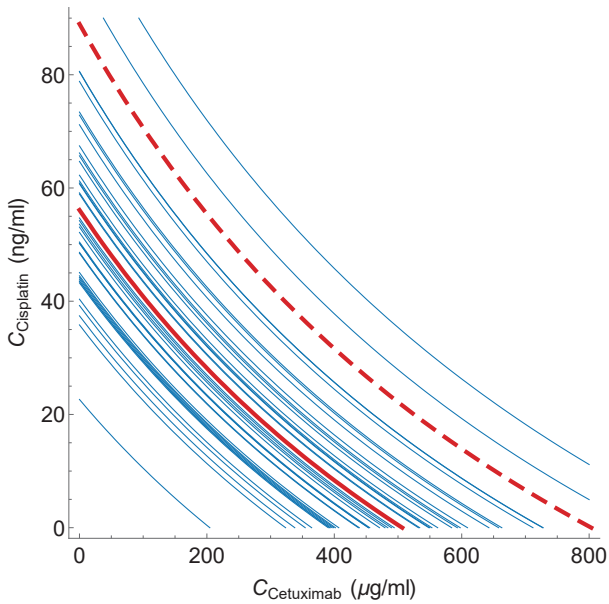

Figure 9: *Individual TSE curves for the data modeled in paper II are shown in blue, whereas the median TSE curve is shown in red. Using these, an approximation of the 95% TSE curve can be constructed (red dashed).*

9.3 Optimization and Ranking of Combinations

Given a cost function $\Psi = \Psi(u)$, we can consider the optimization problem

$$\min_{u \in \mathcal{S}} \Psi(u), \tag{61}$$

where the cost function reflects e.g., the toxicity associated with different exposure combinations. Manuscripts IV and V consider this optimization problem using the simplest possible cost function, namely a linear one: $\Psi(u) = \alpha^T u$, where $\alpha = (\alpha_1, ..., \alpha_m)$ is a vector of weights, reflecting the (relative) costs of the compounds. In general, more complicated cost functions can be considered that include e.g., damage to healthy tissue, body weight, or white blood cell count. Note that convexity of $\mathcal{S}$ is a nice property for this optimization problem since if Ψ is also convex, then any local optimum is also a global one.

Apart from quantifying optima, questions regarding its uniqueness and whether $u^* > 0$, i.e., whether it is optimal to use all compounds, are worthy of consideration. Once an optimum, u^*, has been found, the optimal value $\Psi^* = \Psi(u^*)$ can be used to compare the combination against combinations of different compounds, as we have done in Paper IV for combinations of radiation with three different radiosensitizing agents. Moreover, Paper V considers such an optimization problem for a model with an arbitrary number of compounds, which could be thought of as including a large number of compounds into the same model (which could in principle be done e.g., by simultaneous fitting of data from many different studies), and quantifies the optimum, its uniqueness, and which compounds are used in such an optimum. Thus, besides optimization of a given combination, one could imagine using this optimization problem to deselect compounds from consideration if they are not included in the optimal solution.

10 Summary of Papers

In this section, we provide summaries of the five papers that comprise this work. The complete papers can be found appended at the end of this thesis.

10.1 Summary of Paper I

In the first paper, we construct a tumor growth inhibition (TGI) model for combinations of the drugs cetuximab and cisplatin, and explore the concept of Tumor Static Concentration (TSC) for drug combinations. Cetuximab is a monoclonal antibody and an epidermal growth factor receptor (EGFR) inhibitor. Inactivation of EGFR, an oncogene that is over-expressed in many cancer cases, should prevent uncontrolled cell division and inhibit tumor growth. Cisplatin is a platinum-based chemotherapy that interferes with DNA replication and kills the fastest proliferating cells.

A linear four compartment model with one proliferation state and three damage compartments is used to describe xenograft data from a cetuximab-cisplatin study. Drug action of cetuximab is described as inhibition of tumor growth rate, whereas drug action of cisplatin was described as stimulation of cell death. New to this model was also the inclusion of a natural death rate. Appropriate initial conditions are found by computing the eigenvector associated with the only positive eigenvalue of the system matrix for untreated tumors. Following a mixed-effects approach, model fitting is shown to be adequate and all model parameters were estimated with acceptable precision.

Tumor Static Concentrations are defined as the set of cetuximab-cisplatin concentration pairs (C_{cetux}, C_{plat}) leading to stable disease. Mathematically, this occurs when the largest eigenvalue of the system matrix has a real part of zero. This leads to a curve in the first quadrant of the $C_{cetux}C_{plat}$ concentration plane, called the TSC curve, separating tumor growth from shrinkage. Convexity of the TSC curve for cetuximab and cisplatin, as well as three alternative drug action combinations are established as they are all on the form

$$\alpha C_A C_B + \beta C_A + \gamma C_B = \delta, \tag{62}$$

for two drugs with concentrations C_A and C_B, which describes a hyperbola or a straight line (if $\alpha = 0$), whose intersections with the coordinate axes are given by δ/β and δ/γ. The extension of the TSC curve to three or more compounds is briefly considered, leading to hyper-surfaces in $\mathbb{R}^n$.

10.2 Summary of Paper II

In the second paper, we construct a tumor model for combinations of ionizing radiation (x-rays) and a radiosensitizer, and extend the TSC concept to account for radiation. The radiosensitizer is a small molecule that interferes with the repair of DNA damage caused by radiation, leading to cell death through e.g. apoptosis or mitotic catastrophe.

A system of impulsive differential equations is used to describe data from a xenograft study. The system consists of a proliferating state, three damage damage compartments, and two radiation compartments for lethally irradiated cells. Upon irradiation, a fraction of proliferating cells, chosen according to the linear-quadratic model of radio-biology, are transferred to the first radiation compartment, where they are allowed up to one more cell division before dying through the chain of damage compartments. The extra cell division ensures that the total tumor volume changes smoothly over time. Presence of radiosenzitizer at the time of irradiation was described as enhancing the radiation effect, as though a greater dose of radiation had been given. Following a mixed-effects approach, model fitting is shown to be adequate and all model parameters were estimated with acceptable precision.

TSC is generalized to Tumor Static Exposure (TSE), in order to describe radiation therapy. A TSE curve is derived consisting of all combinations of daily radiation doses and average radiosensitizer concentrations that lead to stable disease. The TSE curve is shown to be convex and on the form

$$\alpha D_{RT}^2 C_{RS} + \beta D_{RT}^2 + \gamma D_{RT} + \delta C_{RS} = \epsilon, \tag{63}$$

where D_{RT} is the daily radiation dose and C_{RS} is the daily average radiosensitizer concentration. The model and TSE curve are validated by using a second data set with different radiosensitizer dose, which, according to the TSE curve, was accurately predicted to lead to slight tumor shrinkage.

10.3 Summary of Paper III

The fourth paper extends the model for radiation and radiosensitizing treatment from paper II to account for long-term effects on tumor growth. The first radiation effect, where a fraction of proliferating cells are allowed up to one more cell division and subsequently died, is retained and a second radiation effect is incorporated into the model whereupon the tumor growth rate, k_g, is decreased depending on the accumulated radiation dose. It is assumed that this effect is modulated depending on the radiosensitizer concentration at the time of irradiation. Model fitting is shown to be adequate and with reasonable parameter estimates and precision, using xenograft data from a study with a treatment schedule of six weeks, similar to what is used in the clinic.

A TSE curve is constructed based on the long-term reduction of growth rate alone, consisting of all total (accumulated) radiation doses and simultaneous radiation concentrations leading to a net growth rate of zero, i.e., when the growth rate k_g was equal to the natural death rate k_k. The equation for the TSE curve can be written on the form

$$\alpha D_{RT} C_{RS} + \beta D_{RT} = \gamma, \tag{64}$$

where D_{RT} is the total radiation dose, and C_{RS} is the radiosensitizer concentration at the time of irradiation. Since the equation contains no terms in C_{RS} alone,

it is not possible to achieve tumor stasis or shrinkage with only radiosensitizer treatment. The TSE curve is clearly convex, with $C_{RT} = 0$ as an asymptote. Beyond the TSE curve, changes in net growth rate depending on combinations of radiation and radiosensitizer are considered and shown as a heat map as well as a three-dimensional plot.

10.4 Summary of Paper IV

The third paper is an application of the radiation and radiosensitizer model from Paper II to describe xenograft data using radiation therapy and three different radiosensitizing compounds, which serves as further validation of the model. The paper also considers the problem of comparing the three combinations (radiation plus radiosensitizer A, B, or C), using TSE curves computed for each combination. For each TSE curve, an optimum exposure pair (D_{RT}, C_{RS}) is computed by minimizing a linear combination of the drug exposures along the TSE curve. The minimum values corresponding to each radiation and radiosensitizer combination is then compared in order to rank the three combination therapies.

The paper also introduces Tumor Shrinkage Exposures (TSE_{dV}) corresponding to those exposure combinations that result in a certain (relative) shrinkage rate, with $dV = 0$ corresponding the the usual Tumor Static Exposure curve, and considers the same optimization problem for different desired shrinkage rates. Results show that the choice of radiosensitizer was independent of the desired shrinkage rate.

10.5 Summary of Paper V

The fifth paper considers a linear cell cycle model with n states subject to combination therapy with m compounds. Drug action is assumed to be linear and may differ depending on the states of the cell cycle. Metabolic pressure associated with chemotherapeutic treatment is represented by a linear combination of the drug exposures, which is then minimized subject to a constraint of stable or regressive disease, i.e., a condition that the largest eigenvalue of the system matrix has zero real part. This constraint describes a TSE hypersurface in $\mathbb{R}^n_+$, which is shown to be convex. Drug actions of the m compounds on the n states can be summarized by the drug action matrix B such that B_{ij} corresponds to the drug action of drug j on state i. It is shown that if B has linearly dependent columns, an optimum exists where at least one of the drug concentrations is zero, whereas if B has linearly independent columns, a solution formula is prescribed. In the special case where each drug targets a different, single, state, the result is similar to one of the classical proofs of the inequality between the arithmetic and geometric means, whereas the more general case is obtained by an invertible affine transformation.

11 Discussion

The research described in this thesis aims to support discovery and development of combination treatments in preclincal oncology using mathematical modeling and simulation. This research is summarized in five appended manuscripts, and in the previous sections of this thesis. The focus has been on three research questions (see section 3) and there is a logical progression - threads connecting the five manuscripts. First, a standard model was adjusted to account for natural cell death, fitted to xenograft data for established compounds, and using this model the TSE concept was derived and analyzed. Next, the model, as well as the TSE concept was extended to radiation therapy, and was later adjusted to account for long-term treatment. Applications of the TSE concept were also explored: to optimize a given combination, and to compare/rank different combinations. Lastly, to reflect tumor heterogeneity, a theoretical investigation of TSE was performed for a cell cycle model, in which combination therapy with an arbitrary number of anticancer drugs was considered.

11.1 Main contributions and research questions

The three research questions (section 3) relate to improving or extending existing models (Q1), what we do with the models (Q2), and how uncertainty and variability is taken into account (Q3). These can be connected to the three main contributions (section 4) in the following way.

The first main contribution, inclusion of natural cell death, is an example of improving, making existing models more biologically feasible (Q1). Similarly, the development of radiation models for short-term and long-term treatment, with and without radiosensitizing treatment, also addresses Q1.

The TSE concept, which is featured in all five manuscripts, is an example of a model-based tool and therefore addresses Q2. How to define and construct TSE curves is addressed in manuscripts I-III, whereas manuscripts IV and V illustrate have TSE can be used. Moreover, the use of nonlinear mixed-effects modeling in general, and the quantification of between-subject variability in TSE in particular, are examples of how this research has addressed Q3.

11.2 Conclusions

The most important conclusions become apparent when looking at the research questions, main contributions, and the discussion above. Throughout this thesis, we have emphasized how relatively simple mathematical models can be used to support preclinical drug discovery and development. Models have been development for different treatment modalities, with a particular focus of radiation, that have a simple and generic structure such that they can be reused for different compounds and combinations without making too many changes.

We have focused on TSE, defined using the appropriate stability notion, as a useful and widely applicable endpoint with a clear biological interpretation. We have explored how to interpret and use the shape of the TSE surface with respect to

compound selection, optimization, and how to account for variability. Moreover, the usefulness of mixed-effects modeling for quantification of between-subject variability, to be able to predict tumor shrinkage in a large portion of the population, has been consistent throughout all model fitting in this thesis.

11.3 Future work and open problems

The work that has been conducted during this project poses a number of new questions, and there are several extensions of existing concepts that have not been fully explored.

Firstly, the main concepts proposed in this thesis, i.e., those related to radiation modeling, and the TSE concept, have yet to be used in practice when designing and analyzing new experiments. Moreover, the predictive power of these concepts could be tested using e.g. published data on combinations with a large number of experiments using different exposures.

Secondly, the usefulness of the TSE concept in translational efforts in vitro - in vivo, as well as across species (with understandable ethical limitations), and, ultimately, to humans, have been treated only superficially in discussion form. A first step in this direction would be to find combinations with the right data (in vitro - in vivo - clinical), such that TSE surfaces can be generated for each type of data and be compared.

The TSE concept itself could likely be extended, particularly with respect to variability. Stability, e.g., the largest eigenvalue of the system matrix, could be treated formally as a function of a random parameter vector such that various properties of the resulting statistical quantity can be examined, including mean, variance, but also e.g., higher moments.

The radiation models proposed in papers II and III could also be extended, particularly to describe the repair process of DNA damage, something that was attempted during this project, but did not seem feasible using only data about tumor volume over time. Analytic expressions and approximations for the chains of damage compartments $(V_2, V_3, ...)$, as well as radiation compartments $(U_1, U_2, ...)$, could also be investigated and tested to see if it makes parameter estimation faster and/or easier.

Lastly, the models used in this thesis have been exclusively ordinary differential equations, but the erratic behavior of the time series for irradiated animals may suggest that stochastic differential equations could help provide a good fit to data.

References

[1] American Cancer Society, Global Cancer Facts & Figures 4th Edition. *American Cancer Society, Atlanta*, 2018.

[2] H. Akaike. A new look at the statistical model identification. *IEEE Trans. Automat. Contr.*, 19(6):716-723, 1974.

[3] M. Akhmet. Principles of Discontinous Dynamical Systems. *Springer, Berlin*, 2010.

[4] J. Almquist, J. Leander, and M. Jirstrand. Using sensitivity equations for computing gradients of the FOCE and FOCEI approximations to the population likelihood. *J. Pharmacokinet. Pharmacodynam.*, 42(3):191–209, 2015.

[5] D. Barbolosi et al. Computational oncology - mathematical modeling of drug regimens for precision medicine. *Nat. Rev. Clin. Oncol.*, 13:242-254, 2016.

[6] S. Benzekry et al. Classical Mathematical Models for Description and Prediction of Experimental Tumor Growth. *PLoS Comput. Biol.*, 10(8):e1003800, 2014.

[7] L. von Bertalanffy. Quantitative laws in metabolism and growth. *Q. Rev. Biol.*,32:217-231, 1957.

[8] C. I. Bliss, The toxicity of poisons applied jointly. *Ann. Appl. Biol.*,26:585-615,1939.

[9] P. L. Bonate. Pharmacokinetic-Pharmacodynamic Modeling and Simulation. *Springer, Berlin*, 2005.

[10] J. F. Bonnans et al. Numerical Optimization. Theoretical and Practical Aspects. *Springer, Berlin*, 2006.

[11] D. J. Brenner et al. The Linear-Quadratic Model and Most Common Radiobiological Models Result in Similar Predictions of Time-Dose Relationships. *Radiat. Res.*, 150(1):83-91, 1998.

[12] J. M . Brown. Tumor hypoxia in cancer therapy. *Methods Enzymol.*, 435:297-321, 2007.

[13] P. Carmeliet. VEGF as a key mediator of angiogenesis in cancer. *Oncology*, 69: Suppl. 3:4-10, 2005.

[14] S. Checkley et al., Bridging the gap between in vitro and in vivo: Dose and schedule predictions for the ATR inhibitor AZD6738. *Sci. Rep.*,27(5):13545, 2015.

[15] D. E. Citrin. Recent Developments in Radiotherapy. *N. Engl. J. Med.*, 377(11):1065-1075, 2017.

[16] J. E. Cohen, Random evolutions and the spectral radius of a non-negative matrix. *Math. Proc. Camb. Phil. Soc.* , 86:345-350, 1979.

[17] M. Davidian, and D. M. Giltinan. Nonlinear Models for Repeated Measurement Data. *Chapman & Hall/CRC, Boca Raton, Florida*, 1998.

[18] L. H. Einhorn. Treatment of testicular cancer: a new and improved model. *J. Clin. Oncol.*, 8(11):1777-1781, 1990.

[19] N. D. Evans et al. Modelling of tumour growth and cytotoxic effect of docetaxel in xenografts. *Comput. Methods Programs Biomed.*, 114(3):e3-13, 2014.

[20] A. Fairchild et al. Hormonal therapy in oncology: a primer for the radiologist. *AJR Am. J. Roentgenol.*, 204(6):W620-630, 2015.

[21] N. Frances et al. Tumor growth modeling from clinical trials reveals synergistic anticancer effect of the capecitabine and docetaxel combination in metastatic breast cancer. *Cancer Chemother. Pharmacol.*, 68:1413-1419, 2011.

[22] J. Gabrielsson, and D. Weiner. Pharmacokinetic and Pharmacodynamic Data Analysis: Concepts and Applications, Fourth Edition. *Swedish Pharmaceutical Press, [Location]*, 2007.

[23] J. Gabrielsson, F. D. Gibbons, and L. A. Peletier. Mixture dynamics: Combination therapy in oncology. *Eur. J. Pharm. Sci.*, 88:132-146, 2016.

[24] J. Gabrielsson et al. Lost in translation: What's in an EC50? Innovative PK/PD reasoning in the drug development context. *Eur. J. Pharmacol.* 835:154-161, 2018.

[25] K. Goteti et al. Preclinical pharmacokinetic/pharmacodynamic models to predict synergistic effects of co-administered anti-cancer agents. *Cancer Chemother. Pharmacol*, 66:245-254, 2010.

[26] R. Gieschke and J. L. Steimer, Pharmacometrics: modeling and simulation tools to improve decision making in clinical drug development. *Eur. J. Drug Matab. Pharmacokinet.*, 25(1):49-59, 2000.

[27] P. H. van der Graaf et al. Topics in mathematical pharmacology. *Dyn. Diff. Equat.*, 28:1337-1356, 2016.

[28] W. R. Greco, G. Bravo, and J. C. Parsons. The Search for Synergy: A Critical Review from A Response Surface Perspective. *Pharm. Rev.*,47(2):331-385, 1995.

[29] D. Hanahan, and R. A. Weinberg. The hallmarks of cancer. *Cell*, 100(1):57-70, 2000.

[30] D. Hanahan, and R. A. Weinberg. Hallmarks of cancer: the next generation. *Cell*, 144(5):646-674, 2011.

[31] J. A. Jacquez. Compartmental Analysis in Biology and Medicine. 3rd edition. *BioMedware, Ann Arbor, Michigan*, 1996.

[32] D. L. Janzén et al. Parameter Identifiability of Fundamental Pharmacodynamic Models. *Front. Physiol.*,7:e590, 2016.

[33] D. G. Johnson, and C. L. Walker. Cyclins and cell cycle checkpoints. *Annu. Rev. Pharmacol. Toxicol.* 39:295,312, 1999.

[34] N. L. Jumbe et al. Modeling the efficacy of trastuzumab-DM1, an antibody drug conjugate, in mice. *J. Pharmacokinet. Pharmacodynam.*, 37:221-242, 2010.

[35] J. Jung. Human Tumor Xenograft Models for Preclinical Assessment of Anticancer Drug Development. *Toxicol. Res.*, 30(1):1-5, 2014.

[36] G. Koch et al. Modeling of tumor growth and anticancer effects of combination therapy. *J. Pharmacokinet. Pharmacodynam.*, 36:179-197, 2009.

[37] G. Koch, J. Schropp, W. J. Jusko. Assessment of non-linear combination effect terms for drug-drug interactions. *J. Pharmacokinet. Pharmacodynam.*, 43(5):461-479, 2016.

[38] S. Kruger et al. Advances in cancer immunotherapy 2019 - latest trends. *J. Exp. Clin. Cancer Res.*, 38(1):268, 2019.

[39] W. Krzyzanski and J. G. C. van Hasselt. Special issue: mathematical pharmacology. *J. Pharmacokinet. Pharmacodynam.*, 45(1), 2018.

[40] W. Krzyzanski Interpretation of transit compartments pharmacodynamic models as lifespan based indirect response models. *J. Pharmacokinet. Pharmacodynam.*, 38(2):179-204, 2011.

[41] J. Leander et al. Mixed Effects Modeling Using Stochastic Differential Equations: Illustrated by Pharmacokinetic Data of Nicotinic Acid in Obese Zucker Rats. *AAPS J.*, 17(3):586-596, 2015.

[42] G. Levy. Pharmacologic target-mediated drug disposition *Clin. Pharmacol. Ther.*, 56:248-252, 1994.

[43] S. Loewe, über Kombination swirkungen. *Arch. für Exp. Pathol.,* 114:313-326, 1926.

[44] D. E. Mager and W. Krzyzanski Quasi-Equilibrium Pharmacokinetic Model for Drugs Exhibiting Target-Mediated Drug Disposition. *Pharm. Res.*, 22, 1589-1596, 2005.

[45] P. Magni et al. A mathematical model to study the effects of drug administration on tumor growth dynamics. *Math. Biosci.*, 200(2):127-151, 2006.

[46] P. Magni et al. A Minimal Model of Tumor Growth Inhibition. *IEEE Trans. Biomed. Eng.*, 55(12):2683-2690, 2008.

[47] P. Magni et al. A Minimal Model of Tumor Growth Inhibition in Combination Regimens Under the Hypothesis of No Interaction Between Drugs. *IEEE Trans. Biomed. Eng.*, 59(8):2161-2170, 2012.

[48] X. Miao et al. Pharmacodynamic modeling of combined chemotherapeutic effects predicts synergistic activity of gemcitabine and trabectedin in pancreatic cancer cells. *Cancer Chemother. Pharmacol.*, 77:181-193, 2016.

[49] L. Michaelis and M. L. Menten, Die Kinetik der Invertinwirkung. *Biochem. Z.*

[50] G. Mo, et al. Lifespan based pharmacokinetic-pharmacodynamic model of tumor growth inhibition by anticancer therapeutics. *PLoS One*, 9(10):e109747, 2014.

[51] H. Moore. How to mathematically optimize drug regimens using optimal control. *J. Pharmacokinet. Pharmacodynam.*, 45(1):127-137, 2018.

[52] D. R. Mould et al. Developing Exposure/Response Models for Anticancer Drug Treatment: Special Considerations. *CPT: Pharmacometrics Syst. Pharmacol.*, 4(1):e00016, 2015.

[53] M. Nedeljkov and M. Oberguggenberger. Ordinary differential equations with delta function terms. *Publ. Inst. Math. (Beograd) (N.S.)*, 91(105):125-135, 2012.

[54] M. Nikanjam et al. Dosing Three-Drug Combinations That Include Targeted Anti-Cancer Agents: Analysis of 37763 Patients. *Oncologist*, 22(5):576-584, 2017.

[55] T. Ozaki, and A. Nakagawara. Role of p53 in Cell Death and Human Cancers. *Cancers (Basel)*, 3(1):994-1013, 2011.

[56] A. Querdani et al. Preclinical Modeling of Tumor Growth and Angiogenesis Inhibition to Describe Paxopanib Clinical Effects in Renal Cell Carcinoma. *CPT: Pharmacometrics Syst. Pharmacol.*, 4:660-668, 2015.

[57] B. Ribba et al. A Review of Mixed-Effects Models of Tumor Growth and Effects of Anticancer Drug Treatment Used in Population Analysis. *CPT: Pharmacometrics Syst. Pharmacol.*, 3:e113, 2014.

[58] R. Rockne et al. A mathematical model for brain tumor response to radiation therapy. *J. Math. Biol.*, 58(4-5):561-578, 2009.

[59] R. K. Sachs, L. R. Hlatky, and P. Hahnfeldt. Simple ODE Models of Tumor Growth and Anti-Angiogenic or Radiation Treatment. *Math. Comput. Model.*, 33:1297– 1305, 2001.

[60] R. M. Savic et al. Implementation of a transit compartment model for describing drug absorption in pharmacokinetic studies. *J. Pharmacokinet. Pharmacodynam.*, 34(5):711-726, 2007.

[61] H. Schättler and U. Ledzewics, Optimal Control for Mathematical Models of Cancer Therapies. *Springer, Berlin*, 2015.

[62] Y. Watanabe et al. A mathematical model of tumor growth and its response to single irradiation. *Theor. Biol. Med. Model.*, 13(6), 2016.

[63] R. M. Webster. Combination Therapies in Oncology. *Nat. Rev. Drug Discov.*, 15(2): 81-82, 2016.

[64] G. H. Williams, and K. Stoeber. The cell cycle and cancer. *J. Pathol.*, 226(2):352-264, 2012.

[65] B.G. Wouters, Cell death after irradiation: how, when and why cells die. In: M. Joiner, and A. van der Kogel, editors. Basic Clinical Radiobiology. Hodder Arnold: London, 2009. p. 27-40.

[66] X. ZU, R. M. Straubinger, and W. J. Jusko. Mechanism-based mathematical modeling of combined gemcitabine and birinapant in pancreatic cancer cells. *J. Pharmacokinet. Pharmacodynam.*, 42:477-496, 2015.