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Clinical implications of germline variations for treatment outcome and drug resistance for small molecule kinase inhibitors in patients with non-small cell lung cancer

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ABSTRACT

Small-molecule kinase inhibitors (SMKIs) represent the cornerstone in the treatment of non-small cell lung cancer (NSCLC) patients harboring genetic driver mutations. Because of the introduction of SMKIs in the last decades, treatment outcomes have drastically improved. Their treatment efficacy, the development of drug resistance as well as untoward toxicity, all suffer from large patient variability. This variability can be explained, at least in part, by their oral route of administration, which leads to a large inter- and intra-patient variation in bioavailability based on differences in absorption. Additionally, drug-drug and food-drug interactions are frequently reported. These interactions could modulate SMKI efficacy and/or untoward toxicity. Furthermore, the large patient variability could be explained by the presence of germline variations in target receptor domains, metabolizing enzymes, and drug efflux transporters. Knowledge about these predictor variations is crucial for handling SMKIs in clinical practice, and for selecting the most optimal therapy. In the current review, the literature search included all SMKIs registered for locally-advanced and metastatic NSCLC by the US Food and Drug Administration (FDA) or European Medicines Agency (EMA) until March 24th, 2022. The *BIM* deletion showed a significantly decreased PFS and OS for East-Asian patients treated with gefitinib, and has the potential to be clinically relevant for other SMKIs as well. Furthermore, we expect most relevance from the *ABCG2* 34 G>A and *CYP1A1* variations during erlotinib and gefitinib treatment. Pre-emptive *CYP2D6* testing before starting gefitinib treatment can also be considered to prevent severe drug-related toxicity. These and other germline variations are summarized and discussed, in order to provide clear recommendations for clinical practice.

Introduction

Lung cancer remains one of the leading causes of mortality. Since the turn of the century, treatment outcomes have drastically improved, largely resulting from the development and introduction of small molecule kinase inhibitors (SMKIs) (Lee et al., 2018). This class of drugs has tremendous advantage over classic chemotherapeutic agents, because these drugs are tailored to act on disease-specific pathways regarding signal transduction (Krause and Van Etten, 2005). As a result of this targeted approach, most SMKIs are registered solely for tumor-specific mutations, and are supposed to be associated with diminished adverse effects. Furthermore, these compounds are administered orally as opposed to chemotherapeutic agents – which are mainly administered intravenously – which is associated with a lower frequency

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of hospital visits, and improved patients' quality of life.

Nonetheless, several important issues have been identified upon SMKIs-based treatment of non-small cell lung cancer (NSCLC). While treatment outcomes are overall better with SMKIs compared to cytotoxic drugs, there is considerable variation in drug resistance and tolerability between patients. This can be explained, at least in part, by their oral route of administration, leading to a large inter- and intra-patient variation in bioavailability based on differences in absorption, whilst drug-drug and food-drug interactions are also frequently reported (van Leeuwen et al., 2014; Veerman et al., 2020). These interactions could modulate SMKI efficacy and/or untoward toxicity. Last, but not least, pharmacogenetics are known to alter SMKI treatment outcomes. Pharmacogenetics is used as an umbrella term for germline variations in genes encoding for enzymes and drug transporters involved in the metabolism of a drug (van Gelder et al., 2004). For example, variations in genes associated with SMKI drug metabolism (e.g. cytochrome P450 (CYP450) enzymes) or with transcellular drug transport (e.g. ABC and SLC transporters) are known to alter the exposure to SMKIs (Mulder et al., 2021), thereby modulating cytotoxicity and survival outcome (Caetano-Pinto et al., 2017). Also, germline variations in genes encoding for membrane transporters have been described to cause increased influx or efflux of SMKIs. This could cause increased tumor drug resistance and toxicity to healthy tissues. Furthermore, germline variations within receptor sites or their molecular pathway may alter the therapeutic activity of SMKIs, rendering patients more prone to drug resistance (Gillis and McLeod, 2016; Juchum et al., 2015; Sarmento-Ribeiro et al., 2019; Saleem et al., 2019).

Germline variations mostly concern single nucleotide polymorphisms (SNPs) but other variations (i.e. repeats of certain nucleotides or deletions) were also reported. Whilst their clinical impact is believed to be substantial, the use of SNPs in clinical cancer care is currently limited (e.g. *DPYD* variations in the prescription of fluoropyrimidines) (Henricks et al., 2018). Therefore, the question that arises is whether or not germline variations should also be considered when prescribing SMKIs, and if so, which germline variations should be tested prior to the treatment. The aim of this review is to provide an overview of the current literature (until March 24th, 2022) on the pharmacogenetic impact of SMKIs treatment, the occurrence of drug resistance and untoward toxicity as well as to provide recommendations for clinicians prescribing SMKIs for NSCLC treatment.

Search strategy and selection criteria

PubMed and Embase databases were searched for clinical research regarding pharmacogenetics and SMKIs registered by the United States Food and Drug Administration (FDA)[13] or the European Medicines Agency (EMA) (EMA, 2021) for the treatment of metastatic NSCLC published until March 24th, 2022. SMKIs considered in the search were afatinib, alectinib, brigatinib, capmatinib, ceritinib, crizotinib, dabrafenib, dacomitinib, entrectinib, erlotinib, gefitinib, larotrectinib, lorlatinib, nintedanib, osimertinib, pralsetinib, selpercatinib, and trametinib. In the PubMed database we searched for 'SMKI name' AND (polymorphism OR transporter). 'Humans' and 'English language' were used as additional filters. In Embase we searched for 'SMKI name' AND ('genetic polymorphism'/exp OR 'genetic polymorphism' OR 'transporter'/exp OR 'transporter') AND [humans]/lim AND [english]/lim AND [clinical study]/lim. All search results were screened for possible relevance based on title, abstract, availability of the full text, but also sample size and statistical power. Articles regarding an association between either germline SNPs or germline polymorphisms and SMKI outcomes, such as pharmacokinetics, exposure, toxicity, the onset of drug resistance expressed as a change in progression-free survival (PFS) and overall survival (OS), were included.

Assessing relevant germline variations in SMKI treatment

In total, our search resulted in 2238 hits for all SMKIs: 840 from Pubmed and 1398 from Embase. After structured title and abstract screening, and full-text reading, 90 articles remained to be included in this review. Evidently, most hits were from the SMKIs which have been registered for the longest period of time (*c.q.* erlotinib and gefitinib). Fewer articles were found on the other, more recently registered SMKIs.

We classified pharmacogenetic studies in SMKI treatment by its focus on either pharmacodynamic or pharmacokinetic influences of germline genetic variation. Pharmacodynamic effects are related to the target of a drug, e.g. by SNPs occurring in the *EGFR*-gene, or to proteins downstream in its molecular pathway. The most important outcomes for these studies are progression-free survival (PFS) and overall survival (OS). Also, in peripheral tissues, the same variations may induce or reduce susceptibility to adverse events, such as the *-216 G>T* SNP in the *EGFR* promotor region, causing increased expression of EGFR receptors, and making patients more prone to SMKI-induced diarrhea and skin rash (McKibbin et al., 2010; Giovannetti et al., 2010)

Meanwhile, germline genetic variations in drug transporters or in drug metabolizing enzymes may affect the pharmacokinetics of drugs (Ahmed et al., 2016). Firstly, polymorphisms in drug transporter genes, most notably *ABCB1* (P-glycoprotein) and *ABCG2* (Breast Cancer Resistance Protein), may influence the distribution of drugs over different body compartments (Caetano-Pinto et al., 2017; Sprowl et al., 2012; Cecchin et al., 2018) This may affect intra-tumoral drug concentrations and, consequently, treatment efficacy (Li et al., 2016; Wang et al., 2021). Alternatively, these same transporters alter drug concentrations in healthy tissues, affecting toxicity (Cusatis et al., 2006). The second group of genetic polymorphisms which affects pharmacokinetics relates to genes involved in drug metabolism including CYP450 or uridine 5′-diphospho-glucuronosyltransferase (UGT) enzymes. When drugs are rapidly metabolized, exposure to drugs is reduced, potentially diminishing therapeutic efficacy. Conversely, when drugs are metabolized slowly, efficacy may be retained, albeit untoward toxicity may increase (Mathijssen et al., 2014).

The European Network of Centers for Pharmacoepidemiology and Pharmacovigilance has made clear guidelines for the design, implementation and analysis of pharmacogenomic studies (ENCEPP, 2022). For implementation of genotyping of a specific germline variation in clinical practice, it is important for variations to have a relevant impact on patient survival, the development of drug resistance or tolerability in multivariable analysis. Preferably, this is confirmed through changes in SMKI pharmacokinetics (ENCEPP, 2022). Additionally, the results must have been validated -including for instance by Bonferroni correction- in an independent second cohort or by thorough statistical bootstrapping, in order to provide solid evidence of the presence and magnitude of the effect. Furthermore, there should be a balance between the impact of a SNP and its prevalence in a population, so genetic testing is (cost-) effective on a large scale (ENCEPP, 2022).

Clinically relevant germline variations in erlotinib treatment

Target variations

Especially in East-Asian patient populations, a thoroughly studied genetic variant is a deletion in intron 2 of the *B-cell Lymphoma-2-like 11* (*BIM*) gene (Table 1 **and** supplementary Table 1) (Lee et al., 2014, 2013, 2015; Ariyasu et al., 2020; Zhao et al., 2014; Sun et al., 2017; Xia et al., 2017; Shuang et al., 2016; Liu et al., 2019, 2012; Cardona et al., 2016; Wang et al., 2019; Winther-Larsen et al., 2015, 2019; Nie et al., 2011; Rudin et al., 2008; Jung et al., 2012; Zhang et al., 2016; Leal et al., 2020; Fukudo et al., 2013; Endo-Tsukude et al., 2018; Ruan et al., 2016; Liao et al., 2020; Hamada et al., 2012; Miyamoto et al., 2020; Chen et al., 2015; Zenke et al., 2016; Wang et al., 2018; Suzumura et al., 2012; Arbitrio et al., 2016; Chiu et al., 2015; Xu et al., 2014) The *BIM* deletion is mostly present in Asian patients, with a prevalence of 13–18%, while

Table 1
The effects of germline variations on PFS, OS, pharmacokinetics and untoward toxicity in erlotinib treatment for NSCLC.

Gene	Variant	rs number	PFS, HR (95% CI)	OS, HR (95% CI)	PK effect	Toxicity	Number of patients	References
BIM	Deletion		NS	–	–	–	197	(Lee et al., 2013)
			NS	NS	–	–	196	(Ariyasu et al., 2020)
			2•09 (1•15–3•82)	–	–	–	166	(Zhao et al., 2014)
			NS	NS	–	–	140	(Sun et al., 2017)
			22 versus 38 months	24 versus 39 months	–	–	245	(Xia et al., 2017)
			NS	–	–	–	295	(Shuang et al., 2016)
			NS	NS	–	–	194	(Liu et al., 2019)
			NS	NS	–	–	117	(Liu et al., 2019)
			NS	NS	–	–	205	(Lee et al., 2015)
			3•0 (1•2–7•6)	3•4 (1•4–8•3)	–	–	89	(Cardona et al., 2016)
BIM	Exon 5 c465C>T		NS	1•81 (1•12–2•81)	–	–	196	(Ariyasu et al., 2020)
EGFR	181946C>T / D994D G>A	rs2293347	0•43 (0•22–0•82)	0•47 (0•24–0•93)	–	–	82	(Winther-Larsen et al., 2015)
			0•35 (0•24–0•93)	0•21 (0•06–0•76)	–	–	73	(Winther-Larsen et al., 2019)
EGFR	R497K A>L	rs11543848	NS	NS	–	–	115	(Nie et al., 2011)
			–	–	AUC NS, C_{max} NS, C_{trough} NS	NS	80	(Rudin et al., 2008)
EGFR	− 216 G>T	rs712829	0•78 (0•44−1•36)	0•82 (0•47–1•43)	–	–	82	(Winther-Larsen et al., 2015)
			5•1 m (2•7–7•5) vs 16•6 m (5•8–27•5)	29•5 m (17•4–41•7) vs 1•4 m (3•7–39•5)	–	–	71	(Jung et al., 2012)
			0•51 (0•33–0•80)	0•54 (0•34–0•84)	–	–	230	(Zhang et al., 2016)
			–	–	AUC NS, C_{max} NS, C_{trough} NS	NS	80	(Rudin et al., 2008)
EGFR	− 191 C>A	rs712830	NS	NS	–	–	82	(Winther-Larsen et al., 2015)
			–	–	AUC NS, C_{max} NS, C_{trough} NS	NS	80	(Rudin et al., 2008)
EGFR	intron 1, CA-SSR1 (repeats)		NS	NS	–	–	71	(Jung et al., 2012)
			–	NS	–	–	115	(Nie et al., 2011)
			–	–	AUC NS, C_{max} NS, C_{trough} NS	NS	80	(Rudin et al., 2008)
EGFR	+ 61 A>G	rs4444903	NS	NS	–	–	111	(Leal et al., 2020)
EGFR	C>T	rs1468727	NS	NS	–	–	230	(Zhang et al., 2016)
AKT1	4 G>A	rs1130233	NS	NS	–	–	184	(Liu et al., 2012)
AKT1	G>T	rs1130214	1•85 (1•09–3•13)	NS	–	–	230	(Zhang et al., 2016)
ABCB1	1236 C>T	rs1128503	NS	NS	AUC NS, C_{trough} NS	NS	88	(Fukudo et al., 2013)
			–	–	↓ 29% Clearance	NS	48	(Endo-Tsukude et al., 2018)
			–	–	–	More rash, OR > 2	134	(Ruan et al., 2016)
			NS	–	C_{trough} NS	NS	87	(Liao et al., 2020)
			–	–	AUC NS, C_{max} NS, C_{trough} NS	NS	50	(Hamada et al., 2012)
			–	–	AUC NS, C_{max} NS, C_{trough} NS	NS	45	(Miyamoto et al., 2020)
ABCB1	2677 G>T/A	rs2032582	NS	NS	AUC NS, C_{trough} NS	NS	88	(Fukudo et al., 2013)
			–	–	AUC NS, C_{max} NS, C_{trough} NS	NS	48	(Endo-Tsukude et al., 2018)
			–	–	AUC NS, C_{max} NS, C_{trough} NS	NS	50	(Hamada et al., 2012)
			–	–	AUC NS, C_{max} NS, C_{trough} NS	NS	45	(Miyamoto et al., 2020)
ABCB1	3435 C>T	rs1045642	NS	NS	C_{trough} NS, Clearance NS	NS	88	(Fukudo et al., 2013)
			NS	–	C_{trough} NS	NS	87	(Liao et al., 2020)
			–	–	AUC NS, C_{max} NS, C_{trough} NS	NS	48	(Endo-Tsukude et al., 2018)
			–	–	AUC NS, C_{max} NS, C_{trough} NS	NS	50	(Hamada et al., 2012)
			–	–	AUC NS, C_{max} NS, C_{trough} NS	NS	45	(Miyamoto et al., 2020)
ABCB1	1236TT-2677TT-3435TT		–	–	↑AUC,↑ C_{min}, C_{max} NS	Earlier onset grade > 1 toxicity: 4 vs 12 days	50	(Hamada et al., 2012)

(*continued on next page*)

Table 1 (*continued*)

Gene	Variant	rs number	PFS, HR (95% CI)	OS, HR (95% CI)	PK effect	Toxicity	Number of patients	References
ABCB1	A>G	rs10248420	–	–	–	Up to 161% more toxicity of any grade	134	(Ruan et al., 2016)
ABCB1	A>G	rs7787082	–	–	–	Up to 54% less toxicity of any grade	134	(Ruan et al., 2016)
ABCG2	421 C>A	rs2231142	NS	NS	↓ 24% Clearance, ↑ C_{trough}	NS	88	(Fukudo et al., 2013)
			–	–	AUC NS, C_{max} NS, C_{trough} NS	NS	48	(Endo-Tsukude et al., 2018)
			NS	–	C_{trough} NS	NS	87	(Liao et al., 2020)
			–	–	AUC NS, C_{max} NS, C_{trough} NS	NS	80	(Rudin et al., 2008)
			NS	NS	–	–	200	(Liu et al., 2012)
			NS	NS	–	–	70	(Chen et al., 2015)
ABCG2	1143 C>T	rs2622604	–	–	AUC NS, C_{max} NS, C_{trough} NS	NS	80	(Rudin et al., 2008)
			–	–	AUC NS, C_{max} NS, C_{trough} NS	NS	48	(Endo-Tsukude et al., 2018)
			NS	–	C_{trough} NS	NS	87	(Liao et al., 2020)
			NS	NS	–	–	70	(Chen et al., 2015)
ABCG2	− 15622 C>T	rs7699188	–	–	AUC NS, C_{max} NS, C_{trough} NS	NS	80	(Rudin et al., 2008)
			–	–	AUC NS, C_{max} NS, C_{trough} NS	NS	48	(Endo-Tsukude et al., 2018)
			NS	–	C_{trough} NS	NS	87	(Liao et al., 2020)
ABCG2	34 G>A	rs2231137	–	–	AUC NS, C_{max} NS, C_{trough} NS	NS	80	(Rudin et al., 2008)
			NS	0•56 (0•38–0•84)	–	–	70	(Chen et al., 2015)
ABCG2	16702 G>A		–	–	AUC NS, C_{max} NS, C_{trough} NS	NS	80	(Rudin et al., 2008)
ABCG2	− 15994 G>A		–	–	AUC NS, C_{max} NS, C_{trough} NS	NS	80	(Rudin et al., 2008)
UGT1A1	*28		–	–	AUC NS, C_{max} NS, C_{trough} NS	NS	48	(Endo-Tsukude et al., 2018)
UGT1A1	*6	rs4148323	–	–	AUC NS, C_{max} NS, C_{trough} NS	NS	48	(Endo-Tsukude et al., 2018)
UGT2B7	211 G>T	rs12233719	–	–	AUC NS, C_{max} NS, C_{trough} NS	NS	48	(Endo-Tsukude et al., 2018)
UGT2B7	802 C>T	rs7439366	–	–	AUC NS, C_{max} NS, C_{trough} NS	NS	48	(Endo-Tsukude et al., 2018)
CYP1A1	*2 A T > C		–	RR 2•36 (1•54–3•64)	–	–	115	(Nie et al., 2011)
CYP1A1	*2 C 2455 A>G	rs1048943	–	NS	–	–	115	(Nie et al., 2011)
			0•23 (0•06–0•92)	–	↑ 61% C_{trough}	NS	87	(Liao et al., 2020)
			–	–	AUC NS, C_{max} NS, C_{trough} NS	NS	48	(Endo-Tsukude et al., 2018)
			–	–	–	NS	134	(Ruan et al., 2016)
CYP1A2	− 3860 G>A	rs2069514	–	–	AUC NS, C_{max} NS, C_{trough} NS	NS	48	(Endo-Tsukude et al., 2018)
CYP1A2	− 2467 T > delT	rs35694136	–	–	AUC NS, C_{max} NS, C_{trough} NS	NS	48	(Endo-Tsukude et al., 2018)
CYP1A2	− 163 C>A	rs762551	–	–	AUC NS, C_{max} NS, C_{trough} NS	NS	48	(Endo-Tsukude et al., 2018)
			–	–	–	57% less severe rash	134	(Ruan et al., 2016)
			NS	–	C_{trough} NS	Less severe rash	87	(Liao et al., 2020)
CYP1A2	5347 T > C	rs2470890	–	–	AUC NS, C_{max} NS, C_{trough} NS	NS	48	(Endo-Tsukude et al., 2018)
CYP1A2	1545 T > C	rs2470890	NS	–	Ctrough NS	NS	87	(Liao et al., 2020)
CYP2C9	99 C>T	rs17885098	–	–	–	Less skin toxicity	134	(Ruan et al., 2016)
CYP2C19	*3	rs4986893	–	–	–	More skin toxicity	134	(Ruan et al., 2016)
CYP2D6	100 C>T	rs1065852	–	–	AUC NS, C_{max} NS, C_{trough} NS	NS	48	(Endo-Tsukude et al., 2018)
			NS	–	C_{trough} NS	NS	87	(Liao et al., 2020)
CYP3A4	*1 G 20230 G>A	rs2242480	–	–	AUC NS, C_{max} NS, C_{trough} NS	NS	48	(Endo-Tsukude et al., 2018)
			NS	–	C_{trough} NS	NS	87	(Liao et al., 2020)
CYP3A4	*1B A>G		–	–	AUC NS, C_{max} NS, C_{trough} NS	NS	80	(Rudin et al., 2008)
CYP3A5	*3 / 6986 A>G	rs776746	–	–	AUC NS, C_{max} NS, C_{trough} NS	NS	80	(Rudin et al., 2008)
			NS	NS	Clearance NS, C_{trough} NS	NS	88	(Fukudo et al., 2013)

(*continued on next page*)

Table 1 (*continued*)

Gene	Variant	rs number	PFS, HR (95% CI)	OS, HR (95% CI)	PK effect	Toxicity	Number of patients	References
			–	–	AUC NS, C_{max} NS, C_{trough} NS	NS	48	(Endo-Tsukude et al., 2018)
			NS	–	C_{trough} NS	50% less any grade diarrhea	87	(Liao et al., 2020)
GSTM1	Deletion		–	–	AUC NS, C_{max} NS, C_{trough} NS	NS	50	(Endo-Tsukude et al., 2018)
GSTT1	Deletion		–	–	AUC NS, C_{max} NS, C_{trough} NS	NS	50	(Endo-Tsukude et al., 2018)

PFS: progression free survival; OS: overall survival; PK: pharmacokinetics; AUC: area under the curve; Cmax: maximum concentration; Ctrough: trough concentration; HR: hazard ratio; OR: odds ratio; RR: relative risk; NS: non-significant.

the deletion does not appear in patients of Caucasian and African origin (Ng et al., 2012). BIM functions as a pro-apoptotic factor in the EGFR-SMKI induced apoptosis pathway. The deletion is therefore believed to cause intrinsic resistance to EGFR-SMKIs, including erlotinib, and consequently impairs response to treatment (Ng et al., 2012). Nonetheless, this remains a highly controversial topic in the literature with studies reporting conflicting results. A recent meta-analysis showed the detrimental effects of the *BIM* deletion in a large EGFR-cohort, albeit this was not SMKI-specific (Lv et al., 2021). Hence, extrapolation of these results for all EGFR-SMKIs is not yet justifiable. For erlotinib, although several studies report no effect on either PFS or OS, others report a significant and clinically relevant impaired PFS and OS in patients with a *BIM*-deletion (up to 16 months' decrease Table 1). As to why only some studies report such a significant impact on survival, remains unclear. Nonetheless, the limited statistical power of studies with a relatively small number of patients who received erlotinib, and lack of correction for confounders, such as *EGFR* mutation status, makes these results prone to bias. Moreover, inclusion of patients treated with other EGFR-SMKIs makes it difficult to interpret the results (Lee et al., 2013; Shuang et al., 2016; Cardona et al., 2016). In conclusion, this *BIM* deletion might negatively influence erlotinib efficacy through intrinsic drug resistance, yet further validation in larger and better designed prospective studies is needed. Therefore, clinical implementation for erlotinib is currently not recommended.

Another often studied germline polymorphism is a SNP in the *EGFR* promotor, *-216 G>T*, which is thought to increase EGFR expression. Survival benefits were found in two studies with erlotinib-treated patients (Jung et al., 2012; Zhang et al., 2016), while a third study found

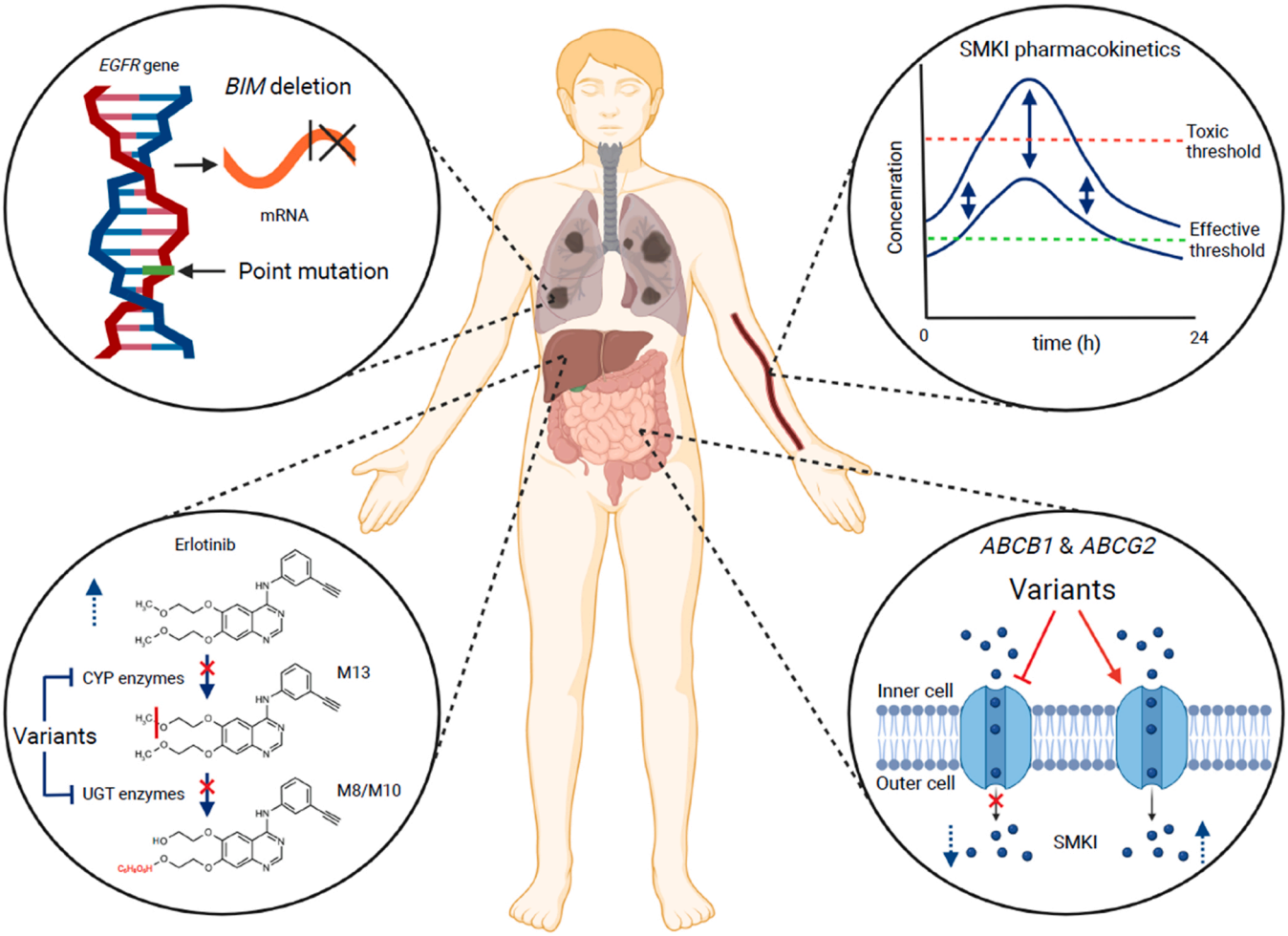

Fig. 1. The influence of germline variations on the pharmacokinetics and pharmacodynamics of erlotinib.

no clear impact of this SNP on either PFS or OS (Winther-Larsen et al., 2015). This last study however found a second SNP in the EGFR-gene, *181946C>T*, which they associated with an increase in PFS and OS, by 2.0 and 1.4 months, respectively (Winther-Larsen et al., 2015). In a validation cohort, these outcome effects were reproduced (Table 1). Winther-Larsen et al., (Winther-Larsen et al., 2019) As to how *181946C>T* mechanistically alters treatment outcomes is yet to be elucidated.

Germline variations in drug transporters

Primarily, erlotinib is transport substrate of both ABCB1 and ABCG2 (FDA, 2021; EMA, 2021) Hence, several SNPs in both of these multidrug resistance efflux transporter genes have been thoroughly investigated. In *ABCB1*, the *1236C>T*, the *2677G>T/A* and the *3435C>T* variations were mostly studied, but did not result in convincing differences in efficacy or toxicity (Table 1). However, less known variations (rs10248420 and rs7787082) were related to erlotinib-induced untoward toxicity of any grade, mostly regarding diarrhea and skin rash (Table 1 and Fig. 1) (Ruan et al., 2016). In our opinion, the evidence for clinical relevant effects of these SNPs is very limited and therefore does not warrant additional research.

In *ABCG2, the 421C>A* variation has been associated with a 24% decrease in erlotinib clearance (Fukudo et al., 2013), however this finding did not translate into an actual increase in survival or toxicity. For *ABCG2 1143 C>T* and *−15622C>T,* multiple studies have been performed (Table 1). Nonetheless, survival, pharmacokinetics, and untoward toxicity are not influenced by either of these two SNPs. Finally, *ABCG2 34 G>A,* has been associated with significantly shorter OS (18 versus 31 months) in a diverse cohort of patients with *EGFR* aberrations (Chen et al., 2015). Although there was no effect on systemic erlotinib pharmacokinetics, this large difference in survival might be explained by lower tumor expression of ABCG2 and subsequently lower erlotinib efflux from the tumor, leading to intra-tumoral accumulation of erlotinib and consequent potentiation of the anti-cancer effect. Clearly, validation of these results has to be performed before genotyping of *ABCG2 34G>A* can be used to identify patients at risk for early treatment failure, but this SNP has the potential to be of clinical relevance.

Germline variations in drug metabolizing enzymes

Erlotinib is primarily metabolized in the liver by several CYP isoenzymes (Fig. 1). In *CYP1A1*, the *3801T>C* germline variant has been associated with a more than 13 months lower OS (Table 1) (Nie et al., 2011). This is in line with previous research demonstrating increased enzyme activity of CYP1A1 in *3801T>C* variations, possibly decreasing tumor exposure to erlotinib (Kiyohara et al., 1996). However, this result has not been validated yet.

Concerning the *CYP1A1 2455A>G* variant, one study reported higher levels of erlotinib (1450 vs 900 ng/mL) compared to patients harboring a wild type *CYP1A1* (Liao et al., 2020). Interestingly, the *2455A>G* genotype is associated with an increase in the PFS by 3 months (Table 1) (Liao et al., 2020). This might be explained by evidence that higher erlotinib concentrations are positively correlated with increased PFS (Steendam et al., 2020). Moreover, the higher baseline concentrations of erlotinib found in the beneficial genotype do not result in increased erlotinib toxicity (Table 1). In summary, if validated, both *CYP1A1 3801T>C* and *2455A>G* might be promising targets for personalized erlotinib treatment recommendations.

Regarding *CYP1A2*, two studies identified a lower risk for erlotinib-induced skin toxicity for those with the *-163C>A* variant (Ruan et al., 2016; Liao et al., 2020), although this was not confirmed by another small study (Endo-Tsukude et al., 2018). No effect of *-163C>A* on treatment outcome or pharmacokinetics has been established (Endo-Tsukude et al., 2018; Ruan et al., 2016; Liao et al., 2020).

For *CYP3A4* no clinical relevant germline variations have been identified yet (Rudin et al., 2008; Endo-Tsukude et al., 2018; Ruan et al., 2016; Liao et al., 2020) For *CYP3A5,* one study reported a lower incidence of diarrhea in patients having at least one *CYP3A5 6986 A>G* variant allele (Liao et al., 2020). This borderline association was not found in other studies (Rudin et al., 2008; Fukudo et al., 2013; Endo-Tsukude et al., 2018), making the evidence for such an association too weak for clinical implementation.

Clinically relevant germline variations in gefitinib treatment

Target variations

Gefitinib is an EGFR-SMKI comparable to erlotinib, and therefore a high degree of overlap between investigated germline variations is seen. As for erlotinib, the *BIM* deletion in gefitinib treatment (Table 2**,** supplementary Table 2 and Fig. 2) (McKibbin et al., 2010; Giovannetti et al., 2010; Cusatis et al., 2006; Lee et al., 2014, 2013, 2015; Ariyasu et al., 2020; Zhao et al., 2014; Sun et al., 2017; Xia et al., 2017; Liu et al., 2019; Wang et al., 2019; Nie et al., 2011; Jung et al., 2012; Zhang et al., 2016, 2013, 2018, 2019, 2014, 2020; Leal et al., 2020; Ruan et al., 2016; Chen et al., 2015; Zenke et al., 2016; Suzumura et al., 2012; Chiu et al., 2015; Xu et al., 2014; Yuan et al., 2018; Isobe et al., 2016; Sasaki et al., 2008a, 2008b, 2009, 2008c; Shitara et al., 2012; Ma et al., 2017, 2009, 2019; Huang et al., 2009; Ichihara et al., 2007; Liu et al., 2008; Gregorc et al., 2008; Nie et al., 2007; Han et al., 2007, 2011; Amador et al., 2004; Tiseo et al., 2008, 2010; Guan et al., 2021; Kobayashi et al., 2016; Kobayashi et al., 2015; Chhun et al., 2009; Tamura et al., 2012; Li et al., 2007; Sakamoto et al., 2020; Lemos et al., 2011; Akasaka et al., 2010; Sugiyama et al., 2015; Yokota et al., 2017; Takimoto et al., 2013; Lei et al., 2020; Chang et al., 2017; Nyberg et al., 2011; Xin et al., 2020; Yokota et al., 2020; Yuan et al., 2017) results in conflicting outcomes with respect to PFS or OS. Interestingly, two studies which solely included gefitinib-treated patients, the *BIM*-deletion was associated with both a reduction in the PFS by 3.8 months and a shortening of OS by 17.6 months (Yuan et al., 2018; Isobe et al., 2016) There has only been one study with predominantly patients treated with gefitinib (135 of 153 patients in total) that prospectively evaluated the impact of the *BIM* deletion on PFS and OS. It showed a significant reduction in PFS and OS, with hazard ratios (HRs) of 2.15 and 1.65, respectively. Lee et al., (Lee et al., 2014) These findings validate the prognostic value of the *BIM* deletion and its potential use in gefitinib treatment in East-Asian patients. These results might also indicate that loss of *BIM* could be more potent in gefitinib compared to erlotinib, but this has to be confirmed in additional studies (e.g. a prospective intervention trial in patients lacking BIM).

Within the *EGFR* gene, a much more studied genetic variant is the CA-dinucleotide repeat in intron 1. The number of CA repeats is inversely associated with the expression of *EGFR* (Amador et al., 2004). It is hence postulated that this CA sequence repeat (CA-SSR1) influences the efficacy of gefitinib, as a higher expression of EGFR is associated with increased tumor sensitivity to gefitinib (Amador et al., 2004). The results of two studies support this theory; both demonstrating a slightly higher PFS and OS in patients with a lower amount of CA repeats (Table 2) (Liu et al., 2008; Han et al., 2007), although others did not (Table 2). It has also been hypothesized that having a lower CA repeat number might result in more toxicity in peripheral tissues, such as the skin and the intestines. This in turn would provide a rationale for the well-known association between gefitinib-induced toxicity and improved survival. Despite this theory, most studies did not find any relationship between CA-SSR1 and toxicity (Table 2) (Huang et al., 2009; Amador et al., 2004) Therefore, we consider the evidence for a clinical relevant impact of the CA-SSR1 germline variation to be inconclusive, and therefore would currently not recommend the pre-treatment testing for CA-SSR1 status.

The polymorphism *181946C>T* in the *EGFR* gene has also been investigated for gefitinib treatment (Fig. 2). Two of three studies did find significant decreases in PFS and OS (Table 2). Another SNP in the *EGFR* gene, rs4947492, has also been associated with extended OS (Table 2). Lastly, the *-216 G>T* in the *EGFR* gene was also associated with better

Table 2
The effects of germline variations on PFS, OS, pharmacokinetics and untoward toxicity in gefitinib treatment for NSCLC.

Gene	Variant	rs number	PFS, HR (95% CI)	OS, HR (95% CI)	PK effect	Toxicity	Number of patients	References
BIM	Deletion		–	1•65 (1•02–2•66)	–	–	204	(Lee et al., 2014)
			2•15 (1•32–3•51)	–	–	–	153	(Lee et al., 2014)
			NS	–	–	–	197	(Lee et al., 2013)
			NS	NS	–	–	196	(Ariyasu et al., 2020)
			2•09 (1•15–3•82)	–	–	–	166	(Zhao et al., 2014)
			NS	NS	–	–	140	(Sun et al., 2017)
			22 versus 38 months	24 versus 39 months	–	–	245	(Xia et al., 2017)
			NS	NS	–	–	194	(Liu et al., 2019)
			NS	NS	–	–	117	(Liu et al., 2019)
			NS	NS	–	–	205	(Lee et al., 2015)
			2•38 (1•30–4•34)	2•53 (1•37–4•65)	–	–	111	(Yuan et al., 2018)
			10•0 versus 24•0 months	44•0 versus 50•8 months	–	–	33	(Isobe et al., 2016)
BIM	Exon 5 c465C>T		NS	1•81 (1•12–2•81)	–	–	196	(Ariyasu et al., 2020)
EGFR	181946 C>T / D994D G>A	rs2293347	NS	NS	–	–	46	(Shitara et al., 2012)
			2•29 (1•30–4•03)	NS	–	–	84	(Ma et al., 2009)
			–	–	–	NS	59	(Ma et al., 2017)
			–	2•44 (1•06–5•56)	–	–	128	(Zhang et al., 2013)
EGFR	R497K A>L	rs11543848	NS	NS	–	–	115	(Nie et al., 2011)
			NS	NS	–	–	46	(Shitara et al., 2012)
			NS	NS	–	7•5x more severe diarrhea	94	(Giovannetti et al., 2010)
			–	NS	–	–	128	(Zhang et al., 2013)
			NS	NS	–	–	46	(Sasaki et al., 2009)
			–	–	–	NS	110	(McKibbin et al., 2010)
			NS	NS	–	NS	92	(Liu et al., 2008)
EGFR	– 216 G>T	rs712829	NS	NS	–	–	46	(Shitara et al., 2012)
			5•1 vs 16•6 months	NS	–	–	71	(Jung et al., 2012)
			–	–	–	66% more rash and 57% more diarrhea	110	(McKibbin et al., 2010)
			NS	NS	–	More severe diarrhea	94	(Giovannetti et al., 2010)
			0•62 (0•38–0•99)	NS	–	More diarrhea; OR 2•63 (1•12–6•17)	92	(Liu et al., 2008)
			0•51 (0•33–0•80)	0•54 (0•34–0•84)	–	–	230	(Zhang et al., 2016)
			–	–	–	NS	52	(Huang et al., 2009)
EGFR	– 191 C>A	rs712830	NS	NS	–	22x more severe diarrhea	94	(Giovannetti et al., 2010)
			–	–	–	NS	110	(McKibbin et al., 2010)
			NS	NS	–	NS	92	(Liu et al., 2008)
EGFR	216GG / 191CC		0•65 (0•42–0•99)	0•54 (0•32–0•91)	–	–	139	(Gregorc et al., 2008)
EGFR	intron 1, CA-SSR1 (repeats)	rs45559542	NS	NS	–	–	71	(Jung et al., 2012)

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Table 2 (*continued*)

Gene	Variant	rs number	PFS, HR (95% CI)	OS, HR (95% CI)	PK effect	Toxicity	Number of patients	References
			–	NS	–	–	115	(Nie et al., 2011)
			NS	NS	–	–	84	(Ma et al., 2009)
			–	–	–	52% decrease of early severe rash	52	(Huang et al., 2009)
			NS	NS	–	NS	94	(Giovannetti et al., 2010)
			–	–	–	NS	110	(McKibbin et al., 2010)
			0•54 (0•33–0•88)	NS	–	NS	92	(Liu et al., 2008)
			NS	NS	–	–	183	(Gregorc et al., 2008)
			0•54 (95%CI: 0•34–0•88)	NS	–	–	86	(Han et al., 2007)
			–	–	–	60% decrease of rash	19	(Amador et al., 2004)
			–	–	–	NS	127	(Cusatis et al., 2006)
			–	0•49 (0•25– 0•97)	–	NS	51	(Tiseo et al., 2008)
			–	0•51 (0•27–0•89)	–	–	76	(Tiseo et al., 2010)
EGFR	+ 61 A>G	rs4444903	NS	NS	–	–	111	(Leal et al., 2020)
EGFR	C>T	rs1468727	NS	NS	–	–	230	(Zhang et al., 2016)
EGFR		rs7809028	–	NS	–	–	128	(Zhang et al., 2013)
EGFR	8227 G>A		–	NS	–	–	46	(Shitara et al., 2012)
EGFR		rs2075102	–	–	–	NS	59	(Ma et al., 2017)
			–	NS	–	–	128	(Zhang et al., 2013)
EGFR	A>G	rs4947492	–	–	–	NS	59	(Ma et al., 2017)
				0•29 (0•10–0•83)	–	–	128	(Zhang et al., 2013)
EGFR		rs11977388	–	–	–	NS	59	(Ma et al., 2017)
			–	NS	–	–	128	(Zhang et al., 2013)
EGFR		rs11568315	–	–	–	NS	59	(Ma et al., 2017)
EGFR	R521K		–	–	–	NS	52	(Huang et al., 2009)
miR-499		rs3746444	NS	NS	–	–	162	(Zhang et al., 2019)
miR-608	G>C	rs4919510	0•63 (0•49–0•81); in validation 0•63 (0•45–0•87)	0•65 (0•51–0•83); in validation 0•59 (0•41–0•85)	–	–	162	(Zhang et al., 2019)
miR-3152		rs13299349	NS	NS	–	–	162	(Zhang et al., 2019)
miR-4513	G>A	rs2168518	0•46 (0•31–0•67); in validation 0•40 (0•24–0•68)	0•45 (0•31–0•66); in validation 0•38 (0•21–0•69)	–	–	162	(Zhang et al., 2019)
miR-4520a	T > C	rs8078913	0•65 (0•45–0•94); in valdation NS	0•64 (0•45–0•93); in validation NS	–	–	162	(Zhang et al., 2019)
AKT1	G>A	rs1130233	3•4 (2•2–5•9)	4•8 (1•2–6•3)	–	NS	94	(Giovannetti et al., 2010)
AKT1	C>T	rs3730350	NS	NS	–	NS	94	(Giovannetti et al., 2010)
AKT1		rs1130214	1•85 (1•09–3•13)	NS	–	–	230	(Zhang et al., 2016)
DACT2	A>G	rs9364433	1•88 (1•34–2•66); in validation 1•61 (1•08–2•41)	1•70 (1•20–2•40); in validation 1•87 (1•16–3•03)	–	–	162	(Zhang et al., 2020)
ABCB1	1236 C>T	rs1128503	–	–	–	More diarrhea; OR 10•78 (1•54–75•40) More rash; OR 15•78 (2•01–124•1)	59	(Ma et al., 2017)
			NS	–	AUC NS, C_{max} NS, C_{trough} NS	–	58	(Ma et al., 2019)

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Table 2 (*continued*)

Gene	Variant	rs number	PFS, HR (95% CI)	OS, HR (95% CI)	PK effect	Toxicity	Number of patients	References
			–	–	–	More severe rash	120	(Guan et al., 2021)
			–	–	AUC NS, C_{max} NS, C_{trough} NS	NS	31	(Kobayashi et al., 2015)
			–	–	AUC NS, C_{max} NS, C_{trough} NS	–	36	(Kobayashi et al., 2016)
ABCB1	2677 G>T/A	rs2032582	–	–	–	NS	59	(Ma et al., 2017)
			NS	–	AUC NS, C_{max} NS, C_{trough} NS	–	58	(Ma et al., 2019)
			–	–	–	NS	120	(Guan et al., 2021)
			–	–	AUC NS, C_{max} NS, C_{trough} NS	NS	31	(Kobayashi et al., 2015)
			–	–	AUC NS, C_{max} NS, C_{trough} NS	–	36	(Kobayashi et al., 2016)
ABCB1	3435 C>T	rs1045642	–	–	–	More skin rash; OR 9•315 (1•227–70•734)	59	(Ma et al., 2017)
			–	–	–	NS	125	(Cusatis et al., 2006)
			NS	–	AUC NS, C_{max} NS, C_{trough} NS	–	58	(Ma et al., 2019)
			–	–	AUC NS, C_{max} NS, C_{trough} NS	NS	31	(Kobayashi et al., 2015)
			–	–	AUC NS, C_{max} NS, C_{trough} NS	–	36	(Kobayashi et al., 2016)
			–	–	AUC NS, C_{max} NS, C_{trough} NS	–	18	(Chhun et al., 2009)
			–	–	–	NS	83	(Tamura et al., 2012)
			–	–	AUC NS, C_{max} NS, C_{trough} NS	–	27	(Li et al., 2007)
ABCB1	G>C	rs10256836	–	–	–	NS	59	(Ma et al., 2017)
			17•40 vs• 10•33 m	–	AUC NS, C_{max} NS, C_{trough} NS	–	58	(Ma et al., 2019)
ABCG2	421 C>A	rs2231142	NS	NS	–	–	70	(Chen et al., 2015)
			–	–	–	NS	59	(Ma et al., 2017)
			–	–	–	3•6 -fold increased diarrhea	125	(Cusatis et al., 2006)
			NS	–	40% lower C_{trough}	–	58	(Ma et al., 2019)
			–	–	AUC NS, C_{max} NS, C_{trough} NS	NS	31	(Kobayashi et al., 2015)
			–	–	AUC NS, C_{max} NS, C_{trough} NS	–	36	(Kobayashi et al., 2016)
			–	–	↓ 24% AUC, ↓ 27% Ctrough	–	61	(Sakamoto et al., 2020)
			–	–	–	NS	83	(Tamura et al., 2012)
			NS	NS	–	NS	94	(Lemos et al., 2011)
			–	–	AUC NS, C_{max} NS, C_{trough} NS	–	27	(Li et al., 2007)
ABCG2	1143 C>T	rs2622604	NS	NS	–	–	70	(Chen et al., 2015)
			NS	NS	–	NS	94	(Lemos et al., 2011)
ABCG2	− 15622 C>T	rs7699188	NS	NS	–	–	70	(Chen et al., 2015)
			NS	NS	–	18 fold more severe diarrhea	94	(Lemos et al., 2011)
ABCG2	34 G>A	rs2231137	NS	0•56 (0•38–0•84)	–	–	70	(Chen et al., 2015)
			–	–	–	NS	59	(Ma et al., 2017)
			NS	–	AUC NS, C_{max} NS, C_{trough} NS	–	58	(Ma et al., 2019)
			–	–	–	3•2x more grade 2 + skin rash	83	(Tamura et al., 2012)
ABCC1		rs129081	–	–	–	NS	120	(Guan et al., 2021)

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Table 2 (*continued*)

Gene	Variant	rs number	PFS, HR (95% CI)	OS, HR (95% CI)	PK effect	Toxicity	Number of patients	References
ABCC4		rs2274405	–	–	–	NS	120	(Guan et al., 2021)
UGT1A1	*28/*28		–	–	–	Grade 3 hepatotoxicity*	1	(Zenke et al., 2016)
UGT1A1	T > G/C	rs10929303	–	–	↓ 50% Ctrough	NS	120	(Guan et al., 2021)
UGT1A1	*6	rs4148323	–	–	–	NS	59	(Ma et al., 2017)
UGT1A1	*6, *7, *27, *28, *60		–	–	–	NS	60	(Sugiyama et al., 2015)
UGT1A7		rs6759892	–	–	–	NS	59	(Ma et al., 2017)
			NS	–	AUC NS, C_{max} NS, C_{trough} NS	–	58	(Ma et al., 2019)
SLC22A8		rs4149179	–	–	–	NS	120	(Guan et al., 2021)
SLC22A1		rs4709400	–	–	↓ 35% AUC, Cmax NS, Ctrough NS	More skin rash; OR: 10•3 (2•1–72•5)	120	(Guan et al., 2021)
SLCO1B3		rs4149117	–	–	–	NS	120	(Guan et al., 2021)
CYP1A1	3801 T > C (*2 A)		–	RR 2•36 (1•54–3•64)	–	–	115	(Nie et al., 2011)
CYP1A1	*2 C 2455 A>G	rs1048943	–	–	–	NS	134	(Ruan et al., 2016)
			–	NS	–	–	115	(Nie et al., 2011)
			–	–	–	NS	59	(Ma et al., 2017)
			NS	–	AUC NS, C_{max} NS, C_{trough} NS	–	58	(Ma et al., 2019)
CYP1A1	C>A	rs2606345	–	–	–	NS	59	(Ma et al., 2017)
			NS	–	AUC NS, C_{max} NS, C_{trough} NS	–	58	(Ma et al., 2019)
CYP1A2	*1 F / − 163 C>A	rs762551	–	–	–	NS	59	(Ma et al., 2017)
			NS	–	AUC NS, C_{max} NS, C_{trough} NS	–	58	(Ma et al., 2019)
CYP2A6	*1, *4, *7, *9		–	–	–	NS	60	(Sugiyama et al., 2015)
CYP2C19	*1, *2, *3		–	–	–	NS	60	(Sugiyama et al., 2015)
CYP2D6	1661 G>C	rs1058164	–	–	↑ up to177% AUC	–	87	(Zhang et al., 2018)
CYP2D6	CYP2D6*2		–	–	–	NS	59	(Ma et al., 2017)
CYP2D6	CYP2D6*2A		–	–	–	NS	59	(Ma et al., 2017)
CYP2D6	all *1,2,5,10,14 versus *5/ *10 + *10/*10		–	–	–	More severe hepatotoxicity; OR 14•50 (95%-CI: 1•56–346•50)	60	(Sugiyama et al., 2015)
CYP2D6	*1/*1 vs *1/*4 or *1/*5		–	–	↓ 33% Clearance	–	18	(Chhun et al., 2009)
CYP2D6	*1/*1, *1/*2 and *2/*2 versus *5/ *10 and *10/*10		–	–	AUC NS, C_{max} NS, C_{trough} NS	NS	31	(Kobayashi et al., 2015)
			–	–	↓88% AUC of O-desmethyl gefitinib	–	36	(Kobayashi et al., 2016)
CYP3A4	*1 G	rs2242480	–	–	–	NS	59	(Ma et al., 2017)
			–	–	AUC NS, C_{max} NS, C_{trough} NS	NS	31	(Kobayashi et al., 2015)
			–	–	–	NS	120	(Guan et al., 2021)
			NS	–	↑ 60% C_{trough}	–	58	(Ma et al., 2019)
CYP3A5	*1 vs *3 / 6986 A>G	rs776746	–	–	–	NS	59	(Ma et al., 2017)
			–	–	AUC NS, C_{max} NS, C_{trough} NS	NS	31	(Kobayashi et al., 2015)
			–	–	–	Grade 3 hepatotoxicity*	1	(Zenke et al., 2016)

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Table 2 (*continued*)

Gene	Variant	rs number	PFS, HR (95% CI)	OS, HR (95% CI)	PK effect	Toxicity	Number of patients	References
			–	–	–	More severe hepatotoxicity; OR 6•84 (1•87–33•15)	60	(Sugiyama et al., 2015)

PFS: progression free survival; OS: overall survival; PK: pharmacokinetics; AUC: area under the curve; Cmax: maximum concentration; Ctrough: trough concentration; HR: hazard ratio; OR: odds ratio; RR: relative risk; NS: non-significant; *: case report.

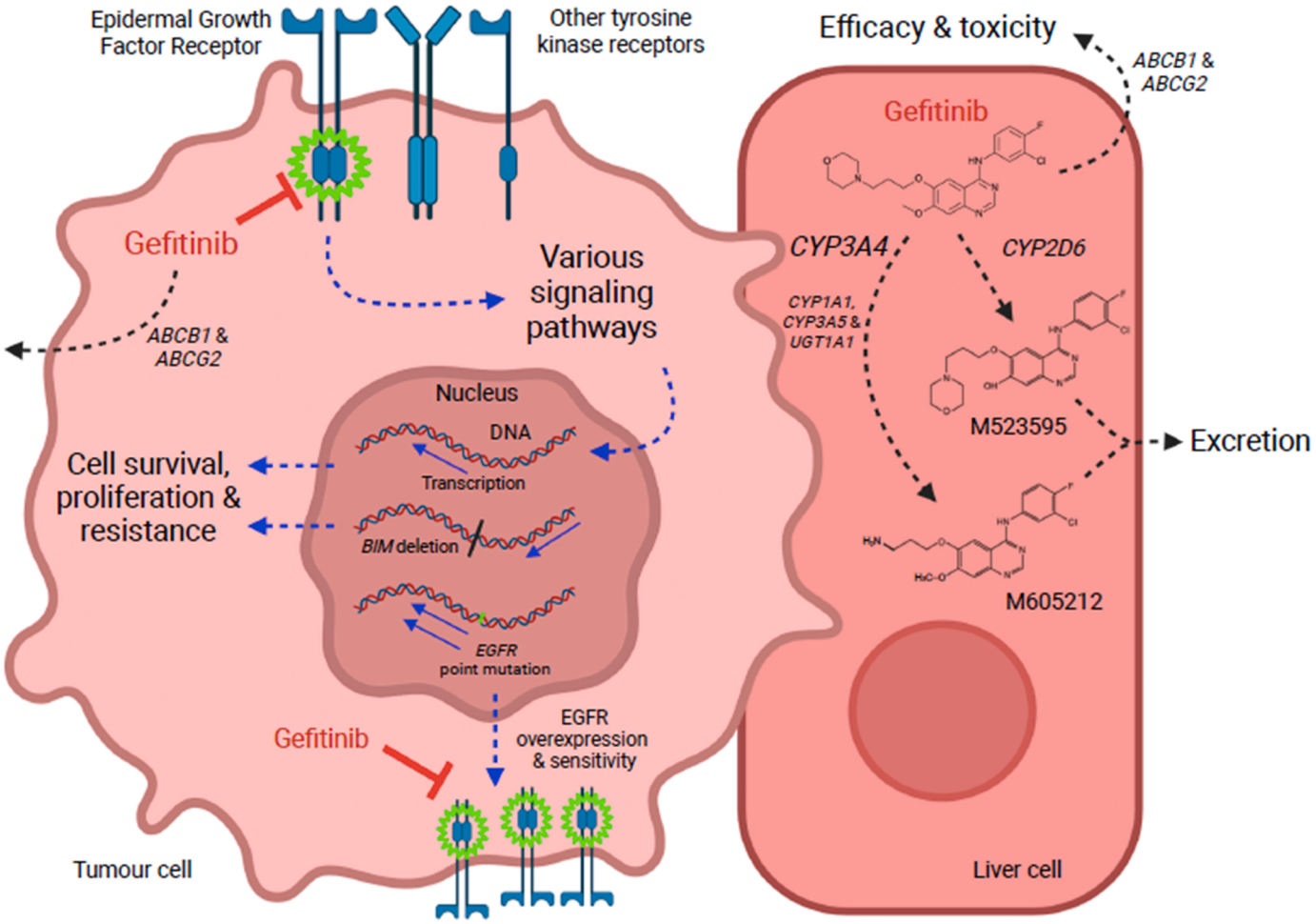

Fig. 2. The influence of germline variations on the pharmacokinetics and pharmacodynamics of gefitinib.

survival. Three studies reported increases in PFS with a mean increase of up to 12 months (Jung et al., 2012; Zhang et al., 2016; Liu et al., 2008); per contra, two studies did not find such an association.(Giovannetti et al., 2010; Shitara et al., 2012) Considering OS, a positive effect on survival i.e. 6 months increase, was only reported in one study (Table 2). Considering these *EGFR* gene polymorphisms *181946C>T, −216G>T* and to a lesser extent rs4947492, all constitute potential targets for personalizing therapy and should be included in future research. If validated prospectively, pre-emptive genotyping might become clinically relevant.

Finally, genetic variations in downstream effectors in the EGFR-pathway were also studied. This group includes genes like *AKT1*, *SMAD3, DACT2* and multiple micro-RNAs (supplementary Table 2). The assumption is that these genes, similar to *BIM*, affect tumor cell proliferation and drug resistance at the molecular level in cancer cells. Correspondingly, germline variations within these genes seem to have a large impact on both initial treatment response and the onset of eventual treatment resistance, largely influencing OS and PFS (Table 2). Whilst their impact is potentially substantial, a major issue is the lack of validation studies for these germline variations. Indeed, if promising SNPs such as rs2168518 in *miR-4513* (Zhang et al., 2019) are validated, clinical implementation could guide the personalized treatment of choice in individual NSCLC patients with driver aberrations.

Germline variations in multidrug efflux transporters

Similar to erlotinib, gefitinib is primarily a substrate of ABCB1 and ABCG2 (Fig. 2). The most studied germline variations in *ABCB1* in patients treated with gefitinib are *1236C>T*, *2677G>T/A,* and *3435C>T* (Table 2). Nonetheless, none of these SNPs (which are in linkage with each other) seem to impact survival, pharmacokinetics or toxicity. Only for the *1236C>T* variant, a potential increase in the occurrence of gefitinib-induced skin rash is described (Ma et al., 2017; Guan et al., 2021) On the contrary, the *ABCB1 G>C* variant has been associated with shorter PFS (17 versus 10 months) (Ma et al., 2019). For *ABCG2*, *421C>A* has been linked to impact the pharmacokinetic parameters of gefitinib (i.e. a 24% decrease in exposure and a 27% lower

concentration) (Ma et al., 2019; Sakamoto et al., 2020). Nevertheless, conflicting results are reported (Kobayashi et al., 2016, 2015; Li et al., 2007) and effects on PFS nor OS (Chen et al., 2015; Ma et al., 2019; Lemos et al., 2011) are seen. A more promising SNP is the *ABCG2 34G>A* variant. As discussed for erlotinib, this SNP was found to increase OS significantly (18 versus 31 months) in a diverse cohort containing various EGFR-SMKIs, including gefitinib (Chen et al., 2015). During gefitinib treatment, this *34G>A* variant was also found to be linked to an increase in the occurrence of skin rash (Tamura et al., 2012), although not always reproduced (Table 2).

Germline variations in drug metabolizing enzymes

The metabolism of gefitinib occurs mostly through CYPs, primarily via CYP1A1, CYP2D6, CYP3A4 and CYP3A5 (Fig. 2) (FDA, 2021; EMA, 2021) Within *CYP1A1*, the **2A 3801T>C* variant has been identified as a predictor of OS: 24 versus 10 months.Nie et al., (Nie et al., 2011) Unlike erlotinib, the *CYP1A1*2C* variant does not seem to influence gefitinib pharmacokinetics or survival (Ruan et al., 2016; Ma et al., 2017, 2019) The *1661G>C* SNP in *CYP2D6* has been associated with higher gefitinib area under the curves (AUCs), with the highest plasma exposure in homozygous variations. Zhang et al., (Zhang et al., 2018) However, it is unclear how this higher exposure translates into either survival or toxicity. Other well-studied *CYP2D6* variations are the **4-*, **5-* and **10*-variations, which are associated with impaired metabolism of gefitinib. Compared to the wild type genotype, patients with at least one **4-* or **5*-variant allele have a 33% lower gefitinib clearance (Chhun et al., 2009). In intermediate metabolizers (IM) (genotype: **5/*10* or **10/*10*)) (Kobayashi et al., 2015), the exposure to the CYP2D6-catalyzed metabolite O-desmetyl-gefitinib, was significantly lower (Kobayashi et al., 2016). These particular genotypes have also been related to a more than 2-fold increased occurrence of skin rash and up to a 15-fold increase of severe hepatotoxicity (Table 2). Based on these results, *CYP2D6* polymorphisms should be assessed in patients before gefitinib treatment initiation. Normal metabolizers can be treated accordingly, but IM and poor metabolizer (PM) patients should be monitored carefully for these toxicities. Alternatively, these patients could safely be switched to erlotinib, as this drug is not metabolized by CYP2D6.

In *CYP3A4*, the ** 1 G* (T > C) variant was reported to alter the trough concentration of gefitinib, with lower concentrations for the CC+CT genotypes, compared to the TT genotype (Ma et al., 2019). Nevertheless, other studies failed to identify any association with either toxicity, pharmacokinetics, or survival (Ma et al., 2017, 2019; Guan et al., 2021; Kobayashi et al., 2015), making it unlikely for the *CYP3A4*1G* variant to have real clinical relevance.

In *CYP3A5*, the PM phenotype might be associated with the occurrence of severe hepatotoxicity. In one report, the PM phenotype -established as a homozygous *3-variant genotype- showed a more than 6-fold higher incidence of gefitinib-induced severe hepatotoxicity compared to others (Sugiyama et al., 2015). Another study initially confirmed this relationship, but after correcting for confounders, this association did not persist (Ma et al., 2017). A third study did not establish a relationship between *CYP3A5* genotype and the incidence of any hepatotoxicity, yet severe hepatotoxicity only occurred in 4 out of 31 patients (Kobayashi et al., 2015). Interestingly in this respect and although only mentioned in a case report, a patient with *UGT1A1*28/*28* (promoter TA repeat) and *CYP3A5*3/*3*, developed grade 3 hepatotoxicity during both gefitinib and subsequent erlotinib treatment (Zenke et al., 2016). As UGT1A1 is necessary for the glucuronidation of bilirubin, and both gefitinib and erlotinib are potent inhibitors of UGT1A1, the combination of the PM-genotype of *UGT1A1* and its SMKI-induced inhibition might potentially provide a high risk environment for the occurrence of hepatotoxicity. In another study, it was reported that the rs10929303 SNP in *UGT1A1* significantly altered gefitinib plasma exposure and concentration in steady state (Guan et al., 2021), which suggests that it could be of interest to study polymorphisms in the phase II metabolism of gefitinib in more detail.

Clinically relevant germline variations in other SMKIs

Afatinib

In contrast to erlotinib and gefitinib, afatinib is not metabolized by the CYP-system. Instead, a small percentage of afatinib is metabolized by the Flavin-containing Mono-Oxygenase 3 (FMO3) enzyme in the liver (FDA, 2021; EMA, 2021) FMO3 is responsible for the oxidative deamination of several drugs, such as amphetamines (FDA, 2021; EMA, 2021). However, as this is a minor metabolic pathway of afatinib, clinical relevant influence of this enzyme is not expected (FDA, 2021; EMA, 2021) This is supported by findings that a SNP constituting a *G15167A* variation in *FMO3* did not alter afatinib concentrations in blood plasma (Table 3) (Sogawa et al., 2020; Tan et al., 2020; Hayashi et al., 2019).

Again, most research has focused on SNPs in the *ABCB1* and *ABCG2* transporter genes, as afatinib is a substrate for both transporters. In *ABCB1*, the variant *2677 G>T/A* has been linked to a vastly higher incidence of diarrhea (Sogawa et al., 2020). No associations with pharmacokinetic parameters or the occurrence of afatinib-induced paronychia were reported for this SNP (Sogawa et al., 2020; Tan et al., 2020). As for the linked *ABCB1* SNPs *1236 C>T* and *3435 C>T*, no influence on pharmacokinetic parameters or toxicity has been established (Table 3). Nonetheless, in a subgroup analysis, patients being wild type for both SNPs, had a 41% higher exposure to afatinib compared to patients who were not wild type for both SNPs (Tan et al., 2020).

In the *ABCG2* gene, the *421 C>A* variant has been associated with a significantly higher concentration of afatinib in plasma, with median concentrations doubling in the variant group (Hayashi et al., 2019). Moreover, patients with this variant had a tendency to experience more severe diarrhea (Hayashi et al., 2019). Both findings however where not reproduced in other studies (Sogawa et al., 2020; Tan et al., 2020), hence no recommendation can yet be given.

Crizotinib

In crizotinib treatment, the *BIM* deletion has been investigated as well. In a study in anaplastic lymphoma kinase (*ALK*)- or *ROS*-positive Asian patients with NSCLC treated with crizotinib, the PFS was decreased with 6 months in those with a *BIM*-deletion (Table 3) (Zhang et al., 2017; Lin et al., 2019; Fujiwara et al., 2016; Xin et al., 2021). This was mainly because of the larger *ALK*-positive subgroup (n = 55 patients) in the study cohort, in which a *BIM* deletion was an independent predictor of decreased PFS (HR: 4.8). In another small study of 30 *ALK*-positive patients with NSCLC, a relationship between *BIM* deletion with PFS or OS could not be assessed (Lin et al., 2019). Crizotinib is mainly metabolized by CYP3A4, and to a lesser extent by CYP3A5, CYP2C8, CYP2C19, and CYP2D6 (van Leeuwen et al., 2014). Although potentially relevant, no germline variations in these genes have been studied.

Nintedanib

Since nintedanib is only minorly (5%) metabolized by CYP3A4 and UGT-enzymes (van Leeuwen et al., 2014), germline variations in these genes are not likely to result in clinically relevant outcomes. However, nintedanib is a transport substrate of ABCB1 (van Leeuwen et al., 2014). A variant could hence potentially alter its pharmacokinetics, toxicity, and efficacy. Germline variations in the treatment with nintedanib have not yet been studied for its registered indication in NSCLC though. However, in a group of 67 malignant pleural mesothelioma patients treated with nintedanib, three SNPs were identified that had a possible weak positive association with survival (Nowak et al., 2017). Two SNPs in *VEGFR3* -rs307821 and rs307826- had a weak association with OS in respectively the G/G and A/A genotype groups, whilst the *VEGFR1* SNP rs9582036 was weakly associated with PFS in the AA genotype group (Nowak et al., 2017). Therefore, further research is warranted to validate these findings and to seek for other genetic variations to influence

Table 3
The effects of germline variations on PFS, OS, pharmacokinetics and untoward toxicity in the treatment of NSCLC with other SMKIs.

SMKI	Gene	Variant	rs number	PFS, HR (95% CI)	OS, HR (95% CI)	PK effect	Toxicity	Number of patients	References
Afatinib	ABCB1	2677 G>T/A	rs2032582	–	–		More any grade diarrhea; OR 46•6 (2•2–989)	38	(Sogawa et al., 2020)
				–	–	AUC NS, C_{max} NS, C_{trough} NS	–	24	(Tan et al., 2020)
Afatinib	ABCB1	1236 C>T	rs1128503	–	–	–	NS	38	(Sogawa et al., 2020)
				–	–	AUC NS, C_{max} NS, C_{trough} NS	–	24	(Tan et al., 2020)
				–	–	Ctrough NS	–	33	(Hayashi et al., 2019)
Afatinib	ABCB1	3435 C>T	rs1045642	–	–	–	NS	38	(Sogawa et al., 2020)
				–	–	AUC NS, C_{max} NS, C_{trough} NS	–	24	(Tan et al., 2020)
				–	–	C_{trough} NS	–	33	(Hayashi et al., 2019)
Afatinib	ABCB1	1236–3435CC		–	–	↑ 41% AUC, ↑ 48% Cmax	–	24	(Tan et al., 2020)
Afatinib	ABCG2	421 C>A	rs2231142	–	–	–	NS	38	(Sogawa et al., 2020)
				–	–	AUC NS, C_{max} NS, C_{trough} NS	–	24	(Tan et al., 2020)
				–	–	↑ Ctrough	More severe diarrhea; OR > 2	33	(Hayashi et al., 2019)
Afatinib	ABCG2	34 G/A	rs2231137	–	–	AUC NS, C_{max} NS, C_{trough} NS	–	24	(Tan et al., 2020)
				–	–	C_{trough} NS	–	33	(Hayashi et al., 2019)
Afatinib	FMO3	G15167A		–	–	C_{trough} NS	–	33	(Hayashi et al., 2019)
Crizotinib	BIM	Deletion		182 vs 377 days	–	–	–	69	(Zhang et al., 2017)
				NS	NS	–	–	54	(Lin et al., 2019)
Crizotinib	ABCB1	1236TT-2677TT-3435TT		–	–	↑ 184% AUC, ↑ 162% Cmax	QTc prolongation grade 3*	8	(Fujiwara et al., 2016)
Crizotinib	STAT1	STAT1 T > C	rs10208033	–	–	–	More any grade hepatotoxicity; OR 5•5 (1•22–24•81)	42	(Xin et al., 2021)
Nintedanib	VEGFR3	GG	rs307821	–	Potentially longer OS	–	–	67	(Nowak et al., 2017)
Nintedanib	VEGFR3	AA	rs307826	–	Potentially longer OS	–	–	67	(Nowak et al., 2017)
Nintedanib	VEGFR1	AA	rs9582036	Potentially longer PFS	–	–	–	67	(Nowak et al., 2017)
Osimertinib	BIM	Deletion		HR 1•63 (1•01–2•63)	NS	–	–	67	(Li et al., 2021)

SMKI: small molecular kinase inhibitor; PFS: progression free survival; OS: overall survival; PK: pharmacokinetics; AUC: area under the curve; Cmax: maximum concentration; Ctrough: trough concentration; HR: hazard ratio; OR: odds ratio; NS: non-significant; *: case report.

outcome, pharmacokinetics and side effects of nintedanib.

Osimertinib

A *BIM* deletion is to date the only published genetic variant which has been studied during osimertinib treatment. One study, initiated after a case report presenting poor survival in a patient harboring a *BIM* deletion (Li et al., 2018), found a significant reduction of 2 months in PFS (Table 3), which is in line with findings for first-generation EGFR-SMKIs (Li et al., 2021). OS was also reduced (16 versus 25 months), although this difference did not reach statistical significance. Since osimertinib is an important SMKI in the treatment of *EGFR*--positive NSCLC, it must be studied in order to explore this effect. If prospectively validated, knowledge of this germline variant could guide clinicians in making better-informed treatment decisions.

Osimertinib is metabolized in the liver by CYP3A4 and CYP3A5 into two metabolites, both of which are bioactive and yield similar anti-neoplastic activities as osimertinib itself. Also, osimertinib is a substrate of both ABCB1 and ABCG2 drug transporters (FDA, 2021; EMA, 2021). Therefore, they provide a rationale for additional research in genes involved in these pharmacokinetic processes.

Currently, the impact of *ABCB1*, *ABCG2*, and *CYP3A4* SNPs on survival and pharmacokinetics of osimertinib in more than 550 patients with NSCLC is being studied in The Netherlands (www.trialregister.nl; Dutch Trial Registry number NL8914). The publication of the results of this study are expected in 2022.

Miscellaneous

Our literature search regarding germline variations in other SMKIs registered for NSCLC did not yield relevant findings, due to the relatively recent registration of most SMKIs. However, all SMKIs are extruded by drug efflux transporters or metabolized by CYP-enzymes (FDA, 2021; EMA, 2021), which implies that genetic variations could potentially have clinical consequences. In Table 4, all SMKIs with a molecular target are presented, together with the drug efflux transporters and CYP-enzymes for which these drugs are substrates. With this table, the rationale of future studies with germline variations and SMKIs is depicted. For example, brigatinib is extruded by ABCB1 and ABCG2, and metabolized by CYP3A4 and CYP2C9 (FDA, 2021; EMA, 2021). There is

Table 4
Overview of SMKIs with their approved target and drug transporters and metabolizing enzymes.

SMKI	Target	ABCB1	ABCG2	CYP3A4	Additional enzymes
Afatinib	EGFR & HER2	+	+	–	–
Alectinib	ALK	–	–	+	CYP2C8
Brigatinib	ALK	+	+	+	CYP2C8
Capmatinib	MET	+	–	+	–
Ceritinib	ALK	+	–	–	CYP3A
Crizotinib	ALK & ROS	+	–	+	CYP2C8/19 & CYP2D6
Dabrafenib	BRAF V600	+	+	+	CYP2C8
Dacomitinib	EGFR	+	+	+	CYP2D6
Entrectinib	NTRK & ROS	–	–	+	–
Erlotinib	EGFR	+	+	+	CYP1A1/2
Gefitinib	EGFR	+	+[a]	+	CYP2D6
Larotrectinib	NTRK	+	+	+	CYP3A
Lorlatinib	ALK & ROS	–	–	+	UGT1A4
Nintedanib		+	–	+	UGT1A
Osimertinib	EGFR	+	+	+	CYP3A5
Pralsetinib	RET	+	+	+	CYP2D6 & CYP1A2
Selpercatinib	RET	+	+	+	CYP3A
Trametinib	BRAF V600	+/-	–	+	CYP2C8

Data were derived from FDA and EMA reports (FDA, 2021; EMA, 2021). [a] derived from reference (Liao et al., 2020). SMKI: small-molecular kinase inhibitor; EGFR: epidermal growth factor receptor; HER2: human epidermal growth factor receptor-2; ALK: anaplastic lymphoma kinase; NTRK: neurotrophic tyrosine receptor kinase; VEGF: vascular endothelial growth factor; RET: rearranged during transfection; +: yes; -: no; +/-: uncertain; CYP: cytochrome P450 enzyme; UGT: Uridine 5′-diphospho-glucuronosyltransferase.

evidence that brigatinib is not only a transport substrate of these efflux pump proteins, but may also induce the expression of these transporters and enzymes.(FDA, 2021; EMA, 2021). This could make the impact of loss of function variations even larger in terms of untoward toxicity and patient outcome. Nevertheless, studies to establish an effect of these variations have not yet been carried out. Probably, this is because of the absence of large patient cohorts in which potential variations can be conclusively studied.

Alectinib on the other hand, is not evidently transported by the abovementioned drug efflux transporters (FDA, 2021; EMA, 2021). Although it is for at least 40% metabolized by CYP3A4 (FDA, 2021; EMA, 2021), a germline variation in this enzyme would theoretically not impair treatment efficacy. This is because the major alectinib metabolite M4 is equally potent as a cytotoxic agent (FDA, 2021; EMA, 2021). Hence, potential alterations of the alectinib:M4 ratio will have limited clinical implications.

When the rationale of genotyping a germline variation is clear, a proper study design maximizes the clinical implementation of the results. Ideally, this would be a prospective intervention study, in which patients are included and genotyped prior to the initiation of the SMKI treatment. Moreover, study endpoints must be directly feasible to be implemented in clinical practice, *c.q.* PFS, OS, or (severe) toxicity. It may also be interesting to study the possible pharmacokinetic effects, especially when a clear exposure-response or exposure-toxicity relationship exists. In addition, the intended study must have sufficient statistical power to establish the net effect of the genetic variation. An important detail in this respect is to correct for the number of germline variations which are tested by e.g. Bonferroni correction. Furthermore, internal or external validation of all findings -by bootstrapping or an independent validation cohort respectively- is essential when an effect is found. Finally, prior to definite implementation of genotyping a particular variation, a thorough population-based cost-effectiveness analysis should be performed.

Conclusion

The current review presents a comprehensive overview of the studied germline variations and which of these are important and promising in the treatment of NSCLC with SMKIs. The currently single prospectively validated mutation is the *BIM* deletion for East-Asian patients treated with gefitinib. The *BIM* deletion is thought to boost intrinsic resistance to therapy by reducing the pro-apoptotic effects of the BIM protein. Carriers of this deletion showed a significantly impaired PFS and OS. The *BIM* deletion has the potential to be clinically relevant for other SMKIs as well. However, before the implementation of most germline variations in clinical practice can be truly recommended, further prospective validation is required. Furthermore, their impact on treatment efficacy or untoward toxicity must be substantial in order to be of clinical relevance. Some variations act through alteration of SMKI pharmacokinetics, indirectly influencing efficacy and toxicity. Based on our findings, we expect most relevance from the *ABCG2 34 G>A* and *CYP1A1* variations during erlotinib and gefitinib treatment. Also preemptive *CYP2D6* testing before starting gefitinib treatment can be considered to prevent severe drug-related toxicity. Additional variations can potentially be implemented though, as research is still expanding. We therefore offer a structured study design and rationale for future research to germline variations, especially for SMKIs which are currently the most prescribed (osimertinib and alectinib). Hence, in the near future, with proper testing for germline variations, treatment with SMKIs could be further optimized to become more effective and safe for patients with NSCLC.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.drup.2022.100832.

References

Ahmed, S., Zhou, Z., Zhou, J., Chen, S.-Q., 2016. Pharmacogenomics of drug metabolizing enzymes and transporters: relevance to precision medicine. Genom. Proteom. Bioinform. 14 (5), 298–313.

Akasaka, K., Kaburagi, T., Yasuda, S., et al., 2010. Impact of functional ABCG2 polymorphisms on the adverse effects of gefitinib in Japanese patients with non-small-cell lung cancer. Cancer Chemother. Pharmacol. 66 (4), 691–698.

Amador, M.L., Oppenheimer, D., Perea, S., et al., 2004. An epidermal growth factor receptor intron 1 polymorphism mediates response to epidermal growth factor receptor inhibitors. Cancer Res. 64 (24), 9139.

Arbitrio, M., Di Martino, M.T., Barbieri, V., 2016. Identification of polymorphic variants associated with erlotinib-related skin toxicity in advanced non-small cell lung cancer patients by DMET microarray analysis. Cancer Chemother. Pharmacol. 77 (1), 205–209.

Ariyasu, R., Yanagitani, N., Tadokoro, K., et al., 2020. Efficacy of EGFR tyrosine kinase inhibitors in patients having EGFR-activating mutations with or without BIM polymorphisms. Cancer Chemother. Pharmacol. 86 (4), 517–525.

Caetano-Pinto, P., Jansen, J., Assaraf, Y.G., Masereeuw, R., 2017. The importance of breast cancer resistance protein to the kidneys excretory function and chemotherapeutic resistance. Drug Resist. Updat. 30, 15–27.

Cardona, A.F., Rojas, L., Wills, B., et al., 2016. BIM deletion polymorphisms in Hispanic patients with non-small cell lung cancer carriers of EGFR mutations. Oncotarget 7 (42), 68933–68942.

Cecchin, E., De Mattia, E., Ecca, F., Toffoli, G., 2018. Host genetic profiling to increase drug safety in colorectal cancer from discovery to implementation. Drug Resist. Updat. 39, 18–40.

Chang, I.S., Jiang, S.S., Yang, J.C., et al., 2017. Genetic modifiers of progression-free survival in never-smoking lung adenocarcinoma patients treated with first-line tyrosine kinase inhibitors. Am. J. Respir. Crit. Care Med. 195 (5), 663–673.

Chen, X., Chen, D., Yang, S., et al., 2015. Impact of ABCG2 polymorphisms on the clinical outcome of TKIs therapy in Chinese advanced non-small-cell lung cancer patients. Cancer Cell Int. 15, 43.

Chhun, S., Verstuyft, C., Rizzo-Padoin, N., et al., 2009. Gefitinib-phenytoin interaction is not correlated with the C-erythromycin breath test in healthy male volunteers. Br. J. Clin. Pharmacol. 68 (2), 226–237.

Chiu, C.-H., Ho, H.-L., Doong, H., et al., 2015. MLH1 V384D polymorphism associates with poor response to EGFR tyrosine kinase inhibitors in patients with EGFR L858R-positive lung adenocarcinoma. Oncotarget 6 (10), 8407–8417.

Cusatis, G., Gregorc, V., Li, J., 2006. Pharmacogenetics of ABCG2 and adverse reactions to gefitinib. J. Natl. Cancer Inst. 98 (23), 1739–1742.

EMA, European public assessment reports, assessment history, and product information. ⟨https://www.ema.europa.eu/en/medicines/human⟩ (Accessed 1 Oct 2021).
ENCEPP, ENCePP Guide on Methodological Standards in Pharmacoepidemiology. ⟨https://www.encepp.eu/standards_and_guidances/methodologicalGuide14_3.shtml⟩ (Accessed on 27 Mar 2022).
Endo-Tsukude, C., Sasaki, J.-i, Saeki, S., 2018. Population pharmacokinetics and adverse events of erlotinib in Japanese patients with non-small-cell lung cancer: impact of genetic polymorphisms in metabolizing enzymes and transporters. Biol. Pharm. Bull. 41 (1), 47–56.
FDA, Drugs@FDA: FDA-approved drugs. ⟨https://www.accessdata.fda.gov/scripts/cder/daf/⟩ (Accessed 1 Oct 2021).
Fujiwara, Y., Hamada, A., Mizugaki, H., et al., 2016. Pharmacokinetic profiles of significant adverse events with crizotinib in Japanese patients with ABCB1 polymorphism. Cancer Sci. 107 (8), 1117–1123.
Fukudo, M., Ikemi, Y., Togashi, Y., et al., 2013. Population pharmacokinetics/pharmacodynamics of erlotinib and pharmacogenomic analysis of plasma and cerebrospinal fluid drug concentrations in Japanese patients with non-small cell lung cancer. Clin. Pharmacokinet. 52 (7), 593–609.
Gillis, N.K., McLeod, H.L., 2016. The pharmacogenomics of drug resistance to protein kinase inhibitors. Drug Resist. Updat. Rev. Comment. Antimicrob. Anticancer Chemother. 28, 28–42.
Giovannetti, E., Zucali, P.A., Peters, G.J., et al., 2010. Association of polymorphisms in AKT1 and EGFR with clinical outcome and toxicity in non-small cell lung cancer patients treated with gefitinib. Mol. Cancer Ther. 9 (3), 581–593.
Gregorc, V., Hidalgo, M., Spreafico, A., et al., 2008. Germline polymorphisms in EGFR and survival in patients with lung cancer receiving gefitinib. Clin. Pharmacol. Ther. 83 (3), 477–484.
Guan, S., Chen, X., Xin, S., et al., 2021. Establishment and application of a predictive model for gefitinib-induced severe rash based on pharmacometabolomic profiling and polymorphisms of transporters in non-small cell lung cancer. Transl. Oncol. 14 (1), 100951.
Hamada, A., Sasaki, J.-i, Saeki, S., et al., 2012. Association of ABCB1 polymorphisms with erlotinib pharmacokinetics and toxicity in Japanese patients with non-small-cell lung cancer. Pharmacogenomics 13 (5), 615–624.
Han, J.Y., Yoon, K.A., Park, J.H., 2011. DNA repair gene polymorphisms and benefit from gefitinib in never-smokers with lung adenocarcinoma. Cancer 117 (14), 3201–3208.
Han, S.-W., Jeon, Y.K., Lee, K.-H., 2007. Intron 1 CA dinucleotide repeat polymorphism and mutations of epidermal growth factor receptor and gefitinib responsiveness in non-small-cell lung cancer. Pharmacogenet. Genom. 17 (5).
Hayashi, H., Iihara, H., Hirose, C., 2019. Effects of pharmacokinetics-related genetic polymorphisms on the side effect profile of afatinib in patients with non-small cell lung cancer. Lung Cancer 134, 1–6.
Henricks, L.M., Lunenburg, C.A.T.C., de Man, F.M., et al., 2018. DPYD genotype-guided dose individualisation of fluoropyrimidine therapy in patients with cancer: a prospective safety analysis. Lancet Oncol. 19 (11), 1459–1467.
Huang, C.L., Yang, C.H., Yeh, K.H., et al., 2009. EGFR intron 1 dinucleotide repeat polymorphism is associated with the occurrence of skin rash with gefitinib treatment. Lung Cancer 64 (3), 346–351.
Ichihara, S., Toyooka, S., Fujiwara, Y., et al., 2007. The impact of epidermal growth factor receptor gene status on gefitinib-treated Japanese patients with non-small-cell lung cancer. Int. J. Cancer 120 (6), 1239–1247.
Isobe, K., Kakimoto, A., Mikami, T., et al., 2016. Association of BIM deletion polymorphism and BIM-gamma RNA expression in NSCLC with EGFR mutation. Cancer Genom. Proteom. 13 (6), 475–482.
Juchum, M., Günther, M., Laufer, S.A., 2015. Fighting cancer drug resistance: opportunities and challenges for mutation-specific EGFR inhibitors. Drug Resist. Updat. 20, 12–28.
Jung, M., Cho, B.C., Lee, C.H., et al., 2012. EGFR polymorphism as a predictor of clinical outcome in advanced lung cancer patients treated with EGFR-TKI. Yonsei Med. J. 53 (6), 1128–1135.
Kiyohara, C., Hirohata, T., Inutsuka, S., 1996. The relationship between aryl hydrocarbon hydroxylase and polymorphisms of the CYP1A1 gene. Jpn. J. Cancer Res. 87 (1), 18–24.
Kobayashi, H., Sato, K., Niioka, T., et al., 2016. Effects of polymorphisms in CYP2D6 and ABC transporters and side effects induced by gefitinib on the pharmacokinetics of the gefitinib metabolite, O-desmethyl gefitinib. Med. Oncol. 33 (6), 57.
Kobayashi, H., Sato, K., Niioka, T., Miura, H., Ito, H., Miura, M., 2015. Relationship among gefitinib exposure, polymorphisms of its metabolizing enzymes and transporters, and side effects in Japanese patients with non-small-cell lung cancer. Clin. Lung Cancer 16 (4), 274–281.
Krause, D.S., Van Etten, R.A., 2005. Tyrosine kinases as targets for cancer therapy. N. Engl. J. Med. 353 (2), 172–187.
Leal, L.F., Laus, A.C., Cavagna, R., 2020. EGF+61 A>G polymorphism does not predict response to first-generation EGFR tyrosine kinase inhibitors in lung cancer patients. Thorac. Cancer 11 (10), 2987–2992.
Lee, J.H., Lin, Y.L., Hsu, W.H., 2014. Bcl-2-like protein 11 deletion polymorphism predicts survival in advanced non-small-cell lung cancer. J. Thorac. Oncol. 9 (9), 1385–1392.
Lee, J.K., Shin, J.Y., Kim, S., et al., 2013. Primary resistance to epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) in patients with non-small-cell lung cancer harboring TKI-sensitive EGFR mutations: an exploratory study. Ann. Oncol. 24 (8), 2080–2087.
Lee, J.Y., Ku, B.M., Lim, S.H., 2015. The BIM deletion polymorphism and its clinical implication in patients with EGFR-mutant non-small-cell lung cancer treated with EGFR tyrosine kinase inhibitors. J. Thorac. Oncol. 10 (6), 903–909.
Lee, Y.T., Tan, Y.J., Oon, C.E., 2018. Molecular targeted therapy: treating cancer with specificity. Eur. J. Pharmacol. 834, 188–196.
Lei, T., Zhang, L., Song, Y., 2020. miR-1262 transcriptionally modulated by an enhancer genetic variant improves efficiency of epidermal growth factor receptor-tyrosine kinase inhibitors in advanced lung adenocarcinoma. DNA Cell Biol. 39 (7), 1111–1118.
Lemos, C., Giovannetti, E., Zucali, P.A., et al., 2011. Impact of ABCG2 polymorphisms on the clinical outcome and toxicity of gefitinib in non-small-cell lung cancer patients. Pharmacogenomics 12 (2), 159–170.
Li, J., Cusatis, G., Brahmer, J., et al., 2007. Association of variant ABCG2 and the pharmacokinetics of epidermal growth factor receptor tyrosine kinase inhibitors in cancer patients. Cancer Biol. Ther. 6 (3), 432–438.
Li, W., Zhang, H., Assaraf, Y.G., 2016. Overcoming ABC transporter-mediated multidrug resistance: molecular mechanisms and novel therapeutic drug strategies. Drug Resist. Updat. 27, 14–29.
Li, X., Wang, S., Li, B., 2018. BIM deletion polymorphism confers resistance to osimertinib in EGFR T790M lung cancer: a case report and literature review. Target Oncol. 13 (4), 517–523.
Li, X., Zhang, D., Li, B., 2021. Clinical implications of germline BCL2L11 deletion polymorphism in pretreated advanced NSCLC patients with osimertinib therapy. Lung Cancer 151, 39–43.
Liao, D., Liu, Z., Zhang, Y., 2020. Polymorphisms of drug-metabolizing enzymes and transporters contribute to the individual variations of erlotinib steady state trough concentration, treatment outcomes, and adverse reactions in epidermal growth factor receptor-mutated non-small cell lung cancer patients. Front. Pharmacol. 11, 664.
Lin, Y.T., Liu, Y.N., Shih, J.Y., 2019. The impact of clinical factors, ALK fusion variants, and BIM polymorphism on crizotinib-treated advanced EML4-ALK rearranged non-small cell lung cancer. Front. Oncol. 9, 880.
Liu, G., Gurubhagavatula, S., Zhou, W., 2008. Epidermal growth factor receptor polymorphisms and clinical outcomes in non-small-cell lung cancer patients treated with gefitinib. Pharmacogenom. J. 8 (2), 129–138.
Liu, G., Cheng, D., Ding, K., 2012. Pharmacogenetic analysis of BR.21, a placebo-controlled randomized phase III clinical trial of erlotinib in advanced non-small cell lung cancer. J. Thorac. Oncol. 7 (2), 316–322.
Liu, S., Li, W., Sun, H., 2019. P1.14-31 lack of association between BIM deletion polymorphism and clinical efficacy of EGFR-TKIs in NSCLC based on NGS. J. Thorac. Oncol. 14 (10), S566.
Lv, F., Sun, L., Yang, Q., Pan, Z., Zhang, Y., 2021. Prognostic value of BIM deletion in EGFR-mutant NSCLC patients treated with EGFR-TKIs: a meta-analysis. BioMed Res. Int. 2021, 3621828.
Ma, F., Sun, T., Shi, Y., 2009. Polymorphisms of EGFR predict clinical outcome in advanced non-small-cell lung cancer patients treated with Gefitinib. Lung Cancer 66 (1), 114–119.
Ma, Y., Xin, S., Huang, M., 2017. Determinants of Gefitinib toxicity in advanced non-small cell lung cancer (NSCLC): a pharmacogenomic study of metabolic enzymes and transporters. Pharmacogenom. J. 17 (4), 325–330.
Ma, Y., Xin, S., Lin, Q., 2019. The analysis of pharmacokinetic and pharmacogenomic impact on gefitinib efficacy in advanced non-small cell lung cancer patients: results from a prospective cohort study. Ann. Transl. Med. 7 (24), 806.
Mathijssen, R.H.J., Sparreboom, A., Verweij, J., 2014. Determining the optimal dose in the development of anticancer agents. Nat. Rev. Clin. Oncol. 11 (5), 272–281.
McKibbin, T., Zhao, W., Tagen, M., 2010. Epidermal growth factor receptor polymorphisms and risk for toxicity in paediatric patients treated with gefitinib. Eur. J. Cancer 46 (11), 2045–2051.
Miyamoto, S., Azuma, K., Ishii, H., 2020. Low-dose erlotinib treatment in elderly or frail patients with EGFR mutation-positive non-small cell lung cancer: a multicenter phase 2 trial. JAMA Oncol. 6 (7), e201250.
Mulder, T.A.M., van Eerden, R.A.G., de With, M., 2021. CYP3A4(*)22 genotyping in clinical practice: ready for implementation? Front. Genet. 12, 711943.
Ng, K.P., Hillmer, A.M., Chuah, C.T.H., 2012. A common BIM deletion polymorphism mediates intrinsic resistance and inferior responses to tyrosine kinase inhibitors in cancer. Nat. Med. 18 (4), 521–528.
Nie, Q., Wang, Z., Zhang, G.C., 2007. The epidermal growth factor receptor intron1 (CA)n microsatellite polymorphism is a potential predictor of treatment outcome in patients with advanced lung cancer treated with Gefitinib. Eur. J. Pharmacol. 570 (1–3), 175–181.
Nie, Q., Yang, X.N., An, S.J., 2011. CYP1A1*2A polymorphism as a predictor of clinical outcome in advanced lung cancer patients treated with EGFR-TKI and its combined effects with EGFR intron 1 (CA)n polymorphism. Eur. J. Cancer 47 (13), 1962–1970.
Nowak, A., Grosso, F., Steele, N., 2017. MA 19.03 Nintedanib + Pemetrexed/Cisplatin in malignant pleural mesothelioma (MPM): phase II biomarker data from the LUME-Meso study. J. Thorac. Oncol. 12 (11), S1884.
Nyberg, F., Barratt, B.J., Mushiroda, T., 2011. Interstitial lung disease in gefitinib-treated Japanese patients with non-small-cell lung cancer: genome-wide analysis of genetic data. Pharmacogenomics 12 (7), 965–975.
Ruan, Y., Jiang, J., Guo, L., 2016. Genetic association of curative and adverse reactions to tyrosine kinase inhibitors in Chinese advanced non-small cell lung cancer patients. Sci. Rep. 6, 23368.
Rudin, C.M., Liu, W., Desai, A., 2008. Pharmacogenomic and pharmacokinetic determinants of erlotinib toxicity. J. Clin. Oncol. 26 (7), 1119–1127.
Sakamoto, S., Sato, K., Takita, Y., et al., 2020. ABCG2 C421A polymorphisms affect exposure of the epidermal growth factor receptor inhibitor gefitinib. Investig. New Drugs 38 (6), 1687–1695.

Saleem, H., Kulsoom Abdul, U., Küçükosmanoglu, A., 2019. The TICking clock of EGFR therapy resistance in glioblastoma: target Independence or target compensation. Drug Resist. Updat. 43, 29–37.
Sarmento-Ribeiro, A.B., Scorilas, A., Gonçalves, A.C., Efferth, T., Trougakos, I.P., 2019. The emergence of drug resistance to targeted cancer therapies: clinical evidence. Drug Resist. Updat. 47, 100646.
Sasaki, H., Okuda, K., Takada, M., et al., 2008. A novel EGFR mutation D1012H and polymorphism at exon 25 in Japanese lung cancer. J. Cancer Res. Clin. Oncol. 134 (12), 1371–1376.
Sasaki, H., Endo, K., Takada, M., 2008. EGFR polymorphism of the kinase domain in Japanese lung cancer. J. Surg. Res. 148 (2), 260–263.
Sasaki, H., Okuda, K., Shimizu, S., et al., 2009. EGFR R497K polymorphism is a favorable prognostic factor for advanced lung cancer. J. Cancer Res. Clin. Oncol. 135 (2), 313–318.
Sasaki, H., Okuda, K., Kawano, O., Yukiue, H., Yano, M., Fujii, Y., 2008. Fibroblast growth factor receptor 4 mutation and polymorphism in Japanese lung cancer. Oncol. Rep. 20 (5), 1125–1130.
Shitara, M., Sasaki, H., Yokota, K., 2012. Polymorphisms in intron 1 of the EGFR gene in non-small cell lung cancer patients. Exp. Ther. Med. 4 (5), 785–789.
Shuang, X., YuanYuan, Z., Yuxiang, M., 2016. Relation of the BIM deletion polymorphism to intrinsic EGFR-TKI resistance of Chinese patients with EGFR mutant advanced non-small cell lung cancer. J. Clin. Oncol. 34 (15_suppl) e14095-e14095.
Sogawa, R., Nakashima, C., Nakamura, T., 2020. Association of genetic polymorphisms with afatinib-induced diarrhoea. In Vivo 34 (3), 1415–1419.
Sprowl, J.A., Mikkelsen, T.S., Giovinazzo, H., Sparreboom, A., 2012. Contribution of tumoral and host solute carriers to clinical drug response. Drug Resist. Updat. Rev. Comment. Antimicrob. Anticancer Chemother. 15 (1–2), 5–20.
Steendam, C.M.J., Veerman, G.D.M., Pruis, M.A., 2020. Plasma predictive features in treating EGFR-mutated non-small cell lung cancer. In: Cancers, 12, p. 3179.
Sugiyama, E., Umemura, S., Nomura, S., et al., 2015. Impact of single nucleotide polymorphisms on severe hepatotoxicity induced by EGFR tyrosine kinase inhibitors in patients with non-small cell lung cancer harboring EGFR mutations. Lung Cancer 90 (2), 307–313.
Sun, S., Yu, H., Wang, H., et al., 2017. Exploratory cohort study and meta-analysis of BIM deletion polymorphism in patients with epidermal growth factor receptor-mutant non-small-cell lung cancer treated with epidermal growth factor receptor tyrosine kinase inhibitors. Onco Targets Ther. 10, 1955–1967.
Suzumura, T., Kimura, T., Kudoh, S., 2012. Reduced CYP2D6 function is associated with gefitinib-induced rash in patients with non-small cell lung cancer. BMC Cancer 12 (1), 568.
Takimoto, T., Kijima, T., Otani, Y., et al., 2013. Polymorphisms of CYP2D6 gene and gefitinib-induced hepatotoxicity. Clin. Lung Cancer 14 (5), 502–507.
Tamura, M., Kondo, M., Horio, M., 2012. Genetic polymorphisms of the adenosine triphosphate-binding cassette transporters (ABCG2, ABCB1) and gefitinib toxicity. Nagoya J. Med. Sci. 74 (1–2), 133–140.
Tan, Y., Cao, K., Ren, G., 2020. Effects of the ABCB1 and ABCG2 polymorphisms on the pharmacokinetics of afatinib in healthy Chinese volunteers. Xenobiotica 50 (2), 237–243.
Tiseo, M., Capelletti, M., De Palma, G., 2008. Epidermal growth factor receptor intron-1 polymorphism predicts gefitinib outcome in advanced non-small cell lung cancer. J. Thorac. Oncol. 3 (10), 1104–1111.
Tiseo, M., Rossi, G., Capelletti, M., 2010. Predictors of gefitinib outcomes in advanced non-small cell lung cancer (NSCLC): study of a comprehensive panel of molecular markers. Lung Cancer 67 (3), 355–360.
van Gelder, T., Hesselink, D.A., van Hest, R.M., Mathôt, R.A.A., van Schaik, R., 2004. Pharmacogenetics in immunosuppressive therapy: the best thing since TDM? Ther. Drug Monit. 26 (4).
van Leeuwen, R.W.F., van Gelder, T., Mathijssen, R.H.J., Jansman, F.G.A., 2014. Drug–drug interactions with tyrosine-kinase inhibitors: a clinical perspective. Lancet Oncol. 15 (8), e315–e326.
Veerman, G.D.M., Hussaarts, K.G.A.M., Jansman, F.G.A., Koolen, S.W.L., van Leeuwen, R.W.F., Mathijssen, R.H.J., 2020. Clinical implications of food–drug interactions with small-molecule kinase inhibitors. Lancet Oncol. 21 (5), e265–e279.
Wang, C., Chen, F., Liu, Y., et al., 2018. Genetic association of drug response to erlotinib in Chinese advanced non-small cell lung cancer patients. Front. Pharmacol. 9, 360.
Wang, F., Diao, X.Y., Zhang, X., 2019. Identification of genetic alterations associated with primary resistance to EGFR-TKIs in advanced non-small-cell lung cancer patients with EGFR sensitive mutations. Cancer Commun. 39 (1), 7.
Wang, J.-Q., Yang, Y., Cai, C.-Y., et al., 2021. Multidrug resistance proteins (MRPs): structure, function and the overcoming of cancer multidrug resistance. Drug Resist. Updat. 54, 100743.
Winther-Larsen, A., Fynboe Ebert, E.B., Meldgaard, P., Sorensen, B.S., 2019. EGFR gene polymorphism predicts improved outcome in patients with EGFR mutation-positive non-small cell lung cancer treated with erlotinib. Clin. Lung Cancer 20 (3), 161–166 e1.
Winther-Larsen, A., Nissen, P.H., Jakobsen, K.R., Demuth, C., Sorensen, B.S., Meldgaard, P., 2015. Genetic polymorphism in the epidermal growth factor receptor gene predicts outcome in advanced non-small cell lung cancer patients treated with erlotinib. Lung Cancer 90 (2), 314–320.
Xia, J., Bai, H., Yan, B., et al., 2017. Mimicking the BIM BH3 domain overcomes resistance to EGFR tyrosine kinase inhibitors in EGFR-mutant non-small cell lung cancer. Oncotarget 8 (65), 108522–108533.
Xin, S., Zhao, Y., Wang, C., et al., 2020. Polymorphisms of NF-kappaB pathway genes influence adverse drug reactions of gefitinib in NSCLC patients. Pharmacogenom. J. 20 (2), 285–293.
Xin, S., Fang, W., Li, J., 2021. Impact of STAT1 polymorphisms on crizotinib-induced hepatotoxicity in ALK-positive non-small cell lung cancer patients. J. Cancer Res. Clin. Oncol. 147 (3), 725–737.
Xu, Y., Pan, Q., Wang, C., 2014. Genetic polymorphisms in oxidative stress-related genes are associated with clinical outcome in patients with advanced non-small cell lung cancer receiving tyrosine kinase inhibitors. Am. J. Cancer Res. 4 (6), 934–942.
Yokota, H., Sato, K., Okuda, Y., 2017. Effects of histamine 2-receptor antagonists and proton pump inhibitors on the pharmacokinetics of gefitinib in patients with non-small-cell lung cancer. Clin. Lung Cancer 18 (6), e433–e439.
Yokota, H., Sato, K., Sakamoto, S., et al., 2020. Effects of STAT3 polymorphisms and pharmacokinetics on the clinical outcomes of gefitinib treatment in patients with EGFR-mutation positive non-small cell lung cancer. J. Clin. Pharm. Ther. 45 (4), 652–659.
Yuan, J., Zhang, N., Yin, L., et al., 2017. Clinical implications of the autophagy core gene variations in advanced lung adenocarcinoma treated with gefitinib. Sci. Rep. 7 (1), 17814.
Yuan, J., Li, B., Zhang, N., 2018. Clinical implications of the BIM deletion polymorphism in advanced lung adenocarcinoma treated with gefitinib. Clin. Lung Cancer 19 (4), e431–e438.
Zenke, Y., Umemura, S., Sugiyama, E., 2016. Successful treatment with afatinib after grade 3 hepatotoxicity induced by both gefitinib and erlotinib in EGFR mutation-positive non-small cell lung cancer. Lung Cancer 99, 1–3.
Zhang, H., Li, Q., Zhu, X., 2018. Association of variability and pharmacogenomics with bioequivalence of gefitinib in healthy male subjects. Front. Pharmacol. 9, 849.
Zhang, L., Li, Q.X., Wu, H.L., et al., 2014. SNPs in the transforming growth factor-beta pathway as predictors of outcome in advanced lung adenocarcinoma with EGFR mutations treated with gefitinib. Ann. Oncol. 25 (8), 1584–1590.
Zhang, L., Jiang, T., Li, X., et al., 2017. Clinical features of Bim deletion polymorphism and its relation with crizotinib primary resistance in Chinese patients with ALK/ROS1 fusion-positive non-small cell lung cancer. Cancer 123 (15), 2927–2935.
Zhang, L., Yuan, X., Chen, Y., Du, X.J., Yu, S., Yang, M., 2013. Role of EGFR SNPs in survival of advanced lung adenocarcinoma patients treated with Gefitinib. Gene 517 (1), 60–64.
Zhang, N., Li, Y., Zheng, Y., et al., 2019. miR-608 and miR-4513 significantly contribute to the prognosis of lung adenocarcinoma treated with EGFR-TKIs. Lab Investig. 99 (4), 568–576.
Zhang, N., Li, Y., Xie, M., et al., 2020. DACT2 modulated by TFAP2A-mediated allelic transcription promotes EGFR-TKIs efficiency in advanced lung adenocarcinoma. Biochem. Pharmacol. 172, 113772.
Zhang, X., Fan, J., Li, Y., et al., 2016. Polymorphisms in epidermal growth factor receptor (EGFR) and AKT1 as possible predictors of clinical outcome in advanced non-small-cell lung cancer patients treated with EGFR tyrosine kinase inhibitors. Tumour Biol. 37 (1), 1061–1069.
Zhao, M., Zhang, Y., Cai, W., et al., 2014. The Bim deletion polymorphism clinical profile and its relation with tyrosine kinase inhibitor resistance in Chinese patients with non-small cell lung cancer. Cancer 120 (15), 2299–2307.