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EXPLORING THE ROLE OF PHARMACOKINETIC ALTERATIONS IN TYROSINE KINASE INHIBITORS (TKIs)-ASSOCIATED TOXICITIES

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DECLARATION

I hereby declare that this thesis is my original work and it has been written by me in its entirety. I have duly acknowledged all the sources of information which have been used in the thesis.

This thesis has also not been submitted for any degree in any university previously.

Teo Yi Ling
03 March, 2015

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Summary

The advent of molecular targeted therapy in the late 1990s marks a major breakthrough in the fight against cancer. The critical role of tyrosine kinases in the control of cancer phenotypes, coupled to the presence of suitable binding domains for small molecules, has led to the development of many tyrosine kinase inhibitors (TKIs) as molecularly targeting anti-cancer agents. While the use of TKIs have largely mitigated the conventional toxicities of chemotherapeutic agents (such as nausea, vomiting, alopecia, myelosuppression), a range of previously unknown and sometimes unpredictable toxicities like cutaneous, cardiac and liver toxicities began to surface. Clearly, such toxicities can impede the wider acceptance of TKIs as a mainstream therapy. Therefore, it is important to find ways to decrease the incidence of these toxicities so that the risk/benefit balance can be further optimized. Furthermore, the introduction of TKIs has also raised several new issues such as the tailoring of cancer treatment to an individual patient's tumor and the economics of cancer care. New approaches to determine optimal dosing, assess patient adherence to therapy and evaluate drug effectiveness and toxicity are also required with these novel targeted therapies.

It is increasingly appreciated that the causes of variability in terms of responses and toxicities observed with TKIs are manifold. Yet, the variability is influenced not only by genetic heterogeneity of drug targets (i.e., pharmacodynamic differences), but also by the patients' pharmacogenetic background. A significant source of variation arises from drug disposition, which includes the different processes of absorption, distribution, metabolism and excretion. Considerable pharmacokinetic (PK)

variability is also evident for virtually all of the TKIs. Current evidences have proposed an association between drug exposure with response or toxicities for several TKIs. Additionally, as a result of germline variation in the genes encoding for these enzymes and transporters, expression and activity of these enzymes and transporters are highly variable and may influence patient's exposure to the drugs and sensitivity to the treatment toxicities. Moreover, cancer patients are susceptible to drug-drug interactions (DDIs) as they receive many medications, either for supportive care or for treatment of therapy-induced toxicity. As the cytochrome P450 3A4 (CYP3A4) enzyme is implicated in the metabolism of almost all of the TKIs, there is a substantial potential for interaction between TKIs and other drugs that modulate the activity of this metabolic pathway.

Therefore, the overall aim of this thesis is to evaluate whether pharmacokinetic alterations in TKIs can contribute to toxicities, by focusing on three themes of drug exposure, genetic polymorphism and drug-drug interactions. It is important that these issues with toxicities are addressed to improve the management of anticancer therapy in patients so as to achieve anticancer efficacy and optimize risk/benefit ratio of these therapies.

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List of acronyms

τ	Dosing interval
AACR	American association for cancer research
ABC	ATP-binding cassette
ABCB1	ATP-binding cassette sub-family B member 1
ABCG2	ATP-binding cassette sub-family G member 2
AD	Attenuated dose
AE	Adverse events
ADME	Absorption, distribution, metabolism and excretion
ADR	Adverse drug reactions
ALT	Alanine transaminase
ALP	Alkaline phosphatase
AST	Aspartate transaminase
ASCO	American society of clinical oncology
ATP	Adenosine triphosphate
AUC	Area under the curve
BCRP	Breast cancer resistance protein
CAM	Complementary and alternative medicine
CI	Confidence interval
Cl	Clearance
C_{max}	Maximum (peak) concentration
$C_{max,ss}$	Maximum (peak) concentration at the steady state
C_{min}	Minimum (trough) concentration
$C_{min,ss}$	Minimum (trough) concentration at the steady state
CML	Chronic myelogenic leukemia

CR	Complete response
CSF-1R	Colony stimulating factor receptor Type 1
CT	Computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
CYP	Cytochrome P450
DDI	Drug-drug interaction
DEX	Dexamethasone
DILI	Drug-induced liver injury
DMEM	Dulbecco's modified eagle medium
DMEM/F-12	Dulbecco’s modified Eagle’s Media/Ham’s F12
DMSO	Dimethyl sulfoxide
E	Patients who receive erlotinib without concurrent dexamethasone
E+D	Patients who receive erlotinib with concurrent dexamethasone
EAP	Expanded-access program
ECOG	Eastern cooperative oncology group
EDTA	Ethylenediaminetetraacetic acid
EGFR	Epidermal growth factor receptor
EGFRI	Epidermal growth factor receptor inhibitor
EQ-5D	EuroQoL Group’s Five Dimensions Questionnaire
EWB	Emotional well-being
FACT-G	Functional Assessment of Cancer Therapy-General
FBS	Fetal bovine serum
FDA	US Food and drug administration
FKSI-15	Functional Assessment of Cancer Therapy-Kidney Symptom Index
FKSI-DRS	FKSI-Disease Related Symptom
FLT	Fms-like tyrosine kinase-3
FWB	Functional well-being

GIST	Gastro-intestinal stromal tumors
HER2	Human epidermal growth factor receptor 2
HPLC	High performance liquid chromatography
HRQoL	Health-related quality of life
IDR	Idiosyncratic drug reaction
IQR	Inter-quartile range
IS	Internal standard
ITS	Insulin, transferrin and selenium mix
k	Elimination rate constant
KIT	Stem cell factor receptor
L	Patients who receive lapatinib without concurrent dexamethasone
L+D	Patients who receive lapatinib with concurrent dexamethasone
LFT	Liver function tests
mRCC	Metastatic renal cell carcinoma
MSKCC	Memorial Sloan-Kettering Cancer Center
MTT	Methylthiazolyldiphenyl-tetrazolium bromide
NCCS	National Cancer Centre Singapore
NSCLC	Non-small cell lung cancers
OR	Odds ratio
$OS_{initiation}$	Overall survival from treatment initiation
OS_{total}	Overall survival from the first documented metastasis
P/S	Penicillin/streptomycin
PBS	Phosphate buffered saline
PCR-RFLP	Polymerase Chain Reaction-Restriction Fragment Length Polymorphism
PD	Progressive disease
PDGFR	Platelet-derived growth factor
PFS	Progression free survival

Pgp	P-glycoprotein
PR	Partial response
PRO	Patient reported outcomes
PK	Pharmacokinetic
PWB	Physical well-being
PXR	Pregnane X receptor
RECIST	Response evaluation criteria in solid tumours
RET	Neurotrophic factor receptor
RCT	Randomized control trials
RM	Reactive metabolite
ROS	Reactive oxygen species
RR	Relative risk
RT-PCR	Real-time quantitative PCR
SD	Stable disease
SM Ratio	Sunitinib to metabolite ratio
SNP	Single nucleotide polymorphism
SWB	Social/family well-being
TAMH	Transforming growth factor α mouse hepatocytes
TB	Total bilirubin
TDM	Therapeutic drug monitoring
TKI	Tyrosine kinase inhibitor
TTO	Time trade off
TTP	Time to progression
ULN	Upper limit of normal
UV	Ultraviolet
VAS	Visual analogue scale
Vd	Volume of distribution

VEGFR	Vascular endothelial growth factor

1 Introduction

The number of people diagnosed with cancer during their lifetime has been steadily increasing. [1] This increase in prevalence across the survivorship trajectory is attributed to improvements in cancer survival rates and the aging population, as cancer incidence rates tend to increase with age. At the same time, there is also a continual development of new anticancer drugs. Clinicians' and patients' hopes for elimination of cancer are renewed with each new class of drug(s); but each is also implicated with a new assortment of toxicities which may impact treatment tolerability and health outcomes. Although the survival trend is optimistic, it may come at a price. The need for routine monitoring, long term effects of the disease, and presence of treatment side effects may place a burden on the cancer patients.

1.1 Introduction to tyrosine kinase inhibitors

The advent of molecular targeted therapy in the late 1990s marks a major breakthrough in the fight against cancer. The significant advancement embodied by such pharmacotherapies is the ability to target specific proteins uniquely regulated in cancer cells or those involved in the mechanism for disease progression, so that off-target effects on healthy tissues can be minimized. Targeted therapies may also be used in combination with conventional cytotoxic chemotherapy or even radiation to provide additive or synergistic anticancer activities as their toxicity profiles generally do not overlap. Thus, targeted therapies such as monoclonal antibodies and tyrosine kinase inhibitors (TKIs) represent a new and promising addition to the anticancer armamentarium.

Tyrosine kinases emerged as a major family of proteins frequently dysregulated in various cancers, either through somatic mutations or overexpression. Activated forms of these enzymes can lead to several biochemical effects such as increase in tumor cell proliferation and growth, induce anti-apoptotic effects, and promote angiogenesis and metastasis. Their critical role in the control of cancer phenotypes, coupled to the presence of suitable binding domains for small molecules, has led to the development of many TKIs as anti-cancer agents. Among them, imatinib, an inhibitor of Bcr-Abl and c-KIT, was the first to be approved and is used for the treatment of chronic myelogenic leukemia (CML) and gastro-intestinal stromal tumors (GIST). [2, 3] Non-small cell lung cancers (NSCLC) that often carry dysregulation, specifically somatic mutations, such as the L858R mutation in the epidermal growth factor receptor (EGFR) pathway were also managed with the use of gefitinib [4], erlotinib [5] and more recently, afatinib. [6] Multi-targeted kinase inhibitors such as sunitinib and sorafenib were approved for the treatment of renal cell carcinoma [7] and hepatocellular carcinoma [8] respectively, and have resulted in unprecedented successes. The growth of this industry is accelerating in two directions: first is through identifying new indications of approved agents and second is through the development of new agents to target tyrosine kinases that are involved in the growth of various cancers.

However, the introduction of targeted therapy has also raised several new issues such as the tailoring of cancer treatment to an individual patient's tumor and the economics of cancer care. Additionally, new approaches to determine optimal dosing, assess patient adherence to therapy and evaluate drug effectiveness and toxicity are also required with these novel targeted therapies. [9]

1.2 Common toxicities associated with tyrosine kinase inhibitors

While the use of TKIs have largely mitigated the conventional toxicities of chemotherapeutic agents (e.g. nausea, vomiting, alopecia, myelosuppression), a range of previously unknown and sometimes unpredictable toxicities began to surface. For example, cutaneous toxicity such as acneiform rash was observed with EGFR inhibitors. Sunitinib and sorafenib have caused hand-foot skin reaction (HFSR), while others have manifested more severe toxicities such as cardiotoxicity and hepatotoxicity as observed after therapy with nilotinib and pazopanib, respectively. [10, 11] In fact, 8 out of the 18 food and drug administration (FDA)-approved agents (as of October 2014) have black box warnings associated with their usage, suggesting the severity of toxicities in these agents. (Table 1) Among them, hepatotoxicity is the most recurrently highlighted toxicity, with black box warnings issued against lapatinib, sunitinib, pazopanib and most recently regorafenib and ponatinib. The sales and marketing of ponatinib has been previously suspended by the FDA due to the risk of life-threatening blood clots and severe narrowing of blood vessels, and which the FDA requires several safety measures to be in place before sale and marketing can be resumed. [12] Clearly, such toxicities can impede the wider acceptance of TKIs as a mainstream therapy. Therefore, it is important to identify strategies to decrease the incidence of these toxicities so that the risk/benefit balance can be further optimized.

Table 1. Overview of FDA-approved tyrosine kinase inhibitors (as of October 2014)

	Year of approval	Indication(s)	Targets	FDA black box warning	Dosing administration	Ref
Afatinib (Gilotrif)	2013	- Metastatic NSCLC with EGFR mutations	EGFR, HER2, HER4		40 mg once daily	[6]
Axitinib (Inlyta)	2012	- Advanced RCC	VEGFR-1, VEGFR-2, and VEGFR-3		5 mg twice daily	[13]
Bosutinib (Bosulif)	2012	- CML	Bcr-Abl, Src		500 mg once daily	[14]
Cabozantinib (Cometriq)	2012	- Thyroid Cancer	RET, MET, VEGFR-1, -2 and -3, KIT, TRKB, FLT-3, AXL, and TIE-2	Hemorrhage	140 mg once daily	[15]
Ceritinib (Zykadia)	2014	- ALK+ NSCLC	ALK		750mg once daily	[16]
Crizotinib (Xalkori)	2011	- ALK+ NSCLC	ALK, MET, RON		250 mg twice daily	[17]
Dasatinib (Sprycel)	2006	- CML - Ph+ ALL	Bcr-Abl, Src		100 mg once daily	[18]
Erlotinib (Tarceva)	2004	- NSCLC - Metastatic pancreatic cancer	EGFR		100 – 150 mg once daily	[19]
Gefitinib (Iressa)	2003	- NSCLC	EGFR		250 mg once daily	[20]
Imatinib (Gleevec)	2001	- CML - GIST - Ph+ ALL	Bcr-Abl		300 – 800 mg once daily*	[21]
Lapatinib (Tykerb)	2007	- Metastatic Breast Cancer	EGFR, HER2	Hepatotoxicity	1250 – 1500 mg once daily*	[22]
Nilotinib (Tasigna)	2007	- CML	Bcr-Abl	QT prolongation	300 mg twice daily	[23]
Pazopanib	2009	- RCC	VEGFR-1, VEGFR-2 and VEGFR-	Hepatotoxicity	800 mg once daily	[24]

(Votrient)		- Soft Tissue Sarcoma	3			
Ponatinib (Iclusig)	2012	- CML - Ph+ ALL	Bcr-Abl	Arterial thrombosis and hepatotoxicity	45 mg once daily	[25]
Regorafenib (Stivarga)	2012	- Metastatic Colorectal Cancer - GIST	VEGFR2 and TIE2	Hepatotoxicity	160 mg once daily	[26]
Sorafenib (Nexavar)	2005	- RCC - Unresectable HCC	KIT, FLT-3, VEGFR-2, VEGFR-3 and PDGFR-B		400 mg twice daily	[27]
Sunitinib (Sutent)	2006	- RCC - GIST - pNET	PDGFR (α,β) VEGFR (1, 2, 3), KIT, FLT3, CSF-1R, RET	Hepatotoxicity	37.5 – 50 mg once daily*	[28]
Vandetanib (Caprelsa)	2011	- Thyroid Cancer	EGFR, VEGFR, RET	QT prolongation	800 mg once daily	[29]

Abbreviations: *ALL*, acute lymphoblastic leukemia*; ALK+*, anaplastic lymphoma kinase; *CML*, chronic myeloid leukemia; *FDA*, Food and Drug Administration; *GIST*, gastrointestinal stromal tumor; *NSCLC*, non-small-cell lung cancer; *RCC*, renal cell carcinoma; *HCC*, hepatocellular carcinoma; *Ph+ ALL*, Philadelphia chromosome-positive acute lymphoid leukemia; *pNET*, progressive, well-differentiated pancreatic neuroendocrine tumors
* Dosing administration depends on indication

1.3 Inter-patient variability in exposure of tyrosine kinase inhibitors

Unlike conventional chemotherapies, TKIs are typically administered orally at fixed doses and often on a daily basis. It is well recognized that equivalent drug doses may result in wide inter-patient variability with regards to drug response, as reflected by differences in drug activity and off-target toxicity and this is similarly observed with TKIs. Although each TKI have their specific targets, not all patients with the target mutation will respond and likewise the nature and severity of adverse events also exhibits extensive variations among patients. Considerable pharmacokinetic (PK) variability is also evident for virtually all of the TKIs. For example, inter-patient variation of area under the curve (AUC) levels is 55% [30], 47% [31] and 71% [32] for imatinib, sunitinib and pazopanib respectively. The variability in drug exposure to TKIs may play a role to the variation in the anti-cancer effects as well as the manifestation of toxicities.

1.4 Sources of inter-patient variability

No two individuals respond to a drug in the same way. A drug may work as expected in one patient, but may fail to exert any effect on another. The side effects of the drug may also be acceptable to most people, but may be harmful or lethal to some others. Certainly, there are many factors attributing to these differences. It is increasingly appreciated that the causes of variability observed with TKIs are manifold. The variability is influenced not only by genetic heterogeneity of drug targets (i.e., pharmacodynamics differences), but also by the patients' pharmacogenetic background.

A significant source of variation arises from the PK processes of drug disposition, which includes the different processes of absorption, distribution, metabolism and excretion (ADME). As such, variations to any of the ADME processes, for example as a result of drug-drug interaction, could affect the drug disposition and consequently, patient's response and toxicity. Since both pharmacokinetics and pharmacodynamics processes contribute to the clinical outcome of efficacy and/or toxicity, and genetic variation may occur in either or both of the process, the final clinical outcome is a result of an intricate relationship between all the processes. (Figure 1) Other identified factors that may also contribute to variability include age, gender, organ function, comorbidities, concomitant medications, environment, lifestyle (e.g. smoking, alcohol), and adherence to treatment. [33-35]

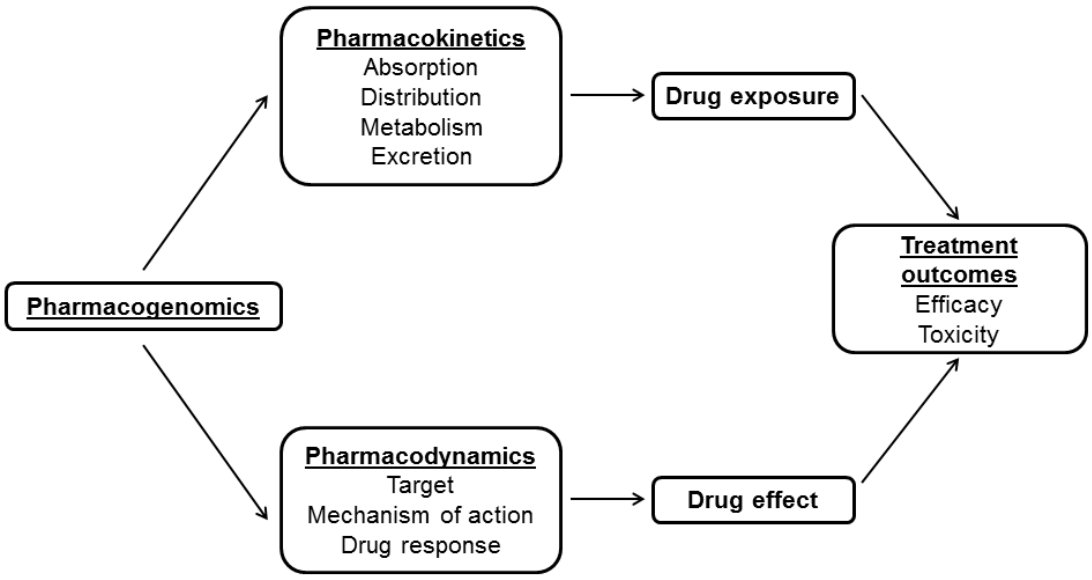


Figure 1. Overview of the processes that influence treatment outcomes

1.4.1 Alterations in absorption

As TKIs are typically taken orally, issues such as compliance, absorption and first-pass metabolism may affect the process of absorption. Variability in intestinal absorption and entero-hepatic circulation, as well as the influence of food and other medications may contribute to the inter-patient variability in drug absorption. Certain diets such as high-fat meals have been known to affect absorption. For instance, the AUC of lapatinib and pazopanib can be increased under the influence of a high-fat meal. [36, 37] However, there are also some TKIs where absorption is not affected by diet, such as imatinib and sunitinib. [38, 39] Furthermore, presence of comorbidities such as gastrointestinal tumors may also affect absorption of drugs. Since absorption limits the amount of drug that goes into the blood stream, any differences in absorption will affect all subsequent processes.

1.4.2 Alterations in distribution

TKIs are extensively distributed into tissues and are highly protein bound, resulting in a large volume of distribution and a long terminal half-life. Hypoalbuminemia secondary to malignant cachexia or liver metastases can increase the amount of free drug, leading to higher risk for toxicity. [34] The distribution process may also be affected by body size. For example, the volume of distribution of sunitinib was affected by body size. [40] Consequently, patients with sarcopenia, low body mass index or low body surface area experienced significantly more dose-limiting toxicities. [41, 42]

1.4.3 Alterations in metabolism

Almost all of the TKIs undergo metabolism by the cytochrome P450 (CYP) family of enzymes, with the CYP3A4 enzyme being the most commonly involved in the metabolism of the majority of the TKIs. Therefore, any alterations to the activity of the enzyme, such as drug-drug interactions (DDIs) or genetic polymorphisms, may have an influence on the drug and metabolite levels.

1.4.4 Alterations in excretion

The majority of TKIs are substrates for drug transporters in the form of efflux pumps (e.g. ABCB1 and ABCG2) or uptake transporters (e.g. SLC22A1). Transport proteins have an important role in regulating the absorption, distribution and excretion of many medications. Similar to drug metabolizing enzymes, the activity of drug transporters may be affected by DDIs or genetic polymorphisms in the transporter.

Although there are various potential causes for such inter-individual variability, differences in metabolism and disposition of drugs, and genetic polymorphism in the drug target receptors may have a large influence on the efficacy and toxicity of medications. [43]

1.5 Association between exposure and response/toxicities

Current evidences have proposed an association between drug exposure with response or toxicities for several TKIs. [34, 44] (Table 2) Drug exposure is commonly measured by trough concentrations (C_{min}) or AUC. For example, imatinib, the

archetypal TKI, clinical response as well as cytogenic and molecular response have been found to be associated with trough concentrations. Toxicities such as hematological toxicities have also been identified to be associated with imatinib concentrations. In another example, sunitinib, it was demonstrated in a meta-analysis that higher sunitinib AUC was associated with a better response, in terms of longer time to progression, longer overall survival and greater reduction in tumor size. It was also shown that higher exposure was associated with an increased risk of several adverse events including hypertension and neutropenia. [45] Consequently, any changes to the drug exposure as a result of the factors mentioned earlier may translate to a deviation in response and toxicities.

Table 2. Correlation of pharmacokinetic parameters, treatment efficacy and toxicity of tyrosine kinase inhibitors

TKI	PK parameter	Clinical response	Ref
Erlotinib	C_{ss}	Improved survival	[46]
Gefitinib	D8/D3 C_{min} ratio	PFS	[47]
	C_{min}	OS	[48]
Imatinib	C_{min}	TTP	[49]
	C_{min}	Molecular response	[50]
	AUC	Response	[51]
	C_{min}	Cytogenic response Molecular response	[52]
Nilotinib	C_{min}	TTP	[53]
Pazopanib	C_{min}	PFS RR	[54]
Sunitinib	AUC	Tumor response PFS OS	[45]
TKI	**PK parameter**	**Toxicity**	**Ref**
Afatinib	AUC/C_{max}	Diarrhea Rash	[55]
Erlotinib	C_{min}	Rash	[56]
	AUC/ C_{max}	Rash	[57]
Gefitinib	C_{min}	Diarrhea	[58]
Imatinib	C_{min}	Hematological toxicity	[50]
	C_{min}	Hematological toxicity	[59]
	AUC	Nunber of adverse events	[51]
Nilotinib	AUC	Anemia Bilirubin elevation	[60]
	C_{min}	QT prolongation	[60]
Pazopanib	C_{min}	Diarrhea Stomatitis HFSR Transaminases elevation	[61]
Sorafenib	C_{min}	Grade 3 toxicity	[62]
	C_{min}	HFSR Hypertension	[63]
	AUC	Grade 3 toxicity	[64]
Sunitinib	C_{min}	Hypertension	[45]
	AUC	Neutropenia	[45]

Abbreviations: *AUC*, area under the curve; C_{max}, peak concentration; C_{min}, trough concentration; C_{ss}, steady-state concentration; *D*, day; *HFSR*, hand-foot skin reaction; *OS*, overall survival; *PFS*, progression free survival; *PK*, pharmacokinetic; *RR*, response rates; *TTP*, time to progression

1.6 Genetic variation of drug exposure

The tumor or somatic genome, mainly determines the features of the tumor such as its aggressiveness and sensitivity to treatment. On the other hand, the patient or germline genome primarily dictates how the body handles and reacts to the chosen treatment. [65] The pharmacokinetics of a drug, which may determine its efficacy and toxicity, is dictated by the latter. As mentioned in the earlier sections, large variations in pharmacokinetics exist between patients. Although there are various potential causes for this variability, inherited differences in metabolism and disposition of drugs, and genetic polymorphism in the drug target receptors may have a large influence on the efficacy and toxicity of medications. [43] For every individual drug, several enzymes as well as transporters are involved in the ADME process. As a result of germline variation in the genes encoding for these enzymes and transporters, expression and activity of these enzymes and transporters are highly variable and may influence patient's exposure to the drugs and sensitivity to the treatment toxicities.

Almost every enzyme involved in drug metabolism exhibits genetic polymorphisms that may contribute to inter-individual variability in drug response. [66] However, not all polymorphisms are clinically relevant. Among the family of CYP enzymes, CYP2D6 represents one of the best studied and understood examples of pharmacogenetic variation in drug metabolism, which can affect drugs like antidepressants and antipsychotics etc. [67] Just about all of the TKIs undergo metabolism by CYP enzymes, with the CYP3A4 being involved in the metabolism of the majority of the TKIs. (Table 3) Additionally, as both CYP3A4 and CYP3A5 share substrate specificity, polymorphism in CYP3A5 would influence the overall CYP3A activity in humans. The defective CYP3A5 enzyme associated with the *3 allele may

cause an accumulation of the parent drug, therefore the single nucleotide polymorphism (SNP) in CYP3A5 is of importance as the mutant CYP3A5*3 allele is highly prevalent in Asians. [68]

Transport proteins play an important role in regulating the absorption, distribution and excretion of many medications. The most extensively studied transporters are members of the adenosine triphosphate (ATP)-binding cassette (ABC) family of transporters, such as the P-glycoprotein (Pgp), which is encoded by the ABC sub-family B member 1 (ABCB1) gene. Pgp affects the pharmacokinetics (PK) of a drug by affecting the processes of oral absorption, renal clearance and uptake into tissues such as the brain. In cases where an individual has an increased expression of Pgp, reduced oral bioavailability, decreased plasma concentrations, increased renal clearance and decreased drug exposure would be expected. [69] Therefore, polymorphisms with the ABCB1 gene may affect the expression of drug transporters and thus bioavailability, although the functional effect of the polymorphism on the Pgp has been heavily debated. [69]

Furthermore, there is also marked heterogeneity in the types and frequencies of the polymorphisms among the different populations and ethnic groups. This means that the optimal dose of medications may also differ among the populations.

Table 3. Metabolism profile of FDA-approved tyrosine kinase inhibitors

	Major CYPs	**Minor CYPs & others**
Afatinib	Negligible	
Axitinib	CYP3A4 CYP3A5	CYP1A2 CYP2C19 UGT1A1
Bosutinib	CYP3A4	-
Cabozantinib	CYP3A4	CYP2C9
Crizotinib	CYP3A4 CYP3A5	-
Dasatinib	CYP3A4	FMO-3 UGT
Erlotinib	CYP3A4	CYP1A2 CYP1A1
Gefitinib	CYP3A4 CYP2D6	-
Imatinib	CYP3A4 CYP2C8	CYP1A2 CYP2D6 CYP2C9 CYP2C19
Lapatinib	CYP3A4 CYP3A5	CYP2C19 CYP2C8
Nilotinib	CYP3A4	CYP2C8
Pazopanib	CYP3A4	CYP1A2 CYP2C8
Ponatinib	CYP3A4	CYP2C8 CYP2D6 CYP3A5
Regorafenib	CYP3A4	UGT1A9
Sorafenib	CYP3A4	UGT1A9
Sunitinib	CYP3A4	-
Vandetanib	CYP3A4	FMO-1 FMO-3
Note: All information was obtained from product information labels [70, 71]		

1.7 Drug-drug interaction in the pharmacokinetic pathway

DDIs occur when a patient's pharmacological or clinical response to the drug is modified by administration or co-exposure to another drug. Pharmacokinetic interactions occur when one drug influences the pharmacokinetic processes such as absorption, distribution, metabolism and excretion, of another drug. This thesis focuses on DDI involving metabolism as altered metabolism is among the most complex of these processes by which DDIs can occur, and induction or inhibition of hepatic enzymes by drugs are often implicated. The clinical consequences of enzyme induction or inhibition depend on the pharmacological and toxic effect of both the parent drug and its metabolite(s). For example, if the parent compound is more active than its metabolite, inhibition of metabolism increases the exposure to the drug and also its therapeutic and/or toxic effects. However, if the parent compound is a pro-drug, inhibition of metabolism may result in a decrease in therapeutic efficacy. More recently, another paradigm of interaction arises when the metabolite is more toxic, hence induction of metabolism down this pathway can exacerbate toxicity.

Central to the metabolism of drugs are the CYP family of enzymes. This consists of numerous enzymes that are responsible for the Phase I metabolism of many drugs, nutrients, endogenous substances, and environmental toxins. The main CYP enzyme, CYP3A4, is responsible for the metabolism of more than 50% of all drugs in the market. It is also implicated in the metabolism of almost all of the TKIs. Therefore, there is a substantial potential for interaction between TKIs and other drugs that modulate the activity of this metabolic pathway. The degree of interaction is also dependent on the extent of hepatic clearance compared to overall clearance, and whether intrinsic clearance is a limiting factor for the drug of concern.

Cancer patients are susceptible to DDIs as they receive many medications, either for supportive care or for treatment of therapy-induced toxicity. [72] For instance, an observational study highlighted that patients were receiving on average 6.8 drugs in addition to sunitinib. Among them, antihypertensive drugs were most commonly prescribed, followed by analgesics, antiemetics and thyroid substitution therapy. [73] In certain cases, a cancer patient's pharmacokinetic parameters may also be altered, for example, edema affecting volume of distribution or impaired drug absorption due to malnutrition or mucositis; these issues may also affect the consequences of DDIs. Since most cancers typically occur at a later age, these patients may also be receiving other drugs for the management of their comorbidities. Differences in DDI outcomes are generally minor because of the wide therapeutic windows of common drugs; however, in cancer chemotherapy with anti-cancer drugs, serious clinical consequences may occur from small changes in drug metabolism and pharmacokinetics. [74]

1.8 Role of therapeutic drug monitoring and individualized therapy

For conventional chemotherapy, therapeutic drug monitoring (TDM) is regarded as impractical for routine use in clinical practice due to various reasons such as the lack of established therapeutic ranges and concentration-effect relationships, the frequent use of multi-drug combinations with overlapping therapeutic and toxic effects, relatively short elimination half-lives and multiple blood samples are needed to adequately define systemic exposure. [35, 75-77] Hence, with the exception of methotrexate, TDM for anticancer drugs are not routinely used in clinical practice. [35] On the other hand, targeted therapy like TKIs are prime candidates for a TDM program. TKIs are administered daily via the oral route and at fixed doses for most of

the time. These drugs possess long elimination half-lives, which makes it possible to estimate drug exposure with a single measurement. TKIs also fulfill most of the traditional prerequisites of TDM, which are long-term therapy, significant inter-individual and relative low intra-individual variability, a narrow therapeutic range, a defined and consistent exposure-response (efficacy/toxicity) relationships, availability of a validated sensitive bioanalytical method and the absence of an easily measurable biomarker for drug effects. [76, 78]

There are several potential benefits of TDM in TKIs. Firstly, under-dosing and over-dosing may be prevented by tailoring the doses to achieve desired drug levels. Secondly, drug concentrations may also be used to delineate whether toxicities are related to the targeted agent or to other causes. Next, it may also be useful in detecting drug-drug interactions as well as to monitor adherence. [35] Although pharmacokinetic targets have been proposed for several TKIs, most remains to be validated in prospective clinical studies. Clinicians should be aware of the limitations of the clinical studies of which the PK targets were based on. The type of patients enrolled, limited cohort of patients, blood sampling protocol as well as method of data analysis should be taken into consideration before adopting the respective PK targets in clinical practice. Although currently available evidences are insufficient to mandate TDM in clinical practice, this option should be further explored due to the favorable PK profile and benefits. The enhanced efficacy and abrogation of toxicity as a result of accurate dosing by TDM will give a competitive advantage for these drugs.

1.9 Research gaps and specific aims

1.9.1 Research gaps and hypothesis

The previous sections summarized the background literature surrounding the main topic of this thesis, which is how alterations in pharmacokinetics may affect the presence of toxicities. TKIs are administered orally and are reported to have large inter-individual differences in their pharmacokinetics. This leads to the following questions: (1) How does this inter-individual variation in pharmacokinetics play a role in the manifestation of toxicity? (2) How does this affect drug and metabolite levels and thus affecting the risk of toxicity? (3) By understanding the pharmacokinetics and its effect on toxicity, can we adopt an individualized therapy regimen, so as to ensure a balance between efficacy and toxicity?

The key research question that this thesis aims to answer is whether pharmacokinetic alterations in TKIs can contribute to toxicities, by focusing on drug exposure, genetic polymorphisms and DDI. Although the exposure-response and pharmacokinetics targets (target drug levels) have been proposed for several TKIs, most of the information was derived through retrospective analysis. [76] Different malignancies were also grouped together to facilitate analysis, although cancer types may enact as a source of pharmacokinetic variation. The somatic genome primarily determines tumor prognosis and response while the germline genome modulates treatment exposure and toxicity. As genetic polymorphisms in the pharmacokinetic pathway may affect exposure, and thus toxicity, it is important to study them in an Asian population, where the frequencies of the various genotypes may be different from other population such as Caucasians or Africans. Cancer patients are at risk of DDIs due to

the large number of medications they receive. DDIs are important in patient receiving TKIs but prospective studies are limited and challenging. Outcomes of DDI of common drugs are broadly unnoticeable due to their wide therapeutic index; however, in anti-cancer drugs like TKIs, serious clinical consequences may occur from small changes in the drug metabolism and pharmacokinetics.

This thesis hypothesizes that pharmacokinetic alterations in TKIs play a role in TKI-associated toxicities.

1.9.2 Specific aims

The overall aim of this thesis is to evaluate whether pharmacokinetic alterations in TKIs can contribute to toxicities. There are three specific aims focusing on the three themes in this thesis – drug exposure, genetic polymorphism and drug-drug interactions.

Most exposure-response correlations are defined by retrospective analysis. Hence, this thesis aimed to explore the association between the manifestation of TKI-associated toxicities and plasma drug levels and consequently, to determine the role of TDM as a possible management strategy for TKI-induced toxicity.

The variability observed with TKIs is influenced not only by genetic heterogeneity of drug targets, but is also contributed by the patients' pharmacogenetic background and also the environmental factors that influence pharmacokinetics. Thus, the disposition of TKIs, which may be affected by variability of proteins in their pharmacokinetic pathway, could play a role in explaining the differences in response and in toxicities

observed. Therefore, the thesis aimed to explore the role of genetic polymorphism in TKI-associated toxicities, by investigating the association between genetic polymorphisms of key drug metabolizing enzyme and drug transporters and their risk for toxicities.

The main CYP enzyme, CYP3A4, is implicated in the metabolism of almost all of the TKIs. Therefore, there is a substantial potential for interaction between TKIs and other drugs that modulate the activity of this metabolic pathway. This thesis aims to assess the effect of metabolism-related pharmacokinetic drug-drug interactions on risk for TKI-associated toxicity. Based on what is known as risk factors for developing drug-induced liver injury (DILI), it is not surprising to observe this with TKIs use, as most of them possess the risk factors such as high daily dose, substrate of P450 enzyme, generation of reactive metabolites and involved in significant hepatic metabolism. Moreover, the liver is the main site of metabolism and any metabolites formed could likely induce a localized damage, thereby convicting the liver at risk for metabolism-related DDI toxicities. Therefore, this thesis will use hepatotoxicity as a case study and will evaluate how the manifestation of DDI affects the risk of hepatotoxicity.

1.9.3 Overall approaches

This project adopts both prospective and retrospective approaches to investigate the role of pharmacokinetic alterations in TKI-associated toxicities in an Asian population. (Table 4)

To address the first and second aims, a prospective, cohort study will be conducted in Asian metastatic renal cell carcinoma (mRCC) patients receiving sunitinib. Drug exposure to sunitinib in these mRCC patients will be determined in chapter 2. The association between the manifestation of sunitinib-associated toxicities and plasma drug levels will be studied in chapter 3. In addition, the in-vitro toxic potential of sunitinib and its active equipotent metabolite, SU12662, will also be evaluated. Further, the relationship between genetic polymorphisms of CYP3A5 and ABCB1 and their risk for toxicities will also be investigated in chapter 4.

For the third aim, the role of metabolism-related DDI in TKI therapy will be explored. The effect of metabolism-related drug interaction on hepatotoxicity will be evaluated in a study involving lapatinib and dexamethasone, where a nested case-control study, as well as a parallel in-vitro cell culture set up will be adopted. Additionally, this thesis will further address why TKIs are at risk for hepatotoxicity and quantify the risk through a meta-analysis.

Table 4. Overall aims, research questions and approaches outlined in this thesis

Aims	Research questions	Approach
1. To explore the association between the manifestation of TKI-associated toxicities and plasma drug levels	What is the association between plasma level of sunitinib and/or active metabolite and the manifestation of toxicity?	Prospective, cohort study
	What is the difference in the in-vitro toxic potential of sunitinib vs. its active metabolite?	In-vitro cell toxicity assays
2. To explore the role of genetic polymorphism in TKI-associated toxicities	What is the association between CYP3A5 and ABCB1 SNPs with the manifestation of toxicity?	Prospective, cohort study
	What is the association between CYP3A5 and ABCB1 SNPs with the plasma level of sunitinib and/or active metabolite?	
3. To assess the effect of metabolism-related pharmacokinetic drug-drug interactions on risk for TKI-associated toxicity	What is the role of metabolism-related DDIs in TKI therapy?	Systematic review
	What is the risk of TKI-induced hepatotoxicity in cancer patients?	Meta-analysis
	Why is TKI at risk for hepatotoxicity?	Systematic review
	Does the CYP3A4 inducer, dexamethasone, affect the incidence of hepatotoxicity of lapatinib?	Nested case-control In-vitro cell toxicity assays

1.10 Scope of thesis

Two TKIs will be selected as drug candidates for investigation in this thesis. Sunitinib will be used for the first and second aims, while lapatinib will be used for the third aim.

1.10.1 Sunitinib

Sunitinib is a small molecule that inhibits multiple receptor tyrosine kinases, such as platelet-derived growth factor (PDGFRα and PDGFRβ), vascular endothelial growth factors (VEGFR1, VEGFR2, VEGFR3), stem cell factor receptor (KIT), Fms-like tyrosine kinase-3 (FLT3), colony stimulating factor receptor Type 1 (CSF-1R), and the glial cell-line derived neurotrophic factor receptor (RET). Sunitinib demonstrates ability to inhibit PDGFRβ- and VEGFR2- dependent tumor angiogenesis in-vivo. The approval of sunitinib has significantly improved the outlooks of patients with mRCC. In the Phase III clinical trial that led to its approval, sunitinib showed significant prolongation of progression-free survival as compared with previous gold standard therapy, interferon-α (11 months vs. 5 months, $p<0.001$). [79] In a recent trial that compared the efficacy of sunitinib with another multi-targeted TKI, pazopanib, both demonstrated similar efficacy, although sunitinib was associated with a higher incidence of toxicities such as severe fatigue, HFSR and thrombocytopenia. [80] Currently, sunitinib is one of the recommended agents for first line treatment of mRCC. [44]

Sunitinib is administered at 50 mg, orally daily for 4-weeks, followed by 2-weeks off, in a repeated 6-week dosing cycle for mRCC. Following administration in humans, sunitinib is primarily metabolized by CYP3A4 to an active N-desethyl metabolite,

SU12662. The metabolite, SU12662, exhibits similar potency as sunitinib in biochemical tyrosine kinase and cellular proliferation assays, acting towards VEGFR, PDGFR and KIT. [28] Sunitinib together with SU12662 form the major drug related compounds in plasma, accounting for 91.5% in pooled samples, with sunitinib having 3 – 4 times higher exposure than SU12662. Combined plasma concentrations of sunitinib and SU12662 ranged from 50 – 100 ng/ml has been shown to be effective for its anti-proliferative effects. [81, 82] Both sunitinib and SU12662 have previously been shown to display linear pharmacokinetics and have prolonged half-lives of about 40 and 80 h, respectively. [28] To a large extent, the exposure to sunitinib accounts for its efficacy in disease management.

Although sunitinib produces favorable clinical outcomes, high incidences of toxicities are associated with its therapy. These toxicities such as HFSR, thrombocytopenia and stomatitis were dose-limiting and resulted in a high rate of dose reductions and discontinuations. [79, 83, 84] Incidence of dose reductions and toxicities were also observed more frequently in Asian populations. Dose reduction was observed to be 46% in a Korean study [84], which is significantly higher than that previously observed in the pivotal phase III trial (32%) [79] and the expanded-access program (EAP) (33%). [83] High grade (more than grade 3) toxicities such as HFSR and thrombocytopenia were also more common in the Korean study (HFSR: 16% and thrombocytopenia: 16%), than in previous phase 3 trial (HFSR: 5% and thrombocytopenia: 8% respectively). In a sub-analysis conducted with the EAP population, incidence of high grade toxicities was more prevalent in the Asian population. [85] For instance, the incidence of high-grade HFSR was 13% in Asians compared with 6% in non-Asians and that of high-grade thrombocytopenia was 26%

and 13% in Asians and non-Asians, respectively. Altogether, these reports suggest a distinct difference in the incidence and severity of some adverse events observed between populations.

Consequently, mRCC patients at our local cancer centre, National Cancer Centre Singapore (NCCS), are receiving an attenuated dose (AD) of sunitinib (37.5 mg daily for 4-weeks, followed by 2-weeks off). [86] In a recent study, Houk *et al* demonstrated that a higher sunitinib exposure was associated with longer time to progression, longer overall survival, greater reduction in tumor size and increased risk of adverse events (fatigue, hypertension and neutropenia). [45] Thus, leading to the question of whether sufficient exposure is achieved with the attenuated dose of sunitinib and how does this attenuated dose reflects in the incidence of toxicities.

Sunitinib is a desirable choice to address both the first and second aims for various reasons. Firstly, since an AD of sunitinib is being used by local mRCC patients, this study seeks to provide an objective measure of effectiveness for this off-label AD regimen. As toxicities and consequently, dose reductions are common in this group of patients, sunitinib is an excellent candidate to study the effect of drug exposure with clinical response and toxicities. Furthermore, sunitinib is one of the TKIs where proposed pharmacokinetic targets are available. [76] Therefore, it would be valuable to study whether the AD provides sufficient exposure for our local patients, by taking reference to the recommended therapeutic target concentration.

In addition, since both sunitinib and SU12662 acts on similar receptors like VEGFR, PDGFR and KIT, toxicities associated with sunitinib therapy could also be likely

attributed to SU12662. As such, could either sunitinib or SU12662 be more toxic than the other? If so, how does variation in the activation pathways affect toxicities?

1.10.2 Lapatinib

Lapatinib is a small molecule dual-kinase inhibitor which inhibits the growth of tumor cells that over-express the human epidermal growth factor receptor 2 (HER2) and EGFR. Lapatinib is indicated in combination with capecitabine, for the treatment of patients with HER2-positive advanced or metastatic breast cancer who have received prior therapy including an anthracycline, a taxane and trastuzumab. [87] It is also indicated in combination with letrozole for treatment of HER2-positive metastatic breast cancer in postmenopausal women. [88]

Side effects of lapatinib are relatively mild, which include diarrhea, rash, pruritus, and nausea. However, a very small subset of patients (less than 1%) succumbs to potentially life threatening liver injury. A black box warning for lapatinib use has been issued to warn prescribers against the potential occurrence of hepatotoxicity. [22] Existing clinical evidence indicates that lapatinib-induced hepatotoxicity is idiosyncratic.

A recent study demonstrated that metabolism of lapatinib generates a reactive metabolite (RM), which has been implicated in other examples of idiosyncratic hepatotoxicity. The generation of lapatinib RM has reported to be from metabolism by CYP3A4 and CYP3A5. [89-91] Many RMs have intrinsic reactivity towards certain types of cellular macromolecules, leading to disruption of protein, lipids, DNA and oxidative stress. Additionally, they may cause mitochondrial dysfunction and loss

of energy production. This impairment of cellular function can result in cell death and possible organ failure. [92] Therefore, factors that affect metabolism and thus generation of RM, such as polymorphism in drug metabolizing enzymes, and DDI could likely affect the manifestation of toxicity. For instance, metabolism of acetaminophen in the liver includes the formation of a RM, the N-acetyl-p-benzoquinoneimine. [93] In the presence of an inducer, RM formation was increased which markedly enhanced hepatotoxicity. [94] Since the metabolism of lapatinib also generates reactive quinoneimine species, factors that increase metabolism may potentially affect toxicity.

The liver is the regulator of chemical homeostasis in the body and is the main site for bioactivation and detoxification of drugs and their metabolites. Hence, any potentially toxic metabolite generated in the process can readily exert a localized damage. Therefore, we propose the use of hepatotoxicity as a model to study the effects of metabolism on lapatinib-induced toxicity.

Lapatinib is a good drug candidate in this context because of its association with a black box warning for hepatotoxicity. The onset of hepatotoxicity appears to carry classical features of idiosyncrasy such as high dosage (> 100 mg) required for clinical use (also seen with acetaminophen, felbamate and procainamide), substrate of P450 enzyme and has significant hepatic metabolism. [95, 96] Although several TKIs have been reported to be capable of generating RM, lapatinib is one of a few TKIs where its metabolites have been extensively characterized (the others being erlotinib, gefitinib and dasatinib).

1.11 Significance of thesis

The number of people diagnosed with cancer during their lifetime has been steadily increasing. [1] Although the survival trend is optimistic, it may come at a price. The need for routine monitoring, long term effects of the disease, and presence of treatment side effects will place a burden on the cancer patients.

Large clinical trials provide efficacy and safety data from a group of carefully selected patients. The characteristics of the population involved in the clinical trials may sometimes be different from the actual patients in clinical practice. Furthermore, although these clinical trials are useful for determining which drug is superior across the patient population, they provide little about which drug is best for an individual. Thus, not surprisingly, there is considerable variation between patients in response to a given drug therapy.

The uncertain nature of patients' response may impose further challenges to a patient's therapy. The new anti-cancer TKIs are generally costly and ineffective therapy is a waste of financial resources. The presence of treatment-related toxicities may also impair the patient's quality of life. Furthermore, an ineffective therapy may encourage tumor resistance, hampering tumor control with further lines of therapy. These costs make it critical that we develop strategies for selecting optimal treatments for individual patients.

Dose reductions of TKIs as a result of toxicity are common, and are often empirical. Under-dosing compromises efficacy, yet determination of the appropriate extent of dose reductions to prevent an ineffective dosage is often difficult. By understanding

this association, a therapeutic window for these agents could be established and with that the role of TDM for TKIs could also be studied as a possibility.

Variations in drug exposure and thus toxicity may be likely due to genetic polymorphisms. These genetic polymorphisms can arise from genes involved in either or both pharmacokinetics and pharmacodynamics of the TKI. Here, we focus on genetic polymorphisms in genes that affect the pharmacokinetics pathway of the TKI. By identifying specific populations of patients who may be at higher risk of toxicity, individualization of therapy could be performed for each patient. Appropriate starting dose and management strategies could be adopted for individual patients with an aim of balancing efficacy and toxicity.

Polypharmacy is common among cancer patients, especially in elderly patients. Since most TKIs undergo metabolism by CYP enzymes, especially CYP3A enzymes, DDIs would be very common as these enzymes are responsible for the metabolism of more than 50% of the drugs available in the market. As DDIs may affect the exposure to TKIs, it is vital to understand how these interactions may affect exposure and thus risk of toxicity. This will be important in managing patients with DDIs.

Since the significant enhancement of the anticancer armamentarium with the introduction of targeted anticancer therapies, cancer has transformed from a death sentence into a chronic disease. Therefore, it is important that we address these issues with toxicities so as to achieve anticancer efficacy and optimize risk/benefit ratio of these therapies. Ultimately, this project seeks to provide evidence for individualization of TKI therapy for cancer patients, to ensure a balance between

efficacy and toxicity. Furthermore, individualized therapy could also lead to cost savings, where a lower dose could be used in a patient who achieves adequate drug exposure with acceptable response and minimal toxicity.

2 Drug exposure in the use of an attenuated dosing regimen of sunitinib in Asian metastatic renal cell carcinoma patients

2.1 Use of attenuated dosing regimen of sunitinib

The approved dosing of sunitinib for mRCC is 50 mg once daily for 4 weeks followed by 2 weeks of rest in a repeated 6-week cycle. One study has demonstrated that an AUC at the steady state of above 0.8 µg•h/ml in mRCC patients is associated with a longer time to progression (TTP), longer overall survival, a higher probability of a response and greater reduction in tumor size. [45] A recent paper proposed a combined minimum sunitinib and SU12662 concentration of more than 50 ng/ml and less than 100 ng/ml as pharmacokinetic targets for efficacy and safety, respectively. [76]

However, high rates of dose modifications, including dose reductions (32–46%) and discontinuations (38%), have been reported with the approved dosing regimen due to systemic toxicities. [79, 83, 84] In view of the high incidences of toxicity and dose modifications, patients at NCCS have been routinely prescribed attenuated doses of sunitinib at 37.5 mg once daily for 4 weeks followed by 2 weeks off in a repeated 6-week cycle (referred to as attenuated dosing, AD) as the first line therapy for treatment of mRCC.

2.2 Evaluation of efficacy and safety outcomes between conventional and attenuated dosing regimen

A retrospective analysis conducted by our collaborators compared the efficacy and safety outcomes between the conventional dosing and AD regimen. Clinical data was retrospectively collected on all patients receiving sunitinib at any line of therapy at the NCCS, Johns Hopkins-International Medical Center, National University Hospital Singapore, and Onco-Care of Gleneagles Medical Center from 2005 to 2012. A total of 160 patients were included in the analysis, where 127 received the AD while 33 patients received the conventional dosing regimen.

The disease control rate between patients on conventional dosing and AD regimens were similar, where 23 of 29 (79.3%) and 79 of 120 (65.8%) patients achieved disease control respectively (p=0.19). Likewise, objective response rates were 12/29 (41.4%) and 39/120 (32.5%) respectively (p=0.36). Overall survival from treatment initiation ($OS_{initiation}$), overall survival from the first documented metastasis (OS_{total}), and progression free survival (PFS) were similar for patients receiving first-line sunitinib for conventional relative to attenuated-dose regimens ($OS_{initiation}$: 18.3 vs. 16.5 months, p=0.68; OS_{total}: 27.4 vs. 21.8 months, p=0.84; PFS: 6.7 vs. 7.9 months, p=0.89 respectively), similar to real-world outcomes in Western studies. Noticeably lower rates of severe toxicities (60% vs. 85%, p=0.008), dose delays (24% vs. 58%, p<0.001) and dose reductions (35% vs. 70%, p<0.001) were also observed with the AD regimen when compared with the conventional dosing regimen. [86]

Therefore, this analysis performed by our collaborators demonstrated that the AD sunitinib regimen has comparable efficacy and improved safety outcomes in comparison with the conventional sunitinib dosing regimen. This result represents the first data supporting an alternative sunitinib dosing regimen in a population differing from the typically Caucasian profiles of most clinical trials. This is particularly important since the prevalence and severity of toxicities in actual usage is commonly different from that observed in Phase III trials. [97] While the results do not represent the methodological equivalent of a randomized controlled trial (RCT) in comparing dosing regimens, it is unlikely that a RCT will ever be conducted to examine this dosing issue, given the high toxicities experienced in the Asian population at conventional-dose regimens reported here and by others. In summary, this regimen could be considered as a standard of care in Asian mRCC patients.

2.3 Pilot study to determine drug exposure to sunitinib and SU12662 in patients receiving the attenuated dosing regimen

To provide an objective measure of effectiveness for this off-label AD regimen, a prospective cohort study was conducted to determine the level of exposure to sunitinib and SU12662 in patients with mRCC receiving the AD regimen. In the following two chapters, further analysis with this cohort of mRCC patients will be conducted. The association between the manifestation of sunitinib-associated toxicities and plasma drug levels will be assessed. (Outlined in Chapter 3) Through the quantification of sunitinib and SU12662, a better understanding can be determined as to whether the parent or metabolite might be a better predictor for toxicity and efficacy. The relationship between genetic polymorphisms of CYP3A5 and ABCB1 as key determinants of sunitinib disposition and their risk for toxicities will also be

investigated. (Outlined in Chapter 4) Ultimately, the results from these studies could provide potential evidence for the role of TDM in patients receiving this therapy.

2.3.1 Methodology

2.3.1.1 Study design

This was a single-centred, prospective, longitudinal study conducted at the NCCS between June 2011 and October 2013. The study was approved by the SingHealth Centralized Institutional Review Board (CIRB 2011-142-B). The procedures were in accordance with the ethical standards of the responsible committee on human experimentation.

2.3.1.2 Patients and follow up

All adult patients with a confirmed diagnosis of mRCC and who are newly starting on sunitinib were invited to participate in the study. Patients need not be treatment-naïve. Written informed consent was sought from the potential subjects who met the inclusion criteria prior to inception of the study. Patients were excluded if they are unable to provide written informed consent, if they were not receiving sunitinib for the treatment of mRCC or if they were currently receiving sunitinib.

During weeks 3–4 of each treatment cycle (when the steady state is achieved), a blood sample was drawn to assess the steady-state plasma levels of sunitinib and SU12662. Patients were followed up for up to a maximum of 3 cycles. Due to the outpatient

setting of this study and taking into account the convenience and compliance of patients, and the feasibility of monitoring, only 1 sample was taken for each cycle. In this study, sampling may occur at any time during office hours, based on patient's convenience. However, the time lapse between sampling time and last dose was noted and was used in the estimation of plasma concentrations.

2.3.1.3 Treatment

Due to the low tolerance of the standard dose in the Asian population, patients were initiated with an AD regimen of sunitinib. Dose discontinuations and adjustments (reductions and increases) of 6.75–12.5 mg were performed by the physician depending on patients' treatment responses and toxicity.

2.3.1.4 Data collection

Treatment-related information was collected from the patients during every clinic visit. The following data was collected through patient interviews, patient case-notes and electronic medical records: demographics, disease characteristics, medical history, treatment records and investigations, such as diagnostic scans and laboratory tests results. Two commercially available databases, Micromedex [98] and Lexicomp [99], were utilized to check for any potential DDIs. Patient's use of complementary and alternative medicine (CAM), if any, was also obtained. In the event when blood sampling was declined, toxicity, clinical response and other relevant study data were collected wherever possible.

2.3.1.5 Processing of blood samples

Blood samples was collected from the patients in ethylenediaminetetraacetic acid (EDTA)-containing blood collection tubes and processed immediately by transfer to a 15 ml centrifuge tube and centrifugation at 2500 rpm, 4 °C, for 10 minutes. Subsequently, plasma was extracted and stored at –80 °C until analysis.

2.3.1.6 Analysis of plasma sample

Plasma samples were analyzed according to the validated methodology adapted from Etienne-Grimaldi et al. [100]

2.3.1.6.1 Chemicals and materials

Sunitinib and vandetanib, the internal standard (IS), were obtained from BioVision (Mountain View, CA, USA), while SU12662 was obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Acetonitrile was from Merck (Darmstadt, Germany) and tert-butyl methyl ether was obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.3.1.6.2 Preparation of calibration curve

The calibration curve was prepared in blank plasma. Final concentrations were 0–2.5–5–10–25–50–100–250 ng/ml for sunitinib and SU12662 (500 µl per data point).

2.3.1.6.3 Extraction procedures

Patients' plasma (500 µl) was added into a 15 ml centrifuge tube, and was spiked with 20 µl of vandetanib (IS) at 0.5 mg/ml. Four milliliter of tert-butyl methyl ether was then added and tubes were vortexed for 30 seconds, after which the tubes were centrifuged at 2500 rpm, 4 °C, for 10 minutes. Next, the resulting organic phase was extracted into a new tube and evaporated to dryness at 50 °C, under a nitrogen stream. The residue was then reconstituted with 200 µl of the mobile phase, vortexed for 10 seconds and subsequently centrifuged at 2500 rpm, 4 °C, for 10 minutes, before being transferred into the microvials.

2.3.1.6.4 High Performance Liquid Chromatography (HPLC) analysis

The Hewlett Packard HP Agilent 1100 system was used for the analysis. The high performance liquid chromatography (HPLC) column was Superiorex ODS 5 µm, 4.6 mm × 250 mm from Shiseido. The mobile phase was composed of 20 mM of ammonium formate at pH 3.25 (adjusted with formic acid) and acetonitrile, following a 60:40 ratio respectively. The flow rate was 0.8 ml/min, injection volume was 20 µl and each run time was 15 minutes. Detection was performed using ultraviolet (UV) at 431 nm, which provided good sensitivity and selectivity for both the IS and the compounds (sunitinib and SU12662). [101] Integration was performed according to peak height. Concentrations were calculated from the calibration curves, normalized by the IS, using the following equation: Concentration (ng/ml) = Slope × [peak height of interest/peak height of IS] + constant.

2.3.1.7 Pharmacokinetic analysis

With one blood sample obtained from the patient during each treatment cycle, patient-specific parameters for sunitinib and SU12662, could not be determined in this study. Hence, clearance (Cl) and volume of distribution (Vd), were estimated by the application of equations reported by Houk et al. This was selected as opposed to the pharmacokinetic parameters reported in the product information as the Cl and Vd estimated from Houk et al accounts for the effects of body weight, gender, cancer type and Asian race. [40] Weight of the patient was obtained from patient's case notes. If weight of the patient was missing for all cycles, the average weight of the patients (male or female) was used in the estimation. If weight of the patient was missing for any cycle, it was assumed there was no change in weight and the value for baseline/previous cycle was used.

Although the models for both sunitinib and SU12662 were two-compartmental model with first-order absorption and first-order elimination [40], further pharmacokinetic parameters such as elimination rate constant and subsequently maximum concentration at the steady state ($C_{max,ss}$) and the minimum concentration at the steady state ($C_{min,ss}$) were determined using a one-compartmental model. Elimination rate constant (k) was approximated from the population Cl and Vd. [40] As blood samples were obtained during week 3–4 of the cycle, which is during "steady state", peak and trough concentration are assumed to be similar for each dosing interval.

After the plasma concentrations of sunitinib and SU12662 had been determined by HPLC, and taking into account the time of blood collection (number of hours since

last sunitinib dose), *k* and the dosing interval (τ, 24 hours), the $C_{max,ss}$ and the $C_{min,ss}$ were then determined by application of the equations found in Table 5.

As sunitinib demonstrates a linear dose–concentration relationship at doses up to 100 mg [28], all plasma $C_{max,ss}$ and $C_{min,ss}$ levels were normalized to 37.5 mg to compensate for differences in doses between cycles and between patients. Total plasma drug (sunitinib and SU12662) concentration and sunitinib to metabolite ratio (SM ratio) were also calculated accordingly.

Table 5. Equations used in the estimation of drug exposure

Clearance (Cl) of sunitinib[a]	$Cl/F_{parent} = 51.8 \cdot (1–0.0876 \cdot sex) \cdot (1–0.13 \cdot race_{Asian}) \cdot (1–0.285 \cdot type_{GIST}) \cdot (1–0.269 \cdot type_{ST}) \cdot (1–0.258 \cdot type_{mRCC})$ Sex is coded 0 for male and 1 for female; Asian race ($race_{Asian}$), presence of GIST ($type_{GIST}$), other solid tumor ($type_{ST}$) and mRCC ($type_{mRCC}$) are coded 0 if not present and 1 if present
Clearance (Cl) of metabolite (SU12662)[a]	$Cl/F_{metabolite} = 29.6 \cdot \left(\frac{\text{weight (kg)}}{77.2}\right)^{0.296} \cdot (1–0.274 \cdot sex) \cdot (1–0.123 \cdot race_{Asian}) \cdot (1– 0.0652 \cdot ECOG) \cdot (1–0.224 \cdot type_{GIST}) \cdot (1–0.287 \cdot type_{ST}) \cdot (1–0.257 \cdot type_{mRCC})$ Sex is coded 0 for male and 1 for female; ECOG is coded 0 for a score of 0 or 1, and 1 for a score of 2 or greater; Asian race ($race_{Asian}$), presence of GIST ($type_{GIST}$), other solid tumor ($type_{ST}$) and mRCC ($type_{mRCC}$) are coded 0 if not present and 1 if present
Volume of distribution (Vd) of sunitinib	$Vd/F_{parent} = 2030 \cdot \left(\frac{\text{weight (kg)}}{77.2}\right)^{0.459}$
Volume of distribution (Vd) of metabolite (SU12662)	$Vd/F_{metabolite} = 3080 \cdot \left(\frac{\text{weight (kg)}}{77.2}\right)^{0.510} \cdot (1–0.241 \cdot sex)$
Elimination rate constant	$k = Cl/Vd$
Maximum concentration at steady state ($C_{max,ss}$)	$C_t = C_{max,ss} (e^{-kt})$ $C_{max,ss} = \frac{C_t}{e^{-kt}}$
Minimum concentration at steady state ($C_{min,ss}$)	$C_{min,ss} = C_{max,ss} (e^{-k\tau})$

[a] [40]

Abbreviations: C_t, concentration at time *t*

2.3.1.8 Assessment of clinical response and toxicity

An assessment of clinical responses was performed through a computed tomography (CT) scan at the end of the second cycle. The investigators objectively evaluated treatment responses using the response evaluation criteria in solid tumors (RECIST). [102] Responses were categorized as a complete response (CR), a partial response (PR), stable disease (SD) or progressive disease (PD).

Treatment-associated toxicity was assessed at the end of each cycle, in conjunction with the scheduled blood sample. Laboratory parameters were collected from the patients' medical records, while other toxicities were assessed through patient interviews. Toxicities were graded according to the Common Terminology Criteria for Adverse Events (CTCAE) criteria version 4.0.2. [103]

2.3.1.9 Definitions

The primary end-point was analyzed according to the intention-to-treat principle. Plasma levels of sunitinib, metabolite (SU12662) and total (combined sunitinib and SU12662) were reported as normalized to 37.5 mg. The SM ratio was the ratio of the level of sunitinib to the level of SU12662. The maximum CTCAE grade observed for each toxicity across the cycles was used for analysis. Grade 2 and above toxicities were considered clinically significant as they could affect patients' daily functioning and living. Henceforth, patients who experienced grade 2 and above toxicities will be defined as those with toxicities, while patients who experienced grade 1 or no toxicity will be defined as those with no toxicities.

2.3.1.10 Statistical analysis

Descriptive statistics were used to summarize the patients' demographics, treatment outcomes and the incidence of toxicity. Data on the drug levels were reported, together with the patients' treatment response. The Mann-Whitney U test was used to compare drug exposure in patients with different outcomes and toxicities. Given the explorative nature of this study, the p-values were not corrected for multiple testing. All tests of statistical significance were two-sided with p values of less than 0.05 and all data analysis was conducted using IBM SPSS Statistics 21.

2.3.2 Results

2.3.2.1 Patient demographics and disease characteristics

A total of 36 patients were recruited in this study. After completion of the first, second and third cycles, 25,21 and 12 patients were available for analysis, respectively. The reasons for exclusion included discontinuation of therapy or patients' refusal to have blood drawn due to reasons such as unable to take time off to return to the study center for blood draw and fear of blood taking (Figure 2). For cycle 1, as one patient provided the blood sample before steady-state drug levels are achieved, this sample was excluded from the pharmacokinetic analysis. For cycle 2, as one patient declared non-adherence of sunitinib throughout the cycle after blood sample collection, this sample was excluded from the pharmacokinetic analysis.

The mean age and weight of the patients were 59 ± 10 years and 66.1 ± 13.5 kg, respectively. The majority of the patients were males (80.6%) and of Chinese ethnicity (86.1%). More than half of the patients (63.9%) had co-morbidities, such as hypertension and hyperlipidemia, and about two-thirds (69.4%) had had a previous nephrectomy. x (Table 6). No DDIs were identified in the patients.

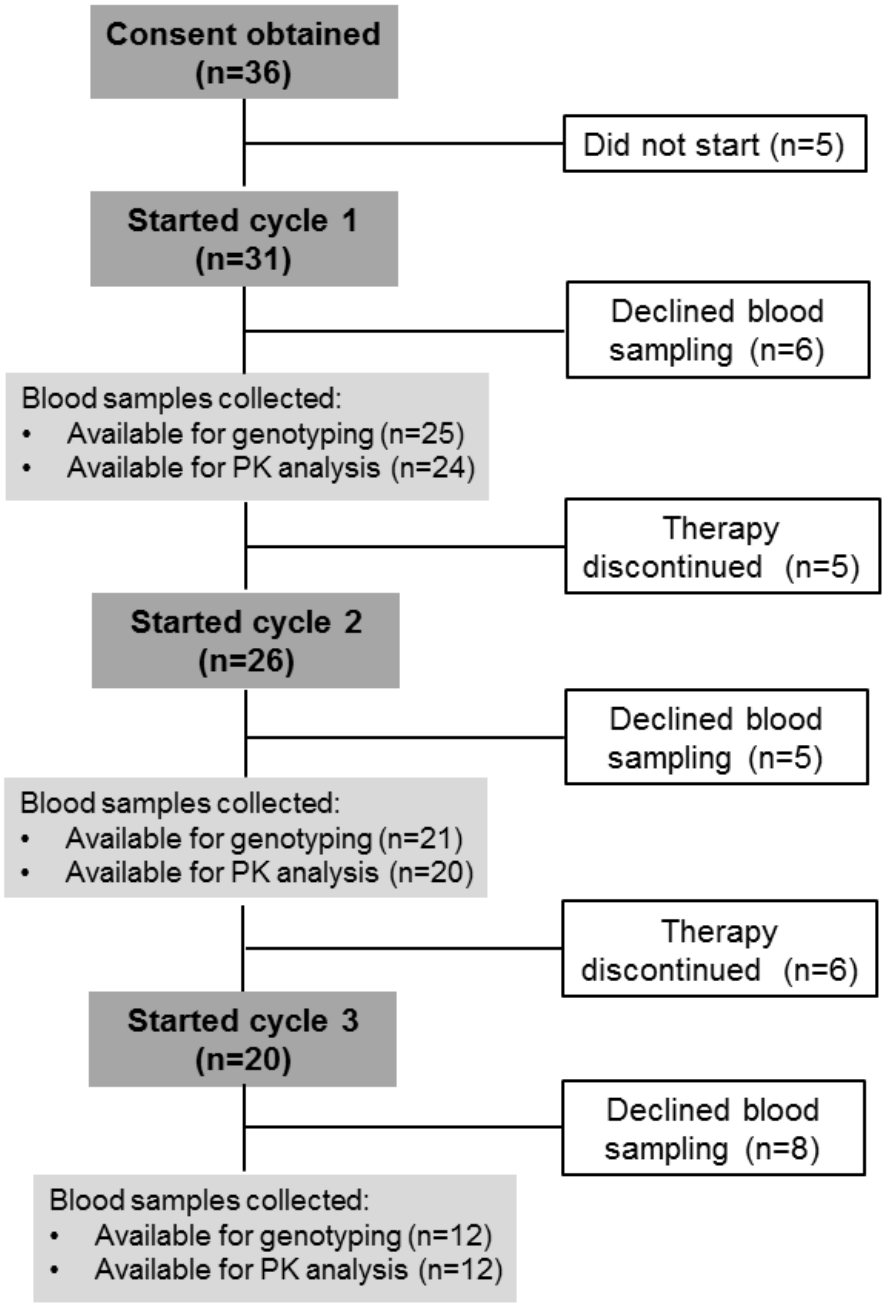


Figure 2. Distribution of patients

Table 6. Patient demographics and disease characteristics (n=36)

	Mean ± SD
Age, years	59 ± 10
Weight, kg (range)	66.1 ± 13.5 (32.4–93.2)
	n (%)
Gender	
Male	29 (80.6)
Female	7 (19.4)
Ethnicity	
Chinese	31 (86.1)
Malay	3 (8.3)
Indian	2 (5.6)
Presence of co-morbidities	23 (63.9)
Previous nephrectomy	25 (69.4)
Site of metastasis	
Lung	27 (75.0)
Bone	14 (38.9)
Adrenals	6 (16.7)
Brain	5 (13.9)
Liver	3 (8.3)
Others	1 (2.8)
No. of MSKCC risk factors*	
0 (favourable)	12 (34.3)
1–2 (intermediate)	18 (51.4)
≥ 3 (poor)	5 (14.3)

Abbreviations: *SD*, standard deviation; *MSKCC*, Memorial Sloan-Kettering Cancer Center
* Risk factors associated with shorter survival according to the MSKCC risk classification

2.3.2.2 Total exposure to sunitinib and SU12662

Most patients were started on 37.5 mg of sunitinib (86.7%), while 4 patients were started on 25 mg (13.3%). No reasons were provided for the 25 mg dosing; but it may be presumed that these patients were thought to be less tolerant to the 37.5 mg dose by their physicians. For the second cycle, 20 (76.9%) patients were given 37.5 mg, with the remaining 6 (23.1%) patients received 25 mg. The doses were reduced due to toxicity. For the third cycle, 1 (5.0%) patient received 18.75 mg, 5 (25.0%) received 25 mg, 13 (65.0%) received 37.5 mg and 1 (5.0%) received 50 mg. All patients were on the 4-week on, 2-week off cycle. The main reasons for dose discontinuations and dose reductions were toxicity and disease progression. One patient had his dose increased from 37.5 to 50 mg daily due to disease progression and the absence of toxicity.

Although most patients were given an AD regimen of sunitinib, the median total exposure levels (normalized) for all 3 cycles were above the therapeutic target of 50 ng/ml. Exposure levels (normalized) were also similar between the 3 cycles. Similar trends were also observed with the actual levels (Table 7). Of the 25 patients who provided blood samples for cycle 1, 84% achieved the minimum target concentration of 50 ng/ml (based on normalized levels). For cycles 2 and 3, 18 of 20 (90%) and 11 of 12 (92%) patients achieved target concentrations, respectively (based on normalized levels). As for the actual levels, the percentage of patients who managed to achieve the minimum target concentration was 76%, 85% and 83% for cycle 1, 2 and 3 respectively.

Table 7. Total exposure levels across 3 cycles of sunitinib therapy

	Cycle 1 (n=25)	Cycle 2 (n=20)	Cycle 3 (n=12)	P
$C_{min,ss}$				
Sunitinib, normalized‡	62.60 (43.15, 85.43)	65.00 (49.88, 87.15)	64.94 (50.11, 102.66)	0.84
Sunitinib, actual¶	56.07 (42.33, 85.48)	55.54 (46.33, 74.34)	62.13 (49.74, 68.10)	0.89
Metabolite, normalized‡	11.12 (8.05, 17.85)	11.16 (7.08, 13.01)	11.97 (8.69, 21.94)	0.81
Metabolite, actual¶	10.09 (6.18, 17.85)	11.16 (7.08, 13.01)	11.85 (5.97, 20.77)	0.93
Total, normalized‡	83.03 (50.75, 104.04)	80.30 (59.94, 108.83)	80.43 (60.48, 116.70)	0.87
Total, actual¶	67.00 (49.33, 104.04)	68.85 (53.90, 91.92)	71.17 (60.22, 99.39)	0.98
SM Ratio	5.56 (4.29, 7.75)	5.18 (4.18, 7.03)	5.20 (4.25, 8.40)	0.92
$C_{max,ss}$				
Sunitinib, normalized‡	96.05 (67.70, 128.11)	99.94 (74.51, 128.46)	98.76 (74.61, 152.68)	0.85
Sunitinib, actual¶	82.34 (66.14, 128.11)	85.04 (71.54, 118.31)	92.20 (75.42, 104.00)	0.92
Metabolite, normalized‡	13.00 (9.45, 20.39)	14.89 (11.96, 20.18)	13.93 (10.08, 25.50)	0.75
Metabolite, actual¶	11.70 (7.25, 20.39)	13.03 (8.26, 15.20)	13.87 (6.91, 24.11)	0.93
Total, normalized‡	119.93 (76.14, 152.30)	119.51 (87.94, 159.00)	116.89 (87.14, 167.22)	0.85
Total, actual¶	95.01 (74.40, 152.30)	102.42 (78.63, 135.31)	101.65 (86.88, 141.27)	1.00
SM Ratio	7.04 (5.62, 10.08)	6.70 (5.50, 9.10)	6.73 (5.56, 10.58)	0.91

Abbreviations: $C_{max,ss}$, steady-state peak concentration; $C_{min,ss}$, steady-state trough concentration

‡ Levels reported as normalized to 37.5 mg (ng/ml/mg) and reported as the median (inter-quartile range).

¶ Actual levels reported (ng/ml) and reported as the median (inter-quartile range).

2.3.2.3 Toxicities observed with sunitinib therapy

Sunitinib-associated toxicities were commonly experienced by the patients. The most common were hypertension (91.3%), fatigue (76.9%), anemia (73.3%), altered taste (73.1%), mucositis (73.1%), dry skin (65.4%) and HFSR (65.4%). The most common grade 2 and above toxicities were mucositis (65.3%), HFSR (61.5%) and hypertension (60.9%). (Table 8)

Table 8. Incidence of toxicities

	N[e]	All grades		Grade 1		Grade 2		≥ Grade 3	
		n	%	n	%	n	%	n	%
Gastrointestinal									
Mucositis	26	19	73.0	2	7.7	16	61.5	1	3.8
Dyspepsia	26	6	23.1	2	7.7	4	15.4	0	0
Dry mouth	26	6	23.1	5	19.2	1	3.8	0	0
Nausea	26	4	15.4	4	15.4	0	0	0	0
Diarrhoea	26	7	26.9	5	19.2	2	7.7	0	0
Constipation	26	3	11.5	2	7.7	1	3.8	0	0
Cardiac									
Hypertension	23	21	91.3	7	30.4	6	26.1	8	34.8
Dermatology									
Dermatological toxicity[a]	26	22	84.6	4	15.4	18	69.2	0	0
Dry skin	26	17	65.4	10	38.5	7	26.9	0	0
HFSR	26	17	65.4	1	3.8	16	61.5	0	0
Rash	26	11	42.3	7	26.9	4	15.4	0	0
Pruritus	26	7	26.9	4	15.4	3	11.5	0	0
Neurology									
Altered Taste	26	19	73.1	11	42.3	8	30.8	0	0
Headache	26	7	26.9	4	15.4	2	7.7	1	3.8
Constitutional									
Fatigue	26	20	76.9	16	61.5	4	15.4	0	0
Dizziness	26	8	30.7	7	26.9	1	3.8	0	0
Fever	26	5	19.2	4	15.4	1	3.8	0	0
Liver									
Hepatotoxicity[b]	30	10	33.3	7	23.3	3	10.0	0	0
Transaminitis[c]	30	9	30.0	7	23.3	2	6.7	0	0
↑ AST	30	7	23.3	6	20.0	1	3.3	0	0
↑ ALT	30	7	23.3	6	20.0	1	3.3	0	0
↑ TB	30	4	13.3	3	10.0	1	3.3	0	0
Renal/ metabolic									
↑ Creatinine	30	17	56.6	10	33.3	7	23.3	0	0
↓ Albumin	30	16	53.3	9	30.0	7	23.3	0	0
Hematology									
Hematological toxicity[d]	30	30	100.0	11	36.7	13	43.4	6	20.0
Anemia	30	22	73.3	13	43.3	8	26.7	1	3.3
Neutropenia	30	14	46.7	5	16.7	7	23.3	2	6.7
Leukopenia	30	14	46.7	8	26.7	6	20.0	0	0
Thrombocytopenia	30	15	50.0	10	33.3	2	6.7	3	10.0

Abbreviations: *HFSR*, hand-foot skin reaction; *AST*, aspartate transaminase; *ALT*, alanine transaminase; *TB*, total bilirubin

a Includes dry skin, HFSR, rash and pruritus

b Includes elevation of TB, ALT and AST

c Includes elevation of ALT and AST

d Includes anaemia, leukopenia, neutropenia and thrombocytopenia

e Lack of blood pressure readings as measurements were not taken routinely by physicians; Lack of patient data for non-laboratory parameters as some patients declined interview for assessment of toxicities.

2.3.3 Discussion

Although an AD was used in this study, 84–92% of the patients managed to achieve the minimum target concentration of 50 ng/ml, which is significantly higher than the 52% reported in a similar study conducted in Caucasians. [104] Differences in the methodology may explain the divergence in the results. Firstly, it should be noted that sampling was performed in week 3–4 in our study, whereas sampling was performed on day 14 in the study by Lankheet et al, which may not be sufficient for both sunitinib and its metabolite to reach a steady state due to their long half-lives. Secondly, body weight, which affects the clearance of sunitinib, was lower in our study (range, 32.4–93.2 kg) and could have resulted in the higher levels. Lastly, because the clearance of sunitinib and its metabolite is most strongly affected by tumor type compared with other factors, such as gender and weight [40], our study included only patients with mRCC to exclude any effect of tumor type on exposure to sunitinib. However, it should be noted that although the target range is achieved, there are no evidence from this study and in the literature thus far to support its association with efficacy.

Although patients receiving 25mg displayed lower exposure levels (sunitinib, metabolite and total levels) than those receiving 37.5mg, the difference was not statistically significant. Similar trends were observed for all three cycles. Despite receiving a lower dose, these patients managed to achieve the minimum target concentration. (Low dose vs. Normal dose: cycle 1 – 100% vs. 82%; cycle 2 – 100% vs. 86%; cycle 3 – 100% vs. 89%) [Data not shown]

It has been previously shown that plasma levels of sunitinib in patients do not vary greatly despite differences in doses [45], suggesting that patient factors that affect the disposition of sunitinib may influence the levels of TKIs and toxicity more than expected. Numerous factors, such as tumor type, Asian race, gender, body weight and elevated Eastern cooperative oncology group (ECOG) performance status, may explain a portion of the variability in the clearance of sunitinib and its metabolite. [40] Exposure to sunitinib and total drugs was predicted to increase by 15% in Asians relative to other races. In females relative to males, exposure was predicted to increase by 17% for total drugs. Furthermore, among Asian females, total drug exposure was predicted to increase by 34% relative to non-Asian male patients. [40] This may provide some explanation for the higher exposure that was observed in our study although a lower dose was used. In addition, it has been suggested that CYP3A4 activity is approximately two-fold higher in women than in men. [105] However, it has also been suggested that the increase in CYP3A metabolism in females compared with males might be a result of their lower Pgp activity rather than gender differences in CYP3A activity. [106] Moreover, CYP3A4 activity varies widely within each gender, and other factors such as environmental factors have been proven to be far more clinically significant than gender differences in CYP3A4. [105]

2.3.4 Limitations of study

A major limitation of the study was that only 1 blood sample was taken for each cycle due to the outpatient setting of the study, to ensure feasibility and minimal inconvenience to the participants. The one-sample strategy may also useful as it is practical and mimics how the TDM of sunitinib may be performed in clinical settings. In addition, the chronic scheduling and long half-lives of most TKIs means that the

steady-state concentration of these agents (and/or their metabolites) has the potential to adequately represent systemic exposure. [35] Furthermore, it has been demonstrated that C_{min} highly correlates with AUC of sunitinib, suggesting that TDM service could be provided based on the C_{min} target. [76] Also, due to the lack of data, a one-compartmental model was used to estimate the drug levels despite both sunitinib and SU12662 assuming two-compartmental models based on literature. However, with a limited number of samples, pharmacokinetic models that describe more accurately the drug profile in the body could not be constructed. If possible, future studies may explore multiple sampling points, such that a more precise population pharmacokinetic models or even pharmacokinetic-toxicity model may be developed to predict the probability of toxicity at a given concentration. The time lapse between the last dose and blood draw were also not strictly controlled as this was to cater for patient's convenience and ensure feasibility of the study. However, this poses a challenge to the analysis of the blood levels as actual levels were extrapolated to $C_{min,ss}$ and $C_{max,ss}$. These PK parameters evaluated in our small study may limit the significance of the findings. The small sample size was another limitation of this study. This may be contributed by the uncommon nature of the disease. Although incidence of kidney cancer constitutes 2.4% of all cancers globally, it contributes to 1.7% of all cancer related deaths. Patients who were receiving sunitinib for other indications like GIST and pancreatic tumors could also have been included. Nonetheless, tumor type (mRCC, GIST and other solid tumors) accounts for a major portion of the variability in the clearance of sunitinib and its metabolite; hence our study included only patients with mRCC. Furthermore, the analysis could have included a comparison of patients who received the AD regimen and those who received standard doses, but this could not be carried out because all of the mRCC

patients at the cancer centre received the AD regimen. Moreover, due to ethical concerns, it is unlikely that a RCT will ever be conducted to examine this dosing issue, given the high toxicities experienced in the Asian population at conventional-dose regimens reported here and by others. As this was an exploratory study with small sample size, p-values were not corrected for multiple testings. Also, due to the outpatient setting, it is challenging to ensure patient compliance, thus it was assumed that patients adhered to the regimen. On the same note, it was unable to control patient's diet as well as use of CAM, although in some cases, patients do provide the type of CAM used. Hence, it was assumed that the patient's diet as well as CAM use does not affect the drug exposure of sunitinib and SU12662.

2.3.5 Summary of important findings

In this chapter, it was demonstrated that the AD regimen of sunitinib in Asian mRCC patients provided sufficient drug exposure. This provides an objective measure of effectiveness for this off-label AD regimen. Taken together with the findings of the evaluation of clinical efficacy and safety outcomes performed by our collaborators, the AD regimen proves to be a feasible choice for the management of mRCC in Asian patients. This regimen could be considered as a standard of care in Asian mRCC patients, as the lower dose not only diminished the incidence of high grade toxicities, but at the same time did not compromise clinical efficacy.

3 Exploring the association between toxicities with drug exposure of sunitinib and SU12662

3.1 Association between toxicity and plasma levels in Asian mRCC patients receiving an attenuated dosing regimen of sunitinib

3.1.1 Methodology

The methodology adopted for this study was previously described under chapter 2.3.1 (methodology)

3.1.1.1 Definitions

Definitions used are previously described under chapter 2.3.1.9 (definitions). As mentioned earlier, patients who experienced grade 2 and above toxicities will be defined as those with toxicities, while patients who experienced grade 1 or no toxicity will be defined as those with no toxicities. Grade 2 and above toxicities were considered clinically significant as they could either require intervention or affect patients' daily functioning and living. [103] With that, the commonly encountered grade 2 and above toxicities were highlighted for comparison. The average exposure levels between cycles were then compared between patients with and without toxicities.

3.1.2 Results

3.1.2.1 Patient demographics and disease characteristics

As the cohort of patients were the same, please refer to chapter 2.3.2.1

3.1.2.2 Toxicities observed with sunitinib therapy

As the cohort of patients were the same, please refer to chapter 2.3.2.3

3.1.2.3 Exposure levels and toxicities

Those grade 2 and above toxicities with a high incidence were highlighted for comparison and they were: mucositis, HFSR, altered taste, anemia, neutropenia and hypertension.

Generally, a trend of higher exposure was observed in patients who manifested toxicities compared with those who had no toxicity, with the exception of neutropenia.

For both $C_{min,ss}$ and $C_{max,ss}$ of sunitinib, patients who experienced mucositis, HFSR, altered taste, anemia and hypertension largely demonstrated higher levels than those who did not experienced the toxicities. Similar trends were observed for actual and normalized levels. The $C_{min,ss}$ (normalized) of sunitinib was significantly higher for those who experienced mucositis than those who did not experienced mucositis (71.69 vs. 47.30 ng/ml, p=0.04). (Table 9 & Table 10)

Exposure (both $C_{min,ss}$ and $C_{max,ss}$) to the metabolite (SU12662) was higher in patients who experienced HFSR, altered taste, neutropenia and hypertension, although the differences were not statistically significant. Similar trends were observed for actual and normalized levels. (Table 9 & Table 10)

Similarly, a trend of higher levels was observed with the total exposure (both $C_{min,ss}$ and $C_{max,ss}$) in patients who experienced mucositis, HFSR, altered taste, anemia and hypertension than those who did not experienced the toxicities. The total maximum exposure (normalized) was significantly higher for those who experienced mucositis and altered taste than for those who had grade 1 or no events (mucositis: 126.46 vs 84.81 ng/ml, p=0.04; altered taste: 159.91 vs 105.22 ng/ml, p=0.05). (Table 9) Likewise, the total minimum exposure (normalized) was also significantly higher for those who experienced mucositis and altered taste than for those who had no events (mucositis: 85.93 vs 57.53 ng/ml, p=0.04; altered taste: 109.37 vs. 71.15 ng/ml, p=0.04). (Table 10) Similar trends were observed for actual and normalized levels.

The SM ratio was largely similar between the two groups of patients, although it was observed to be higher in patients who reported mucositis, HFSR and anemia. However, these differences were not statistically significant. (Table 9 & Table 10)

Table 9. Exposure levels ($C_{max,ss}$) and toxicities

$C_{max,ss}$ (ng/mL)		**Grade ≥ 2**	**Grade ≤ 1**	**P**
Mucositis (n=17 vs. 8)	Sunitinib, N	110.74 (88.90, 144.73)	73.79 (63.33, 112.82)	0.06
	Sunitinib, A	106.11 (80.28, 144.73)	71.72 (51.64, 86.13)	**0.01**
	SU12662, N	13.13 (10.47, 21.65)	13.47 (10.77, 15.67)	0.49
	SU12662, A	13.10 (10.43, 21.58)	12.18 (7.68, 14.23)	0.35
	Total, N	124.46 (104.30, 169.36)	84.81 (76.50, 127.71)	**0.04**
	Total, A	121.83 (89.02, 167.33)	84.52 (60.12, 100.59)	**0.01**
	SM Ratio	7.65 (6.22, 10.08)	6.27 (4.95, 7.31)	0.10
HFSR (n= 16 vs. 9)	Sunitinib, N	101.08 (80.21, 144.42)	90.90 (64.99, 115.09)	0.28
	Sunitinib, A	91.29 (76.25, 137.12)	75.14 (64.99, 115.09)	0.61
	SU12662, N	15.28 (10.41, 19.38)	12.86 (11.30, 15.86)	0.61
	SU12662, A	12.30 (8.80, 18.47)	12.86 (10.79, 15.86)	0.96
	Total, N	124.63 (88.49, 170.67)	103.37 (78.47, 130.46)	0.19
	Total, A	110.85 (87.26, 167.63)	86.64 (78.47, 130.46)	0.46
	SM Ratio	7.19 (5.40, 9.98)	7.01 (5.38, 9.11)	0.78
Altered taste (n=8 vs. 17)	Sunitinib, N	123.33 (78.08, 151.57)	94.65 (70.38, 115.48)	0.22
	Sunitinib, A	107.12 (78.08, 150.08)	85.81 (64.99, 108.43)	0.16
	SU12662, N	19.00 (11.90, 28.51)	12.86 (10.45, 16.63)	0.06
	SU12662, A	14.72 (11.90, 28.51)	11.09 (8.34, 15.07)	0.05
	Total, N	159.91 (96.10, 215.06)	105.22 (82.68, 132.47)	0.05
	Total, A	145.20 (94.27, 180.58)	91.82 (78.47, 120.88)	0.06
	SM Ratio	6.94 (5.48, 8.31)	7.36 (5.41, 9.80)	0.52
Anemia (n=6 vs. 18)	Sunitinib, N	111.05 (64.00, 126.33)	92.78 (71.41, 122.95)	0.79
	Sunitinib, A	95.74 (64.00, 119.02)	87.41 (66.65, 109.29)	0.84
	SU12662, N	12.30 (9.78, 16.87)	13.61 (10.49, 18.78)	0.64
	SU12662, A	11.30 (8.71, 15.81)	13.00 (9.91, 16.45)	0.51
	Total, N	123.14 (74.09, 143.20)	110.85 (82.82, 158.40)	1.00
	Total, A	106.12 (74.09, 134.83)	104.60 (80.43, 144.33)	1.00
	SM Ratio	7.81 (5.74, 9.09)	6.97 (5.22, 9.75)	0.79
Neutropenia (n=9 vs. 15)	Sunitinib, N	87.93 (58.22, 137.93)	106.11 (77.98, 118.83)	0.39
	Sunitinib, A	80.74 (56.60, 124.41)	94.65 (74.75, 111.36)	0.33
	SU12662, N	14.51 (7.79, 21.65)	13.10 (10.56, 17.64)	0.88
	SU12662, A	12.86 (7.24, 19.97)	11.50 (10.48, 14.28)	0.79
	Total, N	103.99 (67.63, 160.21)	121.83 (86.64, 153.88)	0.30

	Total, A	90.41 (63.84, 143.93)	116.49 (86.21, 136.46)	0.30
	SM Ratio	5.58 (5.02, 9.20)	7.36 (6.52, 9.98)	0.20
Hypertension (n=13 vs. 8)	Sunitinib, N	112.12 (76.56, 148.39)	91.29 (67.37, 110.05)	0.19
	Sunitinib, A	102.89 (74.94, 147.39)	84.33 (60.90, 103.25)	0.22
	SU12662, N	17.19 (11.30, 21.68)	11.83 (9.80, 14.40)	0.13
	SU12662, A	13.13 (11.28, 20.79)	10.47 (8.81, 13.84)	0.13
	Total, N	136.46 (84.98, 178.40)	104.60 (78.10, 122.46)	0.08
	Total, A	121.83 (86.43, 169.96)	97.20 (70.81, 113.67)	0.11
	SM Ratio	6.97 (5.29, 10.60)	8.70 (5.90, 9.57)	0.66

Note: reported levels are average exposure levels across the 3 cycles and reported as median (interquartile range)

Abbreviations: A, actual levels; $C_{max,ss}$, steady-state peak concentration; HFSR, hand-foot skin reaction; N, normalized levels; SM ratio, sunitinib to metabolite ratio

Table 10. Exposure levels ($C_{min,ss}$) and toxicities

$C_{min,ss}$ (ng/mL)		**Grade ≥ 2**	**Grade ≤ 1**	**P**
Mucositis (n=17 vs. 8)	Sunitinib, N	71.69 (57.45, 96.85)	47.30 (41.25, 72.33)	**0.04**
	Sunitinib, A	70.15 (51.75, 96.85)	46.59 (33.22, 55.94)	**0.01**
	SU12662, N	11.26 (8.94, 18.79)	11.53 (9.19, 13.39)	0.49
	SU12662, A	11.23 (8.94, 18.79)	10.40 (6.54, 12.13)	0.35
	Total, N	85.93 (69.10, 115.77)	57.53 (52.10, 85.02)	**0.04**
	Total, A	85.55 (61.27, 115.77)	57.53 (40.45, 68.30)	**0.01**
	SM Ratio	5.65 (4.78, 7.89)	4.75 (3.81, 5.44)	0.06
HFSR (n= 16 vs. 9)	Sunitinib, N	66.37 (54.65, 94.13)	59.20 (42.51, 76.81)	0.34
	Sunitinib, A	59.53 (48.73, 90.27)	48.16 (42.51, 76.81)	0.61
	SU12662, N	13.04 (8.92, 17.05)	10.99 (9.70, 13.59)	0.65
	SU12662, A	10.50 (7.57, 16.30)	10.99 (9.29, 13.59)	0.87
	Total, N	84.69 (61.26, 114.17)	69.92 (54.04, 89.35)	0.26
	Total, A	74.15 (58.11, 114.17)	57.98 (53.84, 89.35)	0.57
	SM Ratio	5.46 (4.14, 7.62)	5.32 (4.20, 7.19)	0.82
Altered taste (n=8 vs. 17)	Sunitinib, N	80.12 (50.05, 100.51)	59.29 (45.73, 76.64)	0.20
	Sunitinib, A	69.83 (50.05, 100.51)	57.41 (42.51, 73.06)	0.18
	SU12662, N	16.28 (10.18, 24.47)	10.99 (8.94, 14.20)	0.06
	SU12662, A	12.62 (10.18, 24.47)	9.58 (7.11, 12.91)	**0.04**
	Total, N	109.37 (64.97, 149.75)	71.15 (56.25, 88.00)	**0.04**
	Total, A	101.65 (62.77, 125.00)	64.97 (53.84, 84.29)	**0.04**
	SM Ratio	5.34 (4.24, 6.41)	5.56 (4.16, 7.75)	0.56
Anemia (n=6 vs. 18)	Sunitinib, N	75.32 (41.21, 82.16)	59.24 (46.24, 79.64)	0.74
	Sunitinib, A	63.10 (41.21, 81.16)	58.35 (43.76, 72.02)	0.84
	SU12662, N	10.54 (8.44, 14.42)	11.65 (8.96, 16.04)	0.69
	SU12662, A	9.70 (7.42, 13.61)	11.11 (8.51, 14.08)	0.55
	Total, N	85.74 (49.83, 96.58)	75.10 (56.67, 104.58)	1.00
	Total, A	72.66 (49.83, 93.79)	69.71 (55.26, 98.11)	0.89
	SM Ratio	5.92 (4.52, 7.02)	5.37 (4.01, 7.71)	0.84
Neutropenia (n=9 vs. 15)	Sunitinib, N	57.41 (38.85, 89.54)	70.15 (54.30, 77.66)	0.39
	Sunitinib, A	51.54 (38.75, 82.19)	59.77 (47.79, 75.97)	0.33
	SU12662, N	12.34 (6.72, 18.59)	11.23 (8.98, 15.10)	0.88
	SU12662, A	10.99 (6.25, 17.09)	9.81 (8.98, 12.15)	0.79
	Total, N	71.15 (46.93, 108.58)	85.55 (58.93, 101.38)	0.33

	Total, A	59.76 (45.00, 100.20)	77.15 (57.57, 92.76)	0.33
	SM Ratio	4.26 (3.85, 7.11)	5.50 (4.89, 7.84)	0.25
Hypertension (n=13 vs. 8)	Sunitinib, N	75.97 (51.23, 99.25)	58.35 (45.18, 73.54)	0.17
	Sunitinib, A	64.99 (47.98, 99.25)	54.47 (41.09, 67.43)	0.15
	SU12662, N	14.66 (9.70, 18.70)	10.12 (8.44, 12.27)	0.15
	SU12662, A	11.23 (9.68, 17.87)	8.94 (7.57, 11.87)	0.15
	Total, N	92.76 (58.45, 122.44)	69.71 (54.73, 84.21)	0.08
	Total, A	85.55 (57.77, 117.36)	64.02 (49.62, 77.08)	0.13
	SM Ratio	5.27 (4.08, 8.40)	6.64 (4.55, 7.48)	0.61

Note: reported levels are average exposure levels across the 3 cycles and reported as median (interquartile range)
Abbreviations: A, actual levels; $C_{min,ss}$, steady-state trough concentration; HFSR, hand-foot skin reaction; N, normalized levels; SM ratio, sunitinib to metabolite ratio

3.1.3 Discussion

Higher exposures were observed for patients who developed toxicities (grade 2 or above) than for those who developed no toxicity (grade 0 or 1). The total exposure was observed to be significantly higher for those who experienced mucositis and altered taste than for those who had no events.

In this study, the incidence of grade 3 and above toxicities was generally low and no grade 3 and above HFSR, fatigue or leukopenia were observed. In the Korean study that used the standard sunitinib dose of 50 mg, the most common grade 3 and above toxicities were HFSR (16%), thrombocytopenia (17%), anemia (6%), leukopenia (7%) and neutropenia (7%). [84] However, in our study of Asian patients receiving the AD of 37.5 mg, the incidence of these toxicities was either lower or similar (HFSR, 0%; thrombocytopenia, 11%; anemia, 0%; leukopenia, 0%; and neutropenia, 7%). The higher incidence of HFSR and anemia in the Korean study supports our observation that higher exposure is probably associated with these events.

The study revealed a trend of higher total exposure in patients who reported grade 2 and above toxicity such as mucositis, HFSR, altered taste, anemia and hypertension than those who developed grade 1 or no toxicity. Interestingly, the former group of patients was found to have a sunitinib exposure of more than 100 ng/ml, which is the proposed threshold level for toxicity [76], suggesting that sunitinib may play a more significant role in the manifestation of toxicities than its metabolite. However, the evidence for an association between the SM ratio and the incidence of toxicity remains inconclusive. The higher SM ratio indicated that these patients had relatively

higher plasma concentrations of sunitinib than its metabolite. This may also suggest that these patients have reduced ability to metabolize sunitinib. Although the differences were not statistically significant, this could be due to the small sample size and the large inherent variability in plasma concentrations across patients, which would minimize any differences in concentration. It could also be likely that not toxicities are concentration-dependent.

The SM ratio is a robust measure because it adjusts for changes in the fluctuation of drug concentration. It is also useful for assessing toxicity, because it can help to pinpoint whether sunitinib or its major metabolite SU12662 may have different potential for toxicity. For instance, two patients given the same drug exposure but with a different incidence of HFSR may have different SM ratios; a higher SM ratio would probably be observed in a patient with grade 2 and above HFSR compared with that in a patient with no HFSR.

Not surprisingly, the range of exposure levels achieved was highly variable. The coefficient of variation for the minimum and maximum total concentrations for cycle 1 was 66% and 58%, respectively. Despite the lower dose used in the AD regimen, some patients may still achieve the 100 ng/ml threshold for toxicity and some may even require further dose reductions due to toxicity. Sunitinib engenders considerable and unpredictable inter-patient variability, and TDM could be a means to overcome this. A practical suggestion would be to measure blood levels when sunitinib is initiated for the first cycle, to observe whether therapeutic levels are achieved in relation to efficacy and toxicity. This is particularly important in groups such as

Asians, women and those with a low body weight, because these factors may affect the accumulation of sunitinib and thereby the risk of toxicity. If necessary, doses could also be decreased further to meet the minimum effective plasma concentration without affecting the efficacy, which could potentially lead to cost savings for the patient. Further studies with an active therapeutic drug monitoring component should be conducted to study the feasibility of such a strategy. Total plasma concentration of sunitinib could be used as a marker for therapeutic efficacy, whereas sunitinib levels could be used as a marker for toxicity.

As sunitinib has considerable inter-patient variability, TDM may be considered as a strategy to optimize sunitinib therapy. With the aid of information on drug levels, physicians can adjust doses objectively. However, as pharmacokinetic targets of sunitinib have yet to be validated, and TDM of sunitinib or any TKI has yet to be implemented in clinical practice, we believe that it would be more practical to measure drug concentrations in the event of severe toxicity. This may help to ascertain if the toxicity is exposure-related; if so, dose titration may be performed. Otherwise, supportive management of toxicity may be more appropriate. If necessary, doses could also be reduced further to meet the minimum effective plasma concentration without affecting efficacy, which could potentially lead to cost savings for the patients and the healthcare system.

3.1.4 Limitations of study

Refer to chapter 2.3.4

3.1.5 Summary of important findings

In this section, we have reported a trend of higher exposures in patients who experienced toxicities. The total exposure was observed to be significantly higher for those who experienced mucositis and altered taste than for those who had no events.

3.2 Evaluating the in-vitro dermatological and hepatotoxic potential of sunitinib and SU12662

Although current evidence from the literature as well as from our studies have suggested a probable association between exposure and toxicity, the exact cause of most sunitinib-associated toxic effects is still largely unknown. As both sunitinib and SU12662 act on similar receptors, such as VEGFR, PDGFR and KIT [28], the toxic effects associated with sunitinib therapy may also be attributed to SU12662.

Dermatological toxicity and hepatotoxicity were chosen for this study as they are clinically significant toxicities of sunitinib; dermatological toxicity because of its ubiquity and hepatotoxicity because of its severity. Moreover, these toxicities can be modeled at the in-vitro level using suitable cell culture models.

Currently, data are lacking from studies undertaken to properly elucidate the underlying mechanisms causing the dermatological toxicities. [107, 108] Some had suggested that dermatological toxicities were due to the deregulation of signaling pathways between the different epidermal cells. [107, 109] For instance, HFSR was postulated to be dose-dependent and a consequence of an indirect effect of the inhibition of pro-angiogenic pathways. [110, 111] Inhibition of VEGFR and PDGFR interferes with endothelial cell survival mechanisms, preventing the vascular repair mechanisms from functioning properly, thereby causing HFSR in high-pressure areas, such as the palms and soles, which may be repeatedly exposed to subclinical trauma with daily activities. [111, 112] Others also discussed the possibility of direct toxic effects of sunitinib on keratinocytes. For instance, it has been demonstrated that the

toxicity of sorafenib and sunitinib for keratinocytes was induced by decreased apoptosis suppressors via the inhibition of STAT3 activity. [113] In addition, sunitinib, due to its action on VEGFR, could have interfered with the autocrine loop of VEGF suggested to be present in the epidermis, thus, affecting the survival of keratinocytes. [108, 114] Hence, given that SU12662 is a pharmacologically active metabolite that also inhibits VEGFR, toxicities mediated by the inhibition of VEGFR may arise from exposure to both sunitinib and SU12662. In such event, any differential toxicity between sunitinib and SU12662 may arise from their relative distribution and accumulation in the tissue of concern. Therefore we conducted this study to compare the in-vitro dermatotoxic and hepatotoxic potential of sunitinib and SU12662. This investigation is important as it can help to determine which might be a better marker to monitor in TDM should this be implemented subsequently.

3.2.1 Methodology

HaCaT keratinocytes, 3T3 mouse fibroblast, THLE-2 human hepatocytes and transforming growth factor α mouse hepatocytes (TAMH) were selected in this study.

The immortal human keratinocyte cell line, HaCaT, provided a good representation of the normal keratinocytes with good consistency, durability and availability. [115, 116] HaCaT keratinocyte and 3T3 fibroblast cultures gave an appropriate in-vitro model for skin irritation as keratinocytes and fibroblasts are considered biologically relevant targets for skin irritants and photo-irritants. [117-119] THLE-2 is an immortalized primary human hepatocyte that expresses phenotypic characteristics of normal adult liver epithelial cells. [120] The TAMH cell line was adopted for this study as a stable and metabolically-competent system to support the mechanistic investigation of drug

cytotoxicity. [121] It is of murine liver origin and is capable of maintaining a differentiated phenotype irrespective of the number of passages. [122] It is also non-tumorigenic and expresses drug metabolizing enzymes such as CYP3A and CYP2E1. [123]

3.2.1.1 Cell culture conditions

HaCaT cells were cultured in Dulbecco's modified eagle medium (DMEM) with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (P/S). The 3T3 cells were cultured in DMEM with 5% v/v FBS and 1% P/S and incubated under 5% CO_2 environment at 37 °C. THLE-2 cells were cultured in commercially available LHC-9 media with 10% FBS. TAMH cells (kind gift from Prof. Nelson Fausto, University of Washington) were cultured in serum free Dulbecco's modified Eagle's Media/Ham's F12 (DMEM/F-12) media, supplemented with ITS (5 µg/ml insulin, 5 µg/ml transferrin, 5 ng/ml selenium), 10 mM nicotinamide, 100 nM dexamethasone and 10 µg/ml gentamicin. [59]

Additionally, THLE-2 cells were also cultured with pre-coated flasks and plates. A fibronectin/collagen coating solution (0.01 mg/ml fibronectin, 0.03 mg/ml bovine collagen type I in LHC-9 media) were used for the coating. A volume of the coating solution was introduced (3 ml for T75 flasks and 50 µl per well for 96-well plates), and left to stand for 24 hours in the incubator. Thereafter the coating solution is removed and the coated plates stored in a 4 ºC refrigerator until use.

All cells were incubated under 5% CO_2 environment at 37 °C. Upon reaching 70 – 90% confluence, trypsin was used to passage the cells and was inhibited by fresh

media (HaCaT, 3T3 and THLE-2) or 0.5 mg/ml soybean trypsin inhibitor (TAMH) before the cells were plated.

3.2.1.2 Treatment and cell viability assay

Cells (2,000 – 10,000/well) were seeded in 96-well plates and incubated for 24 hours. Sunitinib or SU12662 dissolved in dimethyl sulfoxide (DMSO), with concentrations ranging from 0.5 to 100 μM were then added and allowed to incubate for another 24 hours (6 wells per concentration). After incubation, media was aspirated and replaced with 50 μl of 2 mg/ml of methylthiazolyldiphenyl-tetrazolium bromide (MTT) dye in phosphate buffered saline (PBS) and 200 μl of serum free media (HEPES-buffered for TAMH). [124, 125] Plates were then incubated in the dark for 2 hours at 37 °C. Subsequently, medium was then aspirated and the residual dye re-dissolved in 25 μl of Sorensen's buffer (0.1 M glycine and 0.1 M NaCl equilibrated to pH 10.5 with 0.1 M NaOH) and 200 μl of DMSO. Plates were read at 570 nm using the Infinite® 200. Experiments were performed in triplicates for each compound. Cell viability was expressed as a ratio normalized to the vehicle-treated control.

3.2.1.3 Statistical analysis

Average percentage viabilities at each concentration were calculated and the IC_{50} was estimated with Prism 6 (GraphPad, La Jolla, USA). T-test was conducted with SPSS 21, to compare IC_{50} of the two compounds. A p-value of less than 0.05 was considered statistically significant.

3.2.2 Results

3.2.2.1 Toxic potential of sunitinib and SU12662

A dose dependent relationship between concentration and cell viability was observed for all cell lines.

HaCaT

The IC_{50} for sunitinib was 23.76, 24.90 and 21.34 μM, while IC_{50} for SU12662 was 37.02, 39.09 and 29.84 μM. The lower mean IC_{50} observed with sunitinib indicated that it was more toxic than SU12662 towards HaCaT cells and this was statistically significant (23.33 ± 1.82 μM vs. 35.32 ± 4.85 μM, p=0.02). (Table 11)

3T3

The IC_{50} for sunitinib was 28.22, 20.08 and 17.65 μM, while IC_{50} for SU12662 was 31.92, 20.55 and 24.06 μM. The mean IC_{50} of sunitinib was 21.98 ± 5.54 μM and for SU12662 25.51 ± 5.82 μM, which were not significantly different (p=0.49), indicating that both sunitinib and SU12662 have an equal potential to cause direct toxic effect to 3T3 cells. (Table 11)

THLE-2

The IC_{50} for sunitinib was 15.29, 15.03 and 9.48 μM, while IC_{50} for SU12662 was 17.87, 16.65 and 10.85 μM. The mean IC_{50} of sunitinib was 13.27 ± 3.28 μM and for SU12662 15.12 ± 3.75 μM, which were not significantly different (p=0.55), indicating that both sunitinib and SU12662 have an equal potential to cause direct toxic effect to THLE-2 cells. (Table 11)

TAMH

The IC_{50} for sunitinib was 27.04, 31.75 and 22.42μM, while IC_{50} for SU12662 was 12.76, 19.70 and 21.80 μM. The mean IC_{50} of sunitinib was 27.07 ± 4.67 μM and for SU12662 18.09 ± 4.73 μM, which were not significantly different (p=0.08), indicating that both sunitinib and SU12662 have an equal potential to cause direct toxic effect to TAMH cells. (Table 11)

Table 11. Mean IC_{50} of sunitinib and SU12662 in various cell lines

	IC_{50} (μM)		
	Sunitinib	**SU12662**	**p**
HaCaT	23.33 ± 1.82	35.32 ± 4.85	0.02
3T3	21.98 ± 5.54	25.51 ± 5.82	0.49
THLE-2	13.27 ± 3.28	15.12 ± 3.75	0.55
TAMH	27.07 ± 4.67	18.09 ± 4.73	0.08

3.2.3 Discussion

The results suggest a differential contribution of sunitinib and metabolite to toxicity. Sunitinib was observed to be more toxic towards keratinocytes while both sunitinib and metabolite were equally toxic to hepatocytes.

Although sunitinib has been shown to be more toxic than SU12662 in keratinocytes, it should also be noted that this statistical difference may not equate to a clinically significant difference. Previous clinical studies reported a correlation with oral adverse events, such as mucositis and HFSR. [126] Although we did not find such a correlation among our patients in the clinical study, our findings that the incidence of mucositis was related to high levels of exposure to sunitinib led us to believe that sunitinib rather than its metabolite may be the major contributor to mucositis. Our clinical study revealed a trend of higher total exposure in patients who reported grade 2 and above toxicity such as mucositis, HFSR, altered taste, anemia and hypertension than those who developed grade 1 or no toxicity. (Table 9 & Table 10) Patients who experience toxicities was also observed to have a high sunitinib exposure of more than 100 ng/ml, providing further indication that sunitinib may indeed be more toxic than its metabolite SU12662. As both in-vitro and clinical findings exhibits a trend where sunitinib was observed to be more toxic, it can thus be postulated that the toxicity arising from sunitinib is an extension of a pharmacological effect on keratinocytes and other skin cells, instead of an idiosyncratic outcome.

The differential toxicity of sunitinib and SU12662 in other cell types has not yet been specified. If differential toxicity exists for diverse cell types, variations in the

activation pathway, such as drug–drug interactions involving enzyme inducers or enzyme inhibitors, could affect the manifestation of toxicity. For example, a patient may experience greater dermatological toxicity when receiving sunitinib concurrently with a CYP3A4 inhibitor, due to the accumulation of the more dermatotoxic parent drug. Moreover, as not all toxicities are likely to be dose-dependent, dose reduction may not be a worthwhile strategy in all cases because efficacy could be compromised with no improvement in the risk of toxicity. Clinicians may have to adopt other strategies, such as providing supportive care or, if toxicity is severe, switching to an alternative agent.

3.2.4 Limitations of study

There may be an issue of longer term damage being done by the test drugs to cells that cannot be easily quantified, and which may not necessarily have a trend in the same direction as direct toxicity. In addition, hepatotoxicity and dermatological toxicity are ultimately organ-level phenomena, so while in-vitro studies like these provide a general indication as to what one may expect, emergent complexities within the organ system may yet lead to a different, unexpected result. Furthermore, only two cell lines per toxicity type was used, which may not be representative of the toxicity as a whole. Although there may be a difference between the IC_{50} of sunitinib and metabolite, the concentrations were much higher than reported human levels. There are also other toxicities relevant to the clinical usage of sunitinib which in-vitro studies are unable to model – such as effects on the nervous and digestive systems, among others. Fatigue, diarrhea and nausea are among the most common side effects of sunitinib therapy, which involve systems too complex for any tissue culture to satisfactorily model. Even should it be the case that hepatotoxicity and dermatological

toxicity are less significant with SU12662 usage as these results suggest, other toxicities may become more significant; as such would be challenging to alter therapy without being able to see the overall toxicity picture.

3.2.5 Summary of important findings

The results of this section suggest a differential contribution of sunitinib and metabolite to toxicity. Sunitinib was observed to be more toxic towards keratinocytes while both sunitinib and metabolite were equally toxic to hepatocytes. As SU12662 has similar clinical efficacy to sunitinib, and the clinical potential of sunitinib therapy is limited by the associated toxicities, modification of the balance between sunitinib and SU12662 has the potential to reduce side effects; this increases the clinical potential of sunitinib therapy in the event that the ratio of active effect to adverse effect between the two clinically active agents are different. By altering metabolism or changing the administered drug, it may possible to change the concentration balance between sunitinib and SU12662 to achieve this effect.

3.3 Supplementary analysis – association of toxicity with health-related quality of life (HRQoL)

Studies investigating health-related quality of life (HRQoL) with sunitinib and other TKIs in patients with mRCC have highlighted the importance of patient reported outcomes (PROs) in determining the overall treatment benefit to the patient. [127] Given the generally poor prognosis of patients with mRCC and the toxicities associated with therapy, PROs have become an increasingly important outcome in this patient population. [128] While some treatment-related toxicities such as HFSR or hypertension may not directly affect survival, they do adversely impact patients' HRQoL. The magnitude of these treatment-related toxicities may be serious enough to warrant dose reductions so as to improve patients' HRQoL, with the possibility to compromise treatment effectiveness or even treatment discontinuation. Hence, a trade-off between the impact of toxicities on patients' HRQoL and treatment efficacy appears inevitable.

It is a concern that toxicities would result in poor treatment adherence and thus poor treatment outcome. Nonetheless, although sunitinib is associated with multiple toxicities, not all of them may lead to poor treatment adherence and not all may warrant interventions. It is likely that only those with an impact on PRO will prompt self-initiated treatment discontinuation among patients. Hence, we conducted additional analysis on the same cohort of mRCC patients, with an objective to evaluate the association between toxicities and PRO. This would help inform

treatment prioritization with regards to the management of toxicities among patients on sunitinib, with higher priority given to those toxicities that impair PRO.

3.3.1 Methodology

3.3.1.1 Patient recruitment and follow up

In addition to the procedures mentioned under chapter 2.3.1.2, questionnaires assessing PRO were administered before treatment commencement, to capture baseline PRO responses. Subsequently, patients were followed-up for PRO responses during week 4 of each treatment cycle.

3.3.1.2 Assessment of patient reported outcomes

Three tools were utilized to measure PRO: (1) EuroQoL Group's Five Dimensions Questionnaire (EQ-5D), (2) Functional Assessment of Cancer Therapy-General (FACT-G), and (3) Functional Assessment of Cancer Therapy-Kidney Symptom Index (FKSI-15).

EQ-5D is a validated and reliable generic measure of health outcome which comprises of a five-level health state classifier and a visual analog scale. [129] The classifier measures five different health dimensions namely, mobility, self-care, usual activities, pain/discomfort and anxiety and depression. Each dimension is described by three different statements, indicating "no problem", "moderate problem" or "extreme problem", with corresponding scores of 1, 2 and 3. The scores are translated into a

single summary index (EQ-5D Index), where a higher index value corresponds to a better HRQoL. The vertical 0-100 point visual analogue scale (EQ-5D VAS) indicates the overall health state of patients. Zero indicates the worst imaginable health state while 100 indicates the best imaginable health state. [76] Clinically significant differences in scores are defined as a minimal 0.08-point change for EQ-5D Index and a minimal 7-point change for EQ-5D VAS. [130]

FACT-G is a validated 27-item scale to assess general HRQoL in cancer patients in the four domains of physical well-being (PWB), social/family well-being (SWB), emotional well-being (EWB) and functional well-being (FWB). [131] Patients rate the extent to which they agree with each statement using the 5-points Likert Scale ("not at all", "a little bit", "some-what", "quite a bit", "very much"). For negatively-phrased questions, scores are reversed. From each individual domain, question scores are summed up to obtain subscale scores. Subsequently, all 4 subscale scores are summed up to obtain the FACT-G Total score. The higher the total score, the better is the HRQoL (score range 0 – 108). [132] Clinically significant differences in scores are defined as a minimal 5-point change in FACT-G Total and a 2-point change in its subscale scores. [133]

FKSI-15 is a validated 15-item symptom index developed specifically to assess disease-related and treatment-related symptoms for patients with advanced kidney cancer. [134] Patients rate the extent to which they agree with each statement using a similar scale and scoring system as FACT-G. Functional Assessment of Cancer Therapy-Kidney Symptoms Index – Disease Related Symptom (FKSI-DRS) is a

subscale derived from 9 of the FKSI-15 questions and measures disease-related symptoms for patients with advanced kidney cancer. The higher the total score, the better is the HRQoL (score range 0 – 60). Clinically significant differences in scores are defined as a minimal 5-point change in FKSI-15 and 3-point change in FKSI-DRS. [134]

3.3.1.3 Statistical analysis

PRO scores for cycle 1 were compared between patients who experienced grade 2 and above toxicities and those who did not were compared using Mann-Whitney U test. PRO were compared between patients with and without Grade 2 and above AEs after cycle 1 as the number of patients is highest at this time point and also to determine the initial impact of the toxicity on QoL. Multiple linear regression was performed to adjust for age, gender and co-morbidities. These factors were selected as they are known to associate with PRO. [135, 136] Due to the small sample size, the number of confounders adjusted for was limited to three. All tests of statistical significance were two-sided with p-value of less than 0.05. A 5-point difference in total FACT-G scores [133] and a 5-point difference in total FKSI-15 scores [134] were considered clinically significant. All data analyses were conducted with IBM SPSS Statistics 21.

3.3.2 Results

3.3.2.1 Association between toxicity and HRQoL

Among the 36 patients recruited, 24, 21 and 13 sets of completed questionnaires were received at the end of cycle 1, 2 and 3 respectively, and analyzed. Patients were excluded for reasons such as did not initiate sunitinib therapy, unable to return for follow up to complete questionnaires and discontinuation of therapy.

In general, across commonly encountered Grade 2 and above toxicities (mucositis, HFSR, altered taste, anemia, neutropenia and hypertension), patients with toxicities reported lower PRO compared to patients with no events, reaching statistical significance for selected PRO in hypertension, neutropenia and altered taste. (Table 12)

Patients with altered taste reported significantly lower FKSI-15 (38.50 vs. 46.50, p=0.45) and FKSI-DRS (26.00 vs. 32.00, p=0.013) scores compared to patients without altered taste. After adjustment with age, gender and co-morbidities, the p-values were 0.09 and 0.03 respectively. Clinically significant poorer scores were observed in patients with altered taste compared to those without, for all scores with the exception of EQ-5D VAS. (Table 12)

Patients with hypertension reported significantly lower EQ-5D Index score compared to patients without (TTO: 0.73 vs. 1.00, p=0.03 and uVAS: 0.69 vs. 1.00, p=0.03).

After adjustment with age, gender and co-morbidities, p-values were 0.05 and 0.04 respectively. The difference in scores for all tools (EQ-5D, FACT-G and FKSI-15) were also clinically significant for hypertension, in other words, patients with hypertension reported clinically poorer scores compared to those without hypertension. (Table 12)

The EQ-5D Index scores were also significantly lower for patients with Grade 2 neutropenia compared to patients without (TTO: 0.66 vs. 0.80, p=0.05 and uVAS: 0.63 vs. 0.76, p=0.05). After adjustment with age, gender and co-morbidities, p-values were 0.05 and 0.13 respectively. Likewise, patients with neutropenia reported clinically poorer scores compared to those without, for EQ-5D index, FACT-G total and FKSI-DRS. (Table 12)

Clinically significant lower scores was also observed in patients with mucositis (EQ-5D VAS and FACT-G total), HFSR (EQ-5D uVAS and FACT-G total) and anemia (FACT-G total). (Table 12)

Table 12. Comparison of PROs at the end of cycle 1 between patients with and without grade 2 and above toxicities

	Grade ≥ 2	Grade ≤ 1	P
EQ-5D Index (TTO)			
Mucositis	0.73 (0.62, 0.88)	0.80 (0.66, 1.00)	0.53
HFSR	0.71 (0.66, 0.82)	0.82 (0.66, 1.00)	0.40
Altered taste	0.67 (0.63, 0.84)	0.80† (0.66, 0.97)	0.27
Anemia	0.85 (0.66, 0.88)	0.80 (0.66, 1.00)	1.00
Neutropenia	0.66 (0.39, 0.83)	0.80† (0.69, 0.94)	0.05/0.05¶
Hypertension	0.73 (0.67, 0.87)	1.00† (0.85, 1.00)	0.03/0.05¶
EQ-5D Index (uVAS)			
Mucositis	0.70 (0.60, 0.81)	0.76 (0.63, 1.00)	0.53
HFSR	0.67 (0.63, 0.77)	0.77† (0.63, 1.00)	0.37
Altered taste	0.64 (0.60, 0.78)	0.76† (0.64, 0.95)	0.27
Anemia	0.77 (0.63, 0.81)	0.76 (0.63, 1.00)	1.00
Neutropenia	0.63 (0.48, 0.81)	0.76 (0.66, 0.91)	0.05/0.13¶
Hypertension	0.69 (0.64, 0.79)	1.00† (0.82, 1.00)	0.03/0.04¶
EQ-5D VAS			
Mucositis	70.00 (65.00, 80.00)	80.00† (70.00, 90.00)	0.25
HFSR	72.50 (65.00, 90.00)	77.50 (68.75, 86.25)	0.67
Altered taste	72.50 (70.00, 78.75)	77.50 (65.00, 90.00)	0.63
Anemia	75.00 (75.00, 80.00)	80.00 (65.00, 90.00)	0.86
Neutropenia	80.00 (72.50, 87.50)	75.00 (65.00, 90.00)	0.65
Hypertension	75.00 (62.50, 85.00)	90.00† (68.75, 100.0)	0.20
FACT-G			
Mucositis	91.00 (71.33, 101.83)	80.33† (67.25, 93.67)	0.57
HFSR	79.50(73.08, 97.83)	91.75† (62.50, 96.46)	0.98
Altered taste	77.00 (60.75, 95.87)	83.34† (72.25, 99.29)	0.53
Anemia	67.17 (58.33, 77.50)	92.25† (71.33, 100.83)	0.05
Neutropenia	67.17 (60.84, 87.67)	92.50† (78.09, 98.83)	0.14
Hypertension	83.00 (65.09, 95.75)	96.75† (83.41, 101.58)	0.30
FKSI-15			
Mucositis	45.00 (34.00, 55.00)	46.00 (42.00, 52.00)	0.82
HFSR	44.00 (42.00, 52.50)	46.50 (40.75, 52.75)	0.47
Altered taste	38.50 (32.25, 44.75)	46.50† (42.45, 53.50)	0.05/0.09¶
Anemia	46.00 (42.00, 46.00)	45.00 (42.00, 54.00)	0.72
Neutropenia	42.00 (32.50, 50.00)	46.00 (44.00, 53.00)	0.10
Hypertension	45.00 (43.00, 52.00)	52.00† (44.00, 54.75)	0.41
FKSI-DRS			
Mucositis	29.00 (26.00, 34.00)	31.00 (28.50, 33.00)	0.42
HFSR	31.00 (26.75, 33.00)	30.00 (26.75, 34.25)	0.67
Altered taste	26.00 (22.25, 28.25)	32.00† (27.75, 33.75)	0.01/0.03¶
Anemia	31.00 (31.00, 34.00)	30.00 (26.00, 33.00)	0.52
Neutropenia	27.00 (23.50, 33.50)	33.00† (28.00, 33.50)	0.40
Hypertension	30.00 (26.50, 33.50)	33.00† (30.75, 34.50)	0.30

Note: All values presented as median (Interquartile range); mucositis (n=24), HFSR (n=24); Altered taste (n=24), Anemia (n=22), Neutropenia (n=22), Hypertension (n=17)
Abbreviations: *EQ-5D Index (TTO)*, EuroQoL Group's Five Dimension Questionnaire Index (Time Trade Off); *EQ-5D Index (uVAS)*, EQ-5D Index (Visual Analogue Scale); *EQ-5D VAS*, EQ-5D Visual Analogue Scale; *FACT-G*, Functional Assessment of Cancer Therapy-General Total score; *FKSI-15*, Functional Assessment of Cancer Therapy – Kidney Symptoms Index 15 items; *FKSI-DRS*, Functional Assessment of Cancer Therapy – Kidney Symptoms Index Disease Related Symptoms
¶ Significant p-values are presented as before adjustment p-value/after adjustment p-value. Variables used for adjustment include age, gender and co-morbidities.
† Clinically significant (i.e. exceed 0.08 points for EQ-5D, 7-points for EQ-VAS, 5-point for FACT-G Total, 2-point for FACT-G subscales, 5-point for FKSI-15 Total and 3-point for FKSI-DRS subscale)

3.3.3 Discussion

Among several common Grade 2 and above toxicities, we found that hypertension, neutropenia and altered taste were most significantly associated with PRO. Instead of responding immediately to the presence of toxicities by dose reducing or discontinuation of treatment, the impact of toxicities on HRQoL may serve as a useful guide to inform the appropriate timing for dose reduction or treatment discontinuation. Our observation with regards to the impact of Grade 2 and above altered taste is novel as we are not aware of any previous reports, although a published systemic review did suggest that altered taste in chemotherapy patients could affect their diet, leading to weight loss and malnutrition, therefore affecting PRO. [137] One study reported that pharmacists who provided guidance and confirmed patients' awareness and knowledge regarding sunitinib toxicities were able to help patients better manage the toxicities and improved their treatment outcomes. [138] As such, our study provided important information on those sunitinib-induced toxicities that most significantly impaired PRO.

With regards to the effects of toxicities and PRO in mRCC patients, there was a published study that evaluated the association between HFSR and PRO among 23 patients treated with sorafenib or sunitinib. [139] The authors reported that HFSR had the most significant impact on the symptoms domain of the Skindex16 questionnaire, where the clinical severity was Grade 2 in 74% of the patients. In our study, patients with Grade 2 and above HFSR experienced only marginally lower PRO scores compared to patients without HFSR. For example, FKSI-DRS score was 31.00 among patients with Grade 2 and above HFSR while the FKSI-DRS score was 30.00 among patients without HFSR. This could suggest that the FKSI-DRS is less sensitive than

the symptoms domain of the Skindex16 questionnaire in detecting the impact of HFSR in patients with mRCC. However, this needs to be confirmed in a head-to-head comparison of the two instruments in the same study. The findings of that study would inform the choice of the optimal instrument for assessing the impact of HFSR on PRO among patients with mRCC.

3.3.4 Limitations of study

As this study was conducted with the same group of patients, limitations of the study have been previously described under chapter 2.3.4. Additionally, as we have terminated follow-up at the end of 3 cycles, we were unable to evaluate the long-term impact of sunitinib on PRO.

3.3.5 Summary of important findings

This is the first study to evaluate the PRO among Asian patients receiving the AD sunitinib for mRCC. This study has incorporated a wide range of tools, including both generic and disease-specific tools, which can provide a better understanding of the PRO. At the same time, this study identified several toxicities that significantly impaired PRO, namely hypertension, neutropenia and altered taste. Furthermore, we previously report that patients who develop altered taste demonstrated higher total exposure than those who did not develop altered taste. Altogether, this suggests that exposure levels as a result of intra-patient factors may affect the manifestation of altered taste, which in turn may adversely impact a patient's HRQoL. Hence, actively monitoring and managing these toxicities in patients taking sunitinib are warranted to prevent self-initiated treatment discontinuation among patients. Since altered taste

significantly impaired PRO, and it may be potentially associated with exposure levels, the impact of altered taste on HRQoL may serve as a useful guide to inform the appropriate timing for dose reduction or treatment discontinuation while a TDM program may serve as an objective guide for the optimization of dose. This can help to tailor the patient's dose of sunitinib such as there will be minimal impact on HRQoL, yet without compromising on treatment efficacy.

4 Exploring the association between genetic polymorphism of CYP3A5 and ABCB1 with the manifestation of toxicities in Asian mRCC patients receiving an attenuated dose of sunitinib

Disposition of sunitinib involves transportation through the ABCB1 transport protein and subsequent metabolism by CYP3A4/5 enzyme to its principal equipotent metabolite, SU12662. Differences in the safety profile of sunitinib in Asian and non-Asian patients allude to the possible role of genetic variability, although environmental differences cannot be excluded. [85]

The variability observed with TKIs is influenced not only by genetic heterogeneity of drug targets (i.e., pharmacodynamic differences), but is also contributed to by the patients' pharmacogenetic background (e.g., CYP450 and ABC drug transporter polymorphisms), adherence to treatment, and environmental factors that influence pharmacokinetics. [85] Thus, the disposition of sunitinib, which may be affected by the variability of proteins including drug metabolizing enzymes and transporters in its pharmacokinetic pathway, could play a role in explaining the differences in response and in toxicities observed. [45]

Highly polymorphic genes that play a significant role in sunitinib's pharmacological action may also play a role in explaining the variability in sunitinib exposure. [140] One enzyme of interest is the CYP3A5 enzyme. Although both CYP3A5 and CYP3A4 enzymes share substrate specificity, the relative importance of CYP3A5 and

CYP3A4 in overall CYP3A-mediated metabolism may differ between substrates. [141] The SNP in CYP3A5 is of importance as the mutant CYP3A5*3 allele is not uncommon in Asians (59 – 77%). [68] The defective CYP3A5 enzyme associated with the *3 allele may cause an accumulation of the parent drug, which has been shown in the previous sections to be more dermatotoxic than its metabolite. Coupled with the fact that Asian race and low body weight decrease sunitinib clearance [40], the defective CYP3A5 enzyme in Asians may exacerbate toxicities by further accumulating the parent drug. Furthermore, it has been previously suggested that there is an increased risk of dose reductions of sunitinib due toxicity associated with CYP3A5*1 allele. [142]

Pgp is a transmembrane efflux pump encoded by the ABCB1 gene. It is expressed in the intestines and liver and is involved in the oral absorption and biliary secretion of drugs. The genotype frequency of the homozygous TT genotype in the ABCB1 gene (C3435T) ranged from 28 to 43% in Asians compared with 0–6% in the African group [143] and 24% in the Caucasians. [144] Although this polymorphism is synonymous and there is no change in the amino acid of the resulting proteins, studies have demonstrated and suggested that it is likely to affect overall expression and function of the Pgp. [144] It has been proposed that the TT genotype could lead to increase activity or affinity of the efflux pump for sunitinib, hence leading to lower sunitinib levels. The TT genotype was also associated with a delay in dose reductions [145] and increased sunitinib clearance. [146]

The effect of SNPs on various response and toxicities has been studied previously. (Table 13) Effects of SNPs for both pharmacodynamics and pharmacokinetics targets have been evaluated. However, because the rates of SNPs differ across various populations, a focused population study was conducted to determine the effect of CYP3A5 and ABCB1 SNPs on the manifestation of toxicities and disposition of sunitinib and SU12662, in Asian mRCC patients.

Table 13. Effects of single nucleotide polymorphisms on sunitinib therapy

	N	**Gene studied**	**Significant findings**
Garcia-Donas et al [142]	95 (mRCC)	VEGFR2 VEGFR3 PDGFR-α VEGF-A IL8 CYP3A4 CYP3A5 ABCB1 ABCG2	• Reduced PFS associated with VEGFR3 • Increased risk of dose reductions due to toxicity associated with CYP3A5*1
van Erp et al [147]	203 (152 mRCC, 46 GIST, 5 others)	NR1l2 NR1l3 CYP3A5 CYP1A1 CYP1A2 ABCG2 ABCB1 PDGFRα VEGFR2 VEGFR3 RET FLT3	• Leukopenia associated with CYP1A1, FLT3, NR1l3 haplotype • Increased risk of developing any toxicity higher than grade 2 associated with VEGFR2, ABCG2 • Increased risk of mucosal inflammation associated with CYP1A1 • Increased prevalence of HFSR associated with ABCB1 haplotype
Van der Veldt et al [148]	136 (mRCC)	ABCG2 ABCB1 NR1I2 NR1I3 CYP3A5 CYP1A1 CYP1A2 VEGFR-2 VEGFR-3 PDGFR-α FLT-3	• PFS associated with CYP3A5, NR1I3 haplotype and ABCB1 haplotype
Beuselinck et al [149]	88 (mRCC)	ABCB1 CYP3A5 NR1l2 NR1l3 HIF1A PDGFRA VEGFR2 VEGFR3 FGFR2 IL8	• PFS and OS were associated with ABCB1, NR1/3 and VEGFR3 • PFS was associated with FGFR2 and NR1/2 • OS was associated with NR1/3 and VEGFR3
Kim HR et al [150]	65 (mRCC)	CYP1A1 CYP3A5 ABCB1 ABCG2 PDGFRα VEGFR2 RET FLT3	• HFSR associated with ABCG2 • Thrombocytopenia, neutropenia also associated with ABCG2

Beuselinck et al [145]	98 (mRCC)	ABCB1 CYP3A5 NR1/2 NR1/3 VEGFR1 VEGFR3	• Increased time-to-dose-reduction associated with ABCB1
Beuselinck et al [149]	91 (mRCC)	VEGFR1	• Poorer response rate associated with VEGFR1
Eechoute et al [151]	255 (mRCC)	VEGFA VEGFR2 EDN1 eNOS	• Greater elevations in SBP and MAP were associated with VEGFA • Hypertension associated with VEGFA And eNOS
Kim J et al [152]	63 (mRCC)	VEGF	• Hypertension and duration of hypertension associated with VEGF
Scartozzi et al [153]	84 (mRCC)	VEGFA VEGFC VEGFR1 VEGFR2 VEGFR3	• PFS associated with VEGFA and VEGFR3
Abbreviations: *GIST*, gastrointestinal stromal tumor; *HFSR*, hand-foot skin reaction; *mRCC*, metastatic renal cell carcinoma; *MAP*, mean arterial blood pressure; *OS*, overall survival; *PFS*, progression free survival; *SBP*, systolic blood pressure			

4.1 Methodology

The methodology adopted in this study was previously described under chapter 2.3.1 (methodology).

4.1.1 Definitions

Definitions used are previously described under chapter 2.3.1.9 (definitions). Patients will be genotyped if they provided at least one blood sample for the whole study, regardless of the number of cycles completed. Two mutually exclusive groups were defined based on patients' genotype. For CYP3A5 SNP, patients were categorized into *1*1/*1*3 genotype versus *3*3 genotype. For ABCB1 SNP, patients were categorized into CC genotype versus CT/TT genotype.

The maximum observed CTCAE grade for each toxicity across the cycles was used for analysis. Patients will be included in the analysis of the relative risk of toxicities if both genotype and toxicities data were available. Relative risk was compared in (1) patients with toxicities (any grade) vs. patients with no toxicities and (2) patients with clinically significant toxicities (grade 2 and above) vs. patients with no clinically significant toxicities (grade 1 and 0). Grade 2 and above toxicities were considered clinically significant as they could either require intervention or affect patients' daily functioning and living. Average exposure between cycles was used to compare these 2 groups of patients. If a patient had completed only 1 cycle, then only data from 1 cycle will be used. Exposure levels were compared between the two groups (*1*1/*1*3 versus *3*3 and CC versus CT/TT). Patients who had provided blood samples for pharmacokinetic analysis and genotyping will be included in the analysis

between genotype and drug exposure. Patients will be included in the analysis of the relative risk of toxicities if both genotype and toxicities data were available.

4.1.2 Genotyping

This study assessed ABCB1 SNPs (C3435T, rs1045642) and CYP3A5 SNPs (CYP3A5*1*3, rs776746) relevant to sunitinib transport and metabolism. DNA was extracted from the buffy coat layer using DNeasy Blood and Tissue Kit (Qiagen, Venlo, The Netherlands), performed according to manufacturer's instructions. Genotyping was conducted with Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP). For CYP3A, the forward primer was 5'-CTT TAA AGA GCT CTT TTG TCT CTC A-3' and the reverse primer was 5'-CCA GGA AGC CAG ACT TTG AT-3'. [154] For ABCB1, the forward primer was 5'-TGT TTT CAG CTG CTT GAT GG-3' and the reverse primer was 5'-AAG GCA TGT ATG TTG GCC TC-3'. [155]

The PCR assay was performed in a 50 µl reaction volume containing 200 ng genomic DNA, 2 µl of 10 µM of forward and reverse primers, 5 µl of 10x hot start buffer, 2 µl of 25 µM $MgSO_4$, 5 µl of dNTPs, 1 µl KOD hot start polymerase, 20 µl of betaine and distilled water. PCR conditions were initial denaturation at 95 °C for 7 minutes, denaturation at 95 °C for 30 seconds, annealing at 60 °C for 20 seconds, elongation at 68 °C for 10 seconds, final elongation at 68 °C for 7 minutes and cooling at 12 °C. DNA was then purified using the DNA purification plate (Merck Millipore, Massachusetts, USA).

For CYP3A5 SNP, digestion of the amplified product with restriction enzyme, DdeI (New England Biolabs, Massachusetts, USA), for 2 hours at 37 °C yielded fragments of 107, 71 and 22 bp in the *3 allele, and fragments of 129 and 71 bp in the *1 allele. For ABCB1 SNP, digestion of the amplified product with restriction enzyme, Sau3A1 (New England Biolabs, Massachusetts, USA) for 2 hours at 37 °C yielded fragments of 158 bp and 39 bp in the mutant allele, and no fragments in the wild type allele. This was followed by separation of the digested DNA fragments on a 2.5% w/v agarose gel in 1X TAE buffer for 30 minutes at 120 V (New England Biolabs, Massachusetts, USA). DNA bands were stained with GelRedTM (Biotium, California, USA) for visualization of bands using Molecular Imager® Gel DocTM XR System (Bio-Rad Laboratories, California, USA).

4.1.3 Statistical analysis

Descriptive statistics was utilized to summarize patients' characteristics, incidence of toxicities and prevalence of SNPs. Chi-square or Fishers' exact test was performed to determine the association between SNPs and toxicities. Relative risks (RR) were also estimated. Genotype frequencies were tested for Hardy–Weinberg equilibrium. Mann-Whitney U test was used for determination of the relationship between SNPs and exposure. Given the explorative nature of this study, the p-values were not corrected for multiple testing. All tests of statistical significance were two-sided with p-values of less than 0.05 and analysis was conducted with the IBM SPSS statistics 21.

4.2 Results

4.2.1 Patient demographics and disease characteristics

As the cohort of patients were the same, please refer to chapter 2.3.2.1

4.2.2 Toxicities observed with sunitinib therapy

As the cohort of patients were the same, please refer to chapter 2.3.2.3

4.2.3 Frequencies of the genotype

The frequency of the CYP3A5*1 allele was 40% and frequency of the ABCB1 CC genotype was 66%. Allelic frequencies were in Hardy-Weinberg equilibrium. The allelic frequencies were also largely similar to a previous paper conducted in the Singaporean population, where the reported frequency of CYP3A5*1 allele was 34% [68] and frequency of the ABCB1 CC genotype was 44%. [156] The difference in the frequencies of the ABCB1 allele could be due to the small sample size in our study.

For the CYP3A5 SNP, the genotype frequencies for *1*1, *1*3 and *3*3 were 20% (n = 5), 40% (n = 10) and 40% (n = 10), respectively. For ABCB1 SNP, the genotype frequencies for CC, CT and TT were 36% (n = 9), 60% (n = 15) and 4% (n = 1), respectively.

4.2.4 Incidence of toxicities and SNPs

There was no significant association between CYP3A5 SNPs and the incidence of toxicities, with the exception of anemia. Patients with the *1*1/*1*3 genotype had a lower risk of all-grade anemia than patients with the *3*3 genotype (RR 0.47, 95% CI 0.27 – 0.80). However, this was not observed for grade 2 and above anemia. Risk of all-grade dermatological toxicities appeared to be similar between patients with the *1*1/*1*3 genotype and patients with the *3*3 genotype (RR 0.98, 95% CI 0.72 – 1.32). Risk of both all-grade (RR 0.90, 95% CI 0.35 – 2.35) and grade 2 and above (RR 0.30, 95% CI 0.03 – 2.86) rash was observed to be lower in *1*1/*1*3 carriers. There was a reduction in risk observed for all-grade anemia, neutropenia and thrombocytopenia, while a reverse trend was observed for leucopenia. There was a trend of *1*1/*1*3 carriers having a lower risk of all-grade transaminitis and elevations of total bilirubin (TB), alanine transaminase (ALT) and aspartate transaminase (AST), however this was not statistically significant. For other general toxicities, *1*1/*1*3 carriers were observed to have a reduced risk for toxicities compared with the other group. (Table 14)

A significant association between ABCB1 SNP and incidence of all-grade rash was observed, with the incidence of rash being 3 times higher in patients who were homozygous CC wild type than in patients who were heterozygous CT or mutant TT (RR 3.00, 95% CI 1.17 – 7.67). CC carriers were also at a higher risk for both all-grade (RR 1.60, 95% CI 1.10 – 2.34) and grade 2 and above (RR 2.00, 95% CI 1.23 – 3.27) mucositis. There was no significant association between ABCB1 SNPs and incidence of other toxicities. Risk of all-grade dermatological toxicities was observed to be higher in patients with CC genotype than in CT/TT patients (RR 1.23, 95% CI

0.97 – 1.56). Risk of grade 2 and above hematological toxicities was lower in CC patients than in CT/TT patients (RR 0.73, 95% CI 0.34 – 1.57). A trend of CC carriers having a higher risk of hepatotoxicity was observed. This trend was consistent for hepatotoxicity as a group, and for individual elevations of TB, ALT and AST, but it was not statistically significant. For other general toxicities, CC carriers were observed to have similar risk for all-grade fatigue, increase in blood pressure, and altered taste, compared with the other group. (Table 15)

Table 14. Incidence of toxicities and CYP3A5 SNPs

	CYP3A5 *1*1/ *1*3 (n = 15) vs. *3*3 (n = 9)	
RR (95% CI)	**All-grade**	**Grade 2 and above**
Dermatological Toxicity [a]	0.98 (0.72 – 1.32)	0.86 (0.52 – 1.41)
Dry Skin	1.00 (0.56 – 1.79)	1.20 (0.27 – 5.29)
HFSR	1.32 (0.68 – 2.55)	1.20 (0.61 – 2.38)
Rash	0.90 (0.35 – 2.35)	0.30 (0.03 – 2.86)
Pruritus	N.A.	N.A.
Hematological Toxicity [b]	N.A.	1.20 (0.61 – 2.38)
Anemia	0.47 (0.27 – 0.80)	0.60 (0.15 – 2.36)
Leucopenia	2.70 (0.74 – 9.81)	3.00 (0.41 – 21.76)
Neutropenia	0.96 (0.45 – 2.04)	2.10 (0.55 – 7.99)
Thrombocytopenia	0.90 (0.35 – 2.35)	1.20 (0.13 – 11.43)
Hepatotoxicity [c]	1.00 (0.31 – 3.22)	N.A.
Transaminitis [d]	0.80 (0.23 – 2.79)	N.A.
Increase in TB	0.60 (0.04 – 8.46)	N.A.
Increase in ALT	0.60 (0.10 – 3.55)	N.A.
Increase in AST	0.40 (0.08 – 1.96)	N.A.
Gastrointestinal		
Mucositis	0.94 (0.59 – 1.50)	1.00 (0.56 – 1.79)
Constitutional		
Fatigue	0.83 (0.56 – 1.21)	0.60 (0.10 – 3.55)
Cardiac		
Increase in BP	1.14 (0.88 – 1.49)	2.00 (0.77 – 5.18)
Neurology		
Altered Taste	0.68 (0.42 – 1.08)	1.00 (0.31 – 3.22)

Abbreviations: *ALT*, Alanine transaminase; *AST*, Aspartate transaminase; *BP*, Blood pressure; *CI*, Confidence interval; *HFSR*, Hand-foot skin reaction; *RR*, Relative risk; *TB*, Total bilirubin

a Includes dry skin, HFSR, rash, pruritus

b Includes anemia, leucopenia, neutropenia, thrombocytopenia

c Includes elevation of TB, ALT, AST

d Includes elevation of ALT, AST

Table 15. Incidence of toxicities and ABCB1 SNPs

	ABCB1 CC (n = 8) vs. CT/TT(n = 16)	
RR (95% CI)	**All-grade**	**Grade 2 and above**
Dermatological Toxicity [a]	1.23 (0.97 – 1.56)	1.09 (0.65 – 1.83)
Dry Skin	1.20 (0.69 – 2.08)	2.00 (0.52 – 7.77)
HFSR	1.20 (0.69 – 2.08)	1.33 (0.74 – 2.40)
Rash	3.00 (1.17 – 7.67)	1.00 (0.11 – 9.44)
Pruritus	2.00 (0.52 – 7.77)	N.A.
Hematological Toxicity [b]	N.A.	0.73 (0.34 – 1.57)
Anemia	0.67 (0.32 – 1.41)	0.40 (0.06 – 2.88)
Leucopenia	0.44 (0.12 – 1.59)	1.00 (0.23 – 4.35)
Neutropenia	0.36 (0.11 – 1.26)	0.57 (0.15 – 2.15)
Thrombocytopenia	1.33 (0.52 – 3.41)	4.00 (0.42 – 37.78)
Hepatotoxicity [c]	2.00 (0.67 – 5.98)	2.00 (0.14 – 27.99)
Transaminitis [d]	2.67 (0.78 – 9.15)	N.A.
Increase in TB	2.00 (0.14 – 27.99)	N.A.
Increase in ALT	2.00 (0.34 – 11.70)	N.A.
Increase in AST	3.00 (0.62 – 14.49)	N.A.
Gastrointestinal		
Mucositis	1.60 (1.10 – 2.34)	2.00 (1.23 – 3.27)
Constitutional		
Fatigue	0.92 (0.58 – 1.47)	N.A.
Cardiac		
Increase in BP	1.08 (0.93 – 1.25)	1.67 (0.88 – 3.14)
Neurology		
Altered Taste	1.09 (0.65 – 1.83)	0.29 (0.04 – 1.94)

Abbreviations: *ALT*, Alanine transaminase; *AST*, Aspartate transaminase; *BP*, Blood pressure; *CI*, Confidence interval; *HFSR*, Hand-foot skin reaction; *RR*, Relative risk; *TB*, Total bilirubin

a Includes dry skin, HFSR, rash, pruritus

b Includes anemia, leucopenia, neutropenia, thrombocytopenia

c Includes elevation of TB, ALT, AST

d Includes elevation of ALT, AST

4.2.5 Exposure levels and SNPs

When exposure levels ($C_{min,ss}$) were compared between the CYP3A5 *1*1/*1*3 genotype and the *3*3 genotype, there was no difference in the normalized and actual sunitinib levels, total levels and SM ratio between the 2 groups for CYP3A5. On the other hand, normalized and actual metabolite SU12662 levels were significantly higher in the *3*3 genotype than the other group (Normalized: 13.74 vs. 9.82 ng/ml, p = 0.05; Actual: 13.74 vs. 9.58 ng/ml, p = 0.05). Similar trends were observed with the $C_{max,ss}$ exposure levels. (Table 16 & Table 17)

Patients who were CC genotype for ABCB1 were observed to have higher normalized and actual sunitinib exposure ($C_{min,ss}$) than those who were CT/TT genotype (Normalized: 76.81 vs. 56.55 ng/ml, p = 0.02; Actual: 76.81 vs. 49.85 ng/ml, p = 0.003). Likewise, the CC genotype group demonstrated higher SM ratio for $C_{min,ss}$ when the 2 groups were compared (7.89 vs. 5.25, p = 0.02). However, no difference was observed between the 2 groups for total and metabolite levels of $C_{min,ss}$. Similar trends were observed with the $C_{max,ss}$ exposure levels. (Table 16 & Table 17)

Table 16. Exposure levels ($C_{min,ss}$) and SNPs

CYP3A5	***1*1/*1*3 (n = 15)**	***3*3 (n = 9)**	**P**
Sunitinib, normalized	59.20 (45.66, 85.58)	74.67 (56.55, 87.19)	0.36
Sunitinib, actual	59.29 (39.79, 75.97)	70.15 (53.62, 87.19)	0.33
Metabolite, normalized	9.82 (8.29, 15.81)	13.74 (11.67, 18.90)	0.05
Metabolite, actual	9.58 (5.98, 12.15)	13.74 (10.08, 18.90)	0.05
Total, normalized	68.27 (53.95, 101.38)	89.65 (75.10, 115.75)	0.09
Total, actual	64.97 (47.13, 85.55)	85.93 (65.46, 115.75)	0.08
SM Ratio	5.50 (4.10, 7.93)	5.32 (4.00, 6.64)	0.42
ABCB1	**CC (n = 8)**	**CT/TT (n = 16)**	**P**
Sunitinib, normalized	76.81 (64.49, 101.46)	56.55 (45.18, 76.65)	0.02
Sunitinib, actual	76.81 (64.49, 101.46)	49.85 (39.84, 63.57)	0.003
Metabolite, normalized	13.16 (9.07, 17.05)	11.13 (8.98, 15.29)	0.85
Metabolite, actual	12.16 (9.07, 16.64)	10.40 (7.57, 13.35)	0.54
Total, normalized	89.15 (80.05, 114.17)	69.10 (54.31, 98.45)	0.11
Total, actual	89.15 (80.05, 114.17)	58.87 (48.10, 83.74)	0.04
SM Ratio	7.89 (5.40, 9.10)	5.25 (4.07, 6.31)	0.02

Note: All values are average exposure ($C_{min,ss}$, ng/ml) between the cycles and reported as median (inter-quartile range)

Abbreviations: *SM ratio,* Sunitinib to metabolite ratio

Table 17. Exposure levels ($C_{max,ss}$) and SNPs

CYP3A5	***1*1/*1*3 (n = 15)**	***3*3 (n = 9)**	**P**
Sunitinib, normalized	90.90 (67.06, 135.31)	111.36 (87.41, 131.56)	0.30
Sunitinib, actual	85.81 (58.43, 110.74)	106.11 (83.82, 130.42)	0.25
Metabolite, normalized	11.50 (9.61, 18.57)	16.06 (13.59, 21.61)	0.06
Metabolite, actual	11.09 (7.01, 14.28)	16.06 (11.74, 21.61)	0.06
Total, normalized	103.37 (76.67, 153.88)	135.62 (110.24, 166.34)	0.13
Total, actual	91.82 (66.95, 121.83)	124.46 (97.20, 166.34)	0.14
SM Ratio	7.36 (5.34, 9.98)	6.97 (5.22, 8.70)	0.46
ABCB1	**CC (n = 8)**	**CT/TT (n = 16)**	**P**
Sunitinib, normalized	114.78 (98.57, 156.00)	87.41 (67.37, 118.67)	0.02
Sunitinib, actual	114.78 (98.57, 151.44)	77.94 (59.24, 100.83)	0.008
Metabolite, normalized	15.39 (10.56, 19.38)	12.98 (10.53, 17.94)	0.85
Metabolite, actual	14.15 (10.56, 18.87)	12.18 (8.80, 15.61)	0.50
Total, normalized	129.15 (117.35, 170.67)	103.68 (78.10, 149.31)	0.11
Total, actual	129.15 (117.35, 167.63)	88.53 (68.85, 122.63)	0.03
SM Ratio	10.08 (7.14, 11.88)	6.91 (5.26, 8.31)	0.02

Note: All values are average exposure ($C_{max,ss}$, ng/ml) between the cycles and reported as median (inter-quartile range)

Abbreviations: *SM ratio,* Sunitinib to metabolite ratio

4.3 Discussion

This study demonstrated that the ABCB1 polymorphism may affect the risk for toxicities and sunitinib levels, but that there was a lack of statistically significant evidence associating the polymorphism of CYP3A5 to the same outcomes. Patients expressing the CC genotype for ABCB1 were more likely than the CT/TT genotype to develop rash and mucositis in the course of their sunitinib therapy. Patients expressing the CC genotype had an increased risk for all-grade rash (3-fold increase), all-grade mucositis (1.6-fold increase) and grade 2 and above mucositis (2-fold increase) as compared with patients expressing the CT/TT genotype. Patients expressing the CC genotype for ABCB1 also achieved higher sunitinib levels, and consequently higher SM ratio, than patients who were CT/TT genotype.

Metabolism of sunitinib was unaffected by variations in the CYP3A5 SNP. This may be attributed to the redundancy between CYP3A5 and CYP3A4 enzymes as the relative metabolizing capacity of CYP3A4 for sunitinib may greatly exceed, and adequately compensate for, any variability in the CYP3A5 enzyme. As sunitinib possesses an intermediate hepatic extraction ratio (0.48), the dependence of the clearance on P450 activity may not be as strong as a low hepatic extraction ratio drug. It has been proposed that sunitinib is a much better substrate of CYP3A4 compared with CYP3A5, and therefore the contribution of CYP3A5 to the metabolism of sunitinib may be low. [141] A recent study has also suggested that CYP3A5 SNPs do not affect the clearance of sunitinib and SU12662. [146] This is in agreement with our findings that the CYP3A5 polymorphism may not be an important contributor to the disposition of sunitinib. One other study has demonstrated that the presence of a copy of the *1 allele in CYP3A5 is associated with an increased risk of dose reduction due

to toxicity. [142] For that reason, we conducted additional analysis to evaluate whether CYP3A5*1 carriers were more likely to have their dose reduced, but did not observe such an association. Although CYP3A5 plays a minimal role in the disposition of sunitinib, several studies have highlighted the contributions of CYP3A4 in sunitinib exposure. Midazolam exposure was found to be highly correlated with sunitinib exposure and this accounted for 51% of the observed inter-patient variability in sunitinib pharmacokinetics. [157] A further study in a Caucasian population has also found that the clearance of sunitinib is affected by the CYP3A4*22 polymorphism. [146] These findings further support CYP3A4 as the main contributor to sunitinib metabolism and the reduced dependence on CYP3A5. However, the CYP3A4*22 polymorphism was not detected in Asians so it cannot explain the differences in exposure in Asians. [158] In addition, patients with the *3*3 polymorphism possessed higher median metabolite levels than the *1*1/*1*3 group. This can be attributed to one patient in the former group displaying an extremely high metabolite level, which skewed the results. In this patient, the level was 144 ng/ml, whereas the metabolite level in the rest of the patients ranged from 4 to 26 ng/ml. When the outlier was excluded from the *3*3 group, the statistical difference was no longer present.

Our results also demonstrated that presence of the ABCB1 SNP is correlated with a higher sunitinib level and SM ratio in patients with the CC genotype compared with those with the CT/TT genotype. The functional effect of the ABCB1 polymorphism on Pgp has been heavily debated. [69] Several studies have provided conflicting results, with the 3435 TT genotype associated with both increased [159-161] and decreased [144, 162-164] expression of Pgp. For sunitinib, it has been shown that the

TT, TC and CC genotypes for ABCB1 affect sunitinib clearance by +7%, +3%, and -11.5%, respectively. [146] This is in line with our results in which the CC group displayed higher levels of sunitinib, possibly due to a decrease in its clearance. Patients exhibiting the CC genotype had an increased risk for all-grade rash (3-fold increase), all-grade mucositis (1.6-fold increase) and grade 2 and above mucositis (2-fold increase) compared with the CT/TT patients. This is not surprising, as the accumulation of sunitinib in the former group of patients could have resulted in the higher exposure and occurrence of toxicity. This is further supported by our previous findings in which sunitinib exhibited greater dermatological toxicity than its metabolite. [165]

Studies conducted in Korea and Japan have demonstrated an association between the ATP-binding cassette sub-family G member 2 (ABCG2) polymorphism, toxicities such as thrombocytopenia, HFSR, and hypertension [150], and the pharmacokinetics of sunitinib. [166] The polymorphic effects of ABCG2, which encodes for the breast cancer resistance protein (BCRP) have also been very recently identified as a significant covariate for the prediction of sunitinib clearance. [166] To confirm such associations with ABCB1, future studies should be performed in our local South-East Asian population, which encompasses different ethnicities of that in Korea and Japan. As all the patients in our study used the AD regimen and demonstrated a lower incidence and severity of toxicities, the effects of SNPs on severe toxicities may have been downplayed. Future studies involving a larger sample should be conducted to confirm these findings. If the role of the ABCB1 polymorphism on exposure and toxicities is confirmed, genotyping for ABCB1 and TDM should be considered in clinical practice to aid in the personalization of drug therapy. Even with the AD

sunitinib, our patients managed to achieve sufficient exposure to sunitinib and experience frequent toxicities, thus further dose reductions may be necessary in this group of patient. Thus, TDM may complement clinical evaluation by providing additional information on efficacy, adherence and toxicity. By ensuring that the optimal dose is prescribed to the right patient according to the genotype, we can achieve maximum efficacy with minimal toxicity.

4.4 Limitations of study

As previously mentioned under chapter 2.3.4, the limitations of our study included limited sampling points and a small sample size. As tumor type (mRCC, GIST and other solid tumor) accounted for a major portion of the variability in the clearance of sunitinib and its metabolite [40], our study only included patients with mRCC and this may have resulted in the small sample size. Furthermore, as this was an exploratory study with small sample size, no statistical adjustments was performed.

4.5 Summary of important findings

In Asian mRCC patients, polymorphism of ABCB1 may be associated with drug exposure and manifestation of toxicities, but the polymorphism of CYP3A5 is not. Although CYP3A5 is highly polymorphic in Asian population, the CYP3A5 polymorphism may be excluded as a cause of high drug exposure and/or toxicity as this enzyme plays a minimal role in the disposition of sunitinib. The ABCB1 polymorphism may affect sunitinib levels and the occurrence of dermatological toxicities, including rash and mucositis.

5 Metabolism-related pharmacokinetic drug-drug interactions in tyrosine kinase inhibitors

5.1 Role of metabolism-related drug-drug interactions in tyrosine kinase inhibitor therapy

DDIs occur when a patient's pharmacological or clinical response to the drug is modified by administration or co-exposure to another drug. Pharmacokinetic interactions occur when one drug influences the pharmacokinetic processes such as absorption, distribution, metabolism and excretion, of another drug. Altered metabolism is among the most complex of these processes by which DDIs can occur, and induction or inhibition of hepatic enzymes by drugs are often implicated. The clinical consequences of enzyme induction or inhibition depend on the pharmacological and toxic effect of both the parent drug and its metabolite(s). For example, if the parent compound is more active than its metabolite, inhibition of metabolism increases the exposure to the drug and also its therapeutic and/or toxic effects. However, if the parent compound is a pro-drug, inhibition of metabolism may result in a decrease in therapeutic efficacy. More recently, another paradigm of interaction arises when the metabolite is more toxic, and hence induction of metabolism down this pathway can exacerbate toxicity. (Figure 3)

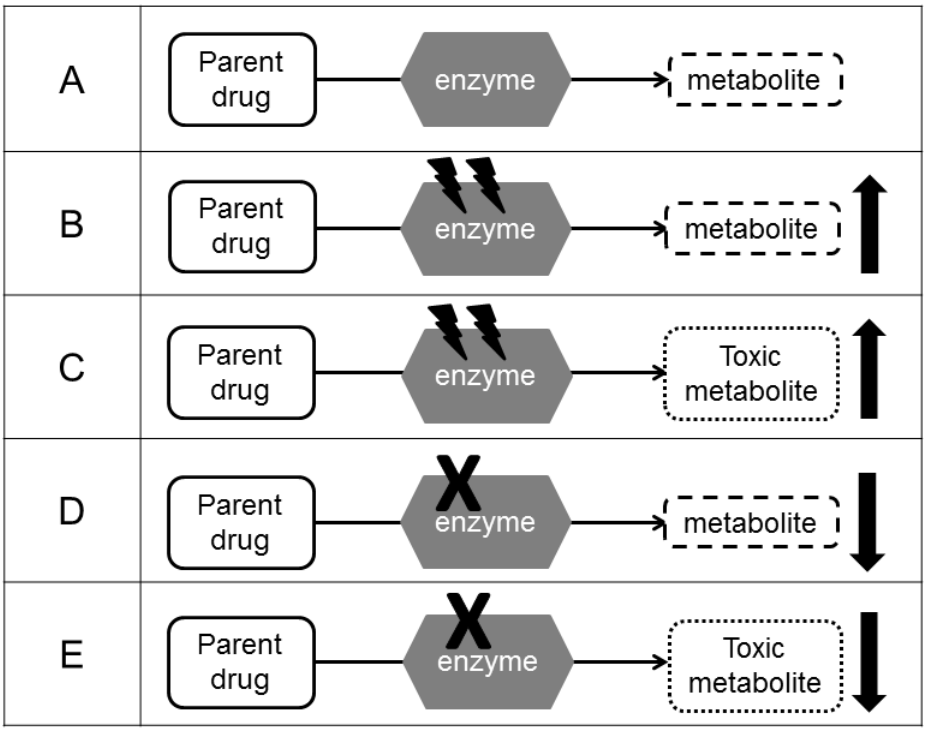


Figure 3. (A) Metabolism of parent drug to metabolite by drug metabolizing enzyme (B) Enzyme induction and increased formation of metabolite (C) Enzyme induction and increased formation of toxic metabolite (D) Enzyme inhibition and decreased formation of metabolite (E) Enzyme inhibition and decreased formation of toxic metabolite

A recent study revealed that co-prescription of drugs that induce or inhibit metabolic pathways used by TKIs was high. Overall co-prescribing rates for DDI drugs that may decrease TKIs effectiveness ranged from 23 – 57%, while co-prescribing rates with drugs that may increase TKI toxicity ranged from 24 – 74%. [167] In another study which studied the pattern of DDI in cancer patients, the frequency of at least one potential DDI occurring was 63%. Among them, almost 62% of the identified DDIs were considered as major, where the effects of the interaction may result in serious consequences such as hospitalization, therapeutic failure, permanent injury or even death. [168] Although many studies highlighted the problem of frequent DDI among TKIs, these studies did not address the clinical consequences of the potential DDIs, such as increased toxicity or therapeutic failure. In some cases, these combinations could have been intentionally prescribed, where physicians may have knowingly prescribed a potentially interacting combination because they considered the potential benefits to outweigh the risks or because the patient had tolerated the combination in the past. [169] As these TKIs are relatively new to the market, the scientific evidence that support their DDI is limited. Therefore, it is not unexpected to observe that oncology professionals are unable to identify TKI DDI pairs which that might have a high probability of causing deleterious effects in cancer patients. [170]

Cancer patients are susceptible to DDIs as they receive many medications, either for supportive care or for treatment of therapy-induced toxicity. [72] For instance, an observational study highlighted that patients were receiving on average 6.8 drugs in addition to sunitinib. Among them, antihypertensive drugs were most commonly prescribed, followed by analgesics, antiemetics and thyroid substitution therapy. [73] In certain cases, a cancer patient's pharmacokinetic parameters may be also altered,

for example, edema affecting volume of distribution or impaired drug absorption due to malnutrition or mucositis; these issues may also affect the consequences of DDIs. Since most cancers typically occur at a later age, these patients may also be receiving other drugs for the management of their comorbidities. Differences in DDI outcomes are generally minor due to the wide therapeutic windows of common drugs; however, in cancer chemotherapy with anti-cancer drugs, serious clinical consequences may occur from small changes in drug metabolism and pharmacokinetics. [74] We described in previous chapters that sunitinib is more dermatotoxic than SU12662, suggesting that patients who receive concomitant CYP3A4 inhibitors would be at a higher risk of dermatological toxicities, due to a lower ability to metabolize sunitinib. Consequently, this may result in an increased risk of non-compliance, dose reduction, or therapy discontinuation and thereby leading to suboptimal therapy.

Due to the substantial potential for interaction between TKIs and other drugs that modulate the activity of metabolic pathways, unwanted clinical consequences may occur from small changes in drug metabolism and pharmacokinetics in cancer patients. Furthermore, it is a challenge to determine the clinical effects of the DDIs due to the large inter-patient variability in the pharmacokinetics of the TKIs. Therefore, the objective of this chapter is to highlight the current understanding of DDIs among TKIs, with a specific focus on DDIs involving metabolism, to identify challenges in the prediction of DDIs and provide some possible recommendations. Henceforth, this chapter provides insights on DDIs among TKIs, which will be helpful in appreciating the following chapters, when actual DDIs cases involving TKIs like lapatinib and also erlotinib are studied.

5.2 Methods

A search was conducted to identify all small molecule TKIs approved by the FDA from January 2000 to February 2014. A comprehensive literature search of articles involving TKIs was performed using the PubMed and Scopus databases. Meetings abstracts presented at American Association for Cancer Research (AACR) and American Society of Clinical Oncology (ASCO) were also reviewed. The search was conducted by using the generic names of all the identified TKIs (afatinib, axitinib, bosutinib, cabozantinib, crizotinib, dasatinib, erlotinib, gefitinib, imatinib, lapatinib, nilotinib, pazopanib, ponatinib, regorafenib, sorafenib, sunitinib, vandetanib), and terms such as ‘‘drug interaction’’, ‘‘metabolism’’ and “pharmacokinetics”. The search was limited to English language articles published between January 1995 and February 2014.

5.3 Results

5.3.1 Metabolic profile of tyrosine kinase inhibitors

Almost all of the TKIs undergo metabolism by CYP enzymes. The CYP3A4 is the CYP enzyme involved in the metabolism of the majority of the TKIs. Some of these TKIs, including imatinib, sunitinib and dasatinib, form an active metabolite upon metabolism. These TKIs can also act as an inducer or inhibitor to the CYP enzymes. All TKIs are primarily excreted in the feces. However, the percentage of unchanged drug recovered in the feces and urine varies widely between the TKIs. For instance, the percentage of unchanged erlotinib recovered in the feces was 1%, compared to almost 70% for nilotinib. (Table 18)

Table 18. Metabolism profile of FDA-approved tyrosine kinase inhibitors

	% of dose recovered (% recovered unchanged)		Metabolism		Induces	Inhibits
	Feces	**Urine**	**Major CYPs**	**Minor CYPs & others**		
Afatinib	85 (N.R.)	4 (N.R.)	Negligible		-	-
Axitinib	41 (12)	23 (N.D.)	CYP3A4 CYP3A5	CYP1A2 CYP2C19 UGT1A1	-	CYP1A2 CYP2C8
Bosutinib	91 (N.R.)	3 (N.R.)	CYP3A4	-	-	-
Cabozantinib	54 (N.R.)	27 (N.R.)	CYP3A4	CYP2C9	CYP1A1	CYP2C8 CYP2C9 CYP2C19 CYP3A4
Crizotinib	63 (53)	22 (2)	CYP3A4 CYP3A5	-	-	CYP3A CYP2B6
Dasatinib	85 (19)	4 (< 1)	CYP3A4	FMO-3 UGT	-	CYP3A4
Erlotinib	83 (1)	8 (< 1)	CYP3A4	CYP1A2 CYP1A1	-	CYP1A1 CYP3A4 CYP2C8
Gefitinib	86 (N.R.)	4 (N.R.)	CYP3A4 CYP2D6	-	-	CYP2C19 CYP2D6
Imatinib	68 (20)	13 (5)	CYP3A4 CYP2C8	CYP1A2 CYP2D6 CYP2C9 CYP2C19	-	CYP2C8 CYP2C9 CYP3A4/5 CYP2D6
Lapatinib	(27)	(< 2)	CYP3A4 CYP3A5	CYP2C19 CYP2C8	-	CYP3A CYP2C8
Nilotinib	93 (69)	N.R.	CYP3A4	CYP2C8	CYP2B6 CYP2C8 CYP2C9	CYP3A4 CYP2C8 CYP2C9 CYP2D6
Pazopanib	Majority in feces	4	CYP3A4	CYP1A2 CYP2C8	-	CYP1A2 CYP3A4 CYP2B6 CYP2C8 CYP2C9 CYP2C19 CYP2D6 CYP2E1
Ponatinib	87 (N.R.)	5 (N.R.)	CYP3A4	CYP2C8 CYP2D6 CYP3A5	-	-
Regorafenib	71 (47)	19 (2)	CYP3A4	UGT1A9	-	CYP2C8 CYP2C9 CYP2B6 CYP3A4 CYP2C19
Sorafenib	77 (51)	19 (N.D)	CYP3A4	UGT1A9	-	CYP2B6 CYP2C8

						CYP2C9 CYP2C19 CYP2D6 CYP3A4
Sunitinib	61 (N.R.)	16 (N.R.)	CYP3A4	-	-	-
Vandetanib	44 (N.R.)	25 (N.R.)	CYP3A4	FMO-1 FMO-3	-	-
Note: All information was obtained from product information labels [70, 71] Abbreviations: *N.R.*, not reported; *N.D.*, not detected						

5.3.2 Potential effect of enzyme inducer/inhibitor on pharmacokinetics of tyrosine kinase inhibitors

As most of these TKIs are substrates of the CYP3A4 enzyme, inducers and inhibitors of this enzyme can affect the exposure to these TKIs. The most common inducer and inhibitor used for the study of the potential pharmacokinetic interaction are rifampicin and ketoconazole respectively. As expected, ketoconazole increases the exposure of TKIs due to the inhibition of metabolism. However, the extent to which the exposure is increased varied widely between the TKIs. For instance, concomitant ketoconazole can result in a slight increase of imatinib AUC by 40%. [171] But for bosutinib, the increase in AUC is more than 8 times with concomitant ketoconazole. [172] It is also interesting to note that exposure to TKI is much increased by concomitant ketoconazole, as CYP3A4 is the only enzyme involved in the TKI's metabolic pathway. This is also supported by a report by Scripture et al, whereby drug interactions are likely to be significant when drug elimination occurs primarily through a single metabolic pathway. [173] Similarly for rifampicin, the combination of drugs resulted in a decrease in exposure to the TKIs. However, the extent to which the exposure is decreased is not as large as that observed with ketoconazole. The decrease in TKI AUC ranged from 40% for vandetanib [174] to 94% for bosutinib. [14] Among all the TKIs, sorafenib seems to be an exception, where studies have consistently demonstrated that clinically important interaction between sorafenib and drugs metabolized primarily by CYPs 3A4, 2C19, or 2D6 are not expected. [175, 176] Although co-administration of sorafenib with capecitabine has been shown to result in a mild increase in capecitabine exposure, these findings were not statistically significant and the mechanism of interaction is unclear. [177] As strong CYP3A4 inducers and inhibitors generally cause a change in exposure, the concomitant use of

such agents with TKIs is not recommended and should be avoided if possible. If such combinations must be used, manufacturers generally recommend that dose increase or decrease may be considered and that patients should be monitored closely following any changes in dosages. (Table 19)

Table 19. Potential effect of enzyme inhibitor/inducer on pharmacokinetics of tyrosine kinase inhibitors

	Changes in PK of TKI	**Recommendations**	**Ref**
Afatinib	Unlikely	Unlikely	[6]
Axitinib	Ketoconazole: ↑ 1.5x C_{max}, ↑ 2x AUC of axitinib Rifampicin: ↓ 71% C_{max}, ↓ 79% AUC of axitinib	Strong 3A4/5 inhibitors → Avoid; Consider alternative agents; Consider ↓ dose of axitinib by half Strong 3A4/5 inducers → Avoid; Consider alternative agents	[178] [179] [13]
Bosutinib	Ketoconazole: ↑ 5.2x $C_{max,}$ ↑ 8.6x AUC of bosutinib Rifampicin: ↓ 86% C_{max}, ↓ 94% AUC of bosutinib	Strong 3A inhibitors → Avoid Strong 3A inducers → Avoid	[172] [14]
Cabozantinib	Ketoconazole: ↑ 38% AUC of cabozantinib Rifampicin : ↓ 77% AUC of cabozantinib	Strong 3A4 inhibitors → Avoid; Consider ↓ daily dose of cabozantinib by 40 mg Strong 3A4 inducers → Avoid; Consider ↑ daily dose of cabozantinib by 40 mg	[15]
Crizotinib	Ketoconazole: ↑ 1.4x C_{max}, ↑ 3.2x AUC of crizotinib Rifampicin: ↓ 69% C_{max}, ↓ 82% AUC of crizotinib	Strong 3A4 inhibitors → Avoid Strong 3A4 inducers → Avoid	[17]
Dasatinib	Ketoconazole: ↑ 4x C_{max}, ↑ 5x AUC of dasatinib Rifampicin : ↓ 81% C_{max}, ↓ 82% AUC of dasatinib	Strong 3A4 inhibitors → Avoid; Consider alternative agents; Consider ↓ dose of dasatinib to 20 mg daily (for patients taking 100 mg) or 40 mg daily (for patients taking 140 mg) Strong 3A4 inducers → Consider alternative agents; Consider ↑ dose of dasatinib	[180] [18]
Erlotinib	Ketoconazole: ↑ 67% AUC of erlotinib Ciprofloxacin: ↑ 17% $C_{max,}$ ↑ 39% AUC of erlotinib Rifampicin: ↓ 58% AUC of erlotinib	Strong 3A4 inhibitors → Use with caution Strong 3A4 inducers → Consider alternative agents; consider ↑ dose of erlotinib (up to maximum of 450 mg)	[19]
Gefitinib	Itraconazole: ↑ 51% C_{max}, ↑ 78% AUC of gefitinib Rifampicin: ↓ 65% $C_{max,}$ ↓ 83% AUC of gefitinib Phenytoin: ↓ 26% C_{max}, ↓ 47% AUC of gefitinib	Strong 3A4 inhibitors → Use with caution Strong 3A4 inducers → Consider ↑ dose of gefitinib to 500 mg daily	[181] [182] [20]

Imatinib	Ketoconazole: ↑ 26% $C_{max,}$ ↑ 40% AUC of imatinib Gemfibrozil[a]: ↓ 56% C_{max}, ↓ 48% AUC of N-desmethylimatinib Rifampicin: ↓ 54% C_{max}, ↓ 74% AUC of imatinib EIAEDs: ↓ 68% C_{trough} of imatinib	Strong 3A4 inhibitors → Use with caution Strong 3A4 inducers → Consider alternative agents	[171] [183] [184] [185] [21]
Lapatinib	Ketoconazole: ↑ 114% C_{max} , ↑ 257% AUC of lapatinib Carbamazepine: ↓ 59% C_{max}, ↓ 72% AUC of lapatinib	Strong 3A4 inhibitor → Avoid; Consider ↓ dose of lapatinib to 500 mg daily Strong 3A4 inducers → Avoid; Consider ↑ dose of lapatinib up to 4500 mg daily (for HER2+ metastatic breast cancer) or 5500 mg daily (for HR+, HER2+ breast cancer)	[186] [22]
Nilotinib	Ketoconazole: ↑ 1.8x C_{max}, ↑ 3x AUC of nilotinib Rifampicin: ↓ 64% C_{max}, ↓ 80% AUC of nilotinib	Strong 3A4 inhibitors → Avoid ; Consider ↓ dose of nilotinib to 300 mg daily (in resistant or intolerant Ph+ CML) or 200 mg daily (newly diagnosed Ph+ CML-CP) Strong 3A4 inducers → Avoid; Consider alternative agents	[187] [23]
Pazopanib	Ketoconazole: ↑ 45% C_{max}, ↑ 66% AUC of pazopanib	Strong 3A4 inhibitors → Avoid; Consider ↓ dose of pazopanib to 400 mg Strong 3A4 inducers → Pazopanib should not be used	[188] [24]
Ponatinib	Ketoconazole: ↑ 47% C_{max}, ↑ 78% AUC of ponatinib	Strong 3A4 inhibitors → Consider ↓ dose of ponatinib to 30 mg daily	[189] [25]
Regorafenib	Ketoconazole: ↑ AUC of regorafenib Rifampicin: ↓ AUC of regorafenib	Strong 3A inhibitors → Avoid Strong 3A4 inducers → Avoid	[26]
Sorafenib	Ketoconazole: no change in AUC of sorafenib Rifampicin: ↓ 37% AUC of sorafenib	 Strong 3A4 inducers → Consider ↑ dose of sorafenib	[27]
Sunitinib	Ketoconazole: ↑ 49% C_{max}, ↑ 51% AUC of combined sunitinib & N-desethyl sunitinib Ritonavir[b]: ↓ 48% C_{max}, ↓ 40% AUC of N-desethyl sunitinib	Strong 3A4 inhibitor → Consider alternative agents; Consider ↓ dose reduction of sunitinib to a minimum of 37.5 mg (GIST & RCC) or 25 mg (pNET)	[28] [190]

	Rifampicin: ↓ 23% C_{max}, ↓ 50% AUC of combined sunitinib & N-desethyl sunitinib Efavirenz[c]: ↑ 410% C_{max}, ↑ 390% AUC of N-desethyl sunitinib	Strong 3A4 inducers → Consider alternative agents; Consider ↑ dose of sunitinib to a maximum of 87.5 mg (GIST & RCC) or 62.5 mg (pNET)	
Vandetanib	Itraconazole: ↑ 9% AUC of vandetanib Rifampicin: ↓ 40% AUC of vandetanib	 Strong 3A4 inducers → Avoid	[174] [29]

Abbreviations: *AUC*, area under the curve; C_{max}, peak concentration; C_{trough}, trough concentration; *EIAEDs*, enzyme-inducing antiepileptic drugs; *GIST*, gastrointestinal stromal tumor; *PK*, pharmacokinetics; *Ph+ CML*, Philadelphia chromosome-positive chronic myeloid leukemia; *Ph+ CML-CP*, Philadelphia chromosome-positive chronic myeloid leukemia in chronic phase; *pNET*, progressive, well-differentiated pancreatic neuroendocrine tumors; *RCC*, renal cell carcinoma; *TKI*, tyrosine kinase inhibitor

a Gemfibrozil inhibits the CYP2C8-mediated formation of N-desmethylimatinib (equipotent metabolite of parent imatinib)

b Ritonavir inhibits the CYP3A4-mediated formation of N-desethyl sunitinib (equipotent metabolite of parent sunitinib)

c Efavirenz induces the CYP3A4-mediated formation of N-desethyl sunitinib (equipotent metabolite of parent sunitinib)

5.3.3 Effect of tyrosine kinase inhibitors as an enzyme inducer/ inhibitor on pharmacokinetics of other drugs

While the effect of enzyme inhibitors or inducers (such as ketoconazole and rifampicin) on pharmacokinetics of TKI has been extensively studied, the reciprocal effect of a TKI acting as an enzyme inducer or inhibitor has been comparatively less investigated. The ability for TKIs to increase or decrease plasma concentration of non-anticancer drugs is mainly unclear, especially within in-vivo conditions. Concomitant imatinib and simvastatin has resulted in a 2-fold increase in simvastatin's C_{max} and 3-fold increase in simvastatin AUC. This is likely due to inhibition by imatinib of the CYP3A4 enzyme, which is responsible for the metabolism of simvastatin to other metabolites. This also suggests that in the presence of imatinib, plasma levels of standard doses of drugs which are degraded by CYP3A4 enzyme may also be increased. As such, caution is required when imatinib is administered with other CYP3A4 substrates with a narrow therapeutic window. [191] Imatinib has also shown to increase the exposure to metoprolol (23% increase in metoprolol AUC) when both agents are used together, due to the inhibition of CYP2D6 enzyme by imatinib. [192] Although the combination of gefitinib and metoprolol also resulted in an increased exposure to metoprolol (35% increase in metoprolol AUC), this change was not statistically significant. Despite this, gefitinib has a potential to increase plasma concentrations of CYP2D6 substrates and caution should be exercised when using CYP2D6 substrates that have a narrow therapeutic window. [181] Concomitant use of two TKIs has also been investigated in certain cases. Clearance of erlotinib was markedly enhanced by sorafenib when the 2 agents were given concurrently, although the potential mechanism for this seeming interaction is not obvious. [193] In another example, co-administration of lapatinib

and pazopanib lead to an increase in pazopanib exposure, and it has been suggested that this might be the result of inhibition of CYP3A4 and/or cellular transporters such as ABCB1 and ABCG2 by lapatinib. [194] The effect of lapatinib on clearance of vinorelbine has also been studied, where lapatinib resulted in a lower clearance of vinorelbine due to the inhibition of CYP3A4. [195] (Table 20)

Table 20. Reported effect of TKIs as enzyme inhibitor/inducer on pharmacokinetics of other drugs

TKI	Interacting drug	Change in PK	Remarks	Ref
Imatinib	Simvastatin	↑ 2-fold simvastatin C_{max} ↑ 3-fold simvastatin AUC		[191]
	Metoprolol	↑ 23% metoprolol AUC		[192]
Erlotinib	Sorafenib	↓ erlotinib AUC & C_{max}	Mechanism unclear	[193]
Lapatinib	Pazopanib	↑ pazopanib AUC & C_{max}	Inhibition of CYP3A4 and/or cellular transporters such as ABCB1 and ABCG2 by lapatinib	[194]
	Vinorelbine	↓ 30–40% vinorelbine clearance	Inhibition of CYP3A4 by lapatinib, resulting in lower clearance of vinorelbine	[195]
Abbreviations: *PK* pharmacokinetics, *TKI* tyrosine kinase inhibitor, C_{max} maximum concentration, *AUC* area under the curve				

5.3.4 Applicability of in-vitro and in-vivo data within clinical practice

Majority of the available pharmacokinetics information are resulting from in-vitro data, preclinical animal studies or from small phase I studies which evaluated healthy volunteers who were administered single dose of the drugs. Emerging methods includes creating a simulator where in-vivo clearances can be predicted from their in-vitro data. For instance, the impact of co-administration of ketoconazole was simulated, and the predicted two-fold increase in erlotinib exposure was found to be consistent with the results of a clinical study. [196] However, in most cases, the prediction may not be entirely accurate, especially when most of these studies evaluate DDIs in the form of two interacting drugs, and these results may not be realistic where multiple drugs are used concurrently. In addition, several reasons have been proposed to highlight the inability of clinical interactions to be accurately predicted. Firstly, the therapeutic concentration of a new drug and its metabolites in specific tissues are not always possible to determine. To further complicate the issue, the multiplicity of enzymes and transporters involved in the disposition of the said drugs and the intricacy of the pathways and interactions, in addition to overlapping substrate specificities of these proteins result in complex and sometimes perplexing pharmacokinetic interactions with multidrug regimens. Large differences in genotype and expression level of each of these contributors can lead to a very complex influence on actual drug disposition. There can also be compensatory responses when one enzyme or transporter is inhibited, “cushioning” any resulting change in metabolism. Each drug has a different level of dependence on intrinsic clearance for its overall clearance. Drugs with a high extraction ratio may be less sensitive to enzyme inhibition and induction, as their clearance is limited by blood flow rather than intrinsic activity. This makes it very challenging to test all of them in an in-vitro

system. Furthermore, the clinical significance of an interaction is unknown even if the in-vitro or in-vivo effect was established. Moreover, underlying disease states may influence the occurrence of an interaction that is unaccounted for by in-vitro studies or by studies involving healthy volunteers alone. [197, 198] Endogenous CYP isoforms expressed in tumor cells also contributes to the metabolism of active drug, thereby playing a role in altering the half-life and pharmacokinetics of the administered TKI. [199] In summary, it is complex and challenging to extrapolate these preliminary results to routine clinical practice, where TKIs are used to treat patients with cancer, many of whom are receiving multiple drugs and many of whom have impaired renal or hepatic function. [200]

5.3.5 Formation of reactive intermediates/ metabolites and implications for toxicity

Several TKIs such as dasatinib, erlotinib, gefitinib, imatinib, lapatinib, nilotinib, pazopanib, sorafenib and sunitinib undergo bioactivation to form reactive intermediates, which has implications in the generation of idiosyncratic adverse drug reactions (ADR). [201] One TKI whose metabolism and implications for toxicity has been extensively studied is lapatinib. Lapatinib has been shown to be extensively metabolized, as exemplified by diverse biotransformations to form metabolites. A number of the metabolites could potentially form reactive electrophilic intermediates that could contribute to hepatotoxicity. [202] It is also worthy of note that the daily dose of these TKIs are high, for example the daily dose of lapatinib is more than 1000 mg. A high daily dose of more than 50 mg has been demonstrated to be a risk factor for ADR [95]; thereby setting 14 out of the 18 approved TKIs at risk. (Table 21) Due to the high dose, there would be high amounts of these reactive intermediates or

metabolites generated, thus increasing the risk for toxicities. [203] A recent study also further demonstrates that a dose more than 100 mg per day and being a substrate of CYP450 enzymes are two important predictors of DILI. [96] This further suggests that TKIs are at a significant risk of DILI due to its high daily dose (Table 21) and being a substrate of CYP450 enzymes (Table 18).

Table 21. Characteristics of TKIs (daily dose and substrate of CYP450 enzymes)

	Daily dose (mg)	**Substrate of CYP450 enzymes**
Afatinib (Gilotrif)	40	No
Axitinib (Inlyta)	10	Yes
Bosutinib (Bosulif)	500	Yes
Cabozantinib (Cometriq)	140	Yes
Ceritinib (Zykadia)	750	Yes
Crizotinib (Xalkori)	500	Yes
Dasatinib (Sprycel)	100	Yes
Erlotinib (Tarceva)	100 – 150	Yes
Gefitinib (Iressa)	250	Yes
Imatinib (Gleevec)	300 – 800*	Yes
Lapatinib (Tykerb)	1250 – 1500*	Yes
Nilotinib (Tasigna)	600	Yes
Pazopanib (Votrient)	800	Yes
Ponatinib (Iclusig)	45	Yes
Regorafenib (Stivarga)	160	Yes
Sorafenib (Nexavar)	800	Yes
Sunitinib (Sutent)	37.5 – 50*	Yes
Vandetanib (Caprelsa)	800	Yes
* Daily dose may differ depending on indication		

5.3.6 Actual drug-drug interaction cases involving tyrosine kinase inhibitors as documented in literature

There have been several reports demonstrating actual DDI cases in clinical practice. (Table 22) The events reported were potentially fatal ones such as hepatotoxicity and anticoagulation abnormalities. The concomitant use of imatinib and voriconazole resulted in markedly elevated levels of imatinib (between 3500 – 4700 ng/ml), than compared to when imatinib was used alone (2000 ng/ml). The raised plasma levels resulted in severe pustular eruption in the patient, and this was deemed due to the inhibition of imatinib metabolism by voriconazole. [204] Transaminase elevations have also been observed in drug pair involving pazopanib and simvastatin. As both drugs are substrates of the same enzymes and transporters, it is plausible that the concomitant administration of pazopanib and statins may alter systemic and/or hepatic exposures, leading to increased toxicities such as liver injury. [205] However, this list of DDI cases is not exhaustive as there remains a possibility that many DDI cases remain unreported in the literature. Even if the drug pair is not listed in this table, it does not indicate that it is entirely safe for use. Healthcare professionals should still be aware of the potential interactions that may occur, and be vigilant in monitoring those patients who are receiving any potentially-interacting drug pairs.

Table 22. Actual drug-drug interaction cases involving tyrosine kinase inhibitors as documented in literature

TKI	Interacting drug	Event	Remarks	Recommendations	Ref
Gefitinib	Anastrozole	Liver toxicity	-	Routinely monitor liver transaminases in all patients treated with gefitinib	[206]
	Warfarin	Coagulation abnormalities (Prothrombin time [PT] & international normalized ratio [INR] abnormalities)	Gefitinib could inhibit the metabolism of warfarin, which is a substrate of CYP1A2, CYP2C9, and CYP3A4. The degree of the inhibitory effect of gefitinib on CYP enzymes varies from patient to patient. This may in part explain the variability of the PT-INR values observed on the coadministration of gefitinib and warfarin	Close monitoring of PT-INR are recommended for patients receiving gefitinib and warfarin, especially during the first 2 weeks in the beginning of warfarin therapy. Appropriate adjustment of the warfarin dose should be done if an altered response to warfarin is observed.	[207]
Imatinib	Voriconazole	Severe pustular eruption	Plasma levels of imatinib markedly elevated during simultaneous administration with voriconazole, possibly due to inhibition of imatinib metabolism by voriconazole	Use of imatinib in association with CYP3A4 inhibitors has to be considered with caution. When such association is considered, the monitoring of imatinib plasma levels in patients may be of help for identifying individuals with high imatinib concentration who are at risk of developing toxicity, including skin lesions.	[204]
	Amlodipine	Peripheral neuropathy	Amlodipine inhibits CYP3A4, which could increase imatinib levels	Therapeutic monitoring of plasma imatinib levels may be useful to investigate unexpected imatinib toxicity.	[208]
	Phenytoin		AUC of imatinib was decreased by about 80%. After phenytoin was discontinued and the imatinib dose was increased to 500 mg/day, a complete hematological response was observed.	-	[209]
	Levothyroxine	Hypothyroidism	Mechanism unclear	Evaluate thyroid function in hypothyroid patients on tyrosine kinase inhibitors	[210]

Lapatinib	Dexamethasone	Hepatotoxicity	Concomitant use may cause an increase in metabolizing capacity by dexamethasone, which in turn increases the formation of lapatinib-derived RM and thereby, elevating the risk of toxicity	Clinicians should be aware of this risk when considering the use of this combination and follow through with close monitoring where necessary.	[211]
Pazopanib	Simvastatin	Transaminase elevations	As pazopanib and statins are substrates for the same key metabolizing enzymes e.g. CYP3A4 and drug transporters, it is plausible that concomitant administration of pazopanib and statins may alter their systemic and/or hepatic exposures, leading to increased toxicities such as liver injury	In addition to implementing the recommended dose modification guidelines for pazopanib, discontinuation of simvastatin should be considered to manage the risk of liver injury in cancer patients receiving both medications	[205]
Sorafenib	Prednisolone		Serum concentration of sorafenib was gradually increased following tapering of prednisolone, possibly due to prednisolone inducing sorafenib metabolism	Therapeutic drug monitoring could be useful during sorafenib therapy in combination with prednisolone and for determining the optimal dosage of sorafenib.	[212]
Sunitinib	Levothyroxine	Hypothyroidism	Mechanism unclear	Evaluate thyroid function in hypothyroid patients on tyrosine kinase inhibitors	[210]

5.3.7 Challenges and recommendations

The large amount of published research into drug interactions might have overwhelmed most healthcare practitioners. As a clinician cannot know all potential clinically significant drug interactions, this emphasizes the need for practical identification and management systems. [72] The inefficiency of updating and maintaining drug labels poses a serious threat to patients. For example, despite the recent evidence supporting a larger contribution of CYP2C8 and a less significant role for CYP3A4 in the metabolism of imatinib, drug labels continue to warn against potential interactions with CYP3A4 inhibitors but fail to mention any risk with a CYP2C8 inhibitor. [213] Few databases and software programs are capable of checking for all potential interactions among multiple medications. More importantly, the absence of reported interactions does not guarantee of a lack of interaction. [214]

Majority of the phase I trials evaluated two drug combinations of therapeutic interest, rather than combinations hypothesized to have a DDI. When DDI studies were performed with a clear rationale, the probability of identifying a DDI increased from 8% to 32%. This demonstrates the importance of understanding the mechanism behind a DDI and the value to which this translates clinically and suggests that DDI studies should only be performed when there is a pre-specified plausible hypothesis. [215]

Most pharmacokinetic studies would report changes in TKI exposure as a result of the enzyme inhibitor or inducer; however it would be useful if the investigators also provided information regarding its potential effect on toxicity or efficacy. [216]

5.3.8 Utilization of therapeutic drug monitoring in drug-drug interactions

The prerequisites of being a candidate for TDM in clinical practice include long-term therapy, significant inter-individual but limited intra-individual PK variability, narrow therapeutic index, a well-defined exposure-response (efficacy/toxicity) relationship, availability of appropriate bio-analytical methods for quantification. [35, 75-77] As most of the TKIs fulfill the traditional criteria for a TDM program, the role of TDM in TKI therapy is increasingly being studied. Furthermore, as TKIs have the potential to be involved in multiple interactions (e.g. drug-drug, drug-food and drug-herb) involving the pharmacokinetic or pharmacodynamics pathways, TDM could complement clinical evaluation by providing additional information on efficacy, adherence and toxicity. [77]

The application of TDM may be useful in DDIs for several reasons: for monitoring of patient when high-risk drug pairs cannot be avoided; for diagnosis of DDIs; for dose adjustments. [75] In such cases, the changes in drug levels, together with patient's response and toxicity, could be used together to make an informed decision, on whether the drug pair can be continued safely or whether dose adjustments should be performed. For example, we have demonstrated that sunitinib is more dermatotoxic than its active metabolite, SU12662. If sunitinib is used concomitantly with a CYP3A4 inducer, the total effective plasma concentration (sunitinib and SU12662) may still be above the therapeutic target, however, patients may experience less toxicity due to the lesser accumulation of the parent drug.

Although TDM of TKIs is still in its infancy, there are growing suggestions that dose adjustments based on pharmacokinetic targets would help to increase efficacy and reduce toxicity of TKIs, and might be beneficial for patients treated with most of the TKIs. [76] Currently, target plasma concentration of TKIs and its respective dose-adaptation strategy are only available for several of the FDA-approved TKIs. Recommended therapeutic targets for efficacy are available for crizotinib, erlotinib, gefitinib, imatinib, nilotinib, pazopanib and sunitinib, and targets for safety are available for dasatinib and sunitinib. [76] Thus, application of TDM may be limited to those TKIs which have a recommended target for and may be challenging in those TKIs which have a lack of information. Although there are recommended therapeutic targets available for some of the TKIs, there has not been prospective studies conducted to validate these targets, and thus applications of TDM may best be reserved for individual situations relating to a lack of therapeutic response, severe or unexpected toxicities, drug-drug interactions or treatment adherence. [77] In summary, future research should focus on the role and benefits of TDM in TKI therapy, especially those with a well-established dose-response relationship and well established pharmacokinetic targets. Prospective, randomized studies should be performed to confirm the benefits of implementation of TDM, such as reductions in toxicity and/or improvement in outcomes.

5.4 Summary

As we are able to achieve a better control of the disease over the longer lifespan of a patient, these TKIs are now being considered as chronic medications taken in an outpatient setting. Due to the large inter-patient variability in pharmacokinetics of these TKIs, any potential DDI could have serious consequences in a patient's therapy.

Thus far, there have been numerous phase I and in-vitro studies conducted to evaluate DDIs. These information have been incorporated into drug labels or drug information databases, to warn prescribers of the risk of such DDIs. However, as previously mentioned, it is somewhat challenging to extrapolate results from phase I and in-vitro studies to routine clinical practice. During the drug development phase, potentially clinically relevant drug interactions are not usually detected. Only after receiving regulatory approval and after widespread usage, new DDI may surface. Therefore, healthcare professionals especially physicians and pharmacist play a vital role in identifying these new interactions. Perhaps scheduled drug utilization reviews can be conducted routinely to identify any common DDI-pairs. Prospective data can then be collected regarding these DDI pairs, to identify any increase in toxicity or lack of efficacy events. Where possible, investigators can also subsequently recreate the interaction in an in-vitro system to determine the mechanisms which may be involved. However, such research takes time and in the meantime, suspected DDI pairs would have to be used cautiously in patients. Nevertheless, using both clinical and in-vitro data to validate claims of potential DDI would ensure accuracy of data as well as clinical relevance. The utilization of TDM in DDIs may be useful when high-risk drug pairs cannot be avoided, especially during initiation of therapy or when there is lack of response or occurrence of severe toxicities. Instead of withholding a beneficial drug therapy from a patient, the high-risk drug pair may be continued safely with regular monitoring if the patient is not experiencing excessive toxicity, even when the drug levels are increased. In these situations, using additional information on drug levels together with patient's response and toxicity data, a more informed decision can be made.

6 Understanding tyrosine kinase inhibitor associated toxicities: a focus on hepatotoxicity

The liver is the regulator of chemical homeostasis in the body and is the main site for bioactivation and detoxification of drugs and their metabolites. Hence, any potentially toxic metabolite generated in the process can readily exert a localized damage. The human liver has immense regenerative and recovery capabilities, such that even after a drastic resection, the liver responds with rapid regrowth to its original size. Unfortunately, it is also this extensive regenerative capacity that threatens it with cytotoxicity from chemotherapy. As such, hepatic injury may be an inadvertent ramification of attempting to kill cancerous cells, but which may also generate more problems if the secondary hepatotoxicity becomes too severe. [217]

6.1 Hepatotoxicity with tyrosine kinase inhibitors

Hepatotoxicity is one of the serious class-related safety issues signaled in pre-approval clinical trials with TKIs and is now gradually being reported relatively more frequently following their wider clinical use. Among the 18 FDA-approved TKIs (as of October 2014), 5 of them have black box warnings issued, namely lapatinib, sunitinib, pazopanib, regorafenib and ponatinib. Frequencies of all grade hepatic adverse events of TKIs varies from 11%, as seen with gefitinib [20] to more than 50% as seen with pazopanib. [24] The frequency of grade 3 and above hepatic adverse events for all FDA-approved TKIs ranges from 1% to 12%. Although these hepatotoxicity events may be self-limiting or even undetected, there have been several fatalities. [218, 219] Therefore, hepatotoxicity is a rare but serious issue with the use

of TKIs and a better understanding of the problem may help in the management of patients and improve the use of TKIs.

6.2 Risk of tyrosine kinase inhibitor-induced hepatotoxicity

Although existing evidence from clinical trials has demonstrated manifestation of hepatotoxicity with the use of TKIs, overall risks among all commercially available TKIs of such events have yet to be reported. By and large, individual studies are designed with the key purpose on testing efficacy outcomes. Consequently, sample sizes of these studies are insufficiently powered to detect the occurrences of adverse events. By conducting a meta-analysis, multiple studies can be pooled to increase the sample size and thus the power to study rare events of interest, which in this case, is hepatotoxicity. Therefore, a meta-analysis was conducted with an objective to determine the risk of hepatotoxicity associated with the use of TKIs, by comparing the occurrence of hepatotoxicity of the TKI arms against that of comparison arms.

6.2.1 Methodology

6.2.1.1 Search strategy

A search was conducted to identify all small molecule TKIs approved by the FDA since January 2000. A comprehensive literature search of RCTs involving TKIs was performed using the PubMed, Scopus and ClinicalTrials.gov databases. The search was conducted by using the generic names of all the identified TKIs, and terms such as “randomized controlled trials”, “phase 2” and “phase 3”. The search was limited to

English language articles of human studies published between January 1995 and June 2012.

6.2.1.2 Study selection

In this analysis, only randomized, double-blind and placebo-controlled phase II or phase III human trials were included. The included studies must involve the comparison of a TKI against placebo, or the comparison of TKI with chemotherapy agent against placebo with the same chemotherapy agent. Since the objective of this analysis was to quantify the differences in occurrence of hepatotoxicity of the TKIs arm compared to the comparison arms, phase I trials, single-arm studies and those studies which did not report any liver adverse events (AEs) were excluded. When encountered with multiple publications of the same trial, only the most recent publication was selected. Study quality was assessed using the Jadad scoring system [220], with poor quality studies, indicated by a score of 2 or less, being excluded from analysis.

6.2.1.3 Data collection

Data collection was performed by the candidate on all the included studies. Data collected were: first author's name, year of publication, name of journal, trial phase, targeted malignancy, TKI involved, treatment arms, line of treatment, number of patients enrolled, name and version of adverse event criteria used, presence of randomization, type of blinding, and data on events of interest. The events of interest in this analysis are all-grades and high-grade (grade 3 and above) liver AEs, which can be further classified into the elevation of ALT, AST and TB.

6.2.1.4 Endpoints

The primary endpoint of this analysis was the risk of all-types high-grade hepatotoxicity. Secondary endpoints include the risk of all-types all-grades hepatotoxicity, high-grade ALT, AST and TB elevation. High-grade ALT, AST and TB elevations were defined as grade 3 or higher elevation of the corresponding liver enzyme, which is an elevation of more than 5 times upper limit of normal (ULN).

6.2.1.5 Data analysis

Data were included and analyzed using the RevMan 5.1 software. [221] The odds of experiencing all-grades and high-grade liver AEs were calculated as the proportion of patients with these events divided by the proportion of patients without these events. The OR and corresponding 95% confidence intervals (CIs) were computed as the ratio of these odds in the TKI arm compared to the comparison arm. Pooled estimates of OR were computed using the Mandel-Haenszel method, which is recommended when there is low event rates and substantial variability in effect sizes. [221] Statistical heterogeneity between trials was assessed using the Cochran's Q statistic, with a p-value of less than 0.1 representing statistically significant heterogeneity. Inconsistency between trials was quantified with the I^2 statistic, which represents the percentage of variability in effect estimate that is due to heterogeneity rather than sampling error. [221] Random-effects model was used for evaluation when there is statistically significant heterogeneity. Effect measures were presented for all studies, as well as pre-determined subgroup and sensitivity analyses. For the subgroup analyses, trials were classified into two subgroups: (1) TKI versus placebo and (2) TKI with chemotherapy versus placebo with chemotherapy. This was to determine whether the administration of chemotherapy would impact the risk of manifesting

hepatotoxicity. Sensitivity analyses were conducted to compare the following: random-effects versus fixed-effects model, phase II versus phase III studies, small sized versus large sized studies, first-line treatment versus non-first-line treatment studies, epidermal growth factor receptor inhibitor (EGFRI) versus non-EGFRI and long duration versus short duration of therapy. Funnel plots were generated to identify for any publication bias within included studies.

6.2.2 Results

6.2.2.1 Literature search results

A total of 14 TKIs were approved by the FDA between January 2000 to June 2012 and they are: axitinib, crizotinib, dasatinib, erlotinib, gefitinib, imatinib, lapatinib, nilotinib, pazopanib, ruxolitinib, sorafenib, sunitinib, vandetanib, vemurafenib. Among them, there are 4 EGFRIs and they are erlotinib, gefitinib, lapatinib and vandetanib. From the literature search, 3269 articles were identified. After duplicates were removed and abstracts were screened, 136 articles were left to be assessed for eligibility. A total of 12 articles were included in the analysis, and reasons for exclusion were lack of liver AEs (n=92), duplicate trials (n=3), single-arm trials (n=9), TKI versus TKI comparison (n=8) and non-placebo controlled (n=12). (Figure 4)

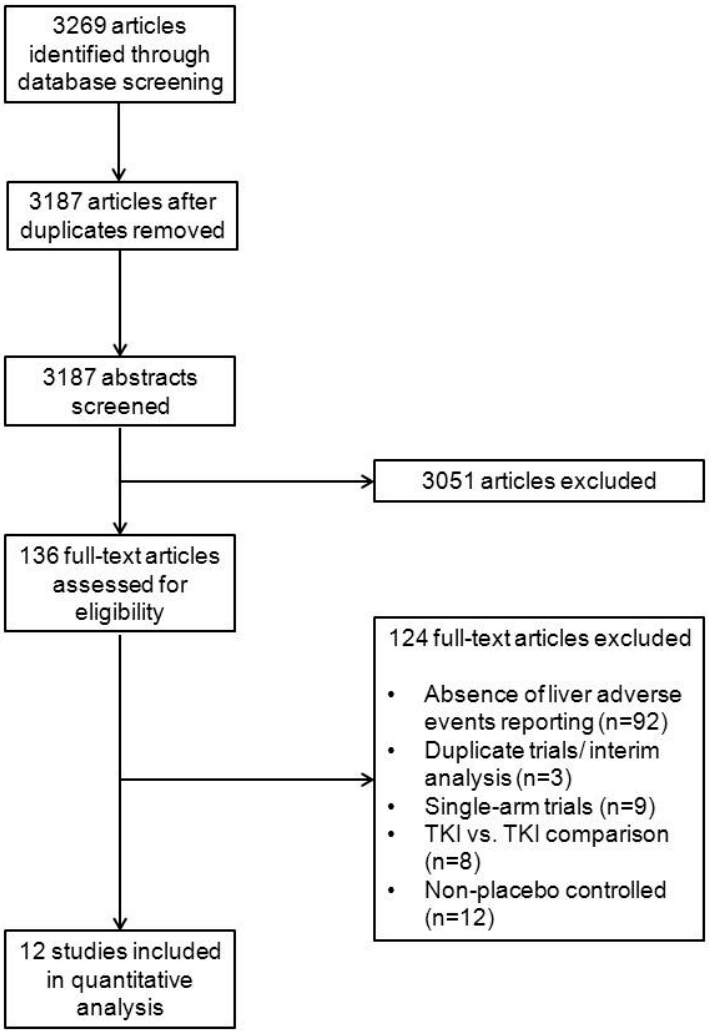


Figure 4. Study flow diagram

6.2.2.2 Study characteristics

Eight studies were categorized under subgroup 1 (TKI versus placebo) [11, 135, 222-227] and 4 studies under subgroup 2 (TKI with chemotherapy versus placebo with chemotherapy). [228-231] TKIs included in the analysis were gefitinib (n=4), lapatinib (n=2), pazopanib (n=2), erlotinib (n=1), imatinib (n=1), sorafenib (n=1) and vandetanib (n=1). As per inclusion criteria, all included studies were randomized, double-blind, placebo-controlled trials, with a Jaded score of 3 or more. Four studies (33%) were investigating the use of TKIs as first-line therapy. [223, 228, 229, 231] There were 5 (42%) phase II [222, 225, 228-230] and 7 (58%) phase III trials. [11, 135, 223, 224, 226, 227, 231] Only 1 out of 12 studies (8%) had a Jadad score of less than 4. [230] Almost all studies adopted the CTCAE criteria for grading of adverse events, with the exception of one study which did not mention the grading criteria used. [228] Nine out of 12 studies [11, 135, 223-225, 227, 229-231] adopted version 3.0 of the CTCAE which another 2 studies [222, 226] adopted version 2.0. Nevertheless, the grading criteria were consistent between the 2 versions. Summarized characteristics of the included studies can be found in Table 23.

Table 23. Characteristics of included studies

Reference	n	Phase	Malignancy	TKI arm	Control arm
(1) TKI alone vs. Placebo					
Arnold, 2007 [222]	105	II	SCLC	Vandetanib	Placebo
De Matteo, 2009 [223]	682	III	GIST	Imatinib	Placebo
Sternberg, 2010 [11]	435	III	Locally advanced or metastatic RCC	Pazopanib	Placebo
Kudo, 2011 [224]	456	III	Unresectable HCC	Sorafenib	Placebo
De Censi, 2011 [225]	60	II	HER2-positive breast cancer	Lapatinib	Placebo
Gaafar, 2011 [226]	171	III	Advanced NSCLC	Gefitinib	Placebo
Zhang, 2012 [135]	295	III	Locally advanced or metastatic NSCLC	Gefitinib	Placebo
Van der graaf, 2012 [227]	362	III	Metastatic soft tissue sarcoma	Pazopanib	Placebo
(2) TKI + Chemotherapy vs. Placebo + Chemotherapy					
Guarneri, 2007 [228]	90	II	Breast cancer	Gefitinib + Chemotherapy	Placebo + Chemotherapy
Mok, 2009 [229]	153	II	Advanced NSCLC	Erlotinib + Platinum doublet chemotherapy	Placebo + Platinum doublet chemotherapy
Viéitez, 2010 [230]	76	II	Metastatic colorectal cancer	Gefitinib + Raltitrexed	Placebo + Raltitrexed
Schwartzberg, 2010 [231]	219	III	HER2- and hormone-receptor-positive advanced or metastatic breast cancer	Lapatinib + Letrozole	Placebo + Letrozole

Abbreviations: *GIST*, Gastrointestinal stromal tumor; *HCC*, Hepatocellular carcinoma; *HER2*, Human epidermal receptor type 2; *TKI*, Tyrosine kinase inhibitor; *NSCLC*, Non-small cell lung cancer; *RCC*, Renal Cell Carcinoma; *SCLC*, Small cell lung cancer

6.2.2.3 Primary endpoint – all-type, high-grade hepatotoxicity

There was a significant overall increase in the odds of developing high-grade hepatotoxicity with the use of TKIs compared to the control arms (Pooled OR 4.35, 95% CI 2.96 – 6.39, p<0.001). However, increase in odds was observed for only subgroup 1 (OR 5.61, 95% CI 3.63 – 8.68, p<0.001). The ORs between the two subgroups were statistically different (p<0.001). (Figure 5)

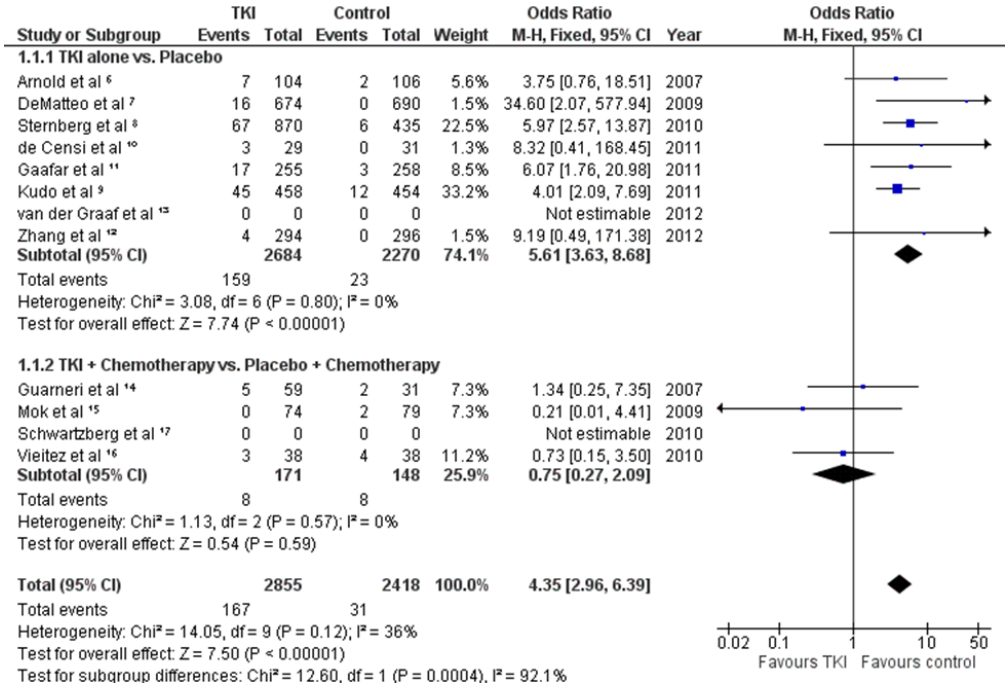


Figure 5. All-types high-grade hepatotoxicity

6.2.2.4 Secondary endpoints

6.2.2.4.1 All-type, all-grade hepatotoxicity

There was a significant overall increase in the odds of hepatotoxicity with the use of TKIs compared to the controls (Pooled OR 2.42, 95% CI 1.52 – 3.85, p<0.001). Increase in odds were observed for both subgroups, although increase in odds for subgroup 2 did not achieve statistical significance (Subgroup 1 vs. Subgroup 2: OR 3.43, 95% CI 2.02 – 5.83, p<0.001 vs. OR 1.13, 95% CI 0.58 – 2.21, p=0.18). (Figure 6)

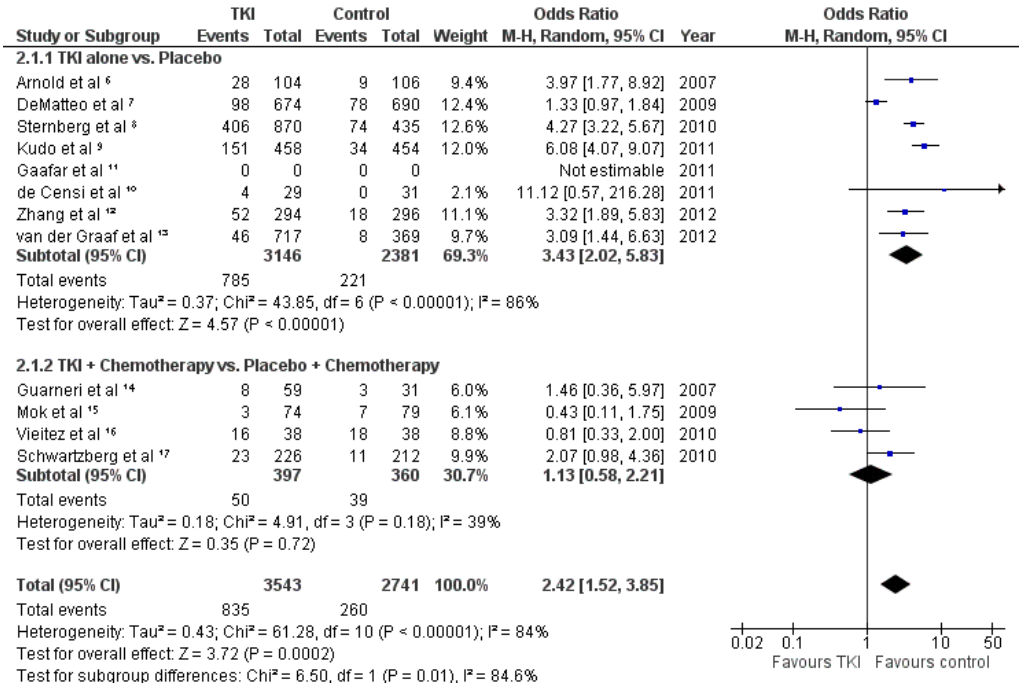


Figure 6. All-types all-grades hepatotoxicity

6.2.2.4.2 High-grade ALT elevation

The odds of developing high-grade hepatotoxicity due to elevation in ALT was higher with the use of TKI than compared to the controls (Pooled OR 5.22, 95% CI 2.88 – 9.46, p<0.001). Similarly, increase in odds was observed for only subgroup 1 (OR 6.35, 95% CI 3.32 – 12.16, p<0.001). The ORs between the two subgroups were statistically different (p=0.03). (Figure 7)

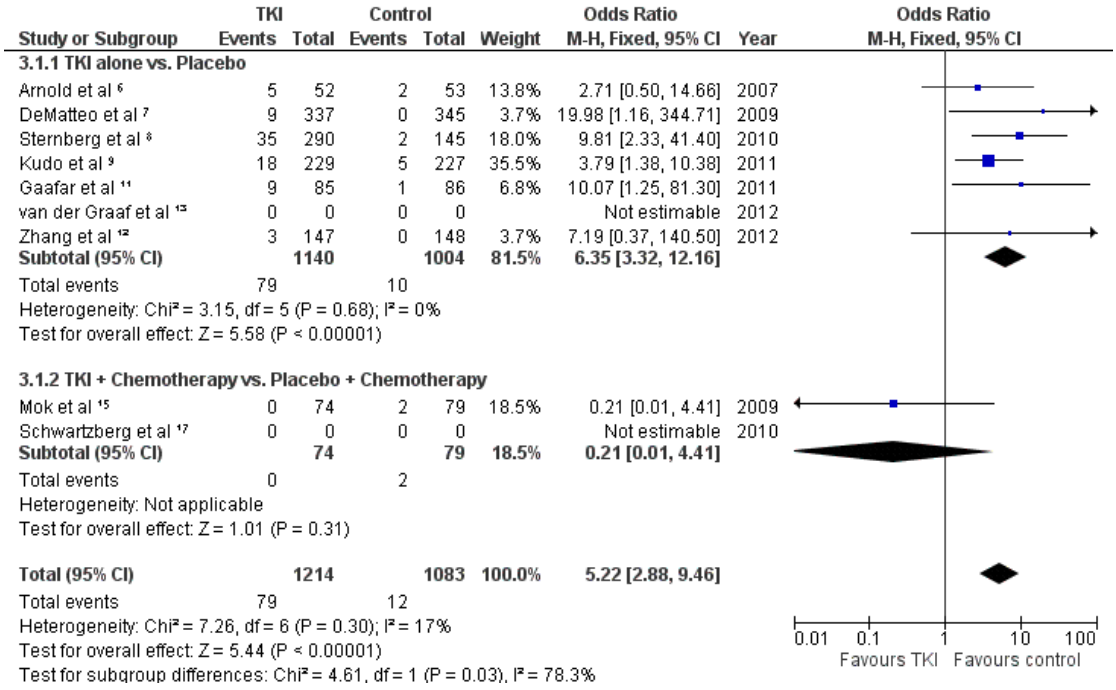


Figure 7. High-grade hepatotoxicity due to ALT elevation

6.2.2.4.3 High-grade AST elevation

The odds of developing high-grade hepatotoxicity due to AST elevation was higher with the use of TKI than compared to the controls (Pooled OR 6.15, 95% CI 3.09 – 12.25, p<0.001). There were no trials in subgroup 2 for this group analysis, hence pooled OR was contributed by studies in subgroup 1 only. (Figure 8)

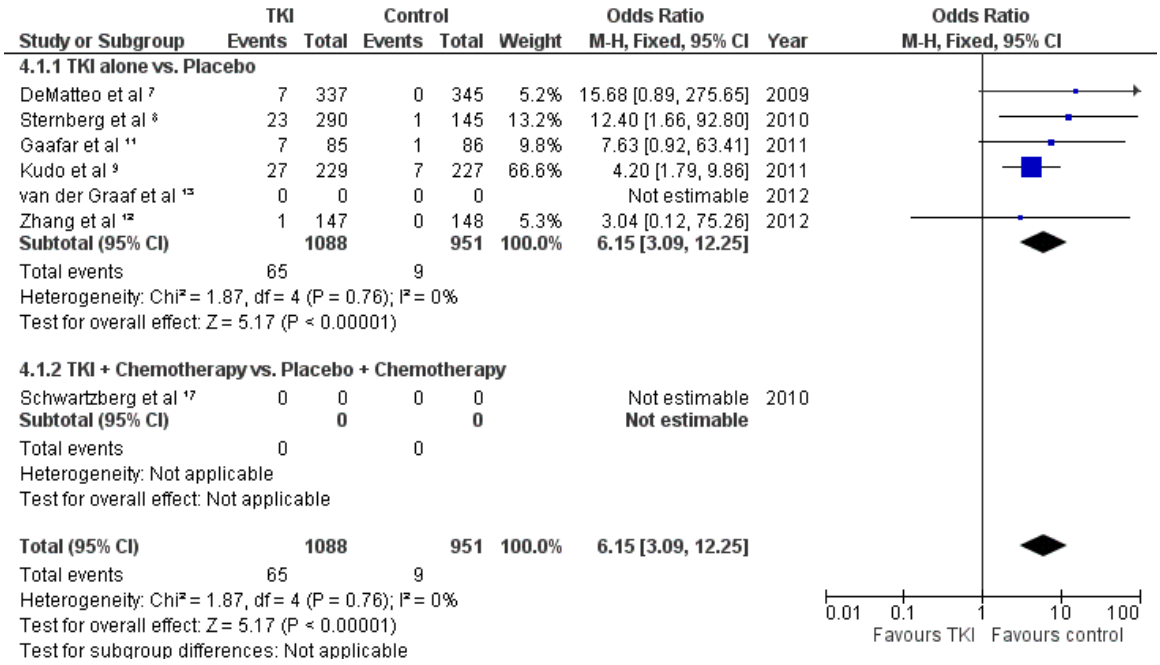


Figure 8. High-grade hepatotoxicity due to AST elevation

6.2.2.4.4 High-grade TB elevation

The odds of developing high-grade hepatotoxicity due to TB elevation was higher with the use of TKI than compared to the controls (Pooled OR 1.76, 95% CI 0.59 – 5.24, p=0.31). However, this was not statistically significant. There were no trials in subgroup 2 for this group analysis, hence pooled OR was contributed by studies in subgroup 1 only. (Figure 9)

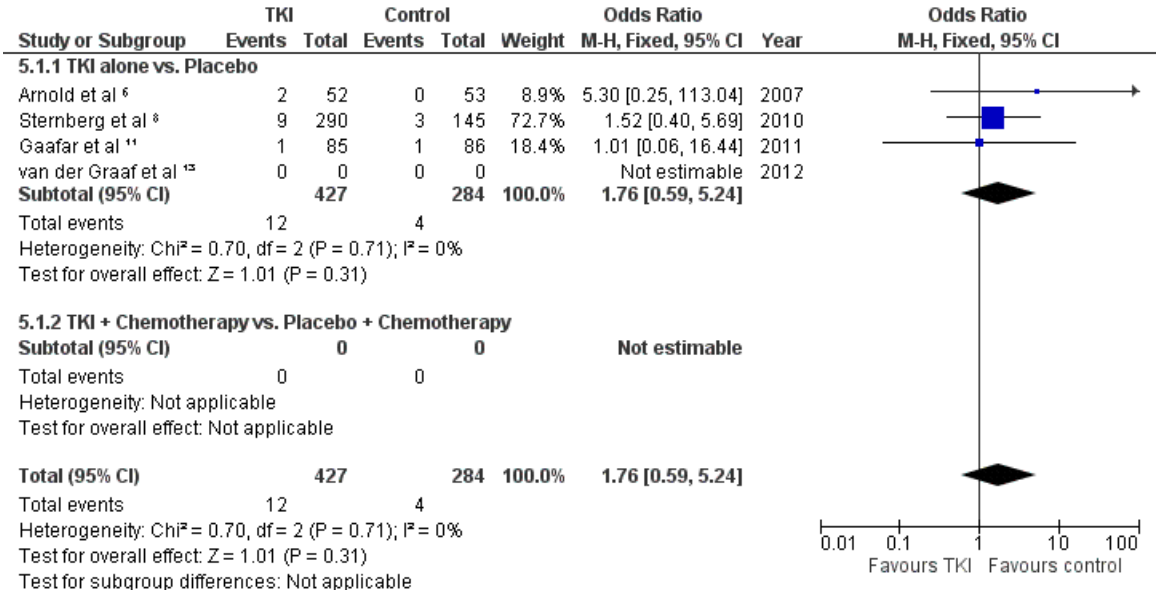


Figure 9. High-grade hepatotoxicity due to TB elevation

6.2.2.5 Sensitivity and bias analysis

There was no statistical difference between using the random-effects model or the fixed effects model, although using the fixed-effects model tends to generate higher ORs. A statistical difference was observed between phase II and phase III studies for all-types, high-grade hepatotoxicity, where phase III studies reported higher ORs (phase II vs. phase III: OR 1.57, 95% CI 0.73 – 3.38 vs. OR 5.7, 95% CI 3.61 – 9.05). A similar trend was observed between phase II and phase III studies for elevation of ALT, where higher OR was observed with phase III studies (phase II vs. phase III: OR 1.28, 95% CI 0.36 – 4.57 vs. OR 7.09, 95% CI 3.49 – 14.41). When small sized studies (defined as sample size 200 or less) were compared against large sized studies (defined as sample size greater than 200), a statistical difference was observed in all-type, high-grade hepatotoxicity, where larger studies had higher OR than smaller studies. (large size vs. small size: OR 5.66, 95% CI 3.45 – 9.29 vs. OR 2.49, 95% CI 1.33 – 4.68). Studies that investigated the use of TKIs as first-line therapy tend to offer higher ORs than studies where TKIs were investigated as non-first-line therapy; nevertheless there was no statistical difference between the groups. Comparing between non-EGFRIs and EGFRIs, the former was associated with a higher risk of all-types, all-grades hepatotoxicity (non-EGFRIs vs. EGFRIs: OR 3.21, 95% CI 1.54 – 6.68 vs. OR 1.90, 95% CI 1.04 – 3.47), although the ORs were not statistically different. The trend of non-EGFRIs associating with a higher risk of hepatotoxicity compared to EGFRIs was similarly observed for all-types high-grade hepatotoxicity as well as high-grade ALT and AST elevation. In terms of the duration of therapy, shorter duration of therapy (defined as median therapy of less than 12 weeks) tend to demonstrated significantly higher ORs than compared to a longer duration (defined as median therapy of 12 weeks or more) for all-types all-grades hepatotoxicity. The

association of higher ORs with shorter duration of therapy was also observed for high-grade elevation of ALT and AST, though not statistically significant. Due to a small number of studies reporting elevations in TB (n=3), sensitivity analysis for TB elevation was not conducted. (Table 24)

The fixed-effects model was used for all-types high-grade hepatotoxicity, as well as for high-grade ALT, AST and TB elevation, due to a lack of heterogeneity within the included studies. The random-effects model was used for all-types all-grades hepatotoxicity due to presence of heterogeneity. Funnel plot for all-types, high grade hepatotoxicity was largely symmetrical, with equal number of studies on each side and 90% of the included studies were within the lines of 95% CI. (Figure 10) This suggests that publication bias is unlikely.

Table 24. Sensitivity analyses

	All-types						ALT-type			AST-type		
	All grades			Grades 3 and above			Grades 3 and above			Grades 3 and above		
	n	**OR**	**95% CI**	**n**	**OR**	**95% CI**	**n**	**OR**	**95% CI**	**n**	**OR**	**95% CI**
Random-effects model	11	2.42	1.52 – 3.85	10	3.63	2.00 – 6.59	7	4.79	2.24 – 10.22	5	5.43	2.71 – 10.89
Fixed-effects model	11	2.94	2.52 – 3.44	10	4.35	2.96 – 6.39	7	5.22	2.88 – 9.46	5	6.15	3.09 – 12.25
Phase II	5	1.53	0.57 – 4.07[*]	5	1.57	0.73 – 3.38[*¶]	2	1.28	0.36 – 4.57[*¶]	-	-	-
Phase III	6	3.03	1.77 – 5.19	5	5.71	3.61 – 9.05[¶]	5	7.09	3.49 – 14.41[¶]	5	6.15	3.09 – 12.25
Small sized (n≤200)	5	1.53	0.57 – 4.07[*]	6	2.49	1.33 – 4.68[‡]	3	2.81	1.04 – 7.62	1	7.63	0.92 – 63.41[*]
Large sized (n>200)	6	3.03	1.77 – 5.19	4	5.66	3.45 – 9.29[‡]	4	6.76	3.17 – 14.38	4	5.99	2.89 – 12.42
First-line	5	1.65	0.94 – 2.90[*]	4	3.83	1.67 – 8.80	3	3.20	1.14 – 8.94	1	15.68	0.89 – 275.65[*]
Non-first-line	6	3.42	2.15 – 5.43	6	4.50	2.91 – 6.94	4	6.35	3.04 – 13.26	4	5.63	2.76 – 11.49
EGFRI	7	1.90	1.04 – 3.47	7	2.73	1.48 – 5.03	4	3.20	1.25 – 8.16	2	6.02	1.05 – 34.55
Non-EGFRI	4	3.21	1.54 – 6.68	3	5.57	3.37 – 9.20	3	6.73	3.08 – 14.69	3	6.17	2.92 – 13.07
Long duration (≥ 12 weeks)	7	1.91	0.97 – 3.74[^]	7	2.88	1.18 – 7.06	5	4.51	2.21 – 9.22	4	5.20	2.50 – 10.83
Short duration (< 12 weeks)	3	4.27	3.27 – 5.57[^]	3	5.53	2.68 – 11.40	2	6.74	2.31 – 19.70	1	12.40	1.66 – 92.80

Abbreviations: *ALT*, Alanine transaminase; *AST*, Aspartate transaminase; *EGFRI*, Epidermal growth factor receptor inhibitor; *OR*, Odds ratio

[*] p>0.05 for OR

[¶] p<0.05 for difference between phase II and phase III

[‡] p<0.05 for difference between small sized and large sized

[^] p<0.05 for difference between long duration and short duration

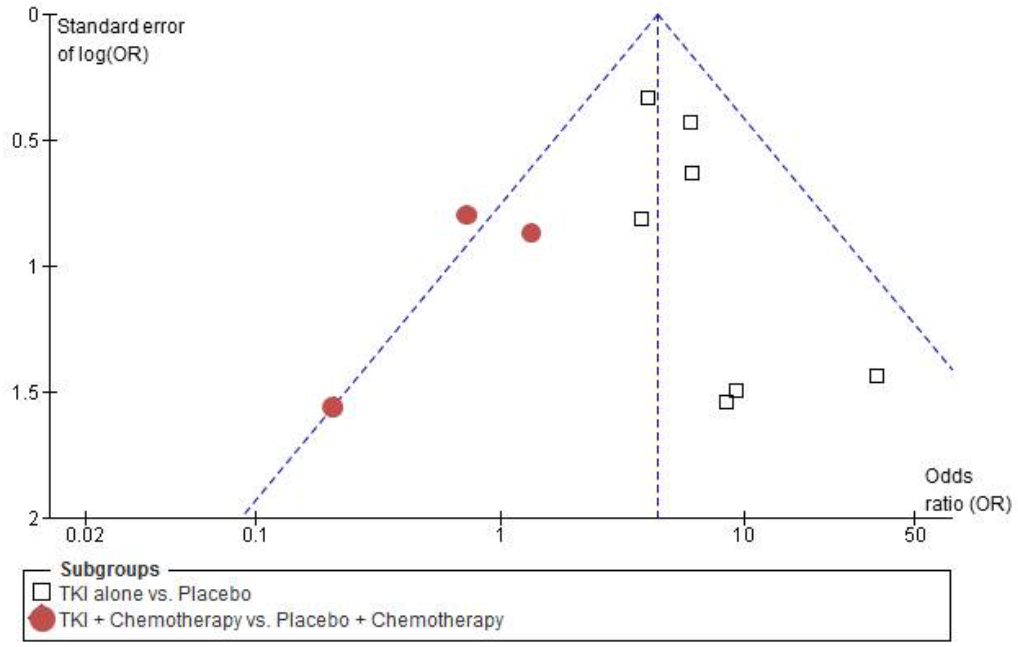


Figure 10. Funnel plot of trials included for analysis for all-types high-grade hepatotoxicity (primary endpoint)

6.2.3 Discussion

It is currently recognized that the use of TKIs can lead to hepatic AEs; however, the risk has yet to be evaluated. This is the first meta-analysis to demonstrate a significantly increased risk of hepatic AEs associated with TKIs use. Overall, the risk of developing hepatic AEs was more than two-fold higher in patients receiving TKIs compared to patients in the control or placebo arms. As for more serious high-grade hepatic AEs, patients receiving TKIs were 4-times more likely to experience such events, compared to patients receiving placebo. On the whole, there was also a significant increase in odds of hepatotoxicity due to the elevation of AST and ALT. There was however a lack of significant effect on elevation of TB. A similar meta-analysis was conducted recently by Ghatalia et al, which evaluated the effect of VEGFR inhibitors on hepatotoxicity. Likewise, an increased risk of all-grades and high-grades ALT, AST and TB elevation was observed. [232]

The use of TKIs increased the risk of hepatotoxicity independently of trial phase, the sequence of therapy and mechanism of action. The OR of subgroup 1 (TKI alone vs. placebo) tend to be higher than that of subgroup 2 (TKI with chemotherapy vs. placebo with chemotherapy). This is expected as the use of chemotherapy contributes to some toxicity itself, and thus leading to a smaller difference in the event rates across the 2 groups. The odds of developing high-grade hepatotoxicity due to ALT and AST elevation were also comparable, at 5-fold and 6-fold respectively. Although non-statistically significant, the risk of hepatotoxicity for non-EGFRI was demonstrated to be higher compared to EGFRIs. There were four non-EGFRIs trials in the analysis, namely pazopanib (n=2), imatinib (n=1) and sorafenib (n=1). High frequencies of ALT and AST elevations have been observed with pazopanib therapy,

where 53% and more than 10% of patients experienced all-grades and high-grade ALT elevation respectively. [24] This could have significantly contributed to the high OR observed with non-EGFRIs. Higher ORs have been demonstrated due to shorter duration of therapy. There are only 3 trials which were categorized as short duration of therapy, and they were vandetanib, lapatinib and pazopanib. Both lapatinib and pazopanib are at a considerably higher risk of hepatotoxicity, as observed with its mandatory black-box warning. As such, this could have influenced the higher ORs observed. Generation of RM upon metabolism of lapatinib have been demonstrated previously [89], and these RM have been implicated in other examples of idiosyncratic hepatotoxicity. [233]

Despite our stringent inclusion and exclusion criteria, we managed to include more than 3000 patients in this meta-analysis, even though a number of phase II or III trials evaluating TKIs were excluded. The most common reason for exclusion was due to lack of reporting of liver AEs, where 89 out of 133 (67%) full articles assessed for eligibility did not report such events. The rigorous criterion offers confidence of good quality data and a purer comparison, whereby the risk of hepatotoxicity can be regarded with assurance to be associated with TKI.

Existing clinical evidence indicates that TKIs are associated with hepatotoxicity, and although we have demonstrated an increased risk of hepatotoxicity with the use of TKIs, the mechanism of toxicity remains largely unknown. Some have attributed the toxicity to generation of RM upon metabolism. [89, 234, 235] These RM are highly reactive and can interfere with cellular molecules and thereby affecting cell function

and cell death. Immune-mediated mechanisms have also been proposed to be a mechanism of toxicity. [236]

6.2.4 Limitations of study

There were several challenges and limitations in this analysis. Firstly, the analysis was based on published aggregated data and not using individual patient's data. Secondly, TKIs of various mechanism of action were pooled and analyzed as a group. However, sensitivity analysis was conducted to assess the effects of the different mechanisms of action. Studies were classified as 'EGFRIs' and 'non-EGFRIs' and although there were some differences observed between the 2 groups, it was not statistically significant. Thirdly, the reporting of hepatic AEs were lacking in many studies, leading to their exclusion from analysis. Unlike efficacy outcomes, AEs are rarely predetermined for systematic data collection in clinical trials. Therefore, reporting of adverse events depends highly on the investigators, and could likely be confounded by other variables as well, such as presence of liver metastasis. Furthermore, it may be possible that in studies that did not report elevations in liver AEs, it may be due to the absence of such events. Hence, the increase in risk could have been inflated. Fourthly, the type of reporting of hepatic AEs were highly variable, where some studies reported hepatotoxicity as a whole, whereas some presented hepatotoxicity in terms of elevation of various liver chemistries such as ALT, AST etc. In spite of that, studies were consistent in grading the adverse events, where the CTCAE criteria were used for grading. A total of two versions of the CTCAE criteria were involved, version 2 and version 3, though classifications of the various liver adverse events were unchanged across the two versions.

6.2.5 Summary of important findings

In summary, this meta-analysis has quantified the risk for TKI-associated hepatotoxicity and has demonstrated that TKI usage is associated with a two-fold and four-fold increased risk of all-types all-grades and all-types high-grade hepatotoxicity respectively. It is currently recognized that the use of TKIs can lead to hepatic AEs; however, the risk has yet to be evaluated. This study provides evidence as well as quantification of this risk. Clinicians should be aware of this risk and provide close monitoring in patients receiving these therapies.

Following the quantification of the risk of hepatotoxicity, in the next few sections, this thesis will seek to understand why these TKIs are at risk for hepatotoxicity as well as to look into the various proposed strategies to managed TKI-associated hepatotoxicity.

6.3 Why tyrosine kinase inhibitors are at risk for hepatotoxicity

A recent study reported that a high daily dose (≥ 100 mg) and being a substrate of P450 enzyme had significantly higher likelihood of causing DILI. [96] In a separate study, compounds with significant hepatic metabolism (> 50%) were found to be more likely to cause elevation of liver enzymes, liver failure and fatal DILI. [237] The authors described that drugs that fulfill both criteria of significant hepatic metabolism and daily doses of more than 50 mg are at the highest risk of hepatic adverse events. [237] Therefore, based on what is known as the risk factors for development of hepatotoxicity, it is not surprising to observe these events with TKIs use, as most of these drugs possess the risk factors for developing DILI.

Genetic characterization of hepatotoxicity caused by TKIs has been well studied in 2 TKIs, lapatinib and pazopanib. Lapatinib-related liver injury has a robust, confirmed association with the class II HLA locus containing DRB1*07:01/DQA1*02:01 alleles [236], while pazopanib-associated ALT elevations have a robust, confirmed association with HFE polymorphisms. [33] A recent review by Spraggs et al highlighted the benefits of genetic characterization of liver safety signals observed for TKI therapies, as it will reveal insights into the mechanisms of DILI, and inform the characteristics and risk. [238] This may allow individualization of therapy according to the severity of the hepatotoxicity and the risk associated with the genotypes. This is particularly important in the use of TKI therapies in cancer treatment, since improved characterization and understanding of the hepatotoxicity may enable continued treatment for many non-susceptible patients, without increasing the risk of serious DILI. This may then lead to more favorable clinical outcomes with improved disease management and patient survival. [238]

Additionally, several of the TKIs were capable of forming reactive metabolites, which constitutes a risk for potential toxicity. In the following subsections, those TKIs which form RM will be highlighted, together with the effect of RM on direct and indirect toxicity.

6.3.1 Tyrosine kinase inhibitors that form reactive metabolites

It has been demonstrated that TKIs such as dasatinib, erlotinib, gefitinib, imatinib, lapatinib, nilotinib, pazopanib, and sunitinib were capable of generating reactive intermediates upon bioactivation. [201] Hence, these agents are at risk for potential

toxicity. However, RM has only been characterized in erlotinib, gefitinib, lapatinib and dasatinib, and they will be highlighted below. (Table 25)

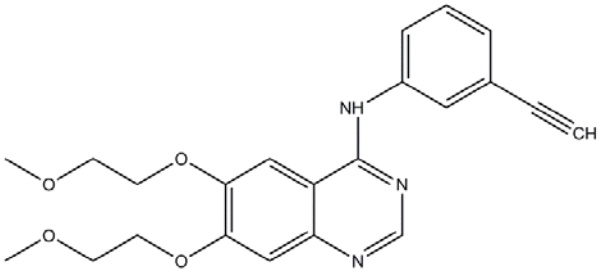
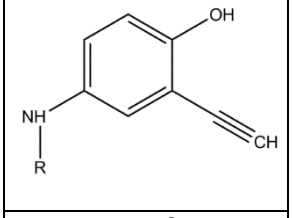
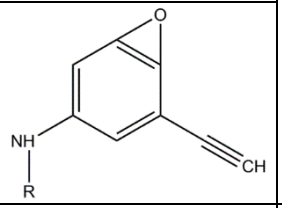
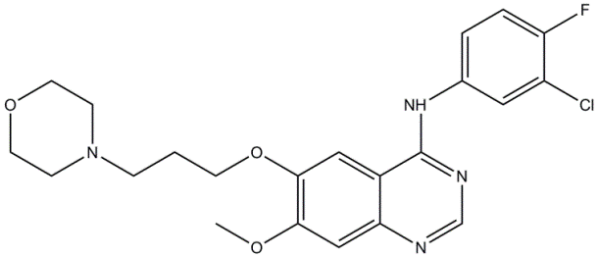
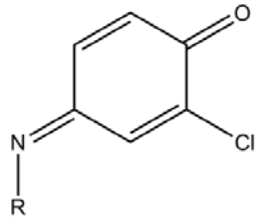
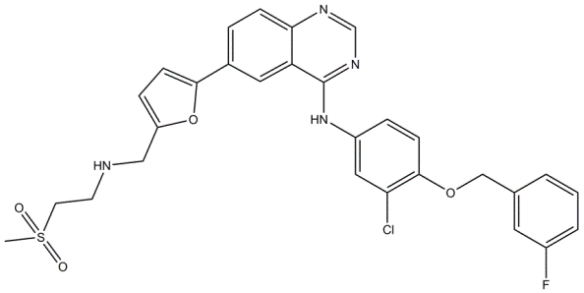
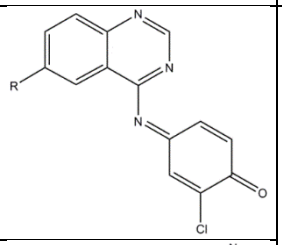
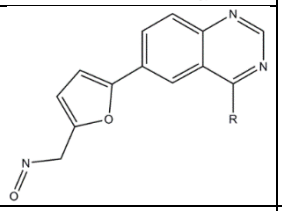
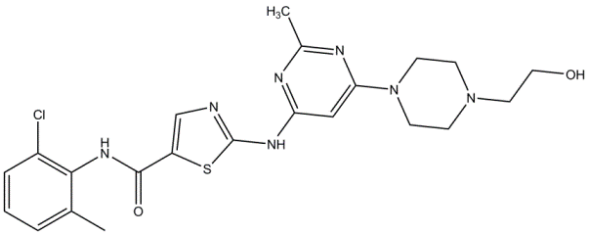
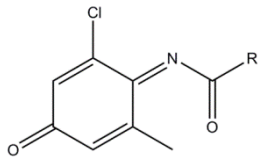
Bioactivation of erlotinib is proposed to be related to the formation of a reactive epoxide and quinoneimine, which may contribute to some of the serious clinical toxicities, such as hepatotoxicity, interstitial lung disease and severe skin disorders such as Stevens-Johnson syndrome and toxic epidermal necrolysis. The CYP3A4 enzyme in the liver and intestines, and the CYP1A1/2 enzyme in the lungs, are the main enzymes responsible for the catalysis of reactive erlotinib metabolites. Although reactive epoxides and quinoneimine metabolites cannot diffuse significant distances from the organ where they were formed, para-hydroxyerlotinib is in general circulation and is the major erlotinib metabolite in human plasma. This may diffuse to other tissues where it is oxidized to generate the reactive quinoneimine. [234]

Similar to erlotinib, the P450-mediated bioactivation of gefitinib generates reactive quinoneimine and epoxide compounds. The CYP3A4 and CYP1A1 were found to be the major enzymes responsible for adduct formation in the liver and intestines and in the lungs respectively. Furthermore, the significant distribution of gefitinib to the liver and lung tissue, where it can be bioactivated to reactive compounds, is likely to increase the incidence of toxicity. [235]

Dasatinib is bioactivated by CYP3A4 to form of both quinoneimine and imine-methide reactive intermediates, indicating that dasatinib is likely to form protein adducts in the liver. [239]

Lapatinib has been shown to be extensively metabolized, as exemplified by diverse biotransformations to form metabolites. A number of the metabolites generated from the metabolism by CYP3A4 and CYP3A5 could potentially form reactive electrophilic intermediates that could contribute to hepatotoxicity. [202] An electrophilic quinoneimine reactive intermediate can be generated from further oxidation of the O-dealkylated lapatinib. [89] Subsequent studies demonstrated that CYP3A4, an enzyme that catalyzes the formation of the reactive quinoneimine metabolite, is inactivated by another lapatinib metabolite that it generated, most likely a nitroso compound. [90] Similarly, the CYP3A5 enzyme can also be inactivated by lapatinib. [91] These studies confirmed the reactivity of lapatinib metabolites and the likely risk they can impose on the liver, where CYP3A4 is the major drug metabolizing enzyme.

Table 25. Tyrosine kinase inhibitors and their reactive metabolites

	Parent compound	Reactive metabolites	Ref
Erlotinib		 	[234]
Gefitinib			[235]
Lapatinib		 	[90]
Dasatinib			[239]

6.3.2 Effect of reactive metabolites on direct and indirect toxicity

During drug metabolism, a drug is converted to a more hydrophilic metabolite, which can be readily excreted from the body. However, in some situations, the process of metabolism gives rise to the formation of chemically reactive species or RM and this includes a diverse group of compounds including unstable conjugates, reactive oxygen species (ROS) and other free radicals, electrophilic metabolites such as epoxides and quinones. [240] These RM can cause direct toxicity or can lead to secondary immune reactions, depending, in part, on the reactivity and formation rate of the reactive metabolites as well as host response.

The RMs are capable of inducing dysfunction through direct adduction to neighboring host proteins or macromolecular targets. The binding to nucleophilic macromolecules in target cells, leads to covalent modifications which are often disruptive to endogenous protein, lipids and DNA function. The presence of RM may lead to DNA damage, induction of proapoptotic proteins, depletion of hepatic glutathione, disruption of intercellular calcium concentrations and ultimately resulting in mitochondrial permeability transition as a hallmark commitment to cell death via both apoptotic and necrotic mechanisms. In addition, oxidative stress as an orthogonal mode of cellular damage may occur as a result of oxidative imbalance between the large production of ROS and the depletion of endogenous antioxidants. This may then result in several sites of cellular damage (i.e. protein damage, lipid peroxidation and nucleotide oxidation), leading to loss of function. Consequently, this alters cellular homeostasis, resulting in impairment of cellular function, which can lead to cell death and possible organ failure. [92, 233, 241-243] Similar mechanisms have been demonstrated to be responsible for other drug-induced toxicities such as

acetaminophen and diclofenac-mediated liver injury. [244, 245] Although most drugs form RM, not all drugs are associated with idiosyncratic drug toxicities. This may be due to the RM being detoxified by cellular defense mechanisms. [233] The covalently modified proteins may be repaired or degraded; otherwise they may impair important cellular functions, which could be directly pathogenic. [233] Moreover, a larger dose of a drug may exacerbate the toxicity, by generating a larger quantity of toxic metabolites, which may likely overwhelm the host defense mechanism.

Some modifications may also elicit a host immune response if the structurally altered proteins are recognized as a foreign macromolecule. This may lead to drug-induced immune toxicities. Such immunological consequences were demonstrated with tienilic acid-induced hepatitis. [246] The formation of a reactive compound that covalently binds to proteins alone might be insufficient to trigger an immune reaction or induce a non-pathogenic immune response. [247] In the presence of a 'danger' signal, such as underlying infection or presence of cytokines due to inflammation and cellular injury, a full-blown immune response may be triggered. [248] The outcome in either direct or indirect toxicity event is cell death caused by either apoptosis or necrosis, depending on several factors like the inherent properties of the drug, dose, duration of exposure, and a variety of environmental and genetic factors that modulate drug metabolism and transport, and the innate and adaptive immune systems. [249]

The liver is the main site for bioactivation and detoxification of drugs and their metabolites. Hence, any potentially toxic metabolite generated in the process can

readily exert a localized damage. As such, the sites of RM formation usually correlate with the major targets of idiosyncratic reactions, in this case, hepatotoxicity.

There also seems to be a correlation between the amount of RM formed and the risk of idiosyncratic reactions. One study demonstrates that a dose more than 100 mg per day and being a substrate of CYP450 enzymes are two important predictors of drug-induced liver injury. [96] High levels of RM may also be due to presence of high levels of enzyme that activates the drug to the RM or low activities of enzymes to detoxify the RM. The activities of the enzymes may be influenced by genetic polymorphisms, such as CYP2D6, or DDIs. For instance, in the case of acetaminophen, an archetypal example of DILI, metabolism of acetaminophen in the liver includes the formation of a RM, the N-acetyl-p-benzoquinoneimine, [93] and in the presence of an inducer, RM formation was increased which markedly enhanced hepatotoxicity. [94]

6.4 Characteristics of tyrosine kinase inhibitors-induced hepatotoxicity

The onset of TKI-induced hepatotoxicity is highly variable. A literature search of published case reports suggests that the median onset is 7 weeks, with a range of 1 – 72 weeks. (Table 26) Although most manufacturers recommend close monitoring of LFTs prior initiation of TKI, liver toxicity can also occur even after long periods of TKI treatment. It is also important to note that drugs that produce severe injury often induce a higher incidence of mild injury. Cases of apparent drug-induced hepatotoxicity may be rare, and they belong to a large subset of patients who display asymptomatic ALT abnormalities, which in certain cases may transform into severe

injury. [250, 251] Take tacrine for example, although overt hepatic injury only occurred in 2% of the patients receiving the drug, half of patients developed minor elevations of liver enzymes and one-quarter had ALT levels that are greater than three times the upper normal limit. [252] The incidence of mild, asymptomatic, and transient ALT abnormalities may occur 10 to 20 times more often than the overt disease [250] and therefore serves as an excellent proxy for the low frequency of severe DILI events which do not frequently occur in clinical practice.

In most instances, TKI-induced hepatotoxicity is mostly reversible. Unfortunately, it has been reported that the liver injury may be fatal in some cases, especially when the liver injury is detected too late and complications such as lactic acidosis have occurred. Based on our literature search, the median time to recovery of LFTs to baseline or normal range was around 6 weeks, with a range of 1 to 44 weeks. (Table 26)

6.5 Overcoming tyrosine kinase inhibitors-induced hepatotoxicity

Currently, there are several strategies that have been adopted to overcome TKI-induced hepatotoxicity. The strategies that have been successful so far are (1) switching to an alternative TKI with similar mechanism of action; (2) using an alternative dosing of the TKI and (3) introduction of steroids for treatment and prevention of hepatotoxicity. However, it should be noted that although these strategies have been successfully introduced to patients without the recurrence of hepatotoxicity, their effect on survival benefits have not been evaluated. Hence, these strategies should be adopted with caution.

6.5.1 Switching tyrosine kinase inhibitors

Substituting with an alternate TKI of similar mechanism of action has been adopted as a strategy in clinical practice. As some TKIs have similar pharmacological action by acting on similar receptors, such as erlotinib and gefitinib, the alternative TKI can be prescribed if patients experience serious toxicities with one TKI. Successful use of erlotinib has been observed in patients who experience gefitinib-associated toxicities, including hepatotoxicity, and vice versa for the use of gefitinib in erlotinib-associated toxicities. [253-258] Although the structures of gefitinib and erlotinib are highly similar, the successful switch between the two TKIs without inducing another episode of hepatotoxicity suggests that minor differences in chemical structure in substituents attached to quinazoline and aniline rings between gefitinib and erlotinib may present different haptens to induce allergic reaction. [255] The positive switches for other classes of TKIs like from imatinib to dasatinib or nilotinib have also been observed. This observation indicates that these TKIs represent an option for maintaining therapeutic responses in patients in whom continuation of imatinib is not possible because of its hepatic toxicity. [259] The successful switch between TKIs to prevent hepatotoxicity also proposes the lack of cross-reactivity between the TKIs. In another words, this implies that the toxicological target is unrelated to the common pharmacological target shared between the TKIs. Also, this effectively eliminates hepatotoxicity as an on-target toxicity related to the inhibition of the target tyrosine kinase pathway shared by the TKIs at risk. [260] (Table 26)

6.5.2 Alternative dosing

Re-challenge of the implicated TKI usually leads to a second episode of hepatotoxicity with a quicker onset, suggesting that the underlying mechanism is

immune-mediated. Nevertheless, re-initiation of the implicated TKI at reduced doses or at reduced dosing frequency have been effective in prevent further hepatotoxicity.

The use of reduced doses of TKIs has been implemented in a few cases. The alteration of the dosing frequency of gefitinib from every day to every other day [261] or every 5 days [262] has been successful to prevent recurrent toxicity. However, as these dosing regimens are neither properly studied nor approved, there is no evidence on its effect on disease response and survival, and this may pose a risk of under-dosing in a patient. Seki et al hypothesized a reduction of hepatotoxicity when gefitinib is administered once every 5 days as the C_{max} and AUC of gefitinib were dependent on the number of consecutive days it was administered and the development of hepatitis depended on the dosage of gefitinib in the IDEAL-1 trial. [262] (Table 26)

6.5.3 Reversibility of toxicities with corticosteroids

Current limited information on TKI-associated hepatotoxicity suggest that it is an idiosyncratic drug reaction (IDR), due to characteristics like rare occurrence, generation of reactive metabolites, dose-independent although a higher daily dose may pose as a risk factor for toxicity etc. If TKI-associated hepatotoxicity is immune-mediated, then corticosteroids may be useful for management of the toxicity. There have been documented cases in the literature where TKI-associated hepatotoxicity has been successfully treated and prevented with the introduction of corticosteroids. Harbaum et al [259] and Ferrero et al [263] demonstrated that when corticosteroids are applied early after the onset of imatinib-induced hepatotoxicity, it may result in rapid and complete hepatic recovery. The concurrent use of corticosteroids with

imatinib has also allowed the continuance of imatinib therapy without hepatotoxicity recurrence in many cases. [263-269] (Table 26)

In most of the cases, the corticosteroid of choice is prednisolone, although methylprednisolone has also been used. Unlike dexamethasone, prednisolone and methylprednisolone do not induce CYP3A4, hence avoiding the potential interactions with TKIs which are mostly CYP3A4 substrates. The dosing regimens as well as the duration of usage of the corticosteroids are also highly variable. For the purpose of treatment, doses of prednisolone can be up to 100 mg/day and duration of usage ranges from several days to 3 months. For the prevention of subsequent episodes of hepatotoxicity, prednisolone has been used at doses ranging from 12.5 – 50 mg daily and for several months of usage. (Table 27)

A danger hypothesis has been suggested by Matzinger, which proposes that presentation of an antigen in the absence of danger results in tolerance, while the presence of a danger signal will result in a full-blown immune response. [248] Therefore, if only a small amount of RM is formed, a significant response is unlikely. However, if the RM formed is more toxic, either due to a larger amount formed or the nature of the RM, cell stress or necrosis can result which may present as an additional 'danger' signal that amplifies the response. This unprogrammed cell death serve as a sign to the immune system that an unplanned destruction is impending, which leads to the activation of a full-fledge immune response. Additionally, in the context of a clear danger signal, such as underlying inflammation, infection or trauma, all of which may constitute the danger signal, the risk of idiosyncratic drug reactions increases. [270]

The usefulness of corticosteroids may be based on their ability to suppress the inflammatory response in the liver as a part of drug-hypersensitivity or an immuno-allergic reaction, and supports the speculation that an allergic mechanism may be involved in the TKI-associated liver injury seen in these patients. [266, 267] Therefore, cautiously re-challenging after complete resolution of the liver injury under steroid administration and with very gradual dose increment may allow patients who have developed severe liver toxicity to continue taking the drug. [269] In summary, corticosteroids are a promising approach for managing TKI-induced hepatotoxicity, in order to avoid the permanent discontinuation of a very effective anti-neoplastic drug. [263] Future studies should evaluate the choice of the corticosteroids, dose and duration of usage for the purpose of treatment as well as prevention of TKI-induced hepatotoxicity. More importantly, the effect of the corticosteroids on the efficacy of TKIs should also be evaluated. As some corticosteroids such as dexamethasone possess CYP3A4-inducing capabilities, pharmacokinetics and safety of the combination of steroids and TKIs should also be assessed.

Table 26. Strategies to overcome TKI-induced hepatotoxicity

	Age, gender	Malignancy	Onset (weeks)	Outcome	Time to recovery (weeks)	Treatment and successful approaches adopted to overcome hepatotoxicity	Reference
(1) Switching tyrosine kinase inhibitor							
ERL	64, F	NSCLC	5	Recovery	2	ERL discontinued Ursodeoxycholic acid for treatment Switched to GEF	[253]
ERL	31, F	NSCLC	4	Recovery	N.A.	ERL discontinued Switched to GEF	[254]
GEF	67, F	Metastatic lung carcinoma	4	Recovery	4	GEF discontinued Switched to ERL	[255]
GEF	83, F	Metastatic lung carcinoma	8	Recovery	8	GEF discontinued Switched to ERL	
GEF	52, M	NSCLC	10	Recovery	4	GEF discontinued Switched to ERL	[256]
GEF	88, M	NSCLC	20	Recovery	5	GEF discontinued Switched to ERL	
GEF	73, M	NSCLC	6	Recovery	N.A.	GEF discontinued Switched to ERL	[257]
GEF	66, F	NSCLC	13	Recovery	7	GEF discontinued Switched to ERL	[258]
IMA	32, F	CML	24	N.A.	N.A.	IMA discontinued Prednisolone for treatment Switched to DASA	[259]
IMA	41, F	CML	24	Recovery	N.A.	IMA discontinued Switched to NILO	[271]
IMA	F	GIST	7	Recovery	9	IMA discontinued Switched to SUNI	[272]
IMA	53, F	GIST	10	Recovery	N.A.	IMA discontinued Prednisolone for treatment	[273]

						Switched to SUNI	
SUNI	76, M	mRCC	2	Recovery	N.A.	SUNI discontinued Switched to SORA	[274]
(2) Alternative dosing							
GEF	69, F	NSCLC	4	Recovery	20	GEF use reduced from daily to every other day at the same dosage	[261]
GEF	61, F	NSCLC	8	Recovery	4	GEF discontinued GEF dosing changed to 250 mg once every 5 days	[262]
IMA	64, M	CML	1	Recovery	6	IMA discontinued IMA restarted at reduced dose	[275]
(3) Use of corticosteroids							
ERL	74, M	NSCLC	4	Recovery	1	ERL discontinued Methylprednisolone for treatment	[276]
IMA	65 M	GIST	4	Recovery	12	IMA discontinued Ursodeoxycholic acid and prednisone for treatment IMA re-initiated with ursodeoxycholic acid and prednisone	[264]
IMA	18, F	CML	2	Recovery	15	IMA discontinued Prednisolone for treatment IMA re-initiated with prednisolone	[265]
IMA	50, M	CML	8	Recovery	8	IMA discontinued IMA re-initiated with prednisolone	[263]
IMA	66, F	CML	32	Recovery	4	IMA discontinued Prednisolone for treatment IMA re-initiated with prednisolone	
IMA	79, F	CML	20	Recovery	12	IMA discontinued Methylprednisolone for treatment IMA re-initiated with prednisolone	
IMA	78, M	CML	24	Recovery	N.A.	IMA discontinued Prednisolone for treatment IMA re-initiated with prednisolone	
IMA	60, F	CML	24	Recovery	4	IMA discontinued	

						Prednisolone for treatment IMA re-initiated with prednisolone	
IMA	20, F	CML	2	Recovery	3	IMA discontinued IMA re-initiated with prednisolone	[266]
IMA	51, F	CML	13	Recovery	N.A.	IMA discontinued Preparation of glycyrrhizin (Stronger Neo-Minophagen C [SNMC]) and ursodeoxycholic acid for treatment IMA re-initiated with prednisolone	[267]
IMA	17, F	CML	72	Recovery	3	IMA discontinued IMA re-initiated with steroids	[268]
IMA	57, F	CML	12	Recovery	4	IMA discontinued Prednisolone for treatment IMA re-initiated with prednisolone	[269]
IMA	32, F	CML	24	N.A.	N.A.	IMA discontinued Prednisolone for treatment Switch to DASA	[259]
IMA	53, F	GIST	10	Recovery	N.A.	IMA discontinued Prednisolone for treatment Switch to SUNI	[273]

Abbreviations: *CML*, chronic myeloid leukemia; *DASA*, dasatinib; *ERL*, erlotinib; *GEF*, gefitinib; *GIST*, gastrointestinal stromal tumours; *IMA*, imatinib; *mRCC*, metastatic renal cell carcinoma; *N.A.*, not available; *NSCLC*, non-small cell lung cancer; *NILO*, nilotinib; *SORA*, sorafenib; *SUNI*, sunitinib

Table 27. The use of corticosteroids to manage TKI-induced hepatotoxicity

	Steroids for treatment/ prevention	Choice of steroids	Dosing regimen	Duration of usage	Reference
ERL	Treatment	Methylprednisolone	IV high dose pulse therapy (1000 mg/day)	3 days	[276]
IMA	Treatment	Prednisolone	100 mg/day	3 days	[259]
IMA	Treatment	Prednisolone	0.8 mg/kg/day	2 months	[273]
IMA	Prevention	Prednisolone	25 mg/day	5 months	[263]
IMA	Prevention	Prednisolone	25 mg/day	2 months	[266]
IMA	Prevention	Prednisolone	20 mg/day	N.A.	[267]
IMA	Prevention	Prednisolone	50 mg/day	N.A	[268]
IMA	Treatment & prevention	Prednisolone	1 mg/kg/day	6 months	[264]
IMA	Treatment & prevention	Prednisolone	(T) 1 mg/kg/day (P) 30 mg/day*	N.A.	[265]
IMA	Treatment & prevention	Prednisolone	(T) 50 mg/day (P) 25 mg and then 12.5 mg/day	(T) 6 weeks (P) 6 months	[263]
IMA	Treatment & prevention	(T) Methylprednisolone (P) Prednisolone	(T) 40 mg/day (P) 25 mg/day	(T) 3 months (P) 8 months	
IMA	Treatment & prevention	Prednisolone	(T) 25 mg/day (P) 12.5 mg/day	(T) 3 weeks (P) N.A	
IMA	Treatment & prevention	Prednisolone	(T) 25 mg and then 37 mg/day (P) 25 mg and then 12.5 mg/day	(T) 4 weeks (P) 2 months	
IMA	Treatment & prevention	Prednisolone	(T) 30 mg/day (P) 30 mg/day	(T) 1 month (P) 4 months	[269]

Note: Outcomes were positive in all cases except in Dhalluin-Venier et al, where ALT and AST elevation occurred again after imatinib was reintroduced with prednisolone and therapy was eventually switched to peg-IFN (*)
Abbreviations: *ERL*, erlotinib; *IMA*, imatinib; *IV*, intravenous; *N.A*, not available; *P*, prevention; *T*, treatment

6.5.3.1 Supplementary analysis – concurrent use of erlotinib and dexamethasone – where steroids help to reduce toxicity

The management of DILI with corticosteroids has often been discussed and has been highlighted in the previous section. Corticosteroids may be useful in patients whose hepatic injury display immune-like features. It is also well recognized that the use of corticosteroids may mask the manifestations of injury in some patients, while hasten the recovery in others. [277] While there are no prospective studies performed to investigate the role of corticosteroids in the management of hepatotoxicity, there are numerous cases in the literature as previously mentioned, where they have been successfully implemented.

Although corticosteroids are able to suppress the immune system and prevent or treat hepatotoxicity, there is also a possible risk of drug-drug interaction. More than 50% of the drugs in the market are metabolized by CYP3A4, and most steroids are inducers of CYP3A4. Hence, a theoretical drug-drug interaction risk exists. In the case of most TKIs, induction of metabolism may increase the formation of reactive metabolites and therefore increase in risk of hepatotoxicity. Therefore, there seems to be a dual and competing role of corticosteroids in hepatotoxicity.

In order to investigate the role of corticosteroids on hepatotoxicity, an observational study was conducted with an objective to evaluate the incidence of dose modification, which is defined as discontinuation, dose reduction or hold and re-challenge of erlotinib due to hepatotoxicity event.

Erlotinib has been selected as it is metabolized by CYP3A4, CYP1A2 in the liver and by the inducible hepatic isoform CYP1A1 in the lungs [278] and current findings suggest that para-hydroxyerlotinib can generate reactive quinoneimine by oxidation in the liver. Due to the electrophilic nature of quinoneimine, it can covalently bind to proteins to trigger oxidative stress, and react with glutathione to form adducts. As cancer patients with brain metastases usually manifest neurologic symptoms associated with increased intra-cranial pressure, corticosteroids have been used to alleviate these symptoms by reducing cerebral edema. [279] Moreover, corticosteroids are generally utilized in managing chemotherapy-induced nausea and vomiting in pancreatic cancer patients. [280] Therefore, concomitant usage of erlotinib with dexamethasone is not uncommon among cancer patients. Due to the co-administration of dexamethasone with erlotinib, a potential theoretical drug-drug interaction may potentially occur, as erlotinib is extensively metabolized by the hepatic CYP3A4 enzyme [234], and dexamethasone induces CYP3A4 via pregnane X receptor (PXR) gene activation in the liver. In addition, there is currently limited evidence on the clinical impact of the interaction between erlotinib and dexamethasone on hepatotoxicity.

6.5.3.2 Methods

6.5.3.2.1 Study design

This was a single-centered, observational, retrospective study conducted in NCCS. Patients who received erlotinib between October 2007 and June 2012 were reviewed for eligibility. Patients who used erlotinib concurrently with CYP3A4 inhibitor drugs,

and drugs that affect absorption of erlotinib were excluded. [98, 99] In addition, this study excluded patients who did not receive baseline LFT assessment before the erlotinib therapy, and at least one follow-up of LFT during or within 6 half-lives after the cessation of erlotinib therapy.

6.5.3.2.2 Definitions and endpoints

Those who received erlotinib without dexamethasone were classified under group E while patients who received erlotinib with dexamethasone were classified under group E+D. Concomitant usage or co-administration was defined as the use of dexamethasone during the period of erlotinib treatment. A hepatotoxicity event was defined as the first clinically significant increase (comparing against baseline levels) of any liver enzyme including TB, ALT, and AST levels by at least 1 grade in the subsequent liver function tests. The grading is according to the CTCAE criteria version 4.0.2. [103] The primary endpoint was the incidence of dose modification, which is defined as discontinuation, dose reduction or hold and re-challenge of erlotinib due to hepatotoxicity event. In addition, the secondary endpoint was the incidence of clinically significant hepatotoxicity event.

6.5.3.2.3 Statistical analysis

For the continuous variables, parametric test such as the independent samples t-test and non-parametric test like Mann Whitney-U test was used to determine statistical significance between the two groups. For the categorical variables, the Chi-square test or Fisher's test was used. Multivariate logistic regression was performed to adjust the relative risk ratio based on clinically important (such as baseline LFTs) and

statistically significant variables. A p-value of less than 0.05 was considered statistically significant. All data was recorded and analyzed using IBM SPSS Statistics 20.0.

6.5.3.3 Results

6.5.3.3.1 Patient demographics and disease characteristics

A total of 202 patients were screened for eligibility. After application of the inclusion and exclusion criteria, 127 patients were excluded due to various reasons such as: lack of LFTs monitoring, drug-drug interactions via absorption and/or CYP enzymes inhibition. Out of the 75 patients included in the analysis, 16 (21%) were classified into group E+D while 59 (79%) were classified into group E. (Figure 11)

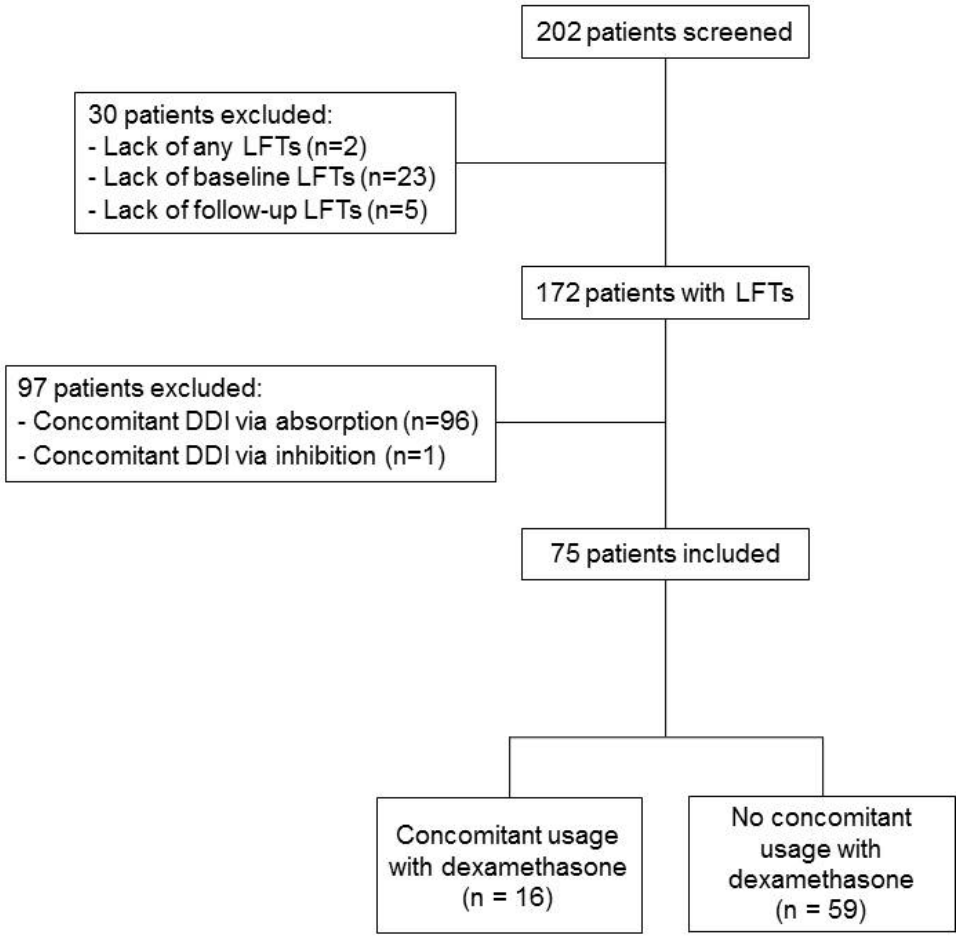


Figure 11. Distribution of patients (erlotinib and dexamethasone)

Majority of the patients were males (56%) and the median age was 63 years old (Inter-quartile range, IQR – 57, 70). Majority of the patients received erlotinib for treatment of NSCLC (88%) and 53 patients (71%) had previously received at least one prior chemotherapy regimen before the usage of erlotinib. Half of the patients (53%) had bone metastasis and 24 (32%) had liver metastasis. Three patients (4%) had underlying liver disease. The overall median baseline LFTs was within the normal range. Twenty-one patients (28%) received erlotinib in combination with chemotherapy. Most of the patients (65%) received 150 mg of erlotinib. In the E+D group, the median dose of dexamethasone used was 4 mg/day (IQR – 4, 8). Meanwhile, the median duration of concomitant usage with erlotinib was 2 days (IQR – 1, 6). (Table 28)

Table 28. Patient demographics and disease characteristics (erlotinib and dexamethasone)

	Total (%) n = 75	**E + D (%) n = 16**	**E (%) n = 59**
Age, years [median, (IQR)]	63 (57, 70)	62 (55, 68)	64 (57, 73)
Body weight*, kg [median, (IQR)]	59 (49, 66)	50 (44, 61)	60 (50, 70)
Male gender	42 (56)	9 (56)	33 (56)
Ethnicity			
Chinese	63 (84)	14 (88)	48 (81)
Indian	0 (0)	0 (0)	0 (0)
Malay	5 (7)	2 (2)	3 (5)
Others	7 (9)	0 (0)	7 (12)
Cancer type			
NSCLC	66 (88)	14 (88)	52 (88)
Pancreatic	6 (8)	2 (13)	4 (7)
Others	3 (4)	0 (0)	3 (5)
Metastasis	73 (97)	16 (100)	57 (97)
Liver	24 (32)	6 (38)	18 (31)
Bone	40 (53)	7 (44)	33 (56)
Brain	23 (31)	5 (31)	18 (31)
Others	25 (33)	6 (38)	19 (33)
Underlying hepatitis	3 (4)	0 (0)	3 (5)
Previous chemotherapy*	53 (71)	15 (94)	38 (64)
Platinum-based drugs	13 (17)	3 (19)	10 (17)
Others	40 (53)	12 (75)	28 (47)
Erlotinib therapy			
Monotherapy*	54 (72)	7 (44)	47 (80)
With gemcitabine*	6 (8)	2 (13)	4 (7)
With other chemotherapy drugs[a]	15 (20)	7 (44)	8 (14)
Erlotinib dose			
100 mg	26 (35)	4 (25)	22 (37)
150 mg	49 (65)	12 (75)	37 (63)
[median, (IQR)]	150 (100, 150)	150 (113, 15)	150 (100, 150)
Duration of erlotinib therapy, days	90 (43, 188)	153 (52, 255)	64 (43, 176)

Abbreviations: *E+D*, coadministration of erlotinib and dexamethasone; *E*, use of erlotinib only; *IQR*, interquartile range; *NSCLC*, non-small cell lung cancer; *TB*, total bilirubin; *AST*, aspartate aminotransferase; *ALT*, alanine transaminase; *ALP*, alkaline phosphatase; *µmol/L*, micromole/liter; *U/L* units/liter

*$p<0.05$ between in the presence of dexamethasone and the absence of dexamethasone

[a] Other chemotherapy drugs include bevacizumab, carboplatin, pemetrexed, cisplatin, and docetaxel.

6.5.3.3.2 Dose modification and concomitant use of erlotinib and dexamethasone

Among all patients, 31 patients (41%) developed all grade hepatotoxicity. Incidence of hepatotoxicity was similar between E+D and E patients (44% vs. 41%). Sixteen (21%), 14 (19%) and 13 (17%) patients showed hepatotoxicity associated with TB, AST and ALT grade increase, respectively. The median onset was 26 days (IQR – 11, 41). Among those patients who demonstrated hepatotoxicity, 10 patients (32%) fully recovered from hepatotoxicity, and the median time for the patients to recover was 43 days (IQR – 33, 65). (Table 29)

Table 29. Evaluation of hepatotoxicity

	Total (%) n = 75	**E + D (%) n = 16**	**E (%) n = 59**
Hepatotoxicity	31 (41)	7 (44)	24 (41)
Elevated TB[a]	16 (21)	5 (31)	11 (19)
Elevated AST[a]	14 (19)	3 (19)	11 (19)
Elevated ALT[a]	13 (17)	1 (6)	12 (20)
Onset, days [median (IQR)]	26 (11, 41)	20 (7, 119)	27 (13, 41)
Outcome of hepatotoxicity			
Full recovery	10 (13)	3 (19)	7 (12)
Time to recovery, days [median, (IQR)]	43 (33, 65)	69 (33, 69)	38 (33, 48)
Hepatic enzymes level, [median (IQR)]			
TB			
Baseline level (µmol/L)		16 (10, 17)	14 (12, 20)
Hepatotoxicity level (µmol/L)		29 (28, 32)	28 (25, 36)
AST			
Baseline level (U/L)		32 (27, 32)	26 (24, 30)
Hepatotoxicity level (U/L)		38 (37, 32)	42 (38, 51)
ALT			
Baseline level (U/L)		23	20 (17, 26)
Hepatotoxicity level (U/L)		49	42 (38, 85)

Abbreviations: *E+D*, coadministration of erlotinib with dexamethasone; *E*, use of erlotinib only; *IQR*, interquartile range; *TB*, total bilirubin; *AST*, aspartate aminotransferase; *ALT*, alanine transaminase; *µmol/L*, micromole/liter; *U/L*, units/liter

*$p<0.05$ between in the presence of dexamethasone and the absence of dexamethasone

a Due to the different mechanisms of liver injury, patients may exhibit different patterns of elevations of liver biochemistries

Among those 31 patients who had developed hepatotoxicity, 17 (55%) received a modified dose of erlotinib. None of the patients in the E+D group and 17 (30%) patients in the E group received dose adjustments ($p < 0.01$). (Table 30)

Table 30. Dose modification and concomitant use of erlotinib with dexamethasone

	E + D (%) n = 16	**E (%) n = 59**
Dose modification due to hepatotoxicity* [a]	0 (0)	17 (30)
Management of adverse events		
Continue with previous dose	5 (31)	6 (10)
Continue with dose reduction (150mg to 100mg) due to:	0 (0)	2 (3)
Hepatotoxicity	0 (0)	2 (3)
Hold and re-challenge due to:	1 (6)	2 (3)
Hepatotoxicity	0 (0)	2 (3)
Skin rash	1 (6)	0 (0)
Discontinue	1 (6)	14 (24)
Hepatotoxicity	0 (0)	13 (22)
Skin rash	0 (0)	1 (2)
Disease progression	1 (6)	0 (0)

Abbreviations: *E+D*, coadministration of erlotinib with dexamethasone; *E*, use of erlotinib only

*$p<0.05$ between in the presence of dexamethasone and the absence of dexamethasone

[a] Dose modification is defined as discontinue or continue with dose reduction or hold and rechallenge of erlotinib

6.5.3.4 Discussion

This study has documented the concurrent use of erlotinib and dexamethasone and the reduced likelihood of developing hepatotoxicity. While there is no reduction in frequency of hepatotoxicity, there were more patients who received erlotinib having experienced dose adjustments due to hepatotoxicity than patients who received dexamethasone concomitantly with erlotinib. This is further supported by the observation that the incidence of recovery from erlotinib-induced hepatotoxicity was higher in patients receiving the combined regimen, compared to those receiving erlotinib without dexamethasone.

Our findings alluded to the possibility that the effect of dexamethasone in suppressing immune response evoked by the reactive metabolite may be more prominent than the CYP3A4 inducing effect in hepatotoxicity events. The dose of dexamethasone recommended for anti-inflammatory indications ranges from 0.75 – 9 mg/day. [281] Hence, at the median dose of 4 mg/day used in the E+D patients, an anti-inflammatory effect will also be observed in addition to its original purpose of antiemesis and cerebral edema. Moreover, dexamethasone exhibits a biphasic induction of CYP3A4 mRNA. At low dexamethasone concentrations (nmol concentrations), CYP3A4 may be transactivated at a low amplitude in a xenobiotic-independent manner. However, at higher levels (> 10 μM), dexamethasone binds to and activates the PXR and producing a high amplitude of induction. [282] In addition, there have been reports in the literature where erlotinib-induced hepatitis and acute interstitial pneumonitis was successfully treated with high-dose corticosteroids. [276] Although RM adduction results in hapten formation, this usually leads to tolerance. However, in the presence of a danger signal, such as underlying inflammation, an

immune response would be prompted, and this may explain the beneficial effects of corticosteroids. Henceforth, suggesting that glucocorticoids, such as dexamethasone, are potentially effective in preventing and treating erlotinib-induced hepatotoxicity. Reproducibility of this clinical finding with a different concomitantly taken steroid would be beneficial to support the findings.

6.5.3.5 Limitations of study

There were a few limitations in this study. Several confounding factors were identified, which include body weight, concurrent use of other chemotherapy agents and baseline LFTs. Additionally, dose reductions of erlotinib may have been influenced by other factors such as physicians' and patients' preference, which were unable to be considered in the analysis due to the lack of data. This study is further challenged by a small sample size (75 patients). After application of several exclusion criteria, more than half of our patients were excluded from the study. In this process, we might have eliminated patients who manifested erlotinib-induced hepatotoxicity but did not have baseline LFT and/or subsequent LFT follow-ups in the database. Consequently, the incidence of hepatotoxicity may be underestimated. However, it is crucial to utilize strict exclusion criteria to ensure the scientific rigor of our results.

6.5.4 Summary of important findings

While the data on formation of RM suggest a possible role in the mechanism of idiosyncratic hepatotoxicity, it is important to note that the role of RM in causality of TKI-induced hepatotoxicity has not been demonstrated and its influence may differ on a case-by-case basis. Numerous events need to occur in an individual patient prior to

the occurrence of an IDR, of which the formation of a reactive intermediate appears to be the prerequisite. The usages of TKIs are associated with a risk of developing hepatotoxicity. Although the mechanism of TKI-induced hepatotoxicity has yet to be clearly established, several strategies have been adopted successfully to overcome TKI-induced hepatotoxicity. However, it should be noted that the effect of these strategies on disease response and survival benefits have not been formally evaluated, and hence they should be adopted with caution. Positive results from our observational study is hypothesis generating; it suggests that future studies can be conducted to examine the role of glucocorticoids such as prednisolone and dexamethasone, in the curative treatment of TKI-induced hepatotoxicity, which may also include the evaluation of the mediators involved in the immune response, such as the level of cytokines and auto-antibodies.

Several strategies are adopted to prevent and treat TKI-induced hepatotoxicity, with the use of corticosteroids being most promising in the treatment and prevention of these toxicities – as there may be a role for the immune system in the development of these IDR. We have observed that there were more cases of dose reductions in patients who received erlotinib only than compared to those who received concomitant erlotinib and dexamethasone, possibly due to the immune suppression effect of dexamethasone. Taken together with our findings from the literature search, the use of glucocorticoids may prevent or even treat hepatotoxicity during the course of TKI therapy. However, further evidence would be required before they can be recommended to the larger populations. Furthermore, the possible risk of increased RM formation through enzyme induction would have to be carefully considered. Drug manufacturers should also look into developing strategies to treat and prevent TKI-

induced hepatotoxicity during drug development, especially when high incidences of elevated liver enzymes are associated with the drug. This could ensure that patient continue to receive treatment, without increasing the risk of serious DILI.

7 Effect of metabolism-related pharmacokinetic drug-drug interaction on risk for TKI-associated hepatotoxicity – a case study of lapatinib and dexamethasone

The use of dexamethasone, a CYP3A4 inducer, together with lapatinib is not uncommon in clinical practice. About one-third of all HER2-positive breast cancer patients would develop brain metastasis [283, 284], and its management involves relieving symptoms of peritumor edema, such as headache, nausea, vomiting, and mental state alterations, which are seen commonly in patients with increased intracranial pressure. These symptoms can be effectively managed with corticosteroids such as dexamethasone [285], with up to 75% of patients demonstrating marked neurological improvement within 24–72 h of dexamethasone initiation [279]. Therefore, both dexamethasone and lapatinib are indicated in metastatic HER2-positive breast cancer patients, which may lead to their concomitant usage. Additionally, the scientific basis of this study is based on the idea that since dexamethasone is a CYP3A4 inducer and lapatinib a CYP3A4 substrate, concomitant use may cause an increase in metabolizing capacity, which in turn increases the formation of lapatinib-derived RM and thereby, elevating the risk of toxicity. Furthermore, in light of the discussion in the previous chapter that dexamethasone possesses enzyme inducing as well as anti-inflammatory effects, it would be challenging to predict the resultant effect of this DDI, which would need to be investigated objectively, using both clinical as well as in-vitro data.

Therefore, this study was conducted with an objective to evaluate the incidence of elevated LFTs in the presence or absence of dexamethasone in clinical setting. The effect of dexamethasone on lapatinib-induced hepatotoxicity was evaluated in a drug utilization review based on patient data obtained from our records. In addition, a parallel in-vitro experimental setup was adopted to provide mechanistic information on the proposed association.

7.1 Drug utilization review

7.1.1 Methodology

7.1.1.1 Study design

This was a single-centered, observational, retrospective, nested case-control study conducted at NCCS. This study was approved by the Institutional Review Board.

7.1.1.2 Data collection

All HER2-positive breast cancer patients who received lapatinib from January 2007 to September 2011 were screened for eligibility. To be included, patients must have baseline LFT monitored and at least one set of LFT follow-up at least 3 days after initiation of lapatinib. Patients were excluded if, during the course of lapatinib therapy, were concomitantly using any CYP3A4 inhibitors or inducers (with the exception of dexamethasone). Two commercially available databases, Micromedex [98] and Lexicomp [99] were utilized to verify the status of CYP3A4 inducers or inhibitors.

Patient information such as demographics, medical history and laboratory parameters were collected through medical records.

7.1.1.3 Endpoints and definitions

Two groups were defined after verification of medication history: Patients who received lapatinib with dexamethasone formed the L+D group, and patients who received lapatinib without dexamethasone, formed the L group. The primary endpoint was the incidence of hepatotoxicity, which was defined by the first clinically significant change (comparing against baseline) of any liver enzyme including TB, ALT or AST by at least 1 grade. Grading of liver enzyme was in accordance of the CTCAE version 4.0.2. [103] Concomitant usage was defined as the use of dexamethasone, during the period of lapatinib therapy. The concomitant usage overlap period was defined as from the day patient starts taking the drug to 6 elimination half-life period after drug discontinuation. Hepatotoxic drugs were defined as drugs with a potential to cause liver injury. [286] Recovery from hepatotoxicity was defined as the normalization of the affected liver enzymes to normal or baseline level at 3 – 4 weeks after the hepatotoxicity event.

7.1.1.4 Statistical analysis

Categorical variables, such as presence of hepatotoxicity, were analyzed with Chi-square or Fisher's exact test. Continuous variables were analyzed using Independent sample t-test or Mann-Whitney U test. Paired data, comparing LFTs at baseline and at hepatotoxicity event, were analyzed using the Wilcoxon Sign Rank test. Univariate analysis was performed to identify variables that are significantly different between

the L+D and L group. Multivariate logistic regression was performed to adjust the odds ratio based on clinically important (e.g. age, presence of liver metastasis, underlying hepatitis, baseline LFT) and statistically significant variables. For 2-tailed tests, a p-value of less than 0.05 was considered significant. Data was analyzed with PASW 18.0.

7.1.2 Results

7.1.2.1 Patient demographics

A total of 120 patients were identified based on lapatinib usage. After assessing inclusion criteria, 23 patients were excluded (3 due to lack of baseline LFT, 14 due to lack of follow up LFT and 6 due to concomitant usage of other CYP3A4 inducer or inhibitor). Ninety-seven patients were included in the analysis, of which 24 (25%) were concomitantly receiving lapatinib with dexamethasone (Figure 12). Median dose of dexamethasone used was 8 mg/day (range 1 – 16), which is within the recommended dosage for management of symptoms of brain metastasis. [279] Median duration of concomitant usage was 11 days (range 2 – 28).

Mean age of the patients was 54 ± 11 years and majority of them were Chinese (77%). Ninety-two patients (95%) had metastatic breast cancer. Only four patients (4%) had underlying liver disease (Table 31). Eighty-seven patients (90%) were using lapatinib as combination therapy, with capecitabine being the most common combination.

Usage of concurrent hepatotoxic drugs was observed in 45 patients (46%). Overall baseline LFT was within normal range (Table 32).

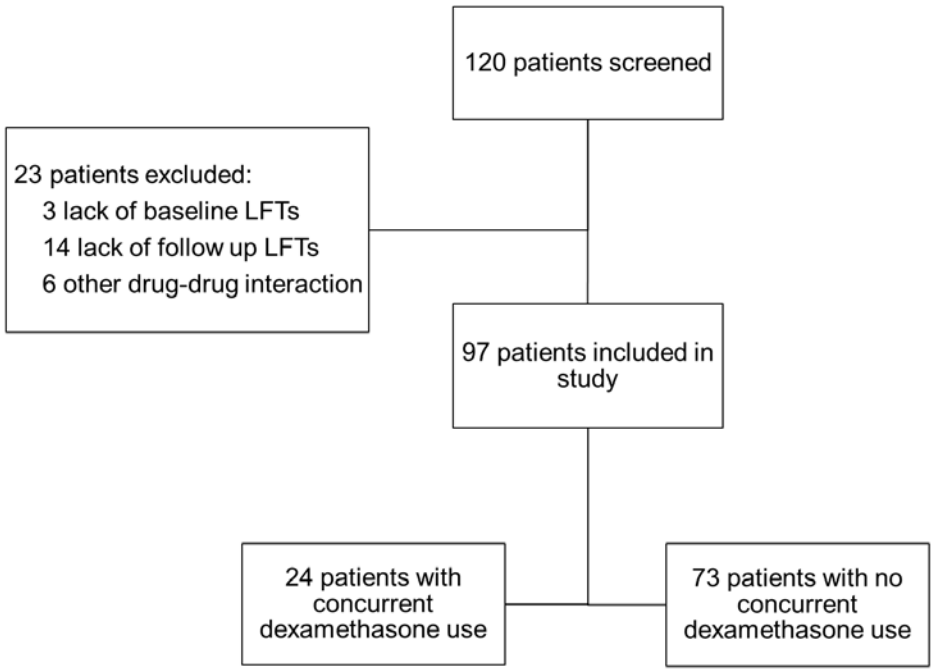


Figure 12. Distribution of patients

Table 31. Patient demographics

	Total (%)	**'L+D' (%)**	**'L' (%)**
	***n*=97**	***n*=24**	***n*=73**
Age, years (mean ± SD) *	54 ± 11	50 ± 10	56 ± 10
Ethnicity			
Chinese	75 (77)	17 (71)	58 (80)
Malay	8 (8)	3 (13)	5 (7)
Indian	6 (6)	1 (4)	5 (7)
Others	8 (8)	3 (13)	5 (7)
Metastasis	92 (95)	24 (100)	68 (93)
Liver	54 (56)	13 (54)	41 (56)
Bone	50 (52)	13 (54)	37 (51)
Brain *	26 (27)	13 (54)	13 (18)
Lung	51 (53)	12 (50)	39 (53)
Others	3 (3)	2 (8)	1 (1)
Hormonal status			
ER+ PR+	39 (40)	11 (46)	28 (38)
ER+ PR-	15 (16)	4 (17)	11 (15)
ER- PR+	12 (12)	1 (4)	11 (15)
ER- PR-	30 (31)	8 (33)	22 (30)
Unknown	1 (1)	0 (0)	1 (1)
Underlying hepatitis	4 (4)	1 (4)	3 (4)
History of chemotherapy	93 (96)	22 (92)	71 (97)
Anthracycline	49 (51)	11 (46)	38 (52)
Taxanes	69 (71)	17 (71)	52 (71)
Vinca alkaloid	36 (37)	6 (25)	30 (41)
History of hormonal therapy	65 (67)	16 (67)	49 (67)
Aromatase inhibitor	49 (51)	13 (54)	36 (49)
Tamoxifen	48 (50)	11 (46)	37 (51)

Abbreviations: *L+D*, Lapatinib and dexamethasone combination; *L*, Lapatinib only; *ER*, Estrogen receptor; *PR*, Progesterone receptor

* $p<0.05$ between with dexamethasone and without dexamethasone group

Table 32. Lapatinib therapy in patients

	Total (%)	**'L+D' (%)**	**'L' (%)**
	***n*=97**	***n*=24**	***n*=73**
Lapatinib therapy			
Monotherapy	10 (10)	4 (17)	6 (8)
Combination therapy	87 (90)	20 (83)	67 (92)
Capecitabine	76 (78)	19 (79)	57 (78)
Hormonal therapy	16 (17)	7 (29)	9 (12)
Vinorelbine	4 (4)	1 (4)	3 (4)
Herceptin	4 (4)	1 (4)	3 (4)
Lapatinib dose, mg (median, (range))	1250 (500, 1500) Mean = 1199	1250 (1000, 1500) Mean = 1229	1250 (500, 1500) Mean = 1188
Concurrent use of hepatotoxic drugs *	45 (46)	20 (80)	25 (34)
Baseline liver function tests *(median, (IQR))*			
TB (U/L)	14 (10, 18)	15 (11, 17)	13 (10, 18)
AST (U/L)	31 (24, 56)	29 (23, 48)	32 (25, 57)
ALT (U/L)	27 (18, 41)	24 (17, 58)	27 (19, 39)
ALP (U/L)	70 (53, 146)	71 (43, 161)	70 (54, 146)

Abbreviations: *L+D*, Lapatinib and dexamethasone combination; *L*, Lapatinib only; *TB*, Total bilirubin; *AST*, Aspartate aminotransferase; *ALT*, Alanine aminotransferase; *ALP*, Alkaline phosphatase; *U/L*, Units/liter

* $p<0.05$ between with dexamethasone and without dexamethasone group

7.1.2.2 Observed differences between L+D and L groups

Patients in the L+D group were younger than those in the L group (50 ± 10 vs. 56 ± 10 years, p=0.03). Brain metastasis was more common in the L+D group (54% vs. 18%, p<0.005). (Table 31) Although more patients in the L+D group were using concurrent hepatotoxic medication (80% vs. 34%, p<0.005), the doses and frequencies of these medications were within normal range. Presence of liver metastasis, underlying hepatitis and baseline LFT were not significantly different between the two groups of patients (p>0.05). (Table 32)

7.1.2.3 Hepatotoxicity evaluation

Sixty-five patients (67%) manifested hepatotoxicity after lapatinib treatment and the median onset was 50 days (range 4 – 528). Generally, hepatotoxicity was associated with a change in AST grading. When compared, the LFT at baseline and at hepatotoxicity event were also significantly different (p<0.005). Twenty-one patients (32%) recovered from the elevated liver enzymes. Among those patients who manifested hepatotoxicity, 46 patients (71%) continued lapatinib use at the same dosage and only 3 (5%) discontinued lapatinib use due to hepatotoxicity. (Table 33)

7.1.2.4 Hepatotoxicity and concomitant use of lapatinib and dexamethasone

Twenty-one patients (88%) in the L+D group developed hepatotoxicity, while it occurred in 44 patients (60%) in the L group (p=0.01). The onset of hepatotoxicity between the groups was also different, with a trend to appear earlier in the L+D group (44 vs. 55 days, p=0.63). Elevation of ALT was observed to be more frequent in the

L+D group compared to the other (42% vs. 19%); and this was significantly different (p=0.03). (Table 33)

Table 33. Evaluation and management of hepatotoxicity

	Total	**L+D**	**L**
	***n*=97**	***n*=24**	***n*=73**
n (%)			
Hepatotoxicity *	65 (67)	21 (88)	44 (60)
Elevated TB †	29 (30)	8 (33)	21 (29)
Elevated AST †	39 (40)	12 (50)	27 (37)
Elevated ALT †*	24 (25)	10 (42)	14 (19)
Onset (days), median (IQR)	50 (22, 110)	44 (22, 107)	55 (20, 110)
Recovery from hepatotoxicity	21 (32)	8 (38)	13 (30)
TB, median (IQR)			
Baseline level (U/L)		15 (11, 27)	
Hepatotoxic event level (U/L)		35 (27, 46)	
AST, median (IQR)			
Baseline level (U/L) ‡		29 (23, 35)	
Hepatotoxic event level (U/L)		50 (38, 107)	
ALT, median (IQR)			
Baseline level (U/L) ‡		24 (16, 32)	
Hepatotoxic event level (U/L)		51 (39, 87)	
Management of hepatotoxicity, *n (%)*			
Continuation of lapatinib therapy			
With the same dose		46 (71)	
With dose reduction		0 (0)	
Discontinuation of lapatinib therapy due to			
Adverse drug reactions			
Hepatotoxicity		3 (5)	
Diarrhea		2 (3)	
Disease progression		14 (22)	

Abbreviations: *L+D*, Lapatinib and dexamethasone combination; *L*, Lapatinib only; *TB*, Total bilirubin; *AST*, Aspartate aminotransferase; *ALT*, Alanine aminotransferase; *U/L*, Units/liter

* $p<0.05$ between with dexamethasone and without dexamethasone group

† Patients may manifest more than one type of hepatotoxicity

‡ $p<0.05$ between baseline and hepatotoxic event level

7.1.2.5 Risk for developing hepatotoxicity

Patients in the L+D group were 4.61 times (95% CI 1.26 – 16.88, p=0.01) more likely to develop hepatotoxicity, than those in the L group. After adjusting with confounding factors such as age, baseline LFT (TB, AST, ALT, ALP), liver and brain metastasis, underlying hepatitis and concurrent usage of hepatotoxic drugs, the odds ratio was 4.57 (95% CI 1.23 – 17.00, p=0.02). (Table 34)

Those in the L+D group were also 3.01 times (95% CI 1.11 – 8.18, p=0.03) more likely to develop a clinically important change in ALT compared to the other group. After adjustment with age, baseline ALT, liver and brain metastasis, underlying hepatitis and concurrent usage of hepatotoxic drugs, the adjusted odds ratio was 3.48 (95% CI 1.24 – 9.80, p=0.02). (Table 34)

Table 34. Risk for developing hepatotoxicity in concomitant usage of dexamethasone

N=97	**OR (95% CI)**	**Adjusted OR (95% CI)**
Hepatotoxicity *	4.61 (1.26 – 16.88)	4.57 ¶ (1.23 – 17.00)
Elevated TB	1.24 (0.46 – 3.33)	-
Elevated AST	1.70 (0.67 – 4.32)	-
Elevated ALT *	3.01 (1.11 – 8.18)	3.48 ¶¶ (1.24 – 9.80)

Abbreviations: TB, Total bilirubin; AST, Aspartate aminotransferase; ALT, Alanine aminotransferase

* $p<0.05$ between with dexamethasone and without dexamethasone group

¶ Adjusted for age, liver metastasis, brain metastasis, baseline LFT (TB, AST, ALT, ALP), underlying liver disease, concurrent use of hepatotoxic medications

¶¶ Adjusted for age, liver metastasis, brain metastasis, baseline ALT, underlying liver disease, concurrent use of hepatotoxic medications

7.2 Cell culture model

7.2.1 Methodology

7.2.1.1 Cell culture conditions

As previously mentioned under 3.2.1.1 Cell culture conditions

7.2.1.2 CYP3A4 induction and RT-PCR

Induction of cyp3a11, the mouse homologue of CYP3A4 was evaluated. TAMH cells (approximately 10,000 – 15,000/well) were seeded in a 6-well plate and were treated with solvent (control) or dexamethasone and incubated for 72 hours. After incubation, cells were harvested using the cell scraper. RNA was extracted and purified using the RNeasy Mini Kit (Qiagen, Venlo, The Netherlands). Subsequently, cDNA synthesis was performed using the SuperScript III First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA). PCR primer for cyp3a11, the CYP3A4 mouse homologue, was used. The primer pair sequences were obtained from literature [287], forward primer TCA CAC ACA CAG TTG TAG GGA GAA and reverse primer GTC CAT CCC TGC TTG TTT GTC. Real-time quantitative PCR (RT-PCR) was performed with the CFX96 Real-Time PCR Detection System (Bio-Rad, Hercules, CA). Data obtained were normalized to reference gene and solvent control to obtain the ΔΔCT value, which was converted to fold change in mRNA expression levels.

7.2.1.3 Treatment and cell viability assay

TAMH cells (approximately 2000/well) were seeded in a 96-well plate. At 24 hours, cells were treated with vehicle-only or dexamethasone (10 and 20 µM) and allowed to incubate for an additional 72 hours. After incubation, various concentration of lapatinib (2.5 to 15 µM) was added and incubated for 24 hours. Thereafter, media was aspirated and replaced with 50 µl of 2 mg/ml of MTT dye (in PBS) and 200 µl of HEPES-buffered DMEM/F12 media, as previously established. [124, 125] The plate was incubated in the dark for 2 hours at 37 °C. Medium was then aspirated and the residual dye re-dissolved in 20 µl of Sorensen's buffer (0.1 M glycine and 0.1 M NaCl equilibrated to pH 10.5 with 0.1 M NaOH) and 200 µl of DMSO. Plates were read at 570 nm using the Infinite® 200 (Tecan, Männedorf, Switzerland). Cell viability is expressed as a ratio normalized to the vehicle-treated control.

7.2.2 Results

7.2.2.1 Evidence of dexamethasone induction

TAMH cells were pre-treated with dexamethasone and RT-PCR was used to quantify the mRNA expression, which was compared against treatment with solvent control. Treatment with 10 µM and 20 µM dexamethasone resulted in a 2-fold and 2.5-fold increase in cyp3a11 mRNA levels respectively. (Figure 13)

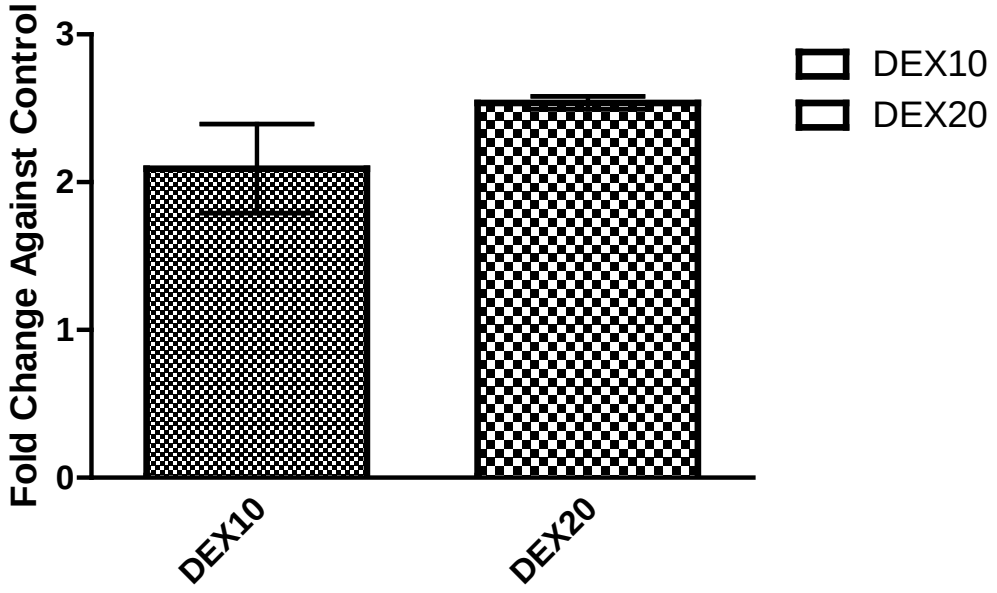


Figure 13. Evidence of dexamethasone induction on TAMH cells

7.2.2.2 Cell viability

The presence of lapatinib (more than 5 µM) as compared to treatment with solvent control, reduced viability in a concentration-dependent manner. The average viability was 91% and 29% for 5 µM and 10 µM lapatinib respectively. There was a further reduction in viability, with the introduction of dexamethasone and this decrease was even more evident with a higher concentration of dexamethasone. At 5 µM lapatinib, the introduction of 10 µM and 20 µM dexamethasone resulted in a 19% and 59% decrease in viability respectively. This result offers complementary in-vitro evidence that the combination of lapatinib and dexamethasone affects the cell viability (Figure 14).

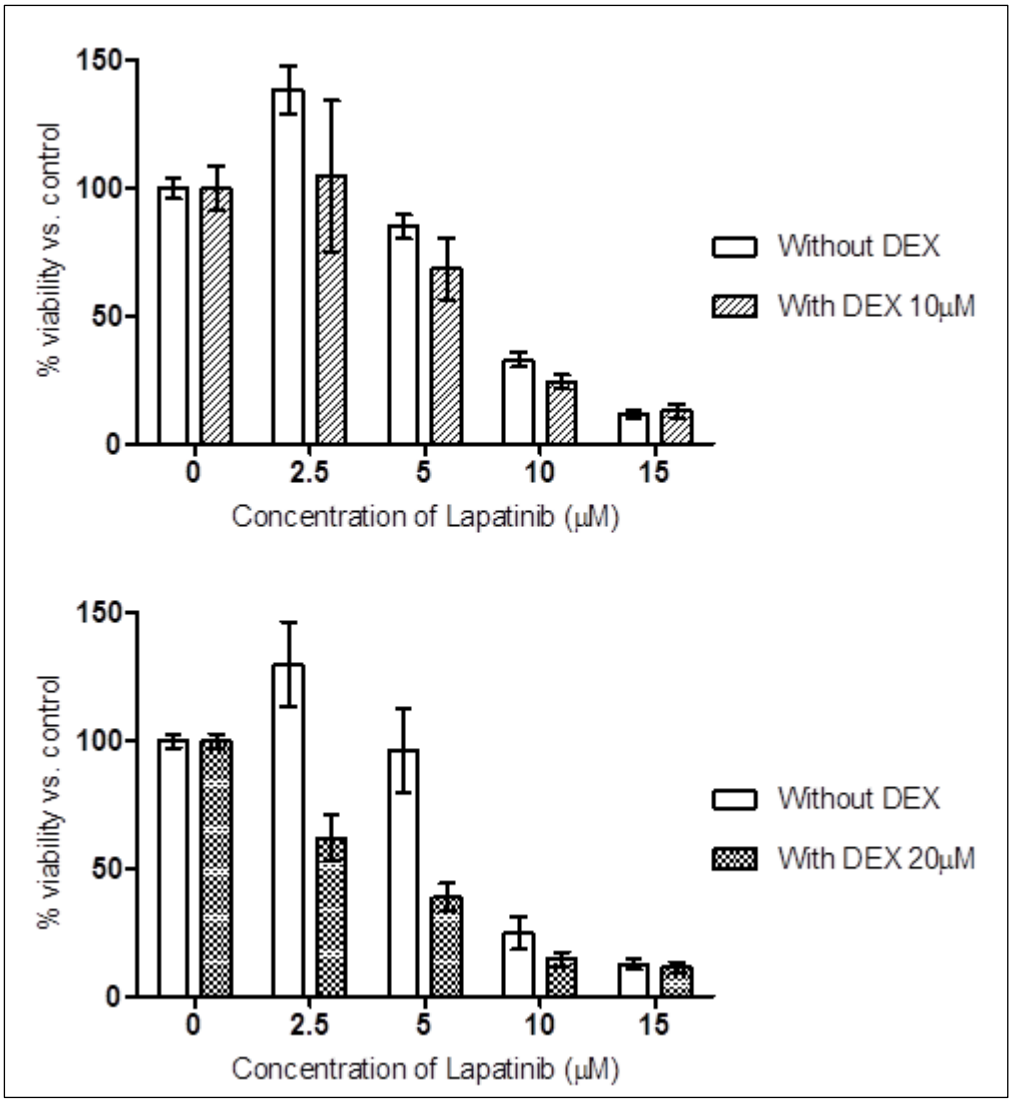


Figure 14. Change in cell viability with treatment of lapatinib and dexamethasone (DEX) (top) 10µM and (bottom) 20µM

7.3 Discussion

This is the first study to document a clinically important interaction between lapatinib and dexamethasone, which associates with an increased occurrence of hepatotoxicity. Patients who were using this combination exhibited about 5-fold higher risk of developing hepatotoxicity than those who were not using the combination.

With the intent to investigate the in-vitro role of dexamethasone in lapatinib-induced hepatotoxicity, a parallel experimental setup was included and the findings have provided substantiating evidence to verify our hypothesis. Treatment of the metabolically-competent TAMH cells with both compounds resulted in a further reduction in viability as compared to treatment with lapatinib alone. In this interaction, dexamethasone induces the enzyme, which increases lapatinib metabolism and RM formation. In addition, this result also provides in-vitro evidence on our first aim, which is to determine the association between TKI plasma levels and toxicities. Treatment of TAMH cells with lapatinib alone resulted in a concentration-dependent reduction in viability, suggesting a possible relationship between exposure and toxicity. Moreover, since this work was published, further corroboration was observed with a similar study in HepaRG using rifampicin as an inducer. [288]

Our study alluded to the possibility that this increase in risk of toxicity could similarly be observed in other CYP3A4 inducers [71]. The usage of CYP3A4 inducers in cancer patients is not uncommon, where they can be part of anticancer therapy, supportive care or management of co-morbidities. The impact of this interaction has also led us to contemplate if other co-administered drugs could be accountable for the

onset of idiosyncratic toxicities with lapatinib. Lapatinib, together with letrozole, is indicated for use in HER2-positive metastatic breast cancer in postmenopausal women. Lapatinib is an inhibitor of CYP3A4, while letrozole is a CYP3A4 substrate, thus leading to the possibility for an interaction where lapatinib could inhibit letrozole metabolism. Interestingly, the expected interaction was not observed, but instead serum levels of both drugs decreased. [289] With that in mind, strong CYP3A4 inducers and inhibitors should be used cautiously with lapatinib. The effect of altering metabolism and toxicity by dexamethasone suggests that genetic differences among individuals may also impact the occurrence of lapatinib-induced hepatotoxicity.

In the previous chapter, it was demonstrated that there was a decrease in risk of hepatotoxicity with the concomitant use of erlotinib and dexamethasone, which is contrasting with the observations in this study. One possible reason may be due to the doses of the drugs. The median dose of lapatinib in the study was 1250 mg, compared to the median dose of 150 mg of erlotinib in that study. Although a dose-toxicity association has yet to be established, a higher dose is usually associated with a higher risk of IDR due to the increase exposure of both drug and RM. The remarkably large load of RM formed may generate a danger signal which may then trigger off a response. Secondly, it could also be due to the innate toxicity of the RM, where RM of lapatinib could be highly toxic compared to the RM of erlotinib. It may also be attributed to the two agents being metabolized by a different panel of P450 enzymes. Erlotinib is also metabolized by CYP1A2 to a large extent, as compared to lapatinib. Hence, the effect of dexamethasone on the expression of these enzymes may be slightly different. As such, the difference in toxicity may not be due to the effects of

dexamethasone alone, but rather a combination of that with the inherent toxicity of the drug.

7.4 Limitations of study

There were several limitations in this study. Several confounding factors were identified, which include age, liver metastasis status, baseline LFT, underlying liver disease and concurrent usage of hepatotoxic medications. These were either clinically important and/or statistically significant variables between the groups and were all included in the multivariate model for adjustment of the odds ratio. These variables had minimal impact on the risks, where the odds of developing hepatotoxicity in patients receiving dexamethasone were 4.61 (95% CI 1.26 – 16.88) and 4.57 (95% CI 1.23 – 17.00), before and after adjustment respectively. Due to the retrospective nature of this study, HLA allele type (DQA1*02:01 and DRB1*07:01 are identified as risk factors for lapatinib-associated hepatotoxicity [290]), alcohol usage and nutritional status were lacking, which undermined the contribution of additional risk factors for developing hepatotoxicity. [291] Patients may also be using medications or CAM such as St John's Wort obtained elsewhere such as community pharmacies, and these were not captured in this study due to the retrospective nature. Next, it is challenging to define and diagnose drug-induced hepatotoxicity, due to the absence of definitive signs and symptoms, biomarkers and findings such as liver biopsy. [292] This is further complicated by the highly variable onset of injury. Since this was a retrospective study, the schedule for laboratory tests was not standardized and hence highly variable. The robust hepatic responses of repair and regeneration may allow seemingly life-threatening liver injury to subside without clinical consequences, despite continuation of drug therapy [293], making it even more challenging to detect

the onset and recovery of drug-induced liver injury. This study also used a definition of hepatotoxicity which is more lenient than the standard DILI working group criteria, and hence the number of hepatotoxicity events observed could have been inflated. To identify patients who experience full frank DILI, the definition of hepatotoxicity from the DILI Expert Working Group Criteria is defined as any one of the following: more than or equal to five-fold elevation above the ULN for ALT, or more than or equal to two-fold elevation above the ULN for ALP (particularly with accompanying elevations in concentrations of 5'-nucleotidase or γ-glutamyl transpeptidase in the absence of known bone pathology driving the rise in ALP level) or more than or equal to three-fold elevation in ALT concentration and simultaneous elevation of bilirubin concentration exceeding two-fold above the ULN. [294] To capture patients who experience clinically significant changes in hepatic function, we defined hepatotoxicity as patients who have experienced at least one grade of elevation of any liver enzymes compared against baseline, based on the CTCAE criteria. However, the 'milder form' of hepatotoxicity serves as an excellent proxy for the more severe DILI events as they do not frequently occur in clinical practice. This is suggested in literature as the 'adaptive' characteristic of idiosyncratic hepatic injury, where patients who develop full blown hepatotoxicity belongs to a subset of a larger group of patients who develop elevated liver enzymes, and as a consequence of failure to 'adapt' to the initial injury. [251]

7.5 Summary of important findings

This chapter has provided clinical evidence that concomitant usage of lapatinib and dexamethasone increases the risk for hepatotoxicity by five-fold, possibly through the induction of metabolism. The in-vitro findings have also provided substantiating

evidence and insights on the role of dexamethasone in lapatinib-induced hepatotoxicity. Since it is not uncommon for them to be prescribed concomitantly, clinicians should be aware of this risk when considering the use of this combination and follow through with close monitoring where necessary. In addition, as supported by the parallel effects of both dexamethasone and rifampicin, caution should be exercised when lapatinib is used with other P450 enzyme inducers. On the other hand, the effect of each DDI may be TKI-specific, as contrasting outcomes of the potential DDI was observed for the combination of lapatinib-dexamethasone and erlotinib-dexamethasone. The difference in outcomes and toxicity may not be due to the effects of the enzyme inducer dexamethasone alone, but rather a combination of that with the inherent toxicity of the drug.

8 Concluding remarks and recommendations for future studies

This thesis aimed to investigate whether pharmacokinetic alterations in TKIs can affect the occurrence of toxicities. Looking at the different aspects of pharmacokinetic alterations such as drug exposure, genetic polymorphisms and drug-drug interactions, this thesis has demonstrated that these factors may play a role in the manifestation of TKI-associated toxicities. Table 35 provides a summary of the important findings in this thesis.

The first aim of this thesis was to explore the association between the manifestation of TKI-associated toxicities and plasma drug levels and consequently, to determine the role of TDM as a possible management strategy for TKI-induced toxicity. In this thesis, a trend of higher drug exposures in patients who experienced toxicities such as mucositis and altered taste was observed. More importantly, several of these toxicities have a significant impact on patient's quality of life. As a drug, sunitinib engenders considerable and unpredictable inter-patient variability; hence therapeutic drug monitoring could be a mean to improve its safety and efficacy. A practical suggestion would be to measure blood concentrations when sunitinib is initiated for the first cycle, to observe whether therapeutic levels are achieved in relation to efficacy and toxicity. This is particularly important in groups such as Asians, women and those with a low body weight, because these factors may affect the accumulation of sunitinib and thereby the risk of toxicity. Total plasma concentration of sunitinib could be used as a marker for therapeutic efficacy, whereas sunitinib levels could be used as a marker for toxicity. If necessary, doses could also be decreased further to meet the minimum effective plasma concentration without affecting the efficacy,

which could potentially lead to cost savings for the patient. Further studies with an active therapeutic drug monitoring component should be conducted to study the feasibility of such a strategy. Furthermore, the parent drug, sunitinib, was found in the in-vitro study to be more toxic to the skin cells than its active equipotent metabolite SU12662. Therefore, in-vitro experiments could also be conducted with other cell types to compare the effect of sunitinib and SU12662 on various toxicities.

As the disposition of TKIs could play a role in explaining the differences in response and in toxicities observed, this thesis aimed to explore the role of genetic polymorphism in TKI-associated toxicities, by investigating the association between genetic polymorphisms of key drug metabolizing enzyme and drug transporters and their risk for toxicities. The thesis demonstrated that ABCB1 may be associated with manifestation of toxicities and drug exposure, but corresponding effect with the polymorphism of CYP3A5 is not apparent. Future studies involving a larger sample should be conducted to confirm these findings. If the role of the ABCB1 polymorphism on exposure and toxicities is confirmed in larger studies, genotyping for ABCB1 and therapeutic drug monitoring should be considered in clinical practice to aid in the personalization of drug therapy. By ensuring that the optimal dose is prescribed to the right patient according to the genotype, we can achieve maximum efficacy with minimal toxicity.

Finally, the last aim of this thesis was to assess the effect of metabolism-related pharmacokinetic drug-drug interactions on risk for TKI-associated toxicity. Due to the substantial potential for interaction between TKIs and other drugs that modulate the

activity of metabolic pathways, unwanted clinical consequences may occur from small changes in drug metabolism and pharmacokinetics in cancer patients. In addition, TKIs possess several risk factors for developing DILI. It is imperative that comprehensive and accurate information be collected on use of medications by patients, to increase awareness and familiarity with potential DDIs to ensure patient safety and to aid the development of optimal therapy. [295, 296] To reduce the potential for unexpected drug interactions during therapy, the patient's medical history should be taken thoroughly and interactively, and updated periodically. [297] Patients who have risk factors for potential DDIs should also be monitored more closely. Risk factors for potential DDIs include liver function status, age, tumor type [298], number and type of medications received [299] and using drugs that are metabolized exclusively by only one CYP isoform. [300] When drugs with potential DDIs are considered with TKIs use, clinicians should also consider alternative agents that have no or less interaction potential. However, it is also important to note that in some cases, switching to an alternative agent may not have any significant difference on the pharmacokinetic profile. For instance, azithromycin exhibits a low potential for interaction. Likewise, there was no significant effect of oral clarithromycin or azithromycin on the pharmacokinetic profile of sunitinib after single administration. [301] As the clinical effects of these potential interactions are unknown, when drug pairs with potential DDIs need to be used (e.g. in cases where there is a compelling indication for the potential interacting drug to be used), more intense patient monitoring for interactions is needed. Scripture et al has provided a valuable summary of the conditions where drug interactions are likely to be clinically significant, such as when drug elimination occurs primarily through a single metabolic pathway or when one or both of the interacting drugs has a steep dose-response curve or a narrow

therapeutic range etc. [173] Furthermore, the effect of each DDI may be TKI-specific, as contrasting outcomes of the potential DDI was observed for the combination of lapatinib-dexamethasone and erlotinib-dexamethasone. The difference in outcomes and toxicity may not be due to the effects of the enzyme inducer dexamethasone alone, but rather a combination of that with the inherent toxicity of the drug.

This thesis has also evaluated how a TKI-associated DDI may affect the risk of hepatotoxicity. The findings demonstrated that patients receiving TKIs were 4-times more likely to develop hepatotoxicity compared to patients receiving placebo. Future studies should be conducted to investigate the mechanism of TKI-associated hepatotoxicity, which is largely idiosyncratic, to determine which RM is culpable for the toxicity and how it causes toxicity. It is also evident that formation of RM alone may not be sufficient for the manifestation of toxicity, for instance, genetic polymorphism in HLA genes may be studied as they could play an important role in the development of immune response to trigger an overt toxicity. In addition, this thesis has also demonstrated both clinical and in-vitro evidence suggests that concomitant usage of lapatinib and dexamethasone increases the risk for hepatotoxicity. Future studies will aim to examine the effects on efficacy and toxicity from drug interaction with other agents. Although explicit cases of TKI-induced hepatotoxicity may be rare, they often occur in a background of higher rate of mild, asymptomatic and usually transient liver injury. Patients who do not adapt to this mild injury will then move on to develop progressive liver injury. Notwithstanding patient risk factors such as age, gender, concurrent diseases, other drug exposures and immunological dispositions, the TKI itself with its unique physicochemical properties and propensity for metabolism, is associated with several risk factors for developing

DILI. Hence, periodic and continual monitoring of LFTs are vital for early detection of injury. Although the product information leaflets of these TKIs do provide some recommendations on the monitoring of LFTs, the quality of the information is inconsistent and more extensive details should be provided. For example, information can be provided on what is the recommended threshold of LFTs elevation to discontinue the TKI, when can TKI be restarted in cases of elevated LFTs, or should they be permanently discontinued. A more comprehensive guidance can aid the clinicians in management of their patients and improved confidence in managing the hepatotoxicity cases. Ultimately, by understanding the different hepatic mechanisms underlying the interaction between the drugs involved in a pharmacotherapy may improve the management strategies for co-administration of drugs.

In conclusion, TKIs have successfully transformed many cancers from a death sentence to a chronic disease. Furthermore, with the unceasing discovery of new anticancer drugs, the outlook is promising. However, these new drugs are also a harbinger of more challenges such treatment tolerability and health outcomes. While we cannot have the best of both worlds at the moment, with continual research in the understanding and management of toxicities as well as the development of improved drugs, perhaps someday in the future we can.

Table 35. Summary of important findings

Aims	Research questions	Important findings
1. To explore the association between the manifestation of TKI-associated toxicities and plasma drug levels	What is the association between plasma level of sunitinib and/or active metabolite and the manifestation of toxicity?	Trend of higher exposures in patients who experienced toxicities
	What is the difference in the in-vitro toxic potential of sunitinib vs. its active metabolite?	Sunitinib more toxic towards keratinocytes while both sunitinib and metabolite were equally toxic to hepatocytes
2. To explore the role of genetic polymorphism in TKI-associated toxicities	What is the association between CYP3A5 and ABCB1 SNPs with the manifestation of toxicity?	ABCB1 may be associated with manifestation of toxicities, but the polymorphism of CYP3A5 is not
	What is the association between CYP3A5 and ABCB1 SNPs with the plasma level of sunitinib and/or active metabolite?	ABCB1 may be associated with drug exposure, but the polymorphism of CYP3A5 is not
3. To assess the effect of metabolism-related pharmacokinetic drug-drug interactions on risk for TKI-associated toxicity	What is the role of metabolism-related DDIs in TKI therapy?	Due to the substantial potential for interaction between TKIs and other drugs that modulate the activity of metabolic pathways, unwanted clinical consequences may occur from small changes in drug metabolism and pharmacokinetics in cancer patients
	What is the risk of TKI-induced hepatotoxicity in cancer patients?	Patients receiving TKIs were 4-times more likely to develop hepatotoxicity compared to patients receiving placebo
	Why is TKI at risk for hepatotoxicity?	TKIs possess several risk factors for developing DILI
	Does the CYP3A4 inducer, dexamethasone, affect the incidence of hepatotoxicity of lapatinib?	Both clinical and in-vitro evidence suggests that concomitant usage of lapatinib and dexamethasone increases the risk for hepatotoxicity

9 Publications arising from this work

9.1 Peer-review articles

1. Teo YL, Wee HL, Chue XP, Chau NM, Tan MH, Kanesvaran R, Wee HL, Ho HK, Chan A. Effect of the CYP3A5 and ABCB1 genotype on exposure, clinical response and manifestation of toxicities from sunitinib in Asian patients. Pharmacogenomics. 2015 Mar 17. doi: 10.1038/tpj.2015.13.

2. Teo YL, Chue XP, Chau NM, Tan MH, Kanesvaran R, Wee HL, Ho HK, Chan A. Association of drug exposure with clinical response and toxicity in metastatic renal-cell carcinoma patients receiving an attenuated dosing regimen of sunitinib. Target Oncol. 2014 Dec 13. doi: 10.1007/s11523-014-0349-2

3. Teo YL, Ho HK, Chan A. Formation of reactive metabolites and management of tyrosine kinase inhibitors-induced hepatotoxicity: A literature review. Expert Opin Drug Metab Toxicol. 2014 Nov 15:1-12.

4. Teo YL, Ho HK, Chan A. Metabolism-related pharmacokinetic drug-drug interactions in tyrosine kinase inhibitors: current understanding, challenges and recommendations. Br J Clin Pharmacol. 2014 Aug 14. doi: 10.1111/bcp.12496.

5. Teo YL, Chong XJ, Chue XP, Chau NM, Tan MH, Kanesvaran R, Wee HL, Ho HK, Chan A. Role of sunitinib and SU12662 on dermatological toxicities in

metastatic renal cell carcinoma patients: in-vitro, in-vivo and outcomes investigation. Cancer Chemother Pharmacol. 2014 Feb;73(2):381-8

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7. Teo YL, Saetaew M, Chanthawong S, Yap YS, Chan EC, Ho HK, Chan A. Effect of CYP3A4 inducer dexamethasone on hepatotoxicity of lapatinib: clinical and in vitro evidence. Breast Cancer Res Treat. 2012; 133(2): 703-11.

8. Teo YL, Tan SY, Chue XP, Chau NM, Tan MH, Kanesvaran R, Ho HK, Wee HL, Chan A. Patient-reported outcomes in Asian metastatic renal cell carcinoma (mRCC) patients receiving an attenuated dosing of sunitinib. (under review)

9.2 Published abstracts and conference presentations

1. Teo YL, Chue XP, Chau NM, Tan MH, Wee HL, Ho HK, Kanesvaran R, Chan A. Toxicities and patient reported outcomes in elderly Asian patients receiving an attenuated dosing of sunitinib for metastatic renal cell carcinoma. J Geriatr Oncol. 2014 Jul;5 Suppl 1:S19. SIOG Asia Pacific Conference, Singapore, 2014.

2. Kanesvaran R, Teo YL, Chue XP, Chau NM, Tan MH, Wee HL, Ho HK, Chan A. Association between clinical response and toxicities with drug exposure in an alternative dosing regimen of sunitinib. J Clin Oncol 32, 2014 (suppl 4; abstr 439) Genitourinary Cancers Symposium, San Francisco, CA, USA, 2014.

3. Teo YL, Wee HL, Chue XP, Chau NM, Tan MH, Kanesvaran R, Wee HL, Ho HK, Chan A. Association of CYP3A5 polymorphism with toxicities in metastatic renal cell carcinoma patients (mRCC) receiving an alternative dosing (AD) regimen of sunitinib. Support Care Cancer 21, 2013 (Suppl 1):S220. MASCC/ISOO International Symposium on Supportive Care in Cancer, Berlin, 2013.

4. Teo YL, Chong XJ, Chue XP, Chau NM, Tan MH, Kanesvaran R, Wee HL, Ho HK, Chan A. Determining the role of sunitinib and its active metabolite (SU12662) on hand-foot syndrome (HFS) in metastatic renal cell carcinoma (mRCC) patients. Support Care Cancer 21, 2013 (Suppl 1):S169. MASCC/ISOO International Symposium on Supportive Care in Cancer, Berlin, 2013.

5. Teo YL, Chue XP, Chau NM, Tan MH, Kanesvaran R, Wee HL, Ho HK, Chan A. Association of drug exposure with clinical response and toxicities in metastatic renal cell carcinoma patients (mRCC) receiving an alternative dosing (AD) regimen of sunitinib. J Clin Oncol, 2013 (suppl; abstr e13582). ASCO Annual Meeting, USA, 2013

6. Teo YL, Chue XP, Chau NM, Tan MH, Kanesvaran R, Wee HL, Ho HK, Chan A. Exploration of the association between pharmacokinetics of sunitinib and treatment-related toxicities in the metastatic renal cell carcinoma (mRCC) treatment of Asian patients. Singhealth Duke-NUS Scientific Congress 2012.

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