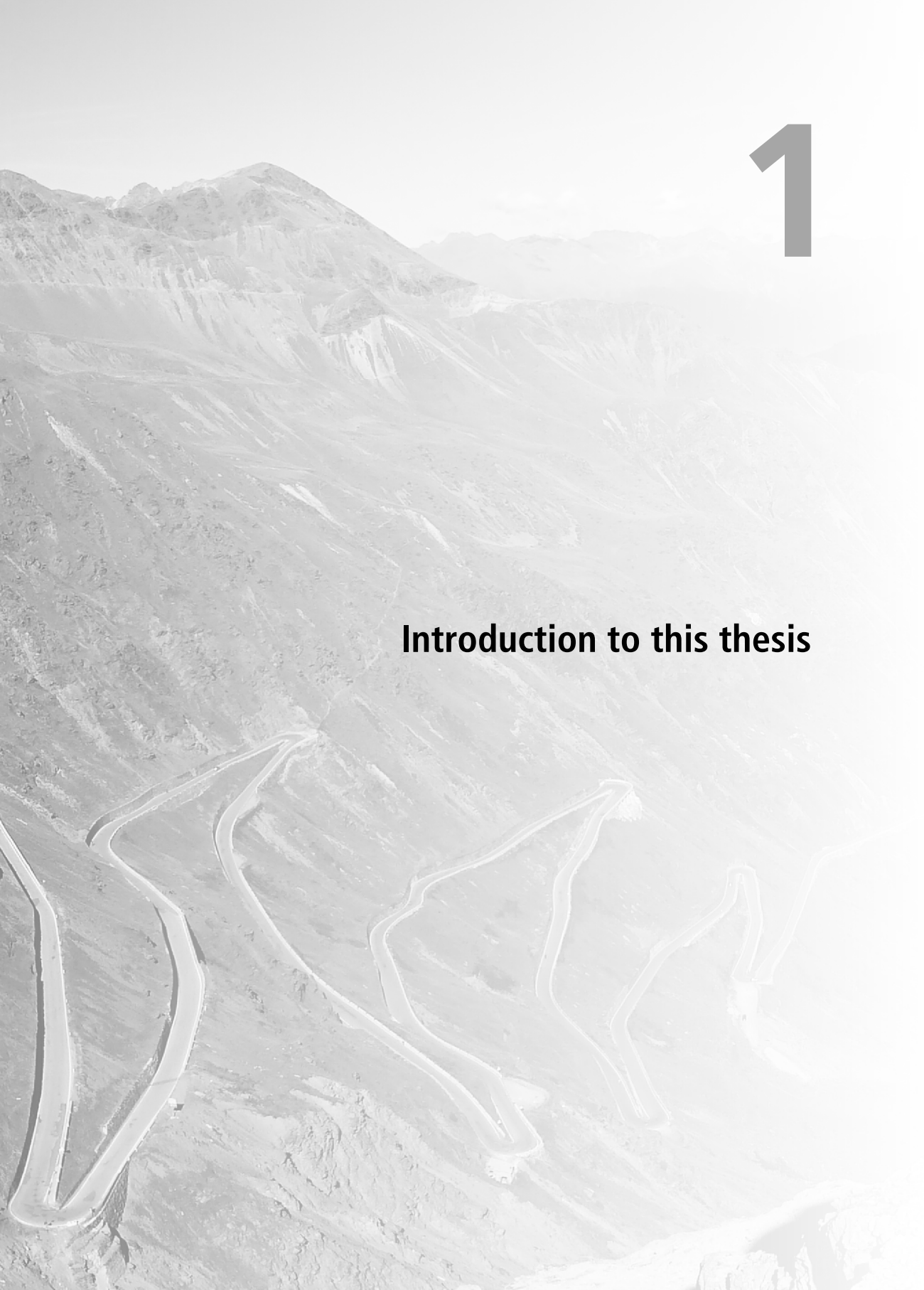

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Introduction to this thesis

The phenotype

The word 'phenotype' has been derived from the Greek and literary means: to show (φαινει) and type (τυποσ), or appearance. The phenotype encompasses a composite of observable characteristics, such as physical appearance, biochemical and physiological properties and products of behaviour [1]. A specific phenotype results from the expression of an individual's genes (the genotype) combined with the influence of environmental factors and the interactions between the two. Therefore, subjects with the same genotype, such as monozygote twins do not look the same and, the other way around, organisms who look the same, do not necessarily harbour the same genotype. Although the genotype-phenotype relationship was first described by Johanson [2] in 1911 to underscore the difference between heredity and the results of heredity in living organisms, the concept of the phenotype was extrapolated to biochemical science to describe cellular processes as a result of the genotype and environmental factors. In such way, drug disposition (pharmacokinetics) in a particular individual could be considered an observable characteristic (i.e. phenotype) as well.

Phenotyping drug disposition

Despite administering equal doses adjusted to body surface, the response to a chemotherapeutic drug is highly variable between individuals and often difficult to predict. Variation in the genotype encoding proteins related to pharmacokinetic and pharmacodynamic properties may thus influence both drug disposition (absorption, metabolism, distribution and excretion) as well as drug effects (target proteins, receptors, signalling pathways) respectively [3]. In general, it is estimated that the genotype accounts for 20–95% of variability in therapeutic response and toxicity dependent upon drug and disease [4]. In oncology however, for only a relatively small number of genetic variants, the relevance for clinical practice, such as *KRAS* status and response to antibodies to epidermal growth factor receptor (EGFR) in colorectal cancer, has been determined [5]. Another example is the presence of polymorphisms of *DYPD*, predicting disposition and toxicity to fluoropyrimidines [22]. Besides the genotype, other factors such as age, gender, race/ethnicity, body composition, renal and liver function, concomitant disease, drug-drug interactions, and lifestyle have been identified to affect drug disposition as well [6,7]. Together, these factors determine the phenotype of drug disposition in an individual cancer patient. Real-time determination of enzyme or drug transporter activity using *in vivo* phenotyping probes [8] or endogenous compounds [9] has a potential advantage over genotyping by taking changing environmental factors into account.

Phenotyping breath tests in oncology

Phenotyping drug metabolizing enzymes and drug transporters may be used to individualize drug therapy. By using labeled (e.g. ^{13}C) probes in phenotyping breath tests (BTs), the amount of exhaled labeled probe metabolite (e.g. $^{13}CO_2$) reflects the clearance of the probe by a specific metabolic route. For example, ^{13}C-dextromethorphan (^{13}C-DM) has been used as a specific CYP2D6 phenotype probe [10]. ^{13}C-Dextromethorphan breath test (DM-BT) is dependent on CYP2D6 mediated *O*-demethylation which results in generation of $^{13}CO_2$ which is measured in expired breath over time (Figure 1.1).

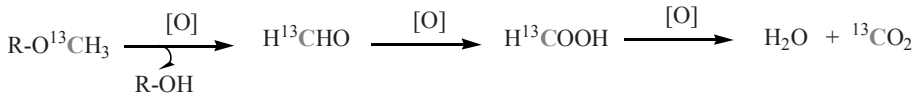

Figure 1.1 The principle of specific CYP2D6 phenotyping ^{13}C-dextromethorphan breath test (DM-BT), which is dependent on CYP2D6 *O*-demethylation. The released methyl group is involved in the formation of $^{13}CO_2$ that is released in expired breath over time [10].

Single point breath tests have been researched, enabling a physician to obtain BT results in 50 minutes.

Most anticancer treatments display high toxicity due to the usage of drugs with a small therapeutic index. BTs are of particular interest because, in theory, they may help to reduce toxicity and improve efficacy through individualizing treatment by means of introducing a phenotype guided treatment and/or dosing.

Tamoxifen metabolism and CYP2D6

Tamoxifen has been used for more than three decades in the adjuvant treatment of localized estrogen receptor positive (ER+) breast cancer. Five years of adjuvant tamoxifen reduces 15-year breast cancer recurrence and mortality by nearly a third in women with ER+ breast cancer. However, in one third of patients disease recurs, suggesting non-response to the antiestrogen action of tamoxifen [11]. Tamoxifen has limited affinity for the ER and is considered to be a prodrug [12]. CYP2D6 and CYP3A are thought to be the most important enzymes for biotransformation of tamoxifen into the active metabolites 4-hydroxy-tamoxifen (4-OH-TAM) and endoxifen (Figure 1.2) [13].

Both 4-OH-TAM and endoxifen have a 30 to 100-fold higher affinity for the ER than tamoxifen and exhibit the same strong anti-estrogen potency [14]. Serum endoxifen levels are 10 to 12

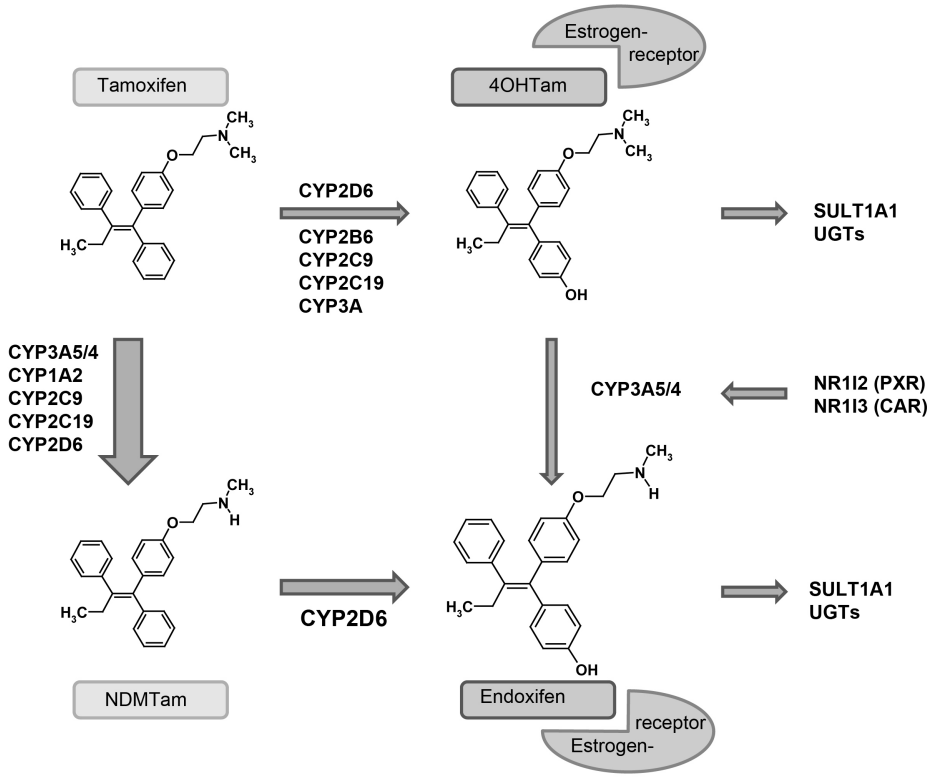

Figure 1.2 Tamoxifen metabolism.
Abbreviations: 4OHTam, 4-hydroxytamoxifen; CYP, cytochrome P450 isoenzyme; SULT, sulfotransferase; UGT, UDP-glucuronosyltransferase; NDMTam, N-desmethyltamoxifen; NR1, nuclear receptor subfamily 1; PXR, pregnane X receptor; CAR, constitutive androstane receptor.

times higher than 4-hydroxy-tamoxifen levels, suggesting that endoxifen is the most important active metabolite of tamoxifen [15]. A minimal threshold concentration has been suggested, above which endoxifen reduces the risk of recurrence of breast cancer [16]. Therefore, endoxifen levels may serve as a predictor for clinical outcome.

Studies correlating the interindividual response to tamoxifen and CYP2D6 pharmacogenetics of the most important enzymes involved in the biotransformation of tamoxifen and metabolites are conflicting: some studies show decreased breast cancer recurrence-free survival in CYP2D6 poor metabolizers (PM) predicted phenotype compared with extensive metabolizers (EM) predicted phenotype, whilst others fail to show any association, One study demonstrated even a better recurrence-free survival in CYP2D6 PMs [17;18].

Variation in response to tamoxifen has been attributed to variations in plasma endoxifen concentrations in carriers of variant alleles. Patients with CYP2D6 PM predicted phenotype have 2 to 4-fold lower endoxifen levels compared to patients with EM predicted phenotype. However, the variation in endoxifen plasma concentrations is explained by *CYP2D6* genotype for only 23% in patients on tamoxifen therapy [19]. This may be explained by the fact that exogenous/ environmental (epigenetic) factors interact and contribute to the CYP2D6 activity. For example, concomitant use of CYP2D6 inhibitors may decrease CYP2D6 activity in such way that efficacy or toxicity of drugs may be altered co-administration of the strong CYP2D6 inhibitor paroxetine decreases plasma concentration of endoxifen by 58–72% in CYP2D6 EMs [20]. Therefore, phenoconversion to PM phenotype might occur in genotypically EMs and IMs when interacting factors such as concomitant use of CYP inhibiting drugs or herbals or infection are present. To determine metabolic activity, phenotype tests using phenotype probes are commercially available or are still in research. One example is a ^{13}C-dextrometorphan breath test (^{13}C-DM-BT), which has been developed to rapidly and selectively assess CYP2D6 phenotype [10].

^{13}C-dextromethorphan breath test for phenotyping CYP2D6 in tamoxifen-treated patients

Dextromethorphan (DM) is an antitussivum and its metabolism has similarities with tamoxifen metabolism and has been used as a phenotyping probe [21]. DM is metabolized to dextrorphan (DXO) through *O*-demethylation by CYP2D6 [21]. Assessing the CYP2D6 phenotype by ^{13}C-DM-BT, which includes both genetic, epigenetic, and environmental factors, would potentially better predict endoxifen levels and breast cancer recurrence in women using tamoxifen in the adjuvant setting than the *CYP2D6* genotype alone. Besides highly variable endoxifen levels in breast cancer patients [16], variability in breath test results occur as well [10], which might be either due to uncertainties in the phenotyping test performance, variation of breath sample analysis and intra- en intersubject variation in ^{13}C-DM metabolism.

Oral uracil loading test and dihydropyrimidine dehydrogenase activity

Fluoropyrimidines are extensively used in the treatment of colorectal, breast, and gastric cancer. The rate-limiting step of degradation of 5-fluorouracil (5-FU) and its prodrug capecitabine to inactive metabolites is determined by dihydropyrimidine dehydrogenase (DPD) activity [22]. Patients with a partial or complete DPD deficiency have a strongly reduced capacity to inactivate 5-FU and capecitabine which can result in severe toxicity [23]. Several methods have been

developed and described to detect patients with reduced DPD activity including a phenotype test [24].

Frailty and phenotype

Most phenotyping studies have been conducted in healthy volunteers and middle aged cancer patients receiving chemotherapy. In daily clinical practice, cancer is more prevalent in the elderly population. In the Netherlands, in 2011, 40% patients who were diagnosed with cancer were between 60 and 75 years old, while 30% was 75+ [25] and therefore patients may be frail and more susceptible to adverse events.

With the increase in the amount of elderly cancer patients, selection of patients in order to minimize toxic effects of anticancer agents is challenging. Frailty is a clinical phenotype that is associated with adverse health outcome and is characterized by an excessive reduction of lean body mass, sarcopenia, chronic under-nourishment, reduced function, and poor endurance [26]. Frailty is possibly contributing to the age-related heterogeneity in the pharmacokinetics and pharmacodynamics of drugs [27]. There are a relatively limited number of studies on the effect of frailty on the pharmacokinetics of drugs in older people and interpretation of these studies is complicated by inconsistent assessment of frailty. From a pathophysiological point of view, there is a clear association between inflammation and frailty. Inflammation has the potential to downregulate drug metabolism and transporter pathways reducing the systemic clearance [28;29]. CYP2D6 is the most important isozyme in the metabolism of a variety of drugs that are commonly used in the elderly such as betablockers, antipsychotics (such as haloperidol) most selective serotonin reuptake inhibitors (SSRIs). CYP2D6 is also important in commonly used antitumor agents such as tamoxifen and gefitinib. There is a need to understand the factors which determine heterogeneity of metabolism in elderly, which might be partly attributed to frailty, in order to individualize pharmacotherapy. To date, no phenotype studies have been reported on CYP2D6 phenotype and frailty.

Aim and outline of this thesis

The general aim of this thesis was to study how phenotype tests may predict cancer drug disposition and to determine whether they are of clinical value in oncology practice.

In **Chapter 2** a systematic review is presented of studies addressing phenotyping methods in oncology. The review discusses drug disposition of anticancer agents and the potential of phenotyping tests to predict activity of drug metabolizing enzymes and transporters.

Chapter 3 gives an overview of studies in which phenotype breath tests were used to predict drug disposition of anticancer agents. The review discuss the analytical and clinical validity as well as the clinical utility of breath tests in oncology practice, some of which are promising to individualize pharmacotherapy.

The ^{13}C-DM-BT for phenotyping CYP2D6 in breast cancer patients using tamoxifen in the adjuvant setting is described in **Chapter 4**. This breath test was developed to account for genetic variants, epigenetic and environmental factors and CYP2D6 inhibitor use that may all influence CYP2D6 phenotype. Results of the ^{13}C-DM-BT are correlated to *CYP2D6* genotype and serum endoxifen concentrations in tamoxifen treated early breast cancer patients in a sub-study of the CYPTAM study.

Chapter 5 investigates the analytical validation of the ^{13}C-DM-BT, which was first described by Leeder [10]. There are a number of uncertainties in ^{13}C-DM-BT results when performing this test in breast cancer patients. The combination of variation in test properties, analysis of breath samples, and physiological variance in DM handling might all contribute to the uncertainty in CYP2D6 phenotype, which could be improved by introducing multiple breath sampling.

It is well known that disease can alter activity of several drug metabolizing enzymes. In **Chapter 6** the impact of metastatic disease on the CYP2D6 phenotype will be described. Comparison of CYP2D6 activity by means of the ^{13}C-DM-BT between patients using tamoxifen in the adjuvant setting and for metastatic breast cancer is studied.

In **Chapter 7** the pharmacokinetic sub-study of the prospective CYPTAM study is described. Tamoxifen treated early breast cancer patients who are CYP2D6 poor metabolizer and intermediate metabolizer predicted phenotype were treated with a temporary pharmacokinetic guided dose escalation. The increased tamoxifen dose was calculated by dividing the individual's baseline plasma endoxifen concentration by the median endoxifen concentration in extensive metabolizers multiplied by 20 mg (120 mg maximum). The aim of this study is to investigate whether such dose escalation in PMs and IMs would increase endoxifen to a level similar to that observed in EMs without increasing short term toxicity.

Chapter 8 describes the results of the KINURA-2 study, in which an oral uracil loading dose is studied to phenotype dihydropyrimidine dehydrogenase (DPD), the enzyme involved in the rate-limiting step of degradation of fluoropyrimidines, in colorectal cancer patients using capecitabine. This drug has been registered for treatment of colorectal patient in the adjuvant as well as in the metastatic setting. It is not known whether DPD activity might be altered by proinflammatory

cytokines associated with metastatic disease. The aim of the study is to investigate whether the DPD phenotype is different in metastatic disease compared to DPD activity in patients receiving capecitabine in the adjuvant setting.

With a rapidly ageing population, it is challenging to select those patients who might benefit from an anticancer treatment without experiencing too much toxicity. More than biological age and multimorbidity together, frailty describes a clinical phenotype that is associated with adverse health outcome and is characterized by an excessive reduction of lean body mass, sarcopenia, chronic under-nourishment, reduced function, and poor endurance [26]. In line with this functional decline, increased incidence of adverse drug reactions in frail subjects compared to non-frail subjects has been reported [30], which might be related do decreased drug metabolism. The aim of this study is to investigate whether CYP2D6 metabolism is altered in frail elderly compared to non-frail elderly subjects. **Chapter 9** describes the results of frailty tests to assess frailty in healthy and frail elderly aged 70–85 using the predefined Fried criteria [26]. CYP2D6 phenotype will be determined by the ^{13}C-DM-BT in all these subjects and will be associated with individual parameters of frailty and to frailty overall. This thesis ends with concluding remarks and future perspectives in **Chapter 10** and a summary of the results is presented in **Chapter 11**.

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