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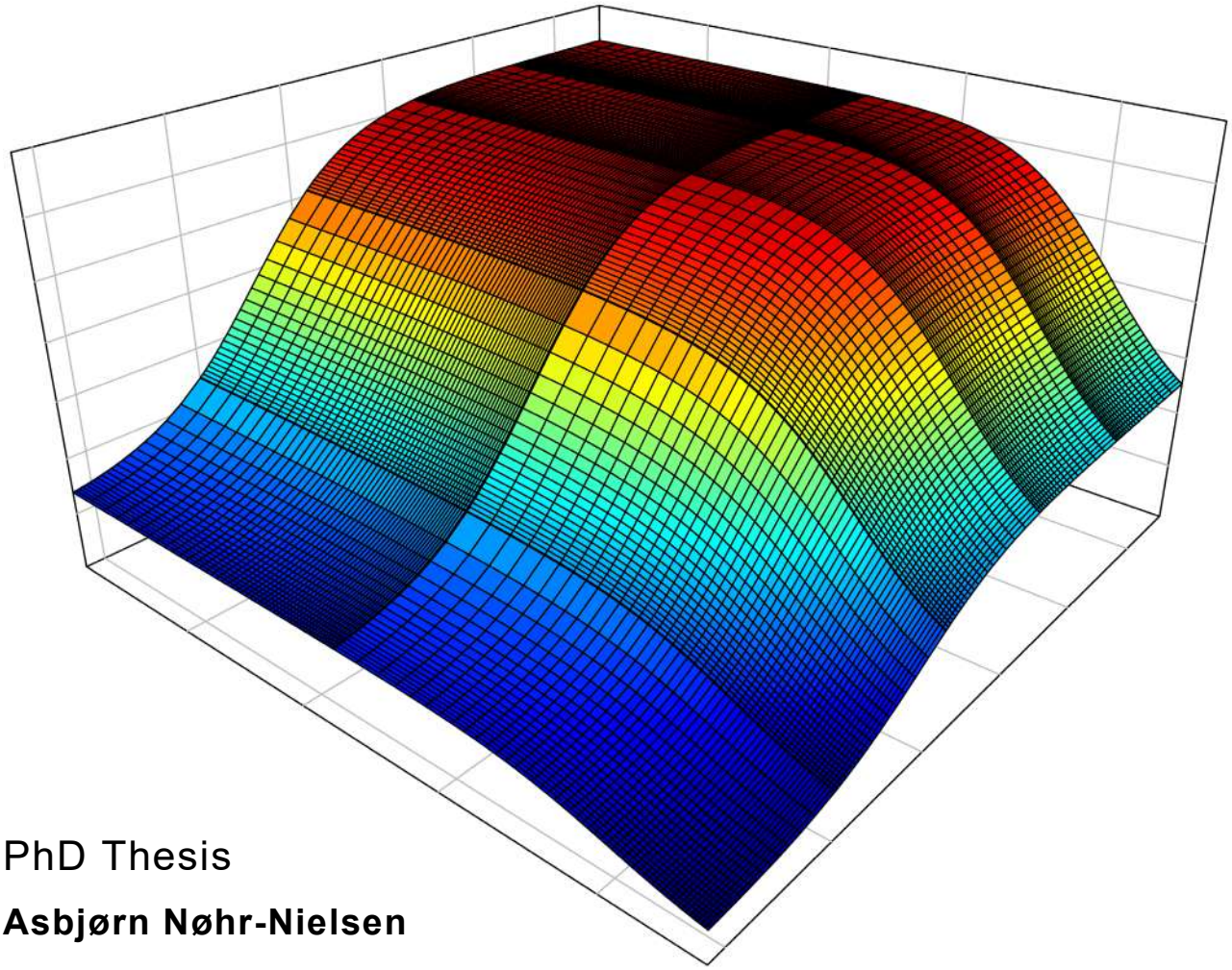

PhD Thesis

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Pharmacokinetic-Pharmacodynamic Modelling of Fixed-Dose Combinations: A Regulatory Perspective

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Preface

This thesis was submitted as part of the requirements for attaining the PhD degree at the Faculty of Health and Medical Sciences, University of Copenhagen.

The thesis is based on work carried out in the period from January 2017 to December 2019.

The PhD project is part of the Copenhagen Centre for Regulatory Science (CORS) located at the University of Copenhagen. The research projects conducted at CORS are focused on "The science of developing new tools, standards, and approaches to evaluate the efficacy, safety, quality and performance of medical products in order to assess benefit-risk and facilitate a sound and transparent regulatory decision making." The project is one of several that are supported by CORS and the collaborative companies: Novo Nordisk, Ferring Pharmaceuticals, H. Lundbeck, and LEO Pharma as well as the patient organization Rare Diseases Denmark.

During the project, I was enrolled at the Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences.

This thesis is based on the following papers:

Nøhr-Nielsen A, De Bruin ML, Thomsen M, Pipper CB, Lange T, Bjerrum OJ, Lund TM. Body of evidence and approaches applied in the clinical development program of fixed-dose combinations in the European Union from 2010-2016. Br J Clin Pharmacol. 2019;(July 2018):1–12.

Nøhr-Nielsen A, Lange T, Forman JL, Papathanasiou T, Foster DJR, Upton RN, Bjerrum OJ, Lund TM. Demonstrating Contribution of Components of Fixed-Dose Drug Combinations Through Longitudinal Exposure-Response Analysis. AAPS J 2020 222 22:1–14.

Nøhr-Nielsen A, Bagger SO, Brünner N, Stenvang J, Lund TM. Pharmacodynamic modelling reveals synergistic interaction between docetaxel and SCO-101 in a docetaxel-resistant triple negative breast cancer cell line. Eur J Pharm Sci 2020 105315.

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Abbreviations

AIC	Akaike information criterion
ATC	Anatomical therapeutic chemical
AUC	Area under the curve
BIC	Bayesian information criterion
CL	Clearance
CWRES	Conditional weighted residuals
EMA	European Medicines Agency
EPAR	European Public Assessment Report
FDA	Food and Drug Administration
FEC	Free equivalent combination
GPDI	General pharmacodynamic interaction model
HAART	Highly Active Antiviral Retrovirus Therapy
HIV	Human immunodeficiency virus
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IVIVE	*In vitro in vivo* extrapolation
LADME	Liberation; Absorption; Distribution; Metabolism; Excretion
OFV	Objective function value
PD	Pharmacodynamics
PK	Pharmacokinetics
PK-PD	Pharmacokinetic-Pharmacodynamic
T2DM	Type 2 diabetes mellitus
TNBC	Triple-negative breast cancer
V	Volume of distribution
VPC	Visual predictive check
WHO	World Health Organization

Glossary

Adherence	Filling or refilling drug prescriptions on time
Compliance	Taking medication on time
Drug development tool	Tools, such as methods, materials, or measures that facilitate drug development
Free equivalent combination	Separate components of a corresponding fixed-dose combination
Pharmacodynamics	The relationship between the concentration of a pharmaceutical drug and the effect of that drug
Pharmacokinetics	The liberation, absorption, distribution, metabolism, and excretion of pharmaceutical drugs
Substance status	The approval state of each component in a fixed-dose combination

Summary (English)

The use of combination drug therapies is central to the successful treatment of several diseases, where monotherapies are not efficacious enough or where resistance to treatment emerges. The development of new drug combinations is, therefore, a major focus. This is also true for fixed-dose combinations, where there has been a rise in approvals in recent years. Developing fixed-dose combinations often involves conducting large factorial design studies to verify the efficacy of the combination. With a greater focus on the personalization of medicines, several dose levels of fixed-dose combinations will need to be available for patients. For a factorial design study, this will result in very costly clinical trials. In order to keep developmental costs low and guide drug development, the validation of existing tools, and the development of new tools is necessary. Such model-based tools used for the analysis of fixed-dose combinations are, however, only in their infancy.

Therefore, it was the overall aim of this PhD thesis to explore, develop, and validate the use of modeling tools for the development of fixed-dose combinations. The conducted research in this thesis resulted in three publications, which addressed the overall aim and objectives of the thesis.

In the first paper, the underuse of model-based approaches in the development of fixed-dose combinations was identified for the fixed-dose combinations approved by the European Medicines Agency (58% of approvals). Additionally, although interesting strategies were employed to reprofile drugs and utilize prior knowledge to avoid dose-finding trials, very few fixed-dose combinations were geared towards personalization or could be considered innovative. Furthermore, the importance of pharmacokinetic modeling in selecting the correct doses was highlighted in the study.

In the second paper, clinical trial simulations were performed to assess the feasibility of performing longitudinal exposure-response modeling of fixed-dose combinations. In a previous study, exposure-response analysis of fixed-dose combinations had been shown to cause an inflated false positive rate. In the present research, this was avoided by employing longitudinal exposure-response to analyze fixed-dose combinations. Thus, longitudinal exposure-response analysis constitutes a new method for fulfilling the regulatory requirement of demonstrating each component's contribution to the overall effect.

In the third paper, combination models were evaluated in a preclinical breast cancer study. A novel combination was analyzed for its potential pharmacodynamic interaction by employing *the general pharmacodynamic interaction model*. Based on this model, the combination was characterized as synergistic with one compound increasing the potency of the other by up to 60% when administered together. Additionally, analysis of the model provided insights into optimal dose ratios, which can be used to guide further investigations of the combination.

In conclusion, this PhD thesis explored the current state of model-based development of fixed-dose combinations, developed a new methodology for the evaluation of fixed-dose combinations, and validated existing tools for the analysis of fixed-dose combinations. Based on the research conducted in this thesis, the overall recommendation is that modeling tools should to a greater extent be incorporated in the development of fixed-dose combinations as they assist in determining efficacy and provide valuable information to guide development.

Summary (Danish)

Brugen af kombinationsbehandling er central for den vellykkede behandling af flere sygdomme, hvor monoterapier ikke er effektive nok, eller resistens overfor behandling opstår. Udviklingen af nye lægemiddelkombinationer er derfor et stort fokus. Dette gælder også for fixed-dose combinations, hvor der har været en stigning i godkendelser i de senere år. Udvikling af fixed-dose combinations involverer ofte udførelse af store faktorielle kliniske studier for at verificere kombinationens effektivitet. Med et større fokus på personalisering af medicin, skal flere dosisniveauer af fixed-dose combinations være tilgængelige for patienter. For et faktorielt design vil dette resultere i meget dyre kliniske forsøg. For at holde udviklingsomkostningerne lave og vejlede lægemiddeludvikling, er validering af eksisterende og udvikling af nye værktøjer nødvendig. Sådanne modelbaserede værktøjer, der bruges til analyse af fixed-dose combinations, er imidlertid kun i deres begyndelse.

Det var derfor det overordnede mål med denne ph.d. afhandling at undersøge, udvikle og validere brugen af modelleringsværktøjer til udvikling af fixed-dose combinations. Forskningen i denne afhandling resulterede i tre publikationer, som opfylder afhandlingens overordnede mål og delmål.

I den første publikation blev manglende brug af modelbaserede tilgange til udvikling af fixed-dose combinations identificeret for fixed-dose combinations godkendt af European Medicines Agency (58% af godkendelser). Det blev vist, at selvom der blev anvendt interessante strategier til at reprofilere lægemidler og anvende forudgående viden for at undgå dose-finding, var meget få fixed-dose combinations rettet mod personalisering eller kunne betragtes som innovative. Desuden blev vigtigheden af farmakokinetisk modellering ved valg af de korrekte doser understreget i studiet.

I den anden publikation, blev simulering af et klinisk studie udført for at vurdere muligheden for at udføre longitudinal exposure-response modellering af fixed-dose combinations. Exposure-response analyse af fixed-dose combinations har i et tidligere studie vist sig at forårsage en forhøjet falsk positiv rate. Forskningen i nærværende studie viste at longitudinal exposure-response var i stand til at analysere fixed-dose combinations uden den forhøjede falsk positive rate, der var set i det tidligere studie. Longitudinal exposure-response giver derfor en ny metode til at opfylde det regulatorisk krav om at demonstrere hver enkelt komponents bidrag til den samlede effekt.

I den tredje publikation blev kombinationsmodeller evalueret i en præklinisk brystkræftundersøgelse. En ny kombination blev analyseret for dens potentielle farmakodynamiske interaktion ved anvendelse af *the general pharmacodynamic interaction model*. Baseret på denne model blev kombinationen karakteriseret som synergistisk hvor den ene komponent øgede den andens styrke med op til 60%, når de blev administreret sammen. Derudover gav analyse af modellen indsigt i optimale dosisforhold, som kan bruges til at guide yderligere undersøgelser af kombinationen.

Denne ph.d. afhandlingen undersøgte den aktuelle tilstand af modelbaseret udvikling af fixed-dose combinations, udviklede en ny metode til evaluering af fixed-dose combinations og validerede eksisterende værktøjer til analyse af fixed-dose combinations. Baseret på den forskning, der er foretaget i denne afhandling, er den overordnede anbefaling, at modelleringsværktøjer i højere grad skal indarbejdes i udviklingen af fixed-dose combinations, da de hjælper med at bestemme effekt og give værdifulde oplysninger til at guide udviklingen.

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1 Aim & Objectives

Combination drug therapy is becoming a central part of the treatment strategies for many diseases where monotherapy so far has not given sufficient efficacy or where resistance to treatment emerges [1–3]. Thus, more drug combination products can be expected to be developed in the future. The standard practice for verification of efficacy for fixed-dose combinations is the factorial design study in which the individual components are compared to the combination across all investigated dose levels [4]. Given the trend towards personalized medicine, it is increasingly important to discover alternatives to the factorial design study [5]. The factorial clinical trial is costly, and it is challenging to perform this type of clinical trial when the number of investigated dose levels increase [5]. To ensure lower developmental costs and easier access to the market, it is important to avoid superfluous investigations by performing the correct and necessary clinical experiments from the beginning. To that end, *in silico* methods provide tools that can assist in parts of the development process and provided valuable information to guide the drug development, thereby saving time and resources [6–9]. However, the tools and models for analyzing fixed-dose combinations are only in their infancy.

Thus, the focus of this project and the overall aim is to evaluate, develop, and validate modeling tools for the development of fixed-dose combination products.

1.1 Objectives

The specific objectives of the project were:

1. To assess the current practice of the use of modeling as a drug development tool for the development of fixed-dose combinations in the European Union, based on the information available in European Public Assessment Reports (EPARs) published by the European Medicines Agency (EMA)
2. To develop new methods that may assist in the drug development process of fixed-dose combinations, thus providing novel methodologies, which may be considered by the relevant regulatory authorities when approving fixed-dose combinations
3. To validate combination models on preclinical data of a combination under investigation and assessing the value of applying these models in guiding further investigations of the combination

2 Introduction

In the present thesis, boundaries were established for the objectives to facilitate focused investigations. Overall, both combination therapies and fixed-dose combinations were considered part of the scope for the project, depending on the objective that was considered. Specifically, when concerning research questions that arose from legislation about drug development, only fixed-dose combinations were considered as part of the scope. This was decided as the development of fixed-dose combinations are addressed in a separate set of guidelines. Thus, for the first and second objectives of the project, only fixed-dose combinations are evaluated. For the third objective, no distinction was made between combination therapies and fixed-dose combinations. Both combination therapies and fixed-dose combinations were included, since the mathematical modeling of two pharmaceuticals in combination is, in general, applied in the same way, regardless of being administered as two separate components or as a fixed-dose combination [10].

Several markets could be selected to assess the first objective of the project. The European market in the form of submission to individual countries, the EMA, and the American market in the form of submissions to the Food and Drug Administration (FDA) were all considered. The EMA was chosen as the focal point of the discussion as the FDA has previously been the subject of several publications on the topic [11–13]. Five previously published studies on the analysis of fixed-dose combination approvals are summarized in Table 1. Furthermore, data from EMA is readily available to the public through the EPARs. Despite not being the aim for this objective, the FDA was used as a comparison for discussion.

Several disease areas could have been considered when analyzing fixed-dose combinations, the most prominent being cardiovascular, metabolic, and infectious diseases [5]. An even wider scope could have been considered when addressing combination therapy in its entirety. Cancer is a disease area in which combination therapy is paramount to treatment success [14]. Here, greater efficacy is in focus, but circumventing the emergence of resistance to treatment is equally or more essential [14].

For the second objective, the regulatory guidelines set forth by the EMA is used as a starting point, similarly to that of the first objective. Furthermore, a combination used for the treatment of diabetes is chosen as it represents a major group of fixed-dose combinations under development.

In the third and final objective, a combination for the treatment of cancer is analyzed, as these represent a complex and multifaceted combination in a field were combination treatment is of major importance.

The topics, which have been briefly introduced above, are presented and discussed in greater detail in the following sections.

Table 1 – Summary of five previously published studies on the analysis of fixed-dose combination approvals

Study	Aim	Main finding(s)
Fixed-Dose Combination Drug Approvals, Patents, and Market Exclusivities Compared to Single Active Ingredient Pharmaceuticals [11]. (2015).	To assess the exclusivity life of fixed-dose combinations and the time between approval of single drugs and the corresponding fixed-dose combination approved by the FDA.	Fixed-dose combinations were approved 5.43 years after the single drugs. They entered the market 2.33 years prior to generic single drugs. Lastly, they added 9.70 years to the exclusivity life.
Fixed-dose combination and single active ingredient drugs: A comparative cost analysis [12]. (2016).	To assess price differences and pricing structures of fixed-dose combinations and corresponding single drugs approved by the FDA.	The fixed-dose combinations were on average 83.3 ± 23.4% of the cost of the single drug, based on average wholesale price. This difference in price was correlated with the year of approval, the number of generics available of components in the combination, and the therapeutic class.
An Analysis of the Fixed-Dose Combinations Authorized by the European Union, 2009-2014 [15]. (2015).	To assess the effects of the negative connotations associated with fixed-dose combinations following the bans in the mid- to late 20th century as well as characterizing the reasoning for the Authorization by the EMA.	Stricter guidelines and regulations were found for fixed-dose combinations following the bans. Examples of regulatory flexibility was seen when given proper justification. The main reason for authorization was increased efficacy.
Analysis of Fixed-Dose Combination Products Approved by the US Food and Drug Administration, 2010-2015: Implications for Designing a Regulatory Shortcut to New Drug Application [13]. (2017).	To assess the development process of fixed-dose combinations approved by the FDA and guide the development of these products in the US.	Approval of the fixed-dose combinations was granted even if the full phases of clinical development was not completed. This highlighted a development strategy where phases can be exempted if proper justification is provided.
Investigation of Approval Trends and Benefits of New Fixed-Dose Combination Drugs in Japan [16]. (2019).	To assess the trends and benefits of fixed-dose combinations approved in Japan using a questionnaire survey	Cardiovascular agents were the largest therapeutic group. Compliance of bronchial asthma patients improved by 30.8% when taking fixed-dose combinations. Prescribers reported a decreased time and effort to prescribe the fixed-dose combinations.

The study title, aim, and the main finding(s) of the study are presented for each of the five previously published studies on the analysis of fixed-dose combination approvals.

2.1 Fixed-dose combinations

Fixed-dose drug combinations are a subset of combination therapies containing two or more active ingredients in a single dosage form. Combining two or more active ingredients in a single dosage form enables distinct advantages and challenges for the development and use of fixed-dose combinations. The following section outlines the current stance on the advantages, use, and development of fixed-dose combinations as well as the rationale for combination therapies.

2.1.1 Combination therapy

Combination therapies involve the concurrent treatment of diseases with two or more pharmacologically active compounds. The underlying principle for this approach is the ability to target several biological pathways simultaneously [1, 17]. Advances in high-throughput genome sequencing, loss- and gain-of-function methods, and several screening approaches have allowed increased ability to describe these underlying biological pathways in a healthy and disease state [18, 19]. This description leads to a greater understanding of the disease and the possible biological redundancies that are in place, as well as the potential for the development of resistance to treatment [17]. Additionally, the understanding of diseases as the product of a perturbed network of biological pathways has enabled the identification of molecular targets for combination therapies [20].

There exist many purposes for targeting multiple pathways simultaneously. Pharmacokinetic drug interactions are, in many cases, undesirable as they can alter the exposure of co-administered drugs and thereby cause adverse events and/or treatment failure [21]. However, some combination therapies are specifically developed to alter the pharmacokinetics of a compound. The combination of cobicistat and darunavir for the treatment of Human immunodeficiency virus (HIV) makes use of this principle. Cobicistat inhibits CYP3A4 and CYP2D6, which are the main metabolizers of darunavir, thereby increasing the exposure of darunavir [22].

Other combination therapies make explicit use of the components not interacting directly. In the treatment of the cancer subtype, diffuse large B-cell lymphoma, the use of a 5-component chemo-immunotherapy agent termed R-CHOP is the current standard of care [23]. The advantage of this combination arises from targeting separate pathways, thereby limiting the cross-resistance that can develop to the treatment [24].

Another approach to take advantage of targeting multiple pathways at the same time is by affecting a specific biomarker from multiple angles. Targeting multiple pathways is often the case for diabetes, where the regulation of blood glucose is central to controlling the disease. In combinations for treating diabetes, pathways that affect the distribution and excretion of glucose are often targeted. In the case of the linagliptin and empagliflozin combination, linagliptin causes lower blood glucose through increased blood concentration of incretins and empagliflozin inhibits reuptake of glucose in the kidneys, thereby increasing the excretion of glucose. By administering the compounds together, a greater efficacy is achieved through a synergistic pharmacodynamic effect [25].

Thus, the development of combination therapies have been subject to increased focus in recent years [2, 3] and has become an essential part of the successful treatment of several diseases [1].

Examples of the importance of combination therapy can be found as far back as the 1950s, where the combination of streptomycin and isoniazid led to greater cure rates of tuberculosis [26]. A more recent example is the extensive use of combination therapies for the treatment of HIV. The introduction of the Highly Active Antiviral Retrovirus Therapy (HAART) caused HIV to be considered a chronic illness rather than a death sentence. However, HAART also causes notable adverse events, and therefore, HAART highlights one of the limitations of combination therapy, namely toxicity [27]. Several other disease areas make use of combination therapies with notable areas, including cancer, cardiovascular disease, and metabolic diseases. An interesting perspective from cardiovascular diseases is the association with metabolic diseases. Due to this association, the combinations are often aimed at addressing the underlying issues for both the cardiovascular and metabolic disease, such as high blood cholesterol [28]. Cancer and metabolic diseases will be addressed in further detail in section 2.2.

Fixed-dose combinations utilize the same principles that make combination therapy essential. The class of compounds can be considered a subset of the overall combination therapy group, with fixed-dose combinations being different by being formulated as a single dosage form. This aspect provides both advantages and disadvantages, which are largely dependent on the disease area.

2.1.2 Fixed-dose combination background

Fixed-dose combinations exist in many different forms. They are present as products ranging from household ware to advanced medicines. One of the most common fixed-dose combinations are probably the multivitamin preparations. These contain upward of 20 active ingredients and represent a rather extreme example of fixed-dose combinations. WHO's list of essential medicines includes approximately 40 drug combinations of which most are available as fixed-dose combinations [29]. However, the WHO states that "not all of the fixed-dose combinations in the WHO treatment guidelines exist, and encourages their development and rigorous testing." [29].

Despite being common, the reputation of fixed-dose combinations suffered a setback in the 1950s [30]. This was brought about by combining diuretics with potassium chloride. The underlying reason for making this combination is that a side effect of diuretics is the removal of potassium in addition to the intended sodium removal. Thus, potassium tablets were often given in conjunction with the diuretic. However, when the fixed-dose combination of the two was administered, it resulted in several cases of punctured stomach lining, which required surgery [30]. Thus, fixed-dose combinations were subsequently associated with negative connotations [30].

More recently, a similar issue related to the reputation of fixed-dose combinations has occurred in India. Here, the development of fixed-dose combinations had been very popular, with a total of 1306 approvals in the period from 1961 till 2019 across all therapeutic areas [31]. The rationale behind a large amount of these approvals have been insufficient, as many combinations were developed either solely for marketing interests or were developed with neither theoretical justification nor evidence [32]. Over the last decade, this had led to 294 licenses being withdrawn, the use of 344 fixed-dose combinations being prohibited, and 328 bans being issued by the central government [31]. The issues in India underlines that while combinations can be a powerful tool;

ensuring the validity of the underlying rationale for combining pharmaceutical compounds is of utmost importance.

In other areas of the world with greater cognizance of the regulatory framework, such as the United States of America with the FDA and the European Union with the EMA, fixed-dose combinations have seen a rise in approvals [5, 11, 13, 16]. The reemergence of fixed-dose combinations and the revitalized focus on developing them could stem from an unmet medical need, as monotherapies provide insufficient efficacy in several disease areas. However, filling this unmet medical need is not exclusive to fixed-dose combinations, as all combination therapies can fill this gap. The following section addresses advantages, which are tied to fixed-dose combinations.

2.1.3 Advantages of fixed-dose combination

There are several areas where fixed-dose combinations show advantages over the free-equivalent combinations (FEC). FEC constitutes the same active ingredients as the fixed-dose combination, but in two or more dosage forms. One of the main clinical advantages is the increased adherence to treatment due to a lower medication burden, which has been demonstrated in numerous studies and across several disease areas [33, 34]. In an example from type 2 diabetes mellitus (T2DM), an analysis of seven studies was conducted, where the fixed-dose combinations were compared to the FEC. Here the fixed-dose combinations were associated with a 13% greater adherence [35]. Another example within T2DM assessed the HbA1c levels in 6000 European patients across multiple nationalities, taking either fixed-dose combination or the FEC [36]. Here the advantage of taking the fixed-dose combination manifested through a 0.25% lower level of HbA1c, thus providing better glycemic control than the FEC counterpart [36]. Another interesting point in the study was a comparison between patients classified as either compliant or non-compliant. Here, the compliant patients were found to be five times more likely to be taking the fixed-dose combination than the non-compliant patients. Similar results for increased adherence has been seen within the treatment of hypertension [34, 37] and cardiovascular disease [38].

So far, only the clinical benefits of fixed-dose combinations have been discussed. Another, less obvious advantage, which creates focus on fixed-dose combination development, is the extension of patents.

When inventing novel drugs, a patent ensures the protection and market exclusivity for the developing company for 20 years [39]. A substantial amount of this period can be spent in the developing phase, during testing and clinical trials. Following development, companies typically have between 7-12 years to make a profit on the investment, before generic drug companies enter the market [40]. Here, fixed-dose combinations can provide an avenue to extend the commercial lifespan of the compound by combining the newly patent expired compound with another non-patented or patented compound. However, this strategy is dependent on either the newly combined product being superior to existing treatment, thereby enabling the option for obtaining a patent for the combination, or convincing prescribers to switch to the combination, which would not currently have competition from generic medicines.

Several strategies exist for minimizing the commercial risk of developing fixed-dose combinations and providing a superior product. Combining patent expired product with the current standard of care treatment is a common approach. For instance, this is the basis for many fixed-dose combinations within diabetes care. Here metformin is combined with a range of different products, resulting in a convenience product consisting of the patent expired best-in-class drug and the standard treatment [5, 41]. Another strategy is the collaboration of competing companies to create new fixed-dose combinations using their patent expired stand-alone products. This approach has been shown to be particularly successful [42]. Examples of this include Atripla®, a three-component fixed-dose combination for the treatment of HIV. Competing companies Gilead and Bristol-Myers Squibb developed Atripla®, which in the first six months after launch, had achieved sales for over $200 million [42]. An example within hyperlipidemia is the joint development of Vytorin® by Merck and Schering–Plough, which combined ezetimibe and simvastatin to lower blood cholesterol concentrations [42].

Allowing the purchase of both the fixed-dose combination and the separate components seems to be an important consideration. This was showcased by the public backlash Pfizer faced in 2006 when they planned to discontinue torcetrapib and only sell it as part of their new fixed-dose combination Lipitor® [43].

Pursuing the option of convincing prescribers to switch to the new combined product will be largely dependent on cost. There are several cost-related advantages of fixed-dose combinations. Firstly, the cost of the fixed-dose combination is commonly comparable to or lower than the total cost of the components [33]. Secondly, the co-pay by the patients may be reduced, as only one co-pay is required for the fixed-dose combination in comparison to the several that may be required for the components separately [33]. An important consideration when considering cost is the different national reimbursement systems that exist within the EU [44]. It is usually local committees, which determine if a fixed-dose combination is eligible for reimbursement [44]. Thus, patients are not directly in control of whether they receive the FEC or fixed-dose combination as they are influenced by the decisions of the local health care systems. The various reimbursement systems are very complex [44], therefore, further discussion of the topic is considered outside the scope of this thesis.

It has been demonstrated within antihypertensive medication that patients on a fixed-dose combination had a lower prescription and total medical cost compared to the patients on the same medication but in FEC [45]. A broader analysis across multiple therapeutic areas found similar results, that is that the average wholesale price of fixed-dose combinations are on average lower than the total cost of the FEC [12]. Interestingly, this analysis highlighted that the difference in cost between the fixed-dose combination and the FEC is highly dependent on the therapeutic area. Furthermore, the study analyzed the price point selection of the fixed-dose combination and determined that it was most often set to the price of the costliest single agent in the combination. Selecting this price point was done in order to shift the demand from generics towards the fixed-dose combination [12].

It has been demonstrated, in some cases, that the immediate out-of-pocket costs of fixed-dose combinations may be higher than for their FEC, but that the total long term or intangible costs were lower [46, 47]. Furthermore, it has been shown that a fixed-dose combination can lead to fewer clinic visits and laboratory tests, which further reduces the overall cost of treatment [47]. However, the opposite pattern has also been observed in some cases. Here the total cost of fixed-dose combinations is initially lower, but once the generics enter the market, the FEC becomes the cheaper option [48]. An important consideration here is that an increase in cost has been shown to associate with lower adherence [49]. Thus, identifying the cheapest option for patients is important for ensuring adequate treatment.

Overall, analyzing the marketing strategy for fixed-dose combinations with regards to costs is multifaceted. Providing a better standard of care through combining the best in class products with the standard care in a fixed-dose combination and making it available at the same or lower cost than the FEC is a major selling point of fixed-dose combinations. However, it can be argued that the strategy of extending patents through the development of fixed-dose combination can lead to a greater degree of questionable marketing strategies. In particular, this could be the case if ensuring the extension of the product lifespan is the sole motivation behind development.

2.1.4 Development of Fixed-dose combinations

The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) is an association that works to harmonize regulatory policy across its 16 members and 32 observers [50]. Consequently, guidelines developed by agencies such as the EMA and FDA are highly influenced by the ICH guidelines. The development of fixed-dose combinations is subject to a separate set of guidelines from the conventional drug development guidelines [51, 52]. Currently, there is no ICH guideline specific to combination drugs, instead, the ICH monotherapy guidelines serve as a framework for the development of fixed-dose combinations [53].

Each of the major agencies, which govern drug development, has its own distinct set of guidelines on the development of fixed-dose combinations, which has been the topic of several publications [41, 54]. In the European Union, the EMA has compiled the guideline “Guideline on clinical development of fixed combination medicinal products”, which came into effect in 2017 [51]. In the United States of America, the FDA provides two guidance documents relating to fixed-dose combinations and combination therapy. For fixed-dose combinations, the document “Fixed Dose Combinations, Co-Packaged Drug Products, and Single-Entity Versions of Previously Approved Antiretrovirals for the Treatment of HIV” from 2006 and for combinations therapy the document “Codevelopment of Two or More New Investigational Drugs for Use in Combination” from 2013 [52, 55]. Lastly, the World Health Organization (WHO) has written a guideline for fixed-dose combinations “Guidelines for registration of fixed-dose combination medicinal products” from 2005 [56].

The overarching theme for all the guidelines on fixed-dose combination is summarized well in the EMA’s three overall requirements [51]:

1. Justification and rationale for the combination

2. Demonstrating the contribution of all active substances to the desired therapeutic effect
3. The evidence presented is relevant to the fixed combination medicinal product (important if the evidence is based on the administration of separate active substances in combination)

Another important consideration made by these agencies is based on the approval status of the components of the fixed-dose combination. Here four scenarios outlined in the WHO guideline provide a summary that matches the consideration made in all the guidelines [56]. The four scenarios of fixed-dose combinations that can be considered for approval are summarized here:

1. Generic fixed-dose combination of an already existing fixed-dose combination
2. Fixed-dose combination in the same dosage as two separate compounds that are administered as part of an existing treatment regimen
3. Fixed-dose combination of two compounds not previously combined or combined in new dosage regimen
4. Fixed-dose combination containing one or more new chemical entities

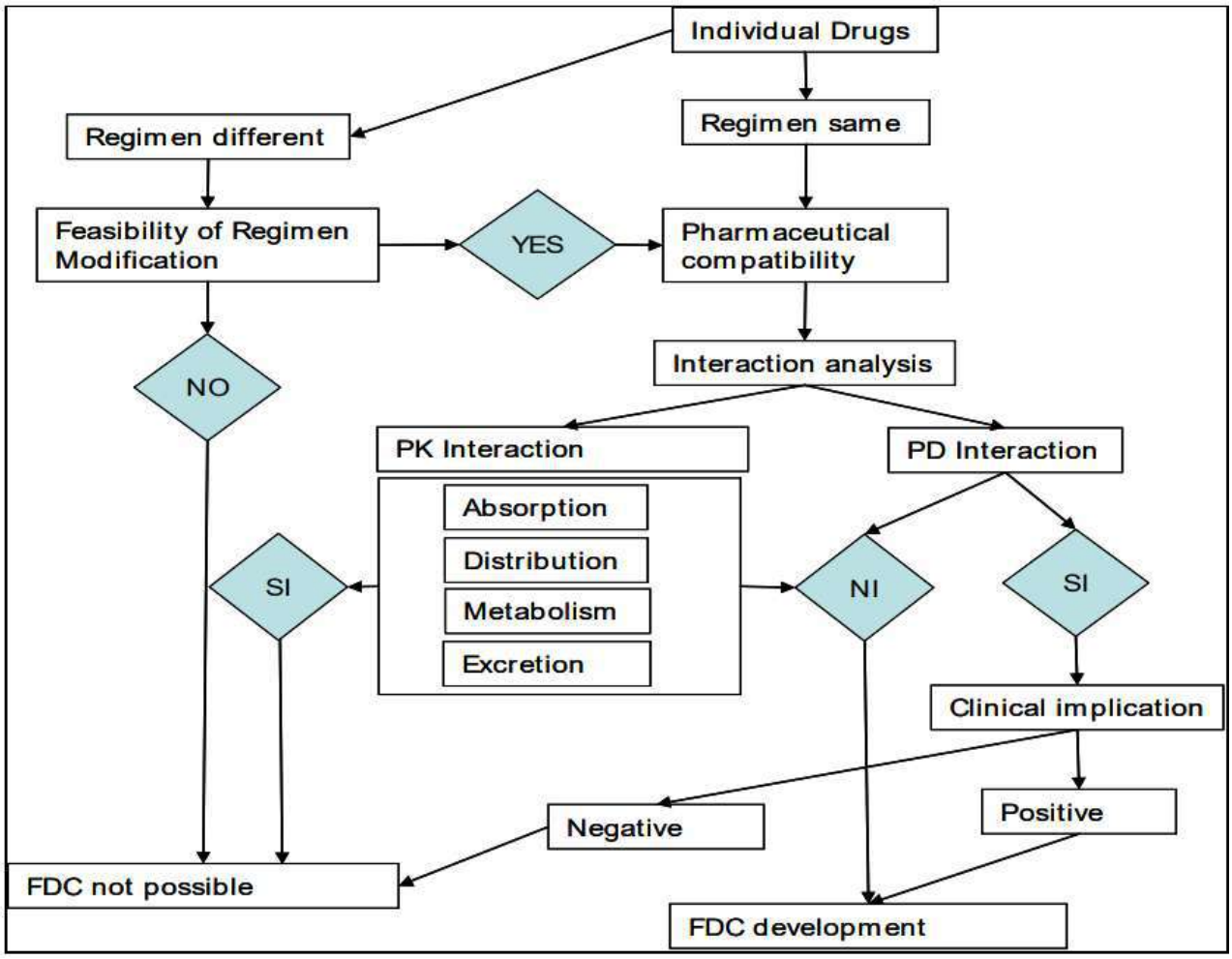

Figure 1 – Schematic to guide the feasibility of fixed-dose combination development with two or more approved compounds. FDC: Fixed-dose combination; NI: No Interaction; PD: Pharmacodynamic; PK: Pharmacokinetic; SI: Significant Interaction. The figure is from [57].

Justification for the combination can range from an improvement in benefit/risk to utilizing drug-drug interactions that, for instance, circumvent resistance development or cause non-efficacious compounds to become effective [51]. Examples of this are mentioned in section 2.1.1. Importantly, simplification of the treatment is not sufficient to constitute a rationale for the combination [51].

Based on each of the four scenarios, and the justification behind combining the components, the evidence base supporting the combination can be required to be appreciably larger or smaller. Specifically, it has been shown that the evidence base, measured as the number of patients, arms, and clinical trials, increases when one or more new molecular entities are included in the fixed-dose combination [5].

The simple scenario involves combining two already approved compounds in a fixed-dose combination. A schematic of a decision-making process for developing this type of fixed-dose combination is shown in Figure 1.

As seen in the schematic, for this type of fixed-dose combination, the most important aspect becomes to determine if drug-drug interactions occur in the pharmacokinetics (PK) or pharmacodynamics (PD) of the compounds. The purpose of investigating drug-drug interactions in PK is to determine if the LADME profiles of the compounds are altered in each other's presence [21, 58]. For fixed-dose combinations, the general expectation is that the presence of both compounds do not affect the PK profiles [57]. However, in some distinct cases, the purpose of the combination is to affect the PK profile of one compound, such as for cobicistat and darunavir discussed in section 2.1.1.

PD interactions are much more complicated to analyze. PD interactions can broadly be categorized into three categories.

- The first describes fixed-dose combination with no PD interactions, which would theoretically provide at higher efficacy than either of the components alone
- The second includes fixed-dose combinations with a negative interaction, which would result in terminating the development process.
- The third group constitutes fixed-dose combinations with a positive interaction, which is the most promising group of drug-drug combinations. This group is arguably the most innovative combinations, which utilize drug-drug interaction to achieve higher efficacy or safety.

A more complex scenario than combining two previously approved compounds is to develop one or more new molecular entities as part of a fixed-dose combination. Of the total two-component fixed-dose combination approved from 2010-2016 by EMA, 36% included one new molecular entity and 5.5% included two new molecular entities in the combination [5]. Ideally, the focus when developing this type of fixed-dose combination is to establish the safety and efficacy of the components separately before analyzing the combination. Thereby, the development follows a similar pattern to using approved compounds after the safety and efficacy are established for the monotherapies. There may however be cases where this is not feasible. This is the case when the components alone cause rapid resistance development in certain diseases such as HIV and microbial diseases [51, 55].

Demonstrating the contribution of each of the components to the overall effect is a central part of adhering to the requirements for fixed-dose combination development. This is the second overall requirement outlined earlier from the EMA and referred to in article §300.50 “Fixed-combination prescription drugs for humans” from the FDA, which is often termed “the combination rule” [51, 59]. The standard approach to achieve this is to perform a factorial clinical trial in which multiple doses of each component is compared to the combination, and the effect of A+B is demonstrated to be greater than that of either A or B [4]. This approach is obviously not feasible for the combined new molecular entities causing resistance when administered separately, and therefore, the approach is here to compare the combination to the standard of care [51, 55].

Other considerations for the development of fixed-dose combination include bioequivalence studies and the dosing intervals of the components. As part of the development package for fixed-dose combinations, it is expected to demonstrate the bioequivalence of the fixed-dose combination to each of the components [51, 52, 57]. For fixed-dose combinations composed of previously approved drugs, the bioequivalence studies can make up a substantial part of the development program, as other parts of the development program, such as dose-finding, can be excluded [5]. Achieving bioequivalence for a fixed-dose combination presents several challenges depending on the differences in dosage forms, formulations, and LADME profiles of the fixed-dose combination and the individual mono-components [60]. Depending on these differences it can be considered significantly more challenging to demonstrate bioequivalence between a fixed-dose combination and the individual mono-components than between two formulations of the same active ingredient [60]. While bioequivalence is important for fixed-dose combination development overall, the topic is not part of any studies conducted in the present thesis and is therefore not discussed in further detail. Dosing intervals present an issue when combining compounds with very different half-lives, as it often results in different frequency of administration. Thus, a modification of the regimen will be necessary for development to continue. Similarly, opposite administration conditions, such as fasting/fed condition, can cause the development to be unfeasible.

2.1.5 European Public Assessment Reports

EPARs are a public compilation of documents published by EMA, which pertain to the evaluation of drug authorizations through the centralized procedures at EMA [61]. The documents include information on “clinical aspects”, “clinical efficacy”, and “clinical safety”, as well as “Product information”, and “Authorized presentations”. While not specifically relating to fixed-dose combinations, the documents were used as the basis for the first paper discussed in the summary of results (section 3.1).

2.2 Combination therapy in diabetes & breast cancer

The present thesis is not limited to specific disease areas; however, during the experimental phase, two disease areas became the focus of the second and third papers. In this section, the two disease areas, diabetes and breast cancer, will be presented briefly in the context of combination therapy with a focus on the areas important to the research conducted in this thesis.

2.2.1 Type 2 diabetes mellitus

T2DM is a metabolic disease characterized by failure to regulate carbohydrate levels in the bloodstream [62]. T2DM constitutes more than 90% of all diabetes cases and is, therefore, the most common subtype [62]. The dysregulation of the carbohydrates arises from several underlying mechanisms, Figure 2. The understanding of these mechanisms is rapidly evolving. Currently, the understanding is that the main cause is a combination of insulin resistance in skeletal muscle, liver, and adipose tissue, coupled with increasingly impaired insulin secretion from the pancreas [63]. This leads to a condition known as prediabetes and predisposes for the development of T2DM, which is hallmarked by hyperglycemia [63, 64].

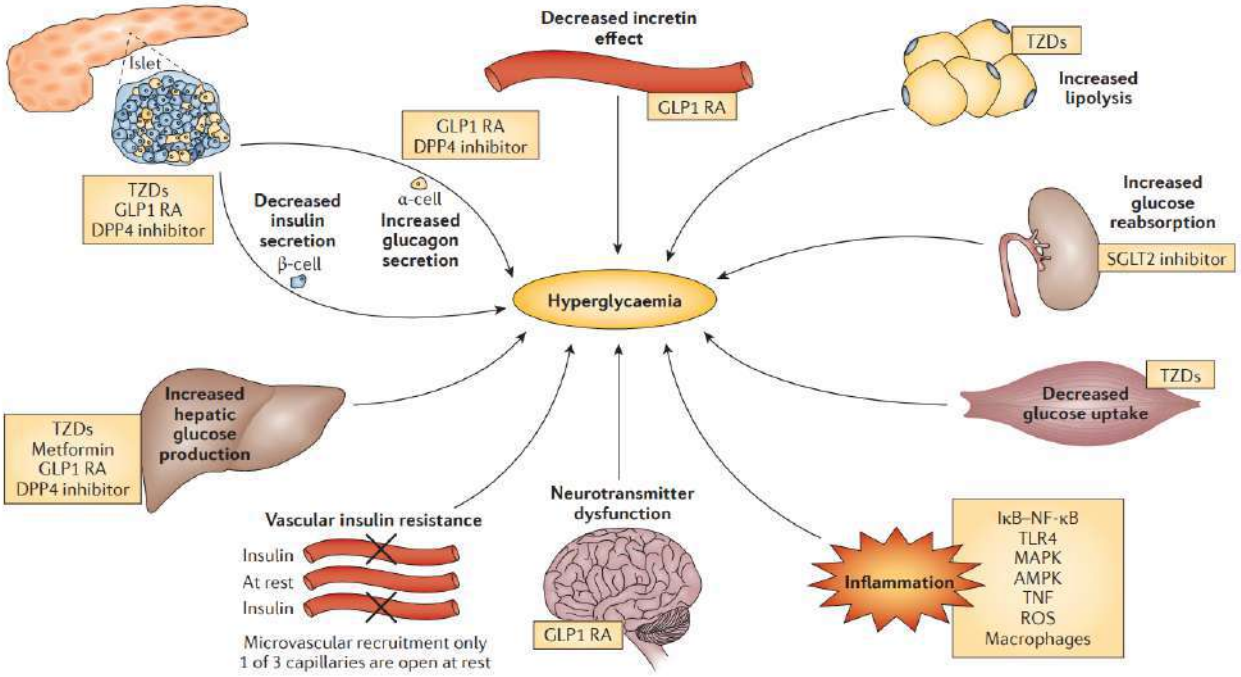

Figure 2 – The origins of hyperglycemia in type 2 diabetes mellitus. Hyperglycemia arises from multiple sources of dysregulated carbohydrate regulation. Each dysregulated function represents potential drug targets for reestablishing the normal carbohydrate levels in the bloodstream. SGLT2, sodium/glucose co-transporter 2; AMPK, AMP-activated protein kinase; DPP4, dipeptidyl peptidase 4; IκB, inhibitor of NF-κB; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor-κB; RA, receptor agonist; ROS, reactive oxygen species; TLR4, Toll-like receptor 4; TNF, tumor necrosis factor; TZDs, thiazolidinediones. The figure is from [62].

As the illustration indicates, the pathogenesis of T2DM is complex and arises from multiple metabolic defects [62, 63]. Hyperglycemia arising from these metabolic defects is known to be a large factor in the risk of complications from diabetes [65]. Therefore, reaching normal blood glucose levels, often measured as the biomarker HbA1c (signifying glycosylated haemoglobin in blood cells), through blood glucose-lowering treatment is incredibly important [66]. Achieving this

glycemic target is the main goal of T2DM treatment through GLP1 analogs, DPP4 inhibitors, SGLT2 inhibitors, thiazolidinediones, and metformin, Figure 2. However, there are issues with reaching the general goal of 7% HbA1c in many cases, as illustrated by a study in the U.S. from 2007-2010, where only 52.5% of individuals reached the target [67]. Hence, initiating therapy with only a single drug has been suggested not to be sufficient to ensure proper glycemic control [63]. Thus, targeting multiple parts of the pathogenic network is of interest, as it has been demonstrated to achieve increased glycemic control [66].

A counterpoint to combination treatment T2DM is the heterogeneity of the disease that arises from the complex pathogenic network. For instance, some patients (5-10%) cannot tolerate metformin treatment [68] and would, therefore, need alternative combination products to achieve their glycemic targets.

2.2.2 Triple-negative breast cancer

Triple-negative breast cancer (TNBC) constitutes 15-20% of all breast cancers and is associated with onset at an early age, aggressive clinical course, and a very poor prognosis in comparison to the hormone receptor and HER2 positive breast cancers [69]. TNBC has a unique pattern of metastasis to other organs compared to the two other subtypes [70]. The pattern is characterized by a larger degree of brain and lung involvement and a lower degree of bone lesions [70]. Subtyping cancers based on protein expression and phenotype is an ongoing process, which for TNBC has led to additional subtyping into categories based on molecular characteristics [71]. Further subtyping is, however, outside the scope of TNBC discussion for this thesis.

In the early stages of TNBC, the cancer cells are generally chemotherapy-sensitive; however, there is no defined optimal treatment strategy [72]. The most common initial step for more advanced or inoperable TNBC is neoadjuvant therapy, which includes anthracyclines, taxanes (such as docetaxel), and cyclophosphamide [72, 73]. Given the severity of the disease, there is a constant effort to identify and utilize new therapies for TNBC patients [69].

As seen from the neoadjuvant therapy, combinations of two or more compounds that target specific pathways in the cancer cell is a cornerstone in cancer therapy [74].

Drug resistance is a multifaceted phenomenon that spans inherent cell heterogeneity, alterations in drug targets, drug efflux, and morphological changes in the form of epithelial-to-mesenchymal transition, Figure 3. Due to the wide range of resistance mechanisms, combination therapy is considered as the best treatment option for all cancer. This is due to a lower risk of drug resistance, and because the combined therapies will generally be more effective than the monotherapies [74]. A general goal of combination therapy in cancer is to utilize drugs with different primary mechanisms, leading to low shared cross-resistance between the compounds [24].

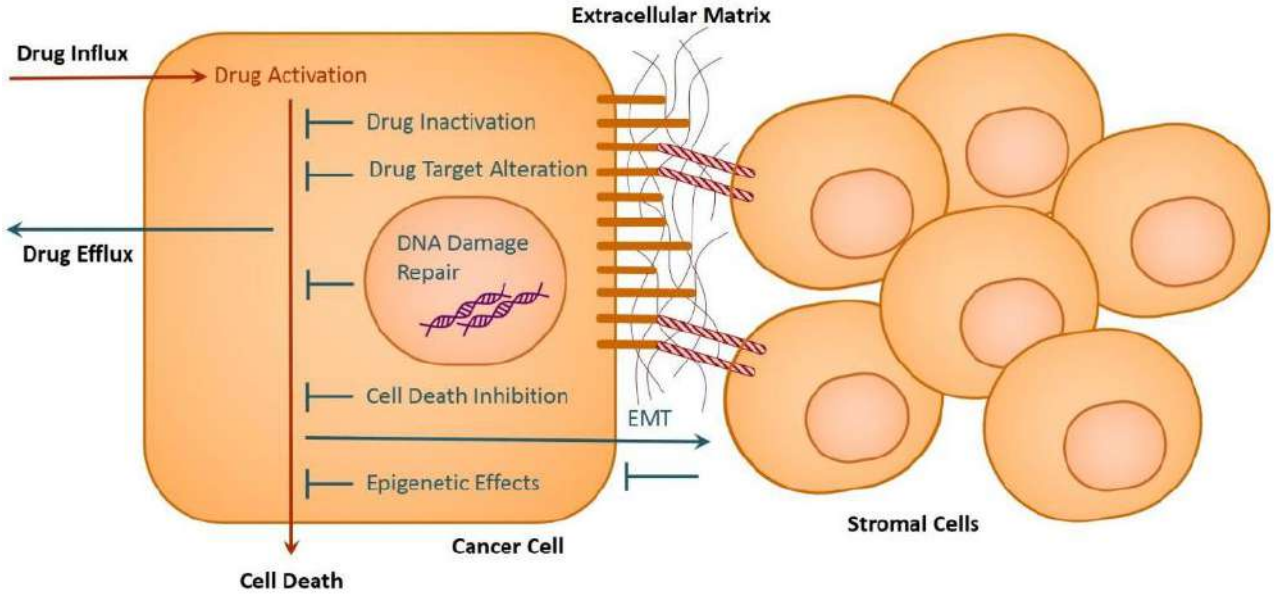

Figure 3 – Primary mechanisms leading to drug resistance of the cancer cells. The primary mechanisms include inherent cell heterogeneity, alterations in drug targets, drug efflux, and morphological changes in the form of epithelial-to-mesenchymal transition. EMT, epithelial to mesenchymal transition. The figure is from [74].

Precision medicine further adds to the complexity of TNBC treatment. Precision medicine aims to provide a tailored treatment to patients through the identification of not only general genetic variants but also specific pathway and network alteration, which can govern highly specific treatments of the individual cancer patient [75].

Based on the complexity and attempt of individualization of cancer treatment, it is no surprise that 0% of approved fixed-dose combinations are within the field of cancer [5, 76]. Fixing the dosage or ratio between components is not feasible for combinations with the need for such a high degree of individualization. However, for the purposes of analyzing and modeling drug-drug combinations, it presents an interesting topic.

2.3 Pharmacokinetic-Pharmacodynamic Modelling & Simulation

Pharmacokinetic-Pharmacodynamic (PK-PD) modeling and simulation were a part of the second and third paper in the present thesis. While standard model building in the form of sequential structural, stochastic, and covariate modeling was not performed, the same principles were applied for simulation and are therefore presented. Finally, analysis and modeling of data was performed using exposure-response modeling and combination modeling, which is discussed in the following sections.

2.3.1 Background

In essence, models provide a simplified and interpretable representation of systems that are of interest to the modeler [77]. In PK-PD modeling, the system that the modeler attempts to describe is the timecourse of drug concentration following administration (PK) as well as the link between that concentration and the effect of the drug (PD) [77].

The value of modeling is substantial in all phases of drug development. In the preclinical phase, the focus is on determining potential drug candidates. Here modeling provides a way to characterize the potency, bioavailability, clearance, toxicity, and drug interactions [78]. In addition, it can be used to guide optimal sampling and dose ranges for further investigation [78]. Establishing a model early in development is essential for successfully utilizing modeling to guide drug development. The importance of establishing the model early on is due to the ability to constantly update the model through an iterative process as data become available [79].

Throughout the clinical development, spanning phase I to III, the scope of modeling expands. In the early phases, there is a focus on accurately describing the PK relationships and estimating the probability of success given assumptions and trial designs [78]. In the later phases, confirmation of a valid PK-PD model and establishing the exposure-response relationship is in focus [78]. Importantly, these are all just examples of the goals that can be achieved using modeling but is not exhaustive.

Within the regulatory agencies EMA and FDA, modeling and simulation are considered an important tool to support rational decision making throughout the development process. The EMA has released guidelines for physiologically based pharmacokinetic modeling and simulation as well as appointed a working party, which focuses on outlining the standards for employing modeling and simulation in drug development [6, 7]. Similarly, the FDA has published guidelines on pharmacokinetic modeling (currently in draft version in 2019) [8]. Additionally, the FDA has presented a talk on the topic termed “Advancing regulatory science with modeling and simulation at FDA”, in which they recognize how modeling and simulation can benefit public health [9].

2.3.2 Population PK-PD modeling

Population PK-PD models consist of three overall components: structural models, stochastic models, and covariate models.

The purpose of the structural model is to describe the timecourse of the PK or PK-PD relationship. This description is achieved either through algebraic or differential equations [77]. In the case of PK

models, the structural model is often described by a compartmental model. A compartmental model typically consists of one to three compartments, with additional absorption compartments for extravascular administration. An example of the differential equation approach of a one-compartment model with first-order absorption and elimination is shown in EQ1.

$$\frac{dA_1}{dt} = -k_A \cdot A_1 \qquad \text{(EQ1)}$$
$$\frac{dA_2}{dt} = k_A \cdot A_1 - \frac{CL}{V} \cdot A_2$$

The differential equation describes the rate of change in drug amount for the absorption "compartment" (1) and the central compartment (2). The equation is solved in very small time increments in order to minimize the computational errors. The estimated population parameters, here absorption rate constant k_A, clearance (CL), and volume of distribution (V), from the structural model, are often termed thetas (θ). Through this approach and developments within algorithms, the difference between the algebraic solution and the differential equation is negligible but can come with a time cost depending on the complexity of the model [77].

The PK is linked to the PD in a specified relationship, and through this relationship, the timecourse of the drug response is described [80]. This relationship can either be direct or delayed depending on the specification. For the direct PK-PD relationships, the plasma concentration is used directly in the description of the drug response, with relationships often being linear, log-linear, or non-linear (e.g., E_{max} relationship and sigmoidal E_{max} relationship), EQ2 [80]. The parameters in the models are: α, the intercept with the y-axis; β, the slope; C_{drug}, the drug concentration; E_{max}, the maximal effect; EC_{50}, the half-maximal concentration, and H_{drug}, the Hill coefficient.

$$E_{drug} = \beta \cdot C_{drug} + \alpha; \quad E_{drug} = \beta \cdot \log(C_{drug}) + \alpha; \qquad \text{(EQ2)}$$
$$E_{drug} = \frac{E_{max,drug} \cdot C_{drug}}{EC_{50,drug} + C_{drug}} \; ; \; E_{drug} = \frac{E_{max,drug} \cdot C_{drug}^{H_{drug}}}{EC_{50,drug}^{H_{drug}} + C_{drug}^{H_{drug}}}$$

For the delayed PK-PD relationships, the effect compartment model and the indirect response model (turnover model) are well established [80, 81].

The effect compartment model is used when the site of drug action is different from the site of measured drug concentration [80, 81]. For instance, this can be the case if the site of drug action is in the brain. Thus, a delay in effect occurs, which is described by the first-order rate constant k_{e0} between the observed concentration and a hypothetical effect compartment [80, 81].

The indirect response attempts to capture the delay in effect that arises from targeting upstream components of a signaling cascade. In this model, the effect is asserted through stimulation or inhibition of the formation constant k_{in} or the elimination rate constant k_{out} of the response (endpoint) [80, 81]. An example where k_{in} is inhibited is shown in Figure 4. This model is used in the simulations for the second paper of this thesis.

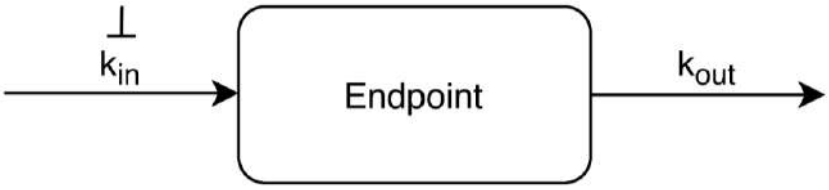

Figure 4 – Overview of indirect response model with inhibition of k_{in}. k_{in}, formation constant of endpoint (response variable), k_{out}, elimination rate constant of endpoint (response variable).

The stochastic model attempts to capture the variability in the estimated parameters by adding random effects to the model. Random effects can span numerous factors, such as inter-individual variability, inter-study variability, and inter-occasion variability. The random effects are termed etas (η), which are usually assumed to be distributed normally or log-normally around zero with a variance (ω).

For simplicity, the following example includes a theoretical parameter P, which varies log-normally between individuals, resulting in the individual parameter P_i, EQ3.

$$P_i = P \cdot e^{\eta_{Pi}}, \qquad \eta_{Pi} \sim N(0, \omega_P^2) \tag{EQ3}$$

Note that while the value of η_{Pi} is normally distributed, applying it exponentially to P produces the log-normal distribution.

While the random effects represent the random unexplained variability, the covariate model attempts to explain the variability. Explaining the variability can be achieved through established relationships, such as that between body weight and volume of distribution or more specific considerations such as genotypes [77]. Covariate modeling is not a focus in this thesis; therefore, the topic is not introduced further.

Determining the optimal model fit is based on model evaluation. Model evaluation can be based on numerous different numerical and graphical approaches. Some of the common numerical considerations are the Akaike information criterion (AIC), the Bayesian information criterion (BIC), and objective function value (OFV) [82, 83]. These values are used for model comparisons in various scenarios and represent how well the model fits the data. Parameter precision, in the form of standard errors, confidence intervals, bias, and shrinkage, are important for assessing model stability and robustness [82, 83]. For instance, high parameter imprecision can be a sign of an overparameterized model [82]. A general consideration for high imprecision is >30% SE for fixed effect and >50% SE for random effects [82]. Graphical diagnostics of the model are employed to identify model misspecification [83]. Examples of graphical diagnostics include conditional weighted residuals (CWRES) against independent variables (e.g., time, concentrations), observed versus population/individual predictions, and simulation-based visual predictive checks (VPC) [82, 83].

Simulation from a PK-PD model is an important tool used for inference and model evaluation. Simulating unobserved data within the bounds of the data for the model can be done with some confidence, whereas simulating data outside the bounds requires confidence in the model used for simulation [77]. Simulating from a model that includes a stochastic model to allow for unexplained variability in the data requires the use of random number generators. An important consideration

here is that when sampling from random distributions, the simulations need to be repeated [77]. In order to summarize the results as confidence intervals, 1000 simulations are usually recommended [77].

2.3.3 Exposure-response analysis

Exposure-response analysis attempts to describe a relationship between an exposure metric and a measured response variable. Several exposure metrics can be considered for this purpose, once the steady-state of the PK is reached. Common metrics include the area under the concentration-time curve (AUC), maximal drug concentration (C_{max}), or the trough concentration, depending on the specific analysis [84]. The response variable is often a clinical endpoint, which is coupled to the exposure metric through linear or non-linear relationships presented in EQ2. Additionally, the exposure-response analysis can study either a single time point or be a timecourse analysis [84]. The single time point is often sufficient and a more common approach. However, timecourse analysis has advantages when inter-occasion variability is high, when the endpoint changes over time at steady-state PK, or if the data includes a high degree of dropouts [84].

Exposure-response analysis has an advantage compared to pairwise comparisons due to the intrinsic variability in the PK that gives rise to a series of exposures for a given dose level and sample size [85]. This variability in exposures results in greater power to identify the exposure-response relationship. However, the variability in the exposures also constitutes a limitation of the analysis, as there is a lack of randomization of the exposures, whereas the dose used in the pairwise comparison is a controlled and randomized variable [85]. Another limitation is the susceptibility to undetected confounders in the model [84]. Confounders in exposure-response analysis is the implicit topic of the second paper in this thesis. Essentially the paper is based on the finding by Zhu & Wang, which demonstrates that exposure-response analyses of fixed-dose combinations lead to inflated false-positive rates [86]. Despite these limitations, exposure-response analysis is becoming a tool with an increasing role in regulatory decisions [87–90].

2.3.4 Combination modeling

Combination modeling is performed to demonstrate that the effect of the combination is superior to the effect of the components [20]. This is typically achieved through characterizing the combination as either synergistic or antagonistic, corresponding to either higher or lower effect, respectively, than the expected additive effect. Defining the additivity criterion can be the most complex part of classifying a combination as synergistic or antagonistic, as opposing results can be obtained depending on the criterion selected [20]. An example of these opposing results is illustrated in Figure 5, where a synergistic combination within Bliss Independence can be considered antagonistic in Response Additivity. This is evident from the combination being below the additivity criterion (dashed line) for Response Additivity and above it for Bliss Independence, Figure 5.

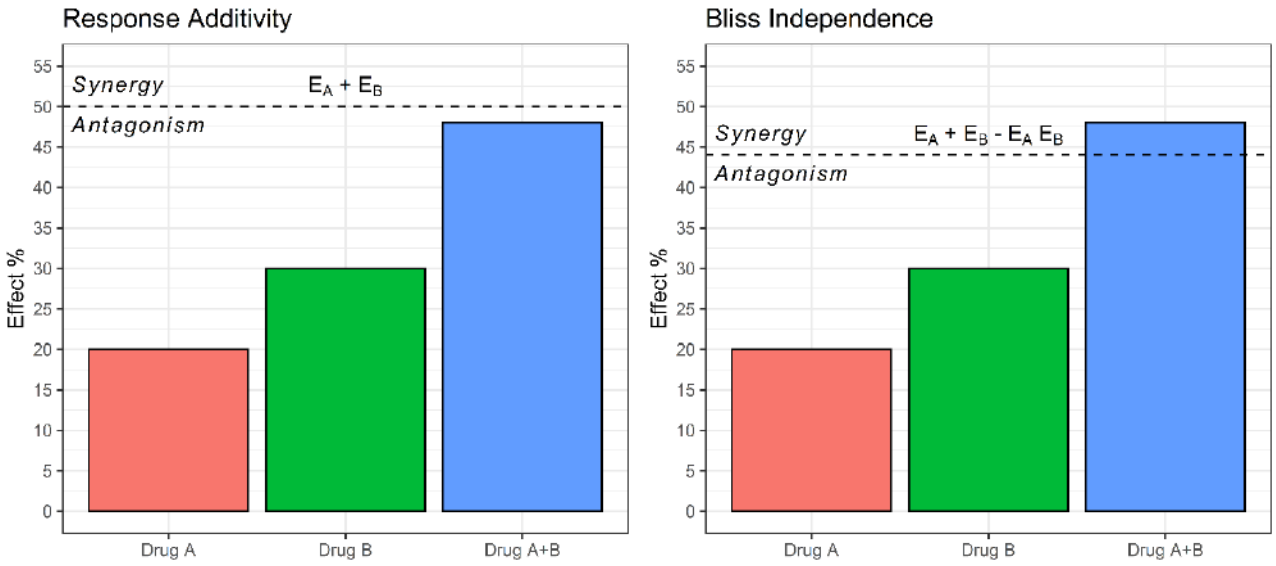

Figure 5 – Overview of two effect-based approaches: Response Additivity (left) and Bliss Independence (right). Drug A effect: 30, Drug B effect: 20, Drug A+B effect: 48.

Response Additivity, Bliss Independence, and Loewe Additivity are three common effect-based approaches for defining the additivity criterion [20, 91–93]. Loewe additivity and Bliss Independence is based on opposing mechanistic assumptions surrounding the compounds in the combination [94]. Loewe Additivity assumes that both compounds share the mechanism of action, thereby making the assumption that one compound can be substituted with the other. For Bliss Independence, the assumption is that the compounds have different mechanisms of action, leading to the addition of the component effects. Response Additivty rests on the principle that the effects of the combination are simply additive and that the dose-effect relationship is linear [20]. While Loewe Additivity is a common approach in combination modeling, it is not utilized in the present thesis, as the investigated combination components do not share the mechanism of action. Loewe Additivity is therefore not considered further. The mathematical equations for a combination of drug A and B using Response Additivity and Bliss Independence is shown in EQ4 and EQ5, respectively.

$$E_{additive,RA} = E_A + E_B \quad \text{(EQ4)}$$

$$E_{additive,BI} = E_A + E_B - E_A \cdot E_B \quad \text{(EQ5)}$$

As given by EQ4, the additivity criterion for Response Additivity is the addition of the effect terms for compound A and B, while any observed effect above or below that level corresponds to synergy or antagonism, respectively [20]. For Bliss Independence, the additivity criterion is the product of the effect term for compound A and B, subtracted from the sum of the two. Furthermore, the effect terms are based on probabilities and are therefore constrained between 0 and 1. Thus, as the effect of the components increase, the $E_{additive,BI}$ in EQ5 will approach a combined effect of 1 [20, 92]. While underlying mechanistic assumptions are made for Bliss Independence, it is important to note that there is no mechanistic basis for the model itself and therefore, it is solely an effect-based approach.

Preclinical modeling of cancer chemotherapeutics has previously been performed [95–98]. While the studies had different approaches to the modeling, they were all based on differential equations describing tumor growth. This approach has the advantage of being semi-physiological in nature, as it attempts to describe the growth cycle of the tumor. However, the drawback of this approach is that the drug effect is difficult to interpret as it is often described through kill rates. Furthermore, the models can be parameter intensive, thereby requiring a large amount of data.

The general pharmacodynamic interaction (GPDI) model, is a relatively new model, which is considered in the third paper of the thesis. The model uses a semi-mechanistic approach and can be combined with both Response Additivity and Bliss Independence [99]. The GPDI model identifies victim and perpetrator drugs in one- or two-way interactions in the potency and/or maximal effect of the compounds. An example of a one-way interaction in the potency where drug A is the victim and drug B is the perpetrator is shown in EQ6. The parameters in the model are: $E_{max,A}$ maximal effect of A, C_A concentration of A, $EC_{50,A}$ half-maximal concentration of A, $INT_{max,B\rightarrow A}$ maximal interaction of B on A, $INT_{50,B\rightarrow A}$ half-maximal interaction concentration, C_B concentration of B.

$$E_A = \frac{E_{max,A} \cdot C_A}{EC_{50,A} \cdot (1 + \frac{INT_{max,B\rightarrow A} \cdot C_B}{INT_{50,B\rightarrow A} + C_B}) + C_A} \quad \text{(EQ6)}$$

The advantage of the GPDI approach is that the estimated interaction parameters provide a way to quantify the interactions between the compounds. Identifying interaction in either the maximal effect or potency leads to greater interpretability of the interaction, which gives a semi-mechanistic understanding of the drug-drug interaction. Furthermore, identification of perpetrator and victim drugs reveals the direction of the interaction, which enables the characterization of drug-drug interaction networks for identifying promising drug combinations.

3 Summary of results

In this section the three papers that were produced as part of this PhD project are summarised and discussed.

Papers:

1. **Nøhr-Nielsen A**, De Bruin ML, Thomsen M, Pipper CB, Lange T, Bjerrum OJ, Lund TM. Body of evidence and approaches applied in the clinical development program of fixed-dose combinations in the European Union from 2010-2016. Br J Clin Pharmacol. 2019;(July 2018):1–12.
2. **Nøhr-Nielsen A**, Lange T, Forman JL, Papathanasiou T, Foster DJR, Upton RN, Bjerrum OJ, Lund TM. Demonstrating Contribution of Components of Fixed-Dose Drug Combinations Through Longitudinal Exposure-Response Analysis. AAPS J 2020 222 22:1–14.
3. **Nøhr-Nielsen A,** Bagger SO, Brünner N, Stenvang J, Lund TM. Pharmacodynamic modelling reveals synergistic interaction between docetaxel and SCO-101 in a docetaxel-resistant triple negative breast cancer cell line. Eur J Pharm Sci 2020 105315.

3.1 Paper 1 - Body of evidence and approaches applied in the clinical development program of fixed-dose combinations in the European Union from 2010-2016

This study was an analysis of the current state of the clinical development programs of fixed-dose combinations. Throughout this summary, the terminology from paper 1 will be used to ensure clarity. The study focused on data from the European Union, specifically the fixed-dose combinations approved through the central procedure at the EMA. The information regarding the approved fixed-dose combinations was obtained from the EPARs. Following a range of selection criteria, outlined in Figure 6, the final pool of fixed-dose combinations included in the analysis was 36. These 36 EPARs included a total of 239 clinical trials and 157.514 patients in their clinical development programs. The purpose of the study was to characterize the approved fixed-dose combinations, identify the strategies employed to gain approval, and assess the volume of evidence in the submissions. An overview of the reviewed EPARs is located in Appendix S2 for paper 1 [5].

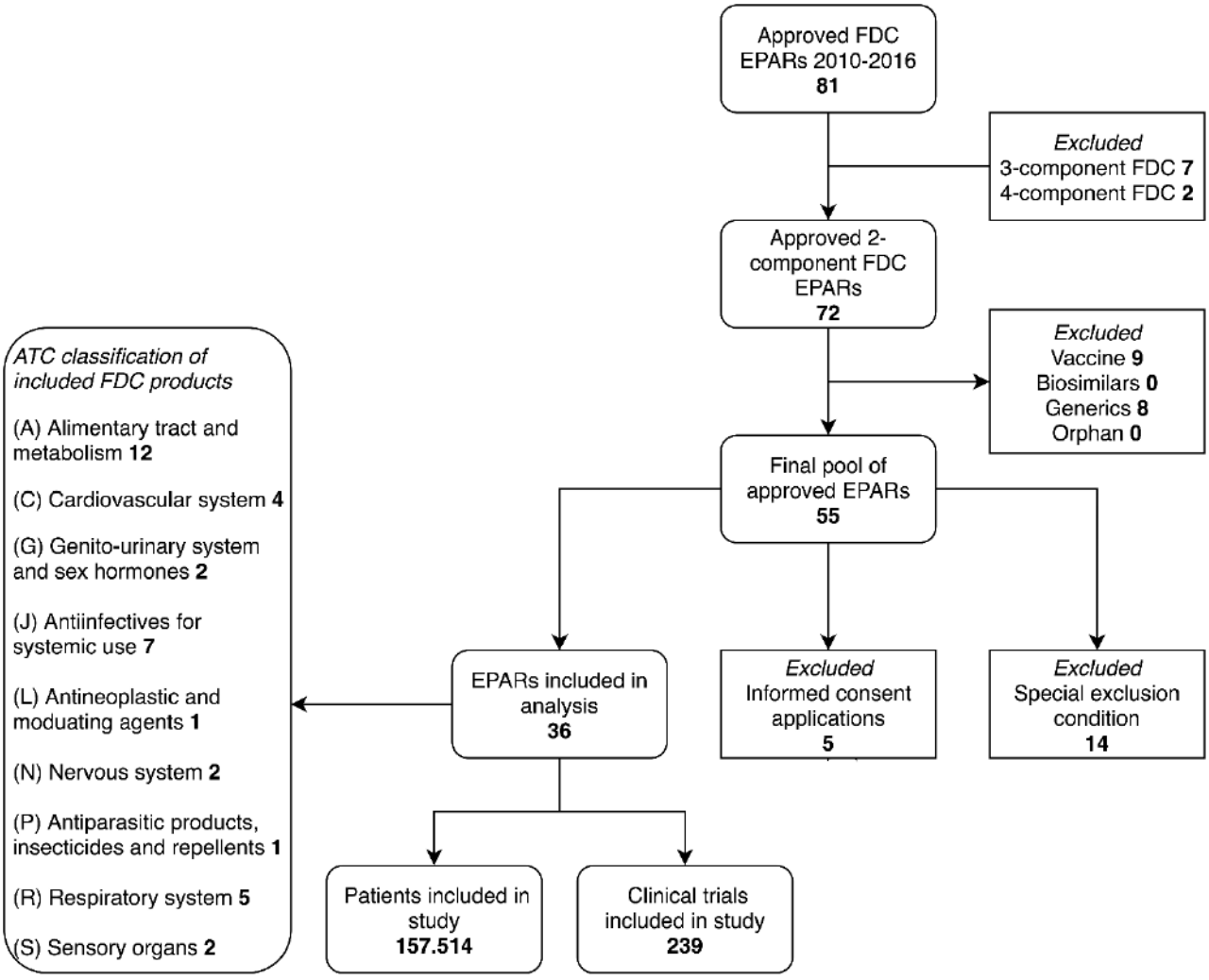

Figure 6 – Flowchart showing the identification of excluded and included European Public Assessment Reports (EPARs) in paper 1. Distribution of the included EPARs in the anatomical therapeutic chemical (ATC) classification system is shown to the left. The condition for "special exclusion condition" are outlined in Appendix S1 of paper 1 [5]*. The figure and caption are from* [5]*.*

3.1.1 Characterization of fixed-dose combinations

The first metric analyzed in the study is the anatomical therapeutic chemical (ATC) codes. This information was extracted from the "Product information and Authorized presentations" documents. Based on the classification, three major groups of fixed-dose combinations were identified, metabolic diseases, anti-infectives, and respiratory diseases. This finding corresponds well with a similar result observed for the FDA, where anti-infectives, metabolic diseases, and cardiovascular diseases were the therapeutic areas of greatest interest [100]. Reprofiling of drugs was assessed by comparing the ATC codes of components with the ATC code of the parent compound. Sharing 0 or 1 level of the ATC code was considered as the drug being reprofiled. A small group (6%) of fixed-dose combinations was composed of reprofiled drugs. Hence, the predominant strategy within the development was to improve treatment within the existing therapeutic area. While small, the group of reprofiled drugs represent an important development path, as it has been shown that the success rate is higher and expenses lower for reprofiled drugs [101].

From the same documents as ATC codes, the number of approved dose levels for each fixed-dose combination was extracted. The largest group (58%) of fixed-dose combinations had one approved dose level, while the remaining fixed-dose combinations were distributed on two, three, and four dose levels. This data was reviewed in the light of the development of personalized medicines, which aim to provide treatment tailored to the individual disease [102]. The initial result is that the inherent limitation of drugs with a fixed drug dose ratio makes fixed-dose combinations and personalized medicines incompatible. However, for the group of fixed-dose combinations with two or more dose levels, some level of personalization can be achieved. This flexibility makes the group particularly important, as personalized medicines are hailed as a transformation of drug development and clinical use [102, 103].

The substance status assessed the approval state of the components in each fixed-dose combination. Substance status of the fixed-dose combinations was classified as either two-approved drugs (AD+AD), one approved and one new molecular entity (AD+NME), or two new molecular entities (NME+NME). The majority (58%) of the fixed-dose combinations were composed of two approved drugs. Consequently, 42% of the fixed-dose combinations contained a new molecular entity. Comparing this distribution to the FDA, where only 25.4% contained a new molecular entity [100], it seems that there is slightly more focus on including new molecular entities in fixed-dose combinations in the European Union.

Furthermore, substance status was analyzed for its influence on the body of evidence and the employed strategies, which is summarized in the following sections.

3.1.2 Body of evidence

The body of evidence was assessed with regards to the number of clinical trials, arms, patients, and dose levels studied included in the submissions. Furthermore, the data were analyzed with a generalized linear model to elucidate the influence of factors such as substance status on the body of evidence.

The data showed that significantly more clinical trials are performed, and significantly more patients are included as part of phase 3 trials than phase 2 trials. For substance status, it was evident that, in general, there was an increased amount of evidence as part of the submission as one or more new molecular entities were included in the fixed-dose combination. This increase was the case for clinical trials, arms, and patients, but interestingly, not for the number of dose levels studied.

The use of PK-PD modeling and the choice of trial design was also considered in relation to the body of evidence. These factors, as employed strategies, will be discussed further in section 3.1.3.

The use of modeling was extracted from the submission and categorized into three categories: No modeling, PK modeling, or PK-PD modeling. Neither the use of PK modeling or PK-PD modeling was found to have had a significant effect on the number of clinical trials, arms, nor patients included in the submission. However, the use of PK modeling did result in a significant reduction in the number of dose levels studied. Based on the result, the study found that understanding the pharmacokinetics of potential drug candidates is essential in selecting the right dose. The lack of influence on the remaining body of evidence is not in accordance with previously published studies [79, 104–106]. This deviation from the literature is thought to arise from the lack of details in the use of modeling in the EPARs. Essentially the modeling was analyzed in a yes/no manner that does not capture the purposes behind the use of modeling, which in turn might not correspond to the purposes investigated in this study.

The choice of clinical trial design in the dose findings or main pivotal trial was categorized as either:

- Factorial design: Two or more combinations (different ratio)
- Ray design: Two or more combinations (same ratio)
- Single combination: One combination tested

The influence of clinical trial choice on the body of evidence was significant for the number of patients and arms. Here, there were significantly more patients and arms when using a *factorial design* compared to *ray design*. Naturally, for the number of doses tested, there was significantly fewer for *single combination* compared to *factorial design*.

3.1.3 Employed strategies in development

Whether dose-finding studies were performed during fixed-dose combination development was found to be affected by the substance status of the fixed-dose combination. For 57% of the fixed-dose combinations consisting of two-approved drugs, no dose-finding study was performed. This approach is described in the relevant guideline for the analyzed period (2009 guidelines) from the EMA [107], and the present study supports that this approach is possible in practice. Additionally, the current 2017 guidelines from the EMA includes a section describing the evidence base, supporting the same approach [51]. A review of the FDA found a similar result, where approval was granted for fixed-dose combinations without completing the full phases of clinical trials [100]. Utilizing the existing dose levels does, however, have a drawback, as the exploration of the drug-drug interaction space could have resulted in more optimal dose levels or ratios.

Clinical trial design was evaluated to assess the strategy for fulfilling the requirement of demonstrating the contribution of components to the overall effect. Approximately half (47%) of the approved fixed-dose combinations had only one dose studied. Furthermore, considering that *Single combination* can be considered a subset of *Factorial design,* the vast majority made use of a factorial design (47+44%). Notably, the 2009 EMA guidelines suggest the use of multilevel factorial design, thus, distinguishing between the *Single combination* and *Factorial design* groups [107]. Finally, few sponsors made use of the *ray design* (8%).

Achieving a personalized treatment with a fixed-dose combination requires several approved combination doses to be available for patients. Consequently, obtaining approval for these combination doses leads to very large factorial design studies. Thus, the extensive use of the factorial design within fixed-dose combination development presents a hurdle for personalization of treatment with fixed-dose combinations. Alternative approaches to the factorial design study may be found in exposure-response modeling or model-based adaptive optimal design, which could reduce the need for patients [86, 104]. However, exposure-response analysis of fixed-dose combinations has been shown to have an inflated false-positive rate, which makes the approach unfeasible [86]. Conversely, longitudinal exposure-response analysis has been shown to provide a modeling approach that can reduce the need for patients while producing reliable results [105].

In the study, it was shown that 42% of the clinical development programs included no modeling, 36% performed PK modeling, and 22% performed PK-PD modeling. Employing PK-PD modeling can utilize the information from early clinical trials to assist in dose selection and provide insights for expected effect sizes [108]. Furthermore, characterization of the PK profile with covariates, such as weight and age, is essential, as it can enable extrapolation to special populations [109]. Therefore, it was surprising that only about half (58%) of the development programs utilized either PK or PK-PD modeling as a development strategy.

3.1.4 Conclusions

In conclusion, the study emphasizes that interesting approaches are being employed in fixed-dose combination development, utilizing prior knowledge, not performing dose-finding trials, and reprofiling of drugs. Personalization of treatment with fixed-dose combinations could be a promising approach to ensure continued increase in the development of fixed-dose combinations, however, the extensive use of the factorial design study presents a hurdle for this approach. Lastly, given the advantages of performing PK-PD modeling and the low use of modeling in the development of fixed-dose combinations shown in this study, developers of fixed-dose combinations should to a greater extent consider incorporating the use of modeling in the development process.

3.2 Paper 2 - Demonstrating Contribution of Components of Fixed-Dose Drug Combinations Through Longitudinal Exposure-Response Analysis

In this study, the focus was on exposure-response modeling of fixed-dose combinations. In a previously published study, it was found that analyzing fixed-dose combinations using exposure-response modeling caused inflated false positive rates, corresponding to finding an effect of a fixed-dose combination component when the component had no true effect [86]. This inflation of false positive rates resulted from confounding factors due to the high correlation between the concentrations of two components administered in fixed ratios across multiple dose levels [86].

3.2.1 Analysis strategy

This study considered an alternative approach in the form of longitudinal exposure-response modeling, which utilizes the entire timecourse of the exposure-response relationship. The hypothesis was that since fixed-dose combinations often have endpoints that have a delay in the concentration-effect relationship, the advantages of longitudinal exposure-response modeling could alleviate the issue with inflation of false positives.

The entire study was based on the simulation of clinical trials in which the exposure-response and longitudinal exposure-response modeling analysis were compared with respect to false positive and false negative rates. The simulated PK was nominally based on models for empagliflozin (compound A) and linagliptin (compound B), while the simulated PD was based on their combination Glyxambi®, which is used for the treatment of T2DM [110–112]. In addition, the influence of the clinical trial parameters on the false positive and false negative rates was investigated by including 432 scenarios that varied across the following: Drug activity, number of patients, duration, sampling frequency, dose distribution, and clinical trial design, Figure 7. Importantly, to evaluate the false positive and false negative rates, the drug activity of one compound in the fixed-dose combination was fixed to 0. The study was performed in R 3.5.1 using the mrgsolve and lme4 packages for simulation and analysis, respectively [113–115].

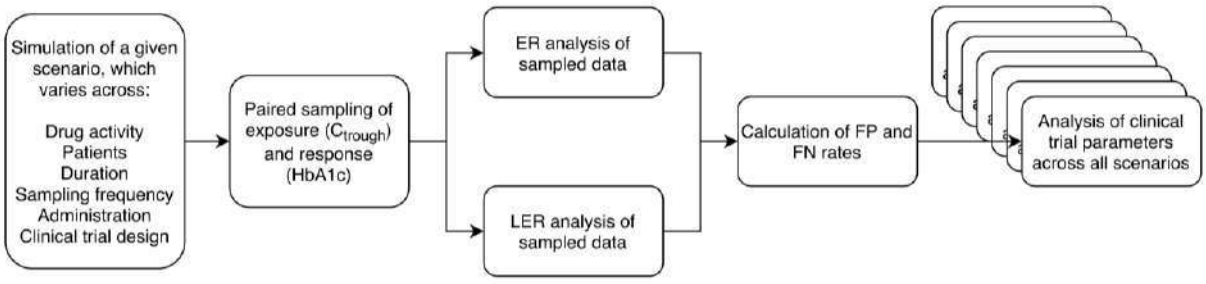

Figure 7 – Flow-chart illustrating the process of the study. Exposure as trough concentrations and response as HbA1c levels are sampled pairwise from the simulated data of each scenario. The samples are then analyzed using exposure-response and longitudinal exposure-response models, and the false positive and false negative rates are computed. Lastly, clinical trial parameters across all scenarios are analyzed. The figure and caption are from paper 2.

3.2.2 Exposure-response analysis

A two-compartment model with first-order absorption and elimination was used to simulate the exposure, sampled as trough concentrations, while an I_{max} model with an inhibitory effect on the formation constant k_{in} constituted the pharmacodynamic model [116].

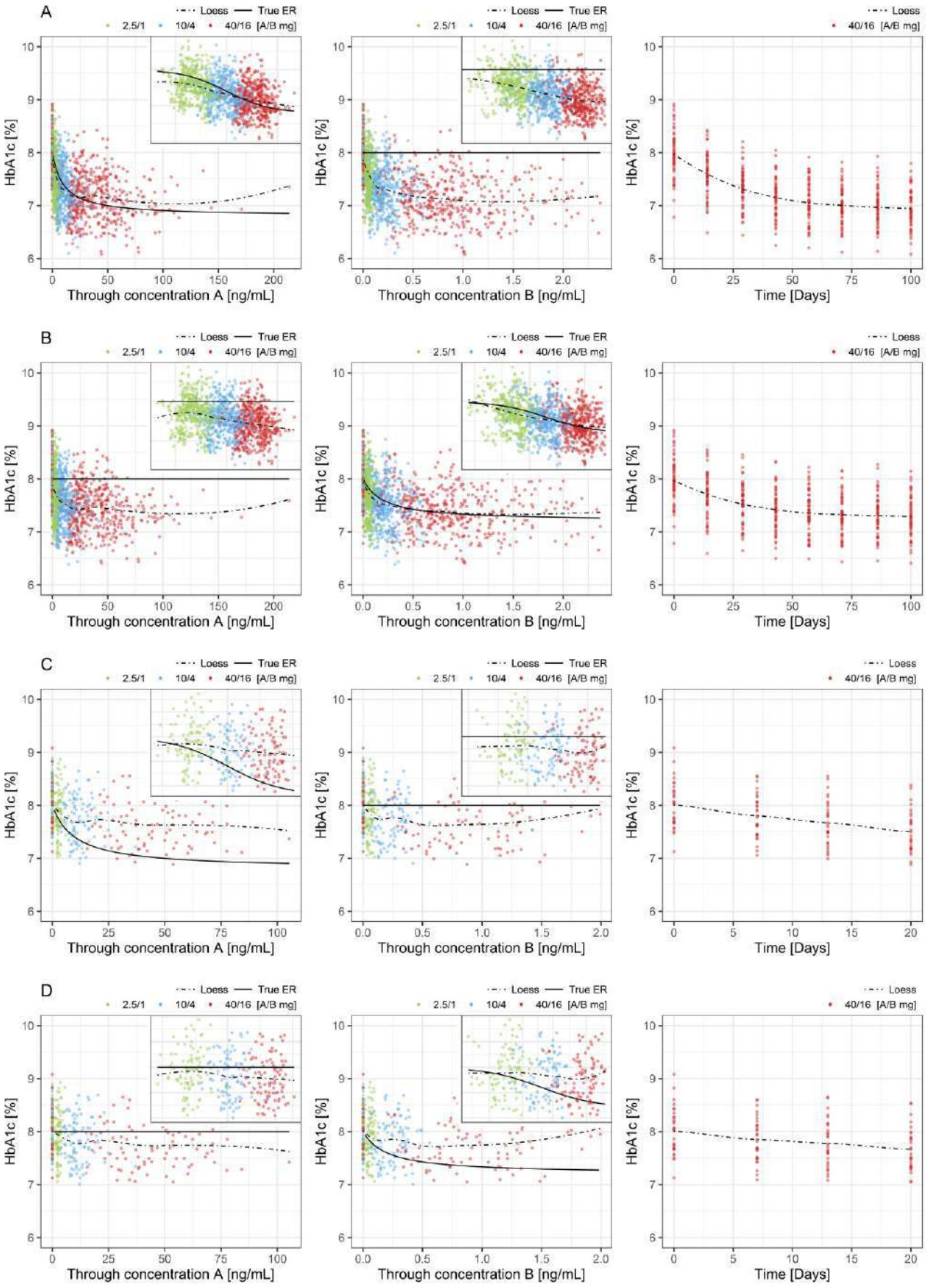
A
B
C
D
Loess
True ER
2.5/1
10/4
40/16 [A/B mg]
HbA1c [%]
Through concentration A [ng/mL]
Through concentration B [ng/mL]
Time [Days]

Figure 8 – Pharmacodynamic response (HbA1c [%]) vs. compound A (left panel) and B through concentration (middle panel) on a linear and logarithmic scale (embedded graph) as well as PD response vs. time (right panel) across four scenarios. Each scenario includes; 60 subjects, 8 samples, and a trial duration of 100 days (A+B), 30 subjects, 4 samples, and a trial duration of 20 days (C+D). All scenarios use a multiple dosing scheme and a dose distribution on either side of IC50. For scenario A+C, compound A is active, while for B+D, compound B is active. Each data pair is represented by dots colored by dose level. The dashed line represents the loess line, and the solid line reflects the underlying relationship between PD response and compound concentration. The figure and caption are from paper 2.

The exposure-response of four simulated scenarios was investigated in detail and is illustrated in Figure 8. These scenarios represented what was considered *high information* and *low information* scenarios in the spectrum of scenarios investigated in the study.

Panels A+B are *high information* scenarios with compound A or B active, respectively. Panels C+D are *low information* scenarios with compound A or B active, respectively. From these results, it was evident that for the active component in the *high information* scenarios, the loess fit, based on visual inspection, closely matched that of the true exposure-response relationship from the simulation model. This was not the case to the same degree for the *low information* scenarios. However, the more interesting part was that while the relationships matched for the active component, the inactive components were more erroneously described for the *high information* scenario. In essence, this demonstrated that as the information increased in the clinical trials, the exposure-response relationship for the active component was better described, but simultaneously, the inactive component was worse described. This occurred due to confounding, through the high correlation between concentrations of drug combinations administered in fixed ratios.

A similar result was derived from the exposure-response and longitudinal exposure-response analysis of false positive and false negative rates. Here the *high* and *low information* scenarios were again considered. For exposure-response analysis, it was clear that the *high information* clinical trials lead to an increase in the observed false positive rates compared to the *low information* clinical trials. Ultimately, based on these results, performing exposure-response trials for fixed ratio drug combinations will cause a false claim of contribution of the components to the overall efficacy.

For longitudinal exposure-response, the false positive rates were generally well-controlled across all scenarios, which essentially showed that longitudinal exposure-response could be used for the analysis of fixed-dose combinations, without risking false claim of efficacy. Except for the *high* information scenario, the false negative rates were generally not well-controlled, especially when the intercept was fixed to 0 in the analysis model. This is a common approach when it is known that the data must pass through the origin. However, this approach caused high false negative inflation for longitudinal exposure-response analysis, which was argued to be caused by an over-simplified regression model. Another misspecification in the analysis was for the non-linear models with regards to time, as these models showed an increase in false positive rates in the *high information scenario.* In the study, exponential time decay was used for the non-linear description of time [84]. The true description of time is more complex, described here [117], than exponential time decay, and this simplification may lead to the inflated false positive rates. Another approach that was considered was a pharmacometric approach, where more mechanistic models could be employed [118]. However, as the simulation study is based on one of these mechanistic models, performing

the analysis with the same model would constitute an artificial best-case scenario. A comparison between the statistical and pharmacometric approaches could be of interest for future research.

3.2.3 Clinical trial parameters

The clinical trial parameters were explored to provide information on how they affect the false positive and false negative rates. For exposure-response, the same pattern was found as previously, showing that more information, through e.g. more patients, led to higher false positive rates. False positive rates for longitudinal exposure-response was not affected by any parameters, demonstrating that regardless of scenario, false positive rates are well-controlled. From the clinical trial parameters, the most important finding was that the inflated false negative rate for longitudinal exposure-response was caused by the scenarios generally providing too little information. Across all parameters, false negative rates decreased as more information was included in the trial. Especially the sequentially administered doses were essential in controlling the false negative rate. In summation, longitudinal exposure-response analysis showed well-controlled false positive rates and, given sufficient information corresponding to the *high information* scenario, also showed well-controlled false negative rates.

3.2.4 Discussion

The hypothesis in this study was that longitudinal exposure-response analysis could be applied to fixed-dose combinations without obtaining results with inflated false positive rates. Based on the results presented in the study, longitudinal exposure-response analysis can be confidently used as the issues with inflated false positive rates observed in the previous study by Zhu and Wang are practically eliminated [86]. However, the analysis does have drawbacks. In particular, the sequential administration of treatment and adequate coverage of the response-time curve were important in identifying the true exposure-response relationship. In combination, these factors will lead to long clinical trials, which have higher costs [119]. Conversely, the gain from this analysis is that the monotherapy arms used in the conventional factorial clinical trial, which is recommended by EMA [51], becomes redundant. By removing these arms, the clinical trial size can either be reduced, thus having lower costs, or more patients can be allocated to the combinations, thereby providing more information on the fixed-dose combination. A general consideration from the study is that the results apply to any fixed ratio drug combination. However, drug combinations given in a defined ratio are a common scenario for fixed-dose combinations [120], and the discussion is therefore focused on fixed-dose combinations.

3.2.5 Conclusion

The conclusion from the study highlights that the previously demonstrated inflated false positive rate in the exposure-response analysis was not present when the longitudinal exposure-response analysis was performed. Thus, longitudinal exposure-response analysis can be recommended for the analysis of fixed-dose combinations or drug combinations in a fixed ratio. It was considered whether the results from this analysis would carry regulatory weight, concluding, that at least it would provide supportive information regarding the claim of contribution of each component to the overall effect. Lastly, carrying out this analysis outside *in silico* studies would help support the validity of longitudinal exposure-response analysis of fixed-dose combinations.

3.3 Paper 3 - Pharmacodynamic modelling reveals synergistic interaction between docetaxel and SCO-101 in a docetaxel-resistant triple negative breast cancer cell line

In the third paper, a drug combination undergoing investigation for its potential use in breast cancer is analyzed using the GPDI model introduced in section 2.3.4. The combination consists of a novel compound under investigation, SCO-101, which is hypothesized to enable the treatment of several drug-resistant cancers, and docetaxel, which is part of the neoadjuvant treatment for TNBC [121]. This paper mainly addresses the modeling and analysis of the data while the cellular, mechanistic, and assay aspects are planned for a separate publication by the lab conducting the experiments.

SCO-101 is hypothesized to enable treatment in drug-resistant cancers through inhibition of the ATP-binding cassette ABCG2, which is a human multidrug transporter [122, 123]. The protein is responsible for the efflux of commonly used pharmaceuticals and thereby contributes to drug resistance in cancers [123]. Furthermore, a phase II clinical study of SCO-101 in combination with FOLFIRI is planned in patients with colorectal cancer using the same rationale [124]. FOLFIRI is an established regiment for the treatment of colorectal cancer [124].

The development of drug resistance is one of the primary barriers in treating patients suffering from cancer [14, 125]. In theory, targeting drug efflux pumps enables several potential substrates. TNBC is outlined in section 2.2.2 and represents a cancer subtype with a very poor prognosis and a high degree of drug resistance. This makes TNBC a good candidate for investigating potential treatment improvement through the addition of SCO-101 to the existing regiment. Therefore, the effects of SCO-101, docetaxel, and the combination was investigated in a docetaxel resistant TNBC cell line, MDA-MB-231.

Pharmacodynamic modeling was conducted as an alternative to more conventional approaches, such as analysis using t-tests or ANOVA. The hypothesis here was that more information could be extracted from the data by employing the GPDI model, which provides a semi-mechanistic understanding of the combination, as opposed to considering whether there are significant differences in cell survival between groups.

3.3.1 Monotherapy models

The initial strategy was to establish the concentration-effect relationship for docetaxel and SCO-101 administered separately. The four relationships, linear, log-linear, I_{max} and sigmoidal I_{max} in EQ7 was considered for both drugs. The parameters in the models are outlined in section 2.3.2. The relationship for docetaxel was best described by an I_{max} model, while the best description was achieved for SCO-101 with a sigmoid I_{max} model based on the OFV. The model fits plotted on top of the observed data from the experiments can be seen in Figure 9.

$$I_{drug} = \alpha \cdot C_{drug} + \beta \quad ; \quad I_{drug} = \alpha \cdot \log(C_{drug}) + \beta \qquad \text{(EQ7)}$$

$$I_{drug} = \frac{I_{max,drug} \cdot C_{drug}}{IC_{50,drug} + C_{drug}} \quad ; \quad I_{drug} = \frac{I_{max,drug} \cdot C_{drug}^{H_{drug}}}{IC_{50,drug}^{H_{drug}} + C_{drug}^{H_{drug}}}$$

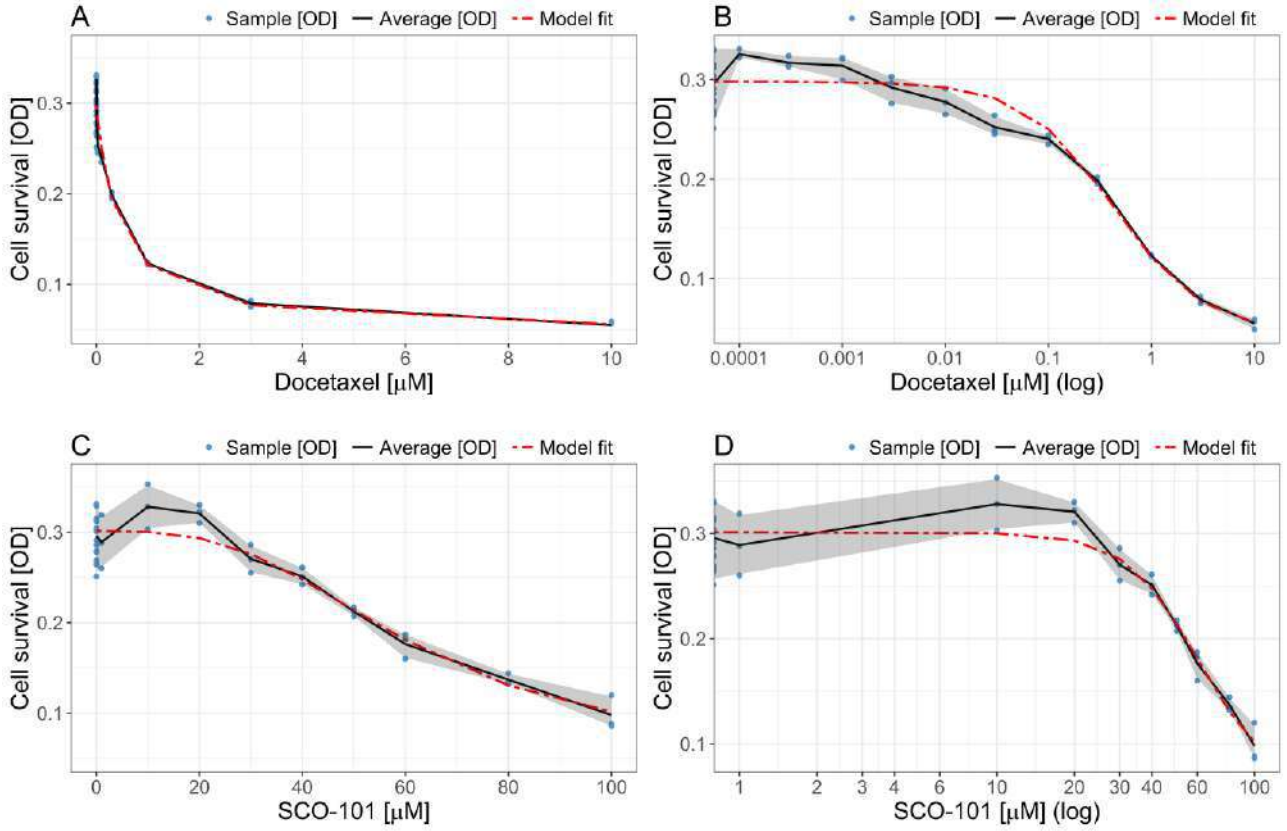

Figure 9 – MDA-MD-231 survival following monotherapy with docetaxel (A+B) or SCO-101 (C+D). The blue dots represent the samples from the experiment, while the black line corresponds to average cell survival. The red dashed line represents the fitted curve for docetaxel (I_{max} model) and SCO-101 (sigmoid I_{max} model). B and D contain the same data as A and C, respectively, but on a logarithmic scale. The figure and caption are from paper 3 [126].

For the data presented in Figure 9, the y-axis represents cell survival measured as optical density, where ~0.3 OD corresponds to ~100% cell survival. The model parameters showed that the two compounds had similar maximal effects, albeit with less certainty of the estimate for SCO-101. The maximal effect for docetaxel was around 80%, with a half-maximal inhibitory concentration of 0.413 µM. These parameter values were in agreement with a previous study of docetaxel in docetaxel resistant MDA-MB-231 cells [127]. The cytotoxicity of SCO-101 alone could be attributed to the inhibition of SRPK1 kinase, which is involved in tumor growth [124].

3.3.2 Combination model

Following the development of the monotherapy models, several combination models were evaluated. These included Response Additivity [20], Bliss Independence [92], and finally, a *general pharmacodynamic interaction model* [99] of the best fit between the two. Bliss Independence provided the better model fit and was used as the basis for the GPDI model, which is outlined in EQ8.

$$I_{Doce} = \frac{I_{max,Doce} \cdot C_{Doce}}{IC_{50,Doce} \cdot \left(1 + \frac{INT_{max,SCO \to Doce} \cdot C_{SCO}}{INT_{50,SCO \to Doce} + C_{SCO}}\right) + C_{Doce}} \; ; \; I_{SCO} = \frac{I_{max,SCO} \cdot C_{drug}^{H_{drug}}}{IC_{50,drug}^{H_{drug}} + C_{drug}^{H_{drug}}} \quad \text{(EQ8)}$$

Other variants of the GPDI model were investigated with a 1-way interaction in the opposite direction of EQ8 and with a 2-way interaction between the drugs. However, these models encountered boundary errors and issues with minimization even with several fixed parameters. Therefore, the model in EQ8 was considered to provide the best fit.

The most interesting parameters extracted from the model were the $INT_{max,SCO\rightarrow Doce}$ designating the maximal interaction effect of SCO-101 on the potency of docetaxel, and $INT_{50,SCO\rightarrow Doce}$ the concentration of SCO-101 for half-maximal interaction with docetaxel. The maximal interaction $INT_{max,SCO\rightarrow Doce}$ was estimated to -0.604, which can be interpreted as an approximately 60% increase in the potency of docetaxel at maximum interaction effect when SCO-101 is administered with docetaxel. The half-maximal concentration of the interaction $INT_{50,SCO\rightarrow Doce}$ was estimated to 30.9 µM, which is approximately half of the IC_{50} for the compound itself, which is 59.4 µM.

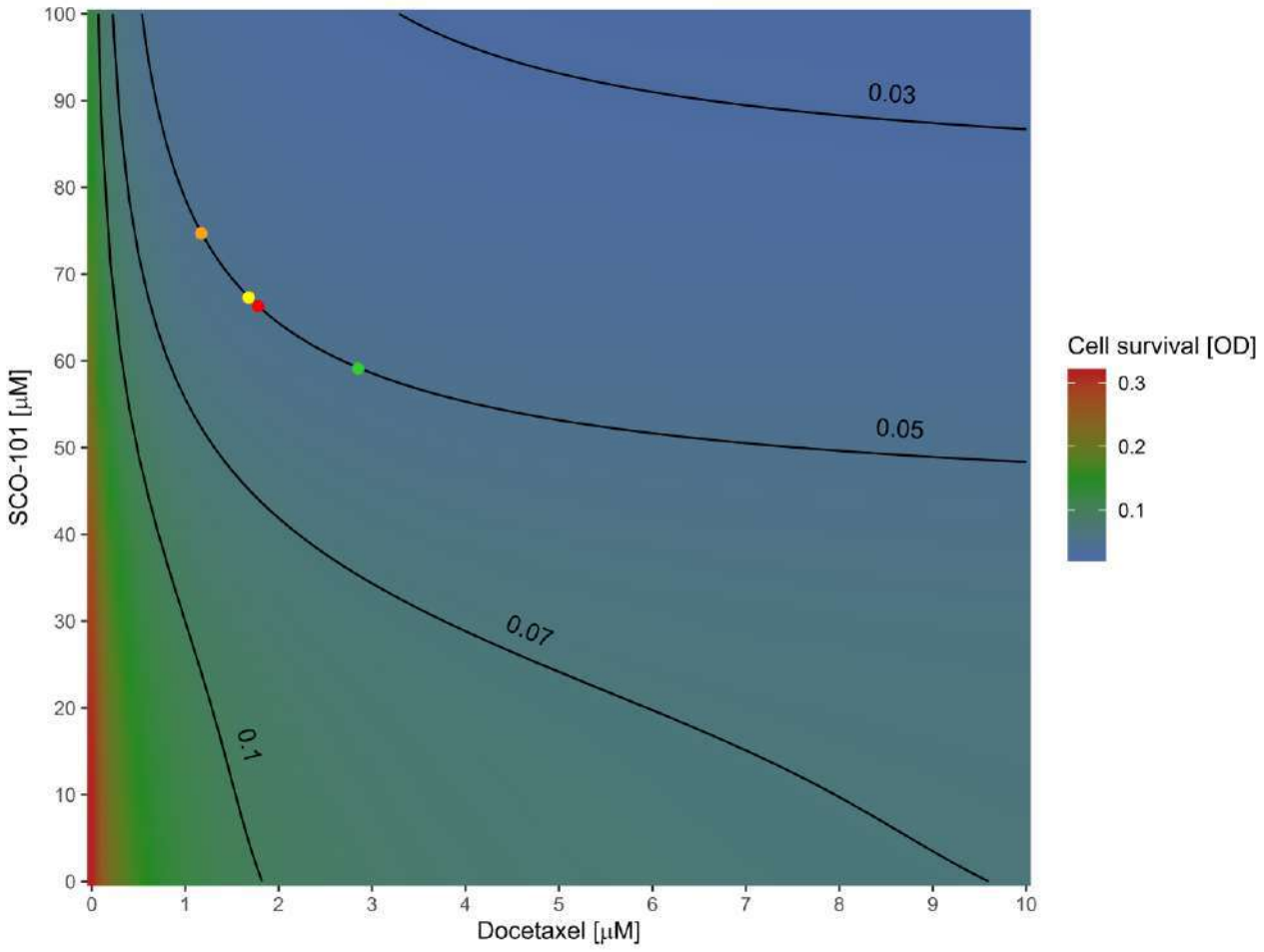

Figure 10 – Contour plot of the final general pharmacodynamic interaction model. The lines indicate four separate response levels of 0.1 OD, 0.07 OD, 0.05 OD, and 0.03 OD across the dose combination space. 0.05 OD corresponds to the 85% reduction in cell viability target. The four dots highlight dose pairs that result in meeting this target, through lowest total dose combination (red), minimized exposure to both compounds (green), and with a weighted penalty factor of 2 on lowest total dose combination (orange) and minimized exposure (yellow). The figure and caption are from paper 3 [126].

The model was used to provide information on dose ratios for further studies. These estimations were based on locating the lowest total drug combination and the minimized exposure to both compounds. The former of the two methods was based on the addition of scaled doses, and the latter was based on Pythagoras' theorem. Lastly, as docetaxel represented the more toxic of the two compounds, a penalty was considered. The resulting contour plot from the model and the optimal dose ratios are illustrated in Figure 10.

The optimal dose ratios were based on the selected effect target of 85% reduction in cell survival and resulted in dose ratios between 1:40 and 1:64 (docetaxel:SCO-101) being promising for further analysis.

3.3.3 Discussion

Preclinical modeling of cancer therapeutics has previously been performed as outlined in section 2.3.4. Some of the core issues that arise from these models are the lack of interpretability of the results and selection of an additivity criterion. The lack of interpretability in these models stems from describing the drug effect through kill rates. Here the empirical nature of the GPDI model provides results expressed as maximal effects and half-maximal concentrations, which can be considered more intuitive. Selecting an additivity criterion causes the classification of a combination as either synergistic or antagonistic to be dependent on assumptions tied to the specific additivity criterion. As an example, selecting Bliss Independence includes base assumptions of the underlying mechanism of the combined drugs, i.e. that they act independently [91, 92]. The GPDI model unifies the interpretation of these additivity criteria in a model-based framework, which addresses the issues with selecting an additivity criterion [99].

A simpler approach would be to employ a more conventional analysis such as t-tests or ANOVA. These methods avoid some of the limitations of the modeling approach, such as selecting an additivity criterion, establishing a model, and the interpretability of the results. However, as highlighted in the following paragraphs, using models such as the GPDI model provides significant insights into drug-drug combinations that go far beyond a comparison of groups.

The potency of the interaction $INT_{50,SCO \rightarrow Doce}$ was estimated to 30.9 µM. This corresponds to half the concentration needed to reach the half-maximal effect on cell survival of SCO-101, which has an IC_{50} of 59.4 µM. The essence of this discovery was that at concentrations near the half-maximal concentration of the interaction, the effect of SCO-101 is primarily mediated through its interaction with docetaxel. Thus, depending on tolerability for SCO-101 the primary treatment capacity of SCO-101 will be its interaction with docetaxel. Usually, the underlying implicit assumption is that the drug effect and interaction share the same potency [127]. Estimating the potency of the interaction is a key trait of the GPDI model, which provided important information on the difference between the potency of SCO-101 and the potency of its interaction with docetaxel.

The maximal interaction effect $INT_{max,SCO \rightarrow Doce}$ was estimated to -0.604. This corresponds to a 60% reduction in the half-maximal concentration of docetaxel when administered in combination with SCO-101 compared to when docetaxel is administered alone. By estimating the maximal interaction, the GPDI model provides a semi-mechanistic understanding of the interaction. Two clinical

implications arise from this parameter. Either the dose of docetaxel can be reduced when given in combination with SCO-101, reducing toxicity but maintaining the same effect, or the dose of docetaxel can be maintained, thus attaining a higher effect with no additional side effects from docetaxel. Identifying victim and perpetrator drugs in the combination is an implicit part of the GPDI model, which enables the identification of interaction networks for large combination analyses [99]. Overall, analysis of the combination data using modeling provided significant insights over conventional approaches such as t-tests or ANOVA.

The clinical relevance of the doses for both SCO-101 and docetaxel should also be considered. SCO-101 was found to have limited toxicity when administered alone, indicating that there will be few potential issues with the doses [122, 128]. However, to the knowledge of the authors, no concrete information on the plasma concentration of SCO-101 is available, thus, evaluation of the clinical relevance of the SCO-101 concentration can be considered speculation. Conversely, the pharmacokinetics of docetaxel are well documented. A pharmacokinetic analysis of docetaxel found the median C_{max} to be 3.7 µM and the range to span 2.6-6.9 µM in patients receiving 100 mg/m^2 docetaxel [129]. These values correspond well with the investigated range of docetaxel concentrations in the present study making them relevant from a clinical perspective. Furthermore, the identified optimal dose pairs in the study all recommend doses of docetaxel below the median C_{max} of 3.7 µM. Thus, the recommendations for the drug-drug ratio of SCO-101 and docetaxel seem sensible.

Assessing the potential of a drug combination in humans, based on *in vitro* experiments, is challenging. The increase in complexity from *in vitro* to *in vivo* is immense. The effects of aspects such as ADME and cellular aspects such as the immune system make extrapolation unreliable. One approach that can assist in utilizing *in vitro* data for extrapolation to *in vivo* is *in vitro in vivo* extrapolation (IVIVE). IVIVE makes use of modeling and simulation to make quantitative extrapolation of drug exposures [130, 131]. The major determinants for this method is establishing a physiologically based model and carrying out *in vitro* studies of solubility in GI fluid and permeability [130]. Overall, this presents a fairly complex process but could provide a method for extrapolating the combination data. However, realistically, to make confident conclusions about the combination in humans, studies *in vivo* and in humans will be required.

3.3.4 Conclusion

In conclusion, a model describing the pharmacodynamics of the combination of SCO-101 and docetaxel was established. Based on the information from this model, it can be concluded that the novel compound SCO-101 has the potential to provide a significant improvement in the treatment of docetaxel resistant TNBC when administered in combination with docetaxel. Modeling the *in vitro* data provided significant insights into the combination compared to conventional approaches through estimation of the maximal interaction and the potency of the interaction. Furthermore, the information from the model enabled recommendations on the optimal drug-drug ratio. Lastly, the study serves as a case study of the GPDI model, which was shown to offer significant advantages over conventional methods.

4 Conclusions and Perspectives for further research

Based on the research conducted in this PhD thesis, three papers were published:

1. **Nøhr-Nielsen A**, De Bruin ML, Thomsen M, Pipper CB, Lange T, Bjerrum OJ, Lund TM. Body of evidence and approaches applied in the clinical development program of fixed-dose combinations in the European Union from 2010-2016. Br J Clin Pharmacol. 2019;(July 2018):1–12.
2. **Nøhr-Nielsen A**, Lange T, Forman JL, Papathanasiou T, Foster DJR, Upton RN, Bjerrum OJ, Lund TM. Demonstrating Contribution of Components of Fixed-Dose Drug Combinations Through Longitudinal Exposure-Response Analysis. AAPS J 2020 222 22:1–14.
3. **Nøhr-Nielsen A,** Bagger SO, Brünner N, Stenvang J, Lund TM. Pharmacodynamic modelling reveals synergistic interaction between docetaxel and SCO-101 in a docetaxel-resistant triple negative breast cancer cell line. Eur J Pharm Sci 2020 105315.

The studies covered in each of the three papers addressed the overall aim and the three objectives outlined in section 1.

The first objective was to assess the current practice of the use of modeling as a drug development tool for the development of fixed-dose combinations in the European Union. This was achieved in paper 1: “Body of evidence and approaches applied in the clinical development program of fixed-dose combinations in the European Union from 2010-2016”. In the study, it was shown that only 58% of approved fixed-dose combinations made use of PK or PK-PD modeling during development. Given the advantages outlined in the discussion of paper 1 (section 3.1.3), this was surprising. The main application of this result is that developers of fixed-dose combinations should to a greater extent consider incorporating the use of modeling in the development process. Other novel results from the study include that:

- Performing PK modeling lead to significantly fewer doses investigated
- Components of the fixed-dose combinations are to a small degree reprofiled (6%)
- Fixed-dose combinations approved by EMA primarily consist of two previously approved drugs (71%) and have a single approved combination dose (71%)
- No dose-finding trial was conducted for more than half of fixed-dose combinations composed of two previously approved drugs (57%)

The main limitation of the study, for achieving the first objective in the thesis, was that the EPARs contain no information on the actual models or the purpose for employing them. Therefore, the study assessed the use of modeling in a yes/no manner. This limitation was highlighted by the fact that PK-PD modeling was shown to have no impact on the number of patients, arms, clinical trials or doses tested, which is not in agreement with previously published studies [79, 104–106].

The continuance of this research would need to address this limitation by analyzing more detailed information regarding the modeling conducted for the development of fixed-dose combinations. Particularly with a focus on the type of model and the purpose for employing it.

In summation, the first objective of the thesis was fulfilled, however, more in-depth knowledge on the use of modeling as a drug development tool for the development of fixed-dose combinations in the European Union can be gained by performing the suggested studies.

The second objective was to develop new methods that may assist in the drug development process of fixed-dose combinations. This was achieved in paper 2: "Demonstrating Contribution of Components of Fixed-Dose Drug Combinations Through Longitudinal Exposure-Response Analysis". In this *in silico* study, the applicability of performing longitudinal exposure-response modeling in the development of fixed-dose combinations was shown. Utilizing longitudinal exposure-response in this setting constituted a new method for demonstrating the contribution of the components to the overall effect without an inflated false positive rate, which had been present for exposure-response analysis in a previous study [86].

The application of this research is that the monotherapy arms used in the conventional factorial clinical trial can be eliminated as they are not required for this type of analysis. The gains from removing these arms are either allocation of more patients to the combination arms or reduction in clinical trial size. Thus, there is either an increase in information on the combination or a reduction in cost and the number of patients needed to treat. Additional novel results included the influence of several parameters on the false positive and false negative rate in both exposure-response and longitudinal exposure-response analysis. In particular, the sequential administration of the doses in the clinical trial and adequate coverage of the response-time curve was essential in identifying the true exposure-response relationship.

The primary limitation of the findings in this study originate from the *in silico* setting. This setting has overarching assumptions about the parameter choices for both the simulated compounds and clinical trials. The most important assumption was that the chosen parameters reflect a realistic setting. To expand upon this research, it will be important to apply longitudinal exposure-response modeling to data from previously conducted clinical trials and assess if the results match those from the original methods. Additionally, defining the longitudinal exposure-response model *a priori* and analyzing clinical trial data will verify the applicability of the method.

Based on the research in paper 2 the second objective has been achieved. While longitudinal exposure-response modeling is not in itself a new method, the application of the method for fixed-dose combinations is novel and will aid in the development of fixed-dose combinations.

The third objective was to validate combination models on preclinical data of a combination under investigation and assessing the value of applying these models in guiding further investigations of the combination. This was achieved in paper 3: "Pharmacodynamic modelling reveals synergistic interaction between docetaxel and SCO-101 in a docetaxel-resistant triple negative breast cancer cell line".

In this *in vitro* study, a combination of the novel compound SCO-101 and docetaxel was used for the treatment of docetaxel resistant TNBC and analyzed using PD modeling. Several models were evaluated and the best model fit was found to be the GPDI model. Based on this model, it was found that SCO-101 interacted with docetaxel, leading to a maximal reduction in the IC_{50} value of docetaxel by 60%. Therefore, the combination was characterized as synergistic. Analysis of the model

identified optimal drug-drug ratios between 1:40 and 1:64 (docetaxel:SCO-101). Furthermore, the study also presents a case study of the GPDI model, which is a fairly recently developed model [99].

The application of these results is that the combination of SCO-101 and docetaxel has the potential to provide a significant improvement in the treatment of docetaxel resistant TNBC and is therefore worth further investigation. Furthermore, the research highlights that the parameters from the GPDI model led to a greater understanding of the drug-drug interaction than conventional approaches and provided valuable information to guide further investigations through dose ratio selection.

A key limitation of modeling *in vitro* studies is the very controlled setting that it represents. The established model showed very low uncertainty in the parameters, which is unfeasible in more variable settings such as *in vivo*. Furthermore, cellular aspects such as the presence of the immune system could potentially influence the efficacy of the combination. Thus, to provide confident conclusions about the application of the combination in humans, more studies of the combination *in vivo* and in clinical trials will be necessary.

Combination models were validated on preclinical data of a combination under investigation and the value of the model in guiding further investigations was assessed in paper 3. Based on this, the third objective of this thesis was achieved.

The work of this PhD thesis and previous works highlights both the pitfalls and potential advantages of utilizing model-based approaches for the development of fixed-dose combinations. The overall aim of this thesis was accomplished as the use of several model-based approaches has been explored, developed, and demonstrated within the field of fixed-dose combinations and the development of these products. Based on the research conducted in this thesis, the overall recommendation is that modeling tools should to a greater degree be incorporated in the development of fixed-dose combinations as they assist in determining efficacy and provide valuable information to guide drug development.

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