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STUDY ON THE ROLE OF PHARMACOKINETIC MECHANISMS OF DRUG

RESISTANCE IN NEW ANTICANCER DRUGS WITH A FOCUS ON SOLID

TUMORS

Doctoral dissertation (article-based)

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Hradec Králové 2021   Dimitrios Vagiannis

AUTHOR'S DECLARATION

I declare that this work is my original authorial work, which I have drawn up separately under the guidance of my supervisor RNDr. Jakub Hofman, Ph.D.. All literature and other sources from which I drew during processing are listed in the list of literature used and duly cited in the work. The work was not used to obtain another or the same title.

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ABSTRACT

Cancer chemotherapy is an important tool for the cure of cancer. Although the development of new anticancer drugs has been rapidly progressing, the phenomenon of multidrug resistance (MDR) continues to be a key issue leading to therapy failure in oncological patients. MDR is based on pharmacodynamic as well as pharmacokinetic mechanisms. Pharmacokinetic MDR includes drug efflux transporters and biotransformation enzymes that decrease the amount of (active form of) a drug in tumors. While the MDR role of transporters has been well understood, the participation of drug metabolizing enzymes is still unclear.

This thesis investigates the role of cytochromes P450 (CYPs) in cytostatic resistance. Furthermore, it focuses on the modulation of pharmacokinetic MDR using pharmacokinetic drug-drug interactions of new targeted antitumor drugs. Finally, it aims to confirm the *in vitro* findings in *ex vivo* patient-derived tumor explants.

In our latest publication, we demonstrate the significant role of CYP3A4 in resistance to docetaxel *in vitro*. In other papers, we report interactions of several small molecule targeted drugs with ATP-binding cassette drug efflux transporters and CYPs. By employing drug combination studies, we show that these interactions could be beneficially exploited for combatting MDR. Finally, using *ex vivo* primary tumor explants, we demonstrate that the response to the dual-activity MDR modulators is closely related to the expression levels of the transporters, confirming thus possible clinical value of this approach. In addition, these results emphasize the importance of adherence to the rules of personalized medicine for this therapeutic strategy.

In conclusion, we provide a mechanistic evidence on the MDR role of CYP3A4 enzyme and suggest possible combination therapies, which, following *in vivo* confirmation, might improve the efficiency and/or safety of anticancer treatment in number of oncological patients.

ABSTRAKT

Protinádorová chemoterapie je důležitým nástrojem při léčbě nádorových onemocnění. Ačkoliv dochází k rapidnímu progresu ve vývoji nových protinádorových léčiv, fenomén mnohočetné lékové rezistence (MDR) i nadále představuje zásadní překážku vedoucí k selhání farmakoterapie u onkologických pacientů. MDR je založena na farmakodynamických i farmakokinetických mechanizmech. Farmakokinetická MDR zahrnuje působení lékových efluxních transportérů a biotransformačních enzymů, které snižují množství (aktivní formy) léčiva v nádoru. Zatímco role transportérů v MDR byla detailně prozkoumána, účast biotransformačních enzymů stále není plně zřejmá.

Tato práce se věnuje studiu role cytochromů P450 (CYPs) v cytostatické rezistenci. Dále se zaměřuje na modulaci farmakokinetické MDR s využitím lékových interakcí nových cílených protinádorových léčiv. V poslední části se věnuje ověření *in vitro* výsledků na *ex vivo* modelech explantů odvozených z nádorových biopsií získaných od pacientů.

V naší nejnovější publikaci demonstrujeme signifikantní roli enzymu CYP3A4 v rezistenci vůči docetaxelu *in vitro*. V ostatních pracích popisujeme interakce několika nízkomolekulárních cílených léčiv s ATP-binding cassette (ABC) lékovými efluxními transportéry a CYPs. S pomocí lékových kombinačních studií ukazujeme, že tyto interakce mohou být výhodně využity pro omezení MDR. Aktivita duálních MDR modulátorů koreluje s expresí transportérů v *ex vivo* primárních nádorových explantech, což potvrzuje možnou klinickou hodnotu tohoto přístupu. Současně toto zjištění zdůrazňuje nutnost dodržování principů personalizované medicíny pro využití této terapeutické strategie.

Závěrem můžeme konstatovat, že jsme poskytli mechanistický důkaz o rezistenční roli enzymu CYP3A4 a navrhli možné kombinační režimy, které by po ověření *in vivo* mohly vylepšit účinnost a/nebo bezpečnost protinádorové léčby u mnoha onkologických pacientů.

LIST OF ABBREVIATIONS

ABC   ATP-binding cassette

ABCB1   P-glycoprotein

ABCC1   multidrug resistance-associated protein 1

ABCG2   breast cancer resistance protein

CYP   cytochrome P450

EMA   European Medicines Agency

FDA   Food and Drug Administration

MDR   multidrug resistance

NSCLC   non-small cell lung cancer

SMTDs   small molecule targeted drugs

Table of Contents

1. INTRODUCTION

Cancer is a severe disease characterized by uncontrolled cell growth (Cooper and Hausman, 2000). Numerous epidemiological studies have demonstrated it as one of the most common causes of death worldwide (Sung et al., 2021). Subsequently, the effort to make a progress in the therapy of cancer attracts the interest of several research groups around the world. The main types of cancer treatment are surgery, radiation therapy and chemotherapy, with the last one to be mostly used especially for late stages of cancer (Baskar et al., 2012). There are two main groups of chemotherapies, the conventional and the targeted. Even though chemotherapies help improve the cure of cancer and the quality of life of cancer patients, they are facing a vast obstacle called multidrug resistance (MDR) of cancer cells. This therapeutic barrier, which is considered a nightmare for clinical oncologists, often leads to therapy failure and eventually death of the patients (Housman et al., 2014).

One of the main types of MDR is the pharmacokinetic, which includes increased efflux of cytostatic drugs from the cell by ATP-binding cassette (ABC) drug efflux transporters and/or deactivation by drug metabolizing enzymes such as cytochromes P450 (CYPs) (Vadlapatla et al., 2013). ABC efflux transporters are membrane transporters that can pump xenobiotics and drugs out of the cells, and which are often overexpressed in tumors (Cerny, 2016, Szakács et al., 2008). CYPs are biotransformation enzymes that participate in drug metabolism and accelerate the excretion phase of pharmacokinetics. In several types of cancer, they have also been found to be overexpressed (Cerny, 2016, Vadlapatla et al., 2013). In this study, we investigated the role of pharmacokinetic mechanisms in MDR and possibilities of their modulation.

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2. THEORETICAL BACKGROUND

2.1. Cancer

Cancer is a genetic disorder characterized by uncontrolled cell growth caused by mutations of the genes (proto-oncogenes and tumor suppressor genes) responsible for cell function (Cooper and Hausman, 2000). These mutations can be either inherited or acquired and caused by carcinogens or viruses; recent studies discuss important roles also for epigenetic mechanisms and miRNAs. During oncogenesis, proto-oncogenes are activated to oncogenes or tumor suppressor genes are inactivated (Motofei, 2018); this disbalance leads to uncontrolled cell proliferation. Common mutations are found in genes encoding growth factor receptors, tyrosine kinases, signaling molecules, and genes activating cyclin genes, or those that inactivate negative regulations of cyclins and cyclin-dependent kinases (Kumar et al., 2013). Cancer pathogenesis also includes stimulus-independent expression of growth factors and its receptors, as well as overproduction or unregulated activity of transcription factors. Carcinogenesis is not a simple process; it is characterized by the accumulation of several genetic or epigenetic changes, which leads to the establishment of cancer cell hallmarks. Hallmarks are functional characteristics of cancer such as excessive growth, avoiding apoptosis, pro-angiogenesis, local invasiveness, and the ability to form distant metastases (Fig. 1) (Kumar et al., 2013, Seyfried and Huysentruyt, 2013, Dembic, 2020).

Fig. 1. Carcinogenesis and cancer phenotypic heterogeneity.

Adopted from: (Kumar et al., 2013)

Globally, cancer is listed as the second major cause of death and is expected to surpass cardiovascular diseases in the next few years (Wang et al., 2016). Last year, cancer accounted for 19.3 million new cases and caused death to 10.0 million people (Sung et al., 2021). The mortality depends on the type of cancer, such as cancer of the pancreas, which is fatal, while Hodgkin tumors, which are easily curable. According to gender, we can find a high probability of some specific subtypes of cancer; the most common type in men is prostate cancer, while breast cancer is primary in women. In both genders, lung and colon cancers have been ranked as the second and third frequently diagnosed types of cancer, respectively (Fig. 2) (Sung et al., 2021, Siegel et al., 2020). Together, these four mentioned types of tumors account for almost half of the cancer deaths, predominantly lung tumors, with only 16.8% of patients surviving 5 years from diagnosis. Breast tumors have a much better prognosis achieving 5-year survival of 80%, but mortality is still high due to very high incidence (Ridge et al., 2013, Smith, 2013).

Estimated New Cases

	Males				Females	
Prostate	191,930	21%		Breast	276,480	30%
Lung & bronchus	116,300	13%		Lung & bronchus	112,520	12%
Colon & rectum	78,300	9%		Colon & rectum	69,650	8%
Urinary bladder	62,100	7%		Uterine corpus	65,620	7%
Melanoma of the skin	60,190	7%		Thyroid	40,170	4%
Kidney & renal pelvis	45,520	5%		Melanoma of the skin	40,160	4%
Non-Hodgkin lymphoma	42,380	5%		Non-Hodgkin lymphoma	34,860	4%
Oral cavity & pharynx	38,380	4%		Kidney & renal pelvis	28,230	3%
Leukemia	35,470	4%		Pancreas	27,200	3%
Pancreas	30,400	3%		Leukemia	25,060	3%
All Sites	**893,660**	**100%**		**All Sites**	**912,930**	**100%**

Estimated Deaths

	Males				Females	
Lung & bronchus	72,500	23%		Lung & bronchus	63,220	22%
Prostate	33,330	10%		Breast	42,170	15%
Colon & rectum	28,630	9%		Colon & rectum	24,570	9%
Pancreas	24,640	8%		Pancreas	22,410	8%
Liver & intrahepatic bile duct	20,020	6%		Ovary	13,940	5%
Leukemia	13,420	4%		Uterine corpus	12,590	4%
Esophagus	13,100	4%		Liver & intrahepatic bile duct	10,140	4%
Urinary bladder	13,050	4%		Leukemia	9,680	3%
Non-Hodgkin lymphoma	11,460	4%		Non-Hodgkin lymphoma	8,480	3%
Brain & other nervous system	10,190	3%		Brain & other nervous system	7,830	3%
All Sites	**321,160**	**100%**		**All Sites**	**285,360**	**100%**

Fig. 2. Expected incidence and mortality for individual types of malignancies by sex in the USA for 2020.

Adopted from: (Siegel et al., 2020)

2.2. Anticancer pharmacotherapy

Surgery is the oldest strategy for cancer treatment firstly recorded in the era of Ancient Egypt, when first attempts to resect breast carcinomas were conducted (Falzone et al., 2018). Since then, many researchers have focused on developing and improving the treatment of cancer. To date, possible tools for cancer treatment are surgery excision, irradiation, and drug therapy referred to as chemotherapy. In clinical practice, chemotherapy is used alone or in combination with other forms of treatment and is the most effective way to treat advanced and

metastatic tumors. Today, chemotherapeutic agents are split into two main categories: the conventional and the targeted agents based on their specificity on the target (Fig. 3).

Fig. 3. Scheme of effects of conventional vs. targeted chemotherapy in cancer patient. Adopted from: (Vrettos et al., 2018)

2.2.1. Conventional anticancer chemotherapeutics

The majority of the conventional cytostatic drugs target the specific phases of the cell cycle, and their action is mainly based on the interaction with synthesis/replication of cellular DNA or RNA and the metabolism of their binding blocks (Schirrmacher, 2019).

Cytostatics are grouped based on their chemical structures and the way they act on cancer cells (alkylating agents, antimetabolites, cytotoxic antibiotics etc.), their cell cycle specificity (specific such as antimetabolites and not specific such as the alkylating agents), and based on their indication (Schellens et al., 2005). In clinical practice, especially for the treatment of sex hormone-dependent tumors, hormones/analogues, antagonists and aromatase inhibitors are used (Rang and Dale, 2007).

Cancer treatment objectives are curative, adjuvant and palliative. Chemotherapy is a complicated treatment method with a narrow therapeutic window (Müller, 2003). Low extent of selectivity (Fig. 3) results in damaging healthy cells along with the cancer tissues, subsequently creating a high chance of toxic adverse drug reactions occurrence. These toxicities can be acute or chronic and are graded from mild to life-threatening/disabling (Koeppen and Stanton, 2017). The mild and moderate toxicities include damages to the gastrointestinal tract, kidney, hair, skin, bone marrow and blood; fortunately, these effects are not usually prolonged or irreversible once chemotherapy is completed. On the other hand, severe or life-threatening/disabling include toxicities that can affect vital organs, such as the brain, heart, lungs, and reproductive system (Schirrmacher, 2019, Lau et al., 2004, Gradishar and Schilsky, 1988). Remarkably, it has been found that the negative outcomes of chemotherapies' side effects are the severe psychological effects they have on the patients (Love et al., 1989).

During the past three decades, progress in anticancer drug development did not abolish the use of conventional cytostatics in clinical practice. For some cancer types, they still remain a gold therapeutic standard. Moreover, novel cytostatic formulations (e.g. liposomal daunorubicin with cytarabine liposomal irinotecan) have being developed (Nehate et al., 2014, Alfayez et al., 2020). These modified formulations aim to improve the pharmacokinetic properties and/or toxicity profiles of the active substances. Beside new formulations, novel cytostatics have been approved for anticancer therapy during the past few years (trabectedin, trifluridine) (D'Incalci and Galmarini, 2010, Kawazoe and Shitara, 2020).

2.2.2. Targeted anticancer pharmacotherapy and current trends in the development of anticancer drugs

Driving progress in anticancer treatment is the main goal for many researchers around the world. At the beginning of the 21st century, the human genetic code was mapped, leading to the

development of new chemotherapy approaches (Pareek et al., 2011). These new approaches have been categorized as targeted therapies because, in contrast to conventional cytostatics, they act by specific targeting of cell molecules responsible for carcinogenesis and cancer growth (Fig. 3). As a result, this feature leads to fewer serious side effects (Scavone et al., 2017). There are several mechanisms that targeted therapies utilize, such as inhibition of enzymes and growth factor receptors responsible for cancer growth, initiation of cancer cell apoptosis, and interference with the activity of proteins responsible for cancer cell activities (Joo et al., 2013). Throughout the targeted chemotherapies, the most common and clinically successful are the small molecule targeted drugs (SMTDs) and the monoclonal antibodies. Up to date, a number of drugs from these groups have been approved by drug regulatory authorities (Food and Drug Administration (FDA) and European Medicines Agency (EMA)), and an abundance of other compounds were recently subjected to evaluation in clinical trials (Baldo, 2016, Dembic, 2020, Jeon et al., 2017).

Due to SMTDs' structures (molecular weight of < 800 Daltons and other favorable physicochemical properties), they have the ability to enter the cancer cells by crossing the cytoplasmic membrane, interacting with its target and causing damage to the cell (Zhang et al., 2009a). As mentioned above, these therapies are beneficial in cancer types that express specific proteins. Protein kinase inhibitors represent the flagship group of SMTDs, with imatinib being the first approved tyrosine kinase inhibitor targeting the oncogenic cytoplasmic kinase Bcr-Abl in 2001 (Jeon et al., 2017). Beside protein kinase inhibitors, several other groups of SMTDs have been approved for use in anticancer pharmacotherapy, including poly-ADP ribose polymerase inhibitors (olaparib), inhibitors of immune checkpoints (venetoclax), isocitrate dehydrogenase inhibitors (enasidenib), exportin-1 inhibitors (selinexor) and Hedgehog pathway inhibitors (vismodegib) (Arora et al., 2021, Juárez-Salcedo et al., 2019, Reed et al., 2019, Syed, 2019, Axelson et al., 2013). Monoclonal antibodies are immunoglobulins that are not able to

cross the cell membrane, thus they bind to target proteins expressed outside of the cancer cell. The first approved monoclonal antibody, rituximab, binds to the calcium channel-forming CD20 protein causing cell lysis (Weiner, 2007). Moreover, the clinical outcome of these therapies showed the importance of employing personalized medicine; as the treatment must be designed based on the unique tumor phenotype of a particular patient or a specific sub-population (Joo et al., 2013).

Furthermore, apart from standard targeted therapies, several other innovative approaches associated with cancer cure exist (Fig. 4). Nanomedicine involves the use of nanoparticles, which are nano-structured systems with actions overlapping between the concept of conventional/targeted drugs, controlled release and targeted delivery (Tinkle et al., 2014). It does not focus on diagnostic or prognostic aspects, but only on the selective delivery of the drug to the tumor cells (Patra et al., 2018). A stand-alone strategy for cancer treatment is gene therapy. In the standard concept of gene therapy, normal copy of a defective gene is introduced in the genome by several tools such as viral or non-viral vectors. Newer strategies include use of various oligonucleotides or genome editing method (Kaufmann et al., 2013). Thousands of clinical trials using gene therapy are ongoing, with the majority of them being focused on cancer treatment (Ginn et al., 2018). To date, only five gene therapeutics have been introduced into clinical practice in the last few years, with talimogene laherparepvec being the first approved (Greig, 2016). Additionally, extracellular vesicles in clinical practice have been found to be important for diagnosis and drug treatment (Martinelli, 2017). Thermal ablation and magnetic hyperthermia are also novel ways of cancer treatment, as cell necrosis is temperature-dependent and cancer cells are more susceptible to high temperatures than healthy ones (Van der Zee, 2002). Moreover, natural antioxidants products have been introduced in cancer therapy. Some of the natural antioxidants that are used in clinical practice are supplements, however, natural products such as curcumin and quercetin are under clinical trials for several types of cancer

(González-Vallinas et al., 2013) (recruiting studies with ClinicalTrials.gov Identifiers NCT03980509, NCT03769766, NCT04731844, NCT01720147 and NCT03476330). Lastly, two innovative features; radiomics and pathomics, use radiology and pathology screening for the prognosis of cancer and design of the proper chemotherapy (Yu et al., 2016).

Fig. 4. Summary of the novel approaches for cancer therapy, diagnosis and therapy outcome prognosis.

Adopted from: (Pucci et al., 2019)

2.3. Pharmacokinetic mechanisms of multidrug resistance

2.3.1. General aspects of drug resistance

Although pharmacotherapy is the irreplaceable and successful way of treating cancer, it faces a severe obstacle of MDR, resulting in a therapy failure and high mortality rate in many patients (Housman et al., 2014). The resistance can be inherited (intrinsic) or may developed during chemotherapy (acquired). The development of chemoresistance arises from

pharmacokinetic or pharmacodynamic mechanisms or their combination (Fig. 5). Pharmacokinetic resistance, which is the subject of this dissertation thesis, arises from changes in the blood/tumor levels of anticancer drugs. Thus, it is mainly the result of enhanced efflux of drugs (e.g. ABC transporters) or drug deactivation by metabolizing enzymes (e.g. CYPs). Pharmacodynamic resistance arising from target mutations is such a significant and common cause that its knowledge has become an integral part of the development of targeted anticancer drugs in the last few years (Mansoori et al., 2017). To reduce the risk of development of resistance, the combination of drugs and adherence to sufficient dosage are the most important rules, which are followed in clinical practice (Mokhtari et al., 2017). It is also worth mentioning that drug resistance is not occurring only in conventional anti-tumor chemotherapeutics, but it also limits the therapeutic use of modern targeted drugs to the same extent (Fujita, 2014, Noguchi, 2017).

Fig. 5. Summary of the main pharmacodynamic and pharmacokinetic MDR mechanisms. Adopted from: (Tomiyasu and Tsujimoto, 2015)

2.3.2. ATP-binding cassette (ABC) transporters

ABC transporters represent a unique superfamily of membrane transporter proteins accounting for 48 genes in the human genome, grouped in seven subfamilies (ABCA-ABCG) based on ATP-binding domains' amino acid sequence (Gottesman, 2002, Wilkens, 2015, Dean and Annilo, 2005). Their physiological function is the protection of physiological tissues against the harmful effects of xenobiotics. By their ATP-dependent activity, they significantly affect the pharmacokinetic behavior of drugs (reduce oral bioavailability, reduce the transfer of drugs to the brain, fetus and sperm, and increase the clearance of drugs excreted in the bile and urine) (Szakács et al., 2008). These transporters are embedded in cellular membranes and have the characteristic structure that consists of two transmembrane domains (e.g. ABCB1) that transfer the substrates outside the cells and two nucleotide-binding domains, in which ATP binds and undergoes hydrolysis (Fig. 6A). In contrast, half transporters (such as ABCG2) contain one of each domain and they need to form either homo- or heterodimers to become active (Fig. 6B) (Gottesman, 2002, Dean, 2009, Beis, 2015).

Fig. 6. Schematic display of (A) full ABC transporter vs. (B) half ABC transporter structures. Adopted from: (Štefková et al., 2004)

ABC transporters are widely distributed throughout the body such as in the lungs, gastrointestinal tract, liver, kidney and body barriers (Fig. 7) (Szakács et al., 2006). They can transport various endogenous and xenobiotic substances, thereby keeping the balanced homeostasis of the organism.

Fig. 7. Summary of the localization of ABC transporters throughout human body. Blood brain barrier (BBB), cerebrospinal fluid (CSF), blood cerebrospinal fluid barrier (BCSFB).

Adopted from: (Szakács et al., 2006)

Considering their important role in pharmacokinetics, the ABCB1 (also termed P-glycoprotein, P-gp, or MDR1), ABCG2 (breast cancer resistance protein, BCRP or

mitoxantrone resistance protein MXR) and ABCC2 (multidrug resistance-associated transporter 2, MRP2) transporters are the site for drug-drug interactions (e.g., clinically significant interactions with macrolides, cyclosporin A, digoxin, statins, or rifampicin) (Gessner et al., 2019, Giacomini and Huang, 2013, Giacomini et al., 2010). Therefore, drug regulatory authorities (EMA and FDA) recommend testing of the effects of novel drugs on these transporters and provide detailed guidelines for this purpose (EMA, 2012, FDA, 2017). Furthermore, loss of some of the ABC transporters' expression/function is associated with several diseases such as Alzheimer's disease, cystic fibrosis, inflammatory bowel disease, and others (Tarling et al., 2013).

ABC transporters are also overexpressed in several tumors, where they reduce the concentration of anticancer drugs within the cell below the cytotoxic level and thus contribute to treatment failure (Muriithi et al., 2020). Up till now, role in anticancer drug transport have been described for 19 ABC transporters, while unambiguous role in MDR *in vitro* and *in vivo* has been confirmed only for three of them - ABCB1, ABCG2 and ABCC1 (MDR-associated protein 1 or MRP1) (Holohan et al., 2013, Szakács et al., 2006, DeGorter et al., 2012). Anticancer drugs acting as susceptible victims of transporter-mediated resistance include taxanes and *Vinca* alkaloids, anthracyclines, camptothecins, epipodophyllotoxins, anthraquinone and targeted low molecular weight drugs such as protein kinase inhibitors (Szakács et al., 2006, Fletcher et al., 2010, Sun et al., 2012, Deng et al., 2014).

Briefly, ABCB1 is the first discovered ABC transporter, localized at the apical membrane of the epithelial cells of several organs. This transporter participates in the failure of almost half of the current cancer chemotherapies, including classical cytostatics along with SMTDs (Avendaño and Menendez, 2015, Gottesman, 2002, Shukla et al., 2011). Although ABCC1 differs significantly from ABCB1, they share several overlapping features such as substrate affinities and expression regulatory mechanisms (Leschziner et al., 2006, Borst et al.,

2000). ABCG2 is a half transporter expressed in the apical membrane of epithelial cells and transport structurally diverse molecules, including drug metabolites (Taylor et al., 2017, Cole et al., 1992). ABCG2 transporter was found to be able to independently mediate drug resistance (Robey et al., 2001). In clinical studies, it was shown that expression of the above-mentioned transporters correlates with poor prognosis, relapses and therapy failure (Kunická and Souček, 2014, Leonard et al., 2003, Gottesman, 2002).

2.3.3. Cytochrome P450 (CYP) enzymes

Biotransformation enzymes catalyze the chemical conversion of endogenous substances and xenobiotics to polar hydrophilic metabolites, which can be more easily excreted from the body in bile or urine in comparison with the parent drug. Biotransformation is divided into two phases, CYPs are the most important enzymes of the first phase (Michael and Doherty, 2005). CYPs constitute a superfamily of heme-containing enzymes that participate in the metabolism of several endogenous and exogenous substrates, including many clinically used drugs (Fig. 8). Similar to ABC transporters, they are known to perpetrate clinically relevant drug-drug interactions.

Fig. 8. Participation of the individual biotransformation systems in the metabolism of 125 orally or intravenously administered SMTDs. The similar proportion is reported for all of the clinically used drugs.

Adopted from: (Cerny, 2016)

Until now, there have been described more than 57 human CYP isoenzymes categorized in 18 families (Karlgren et al., 2005, Sim and Ingelman-Sundberg, 2010). CYPs are localized in mitochondria as well as in the endoplasmic reticulum, and are widely distributed in body organs such as in the liver, kidneys, lungs, gastrointestinal tract, lungs, and brain (Liu et al., 2004, Du et al., 2004, Zhao and Imig, 2003, Ding and Kaminsky, 2003). From all of the CYPs, 8 of them (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4, and CYP3A5) have been found to play an essential role in drug-drug interactions and are included in interaction testing guidelines of the drug regulatory authorities (Vadlapatla et al., 2013, FDA, 2017, EMA, 2012). CYPs are closely related to cancer as they have been found to participate in carcinogenesis, affecting the efficacy of cancer treatment, chemoprevention and metastasis. Thus, regulation of CYPs' function could be a novel tool for improving cancer treatment (Fig. 9) (Bruno and Njar, 2007).

Fig. 9. Effects of CYP inhibition on carcinogenesis and activation of prodrugs.
Adopted from: (Bruno and Njar, 2007)

CYPs, especially the CYP2A, CYP2B, CYP2C, CYP2D, and CYP3A subfamilies, also metabolize standard cytostatics (e.g. tamoxifen, taxanes and *Vinca* alkaloids), while metabolic conversions either increase or decrease the pharmacodynamic activity of a parent drug. Elevated intratumoral expression of CYPs has been described in several cancer types, leading

to the hypothesis that biotransformation enzymes could be involved in pharmacokinetic resistance along with drug transporters (Rochat, 2009, Vadlapatla et al., 2013, Oyama et al., 2004).

Among phase I enzymes, the CYP superfamily was the most studied regarding their possible role in resistance; however, still not in sufficient detail. The majority of studies investigating this topic provides indirect pieces of evidence, using multi-enzymatic models, which hinders possible estimation on the role of individual enzymes. CYP3A4 is the most important enzyme from the CYP group, which participates in the metabolism of more than half of the commercially available drugs (Bu, 2006). Some studies suggest that when overexpressed, it causes an increase in detoxification of some chemotherapeutics (*Vinca* alkaloids, docetaxel, etoposide etc.) and results in the initiation of MDR. Overexpression of CYP3A4 in patients with breast cancer has been reported to correlate with the resistance to docetaxel (Miyoshi et al., 2005). Another study suggested the possible role of CYP3A4 in resistance to vincristine and vinblastine (Yao et al., 2000). However, this study's results are speculative, as the major metabolite of vinblastine does not show lower but higher antiproliferative activity than the parent compound (Owellen et al., 1977). Besides, the overall metabolism rate in vinblastine excretion is negligible (metabolites represent only 1% excretion, while in vincristine, they account for 40%) (Levêque and Jehl, 2007, Castle et al., 1976). Furthermore, CYP2C8 metabolizes a remarkable number of drugs and is able to metabolically inactivate paclitaxel (Lai et al., 2009, Jamis-Dow et al., 1995, Sonnichsen et al., 1995). *In vitro* studies showed induction of the CYP2C8 enzyme following long-term exposure to paclitaxel, leading the authors to hypothesize that CYP2C8 may represent a resistance mechanism for this drug (García-Martín et al., 2006). However, there is no direct evidence on the role of CYP2C8 in paclitaxel resistance. Finally, some isoforms such as CYP1B1 and CYP2W1 have been reported to be almost selectively overexpressed in cancer cells, characterizing them as possible

diagnostic markers (Rochat et al., 2001, Karlgren et al., 2006). However, they do not deactivate any cytostatic drug, thus their role in chemotherapeutic resistance is unlikely.

2.4. Modulation of pharmacokinetic multidrug resistance (MDR)

As mentioned above, unfortunately, many patients develop drug resistance during therapy and initially sensitive tumor cells cease to respond to the treatment. This phenomenon is not a privilege of classical cytostatics, but also SMTDs (Holohan et al., 2013). This resistance can be developed via many pathways, including efflux transporters and metabolizing enzymes (Michael and Doherty, 2005). Clarification of the pharmacokinetic mechanisms of MDR has inspired the development of strategies that could help overcome it (Choi, 2005). Several attempts have been accomplished to overcome ABC transporter-mediated resistance by applying ABC transporter inhibitors in combination with standard cytostatics, but following initial success, these compounds have failed in clinical trials due to either insufficient efficacy and/or provoking toxicity (Fletcher et al., 2010). Use of the first-generation chemosensitizers cyclosporine A and verapamil showed low effects and high toxicity (provoked by impaired elimination of MDR victim cytostatic) in clinical trials. Second (valspodar) and third (zosuquidar, elacridar, tariquidar) generation ABCB1 modulators showed increased potency and decreased toxicity yet their clinical development was terminated due to insufficient efficacy (Twentyman and Bleehen, 1991, Fox and Bates, 2007, Fletcher et al., 2010).

Similar problems were reported in enzyme-area; a study combining the CYP3A4 inhibitor, ketoconazole, with docetaxel had a similar outcome. Although the combination reduced docetaxel's clearance by almost 50%, the risk of febrile neutropenia increased many-fold, which had to be compensated by reducing the dose of docetaxel (Engels et al., 2004).

From these pieces of information, it is apparent that the overcoming of pharmacokinetic MDR with drugs lacking intrinsic anticancer properties is a dead end. However, apart from

using inhibitors without anticancer effects, targeting pharmacokinetic MDR mechanisms still possess an attractive potential. In particular, the combination of novel ABC transporter/drug metabolizing enzyme modulators from the group of SMTDs with conventional anticancer drugs have a potential to become a new treatment option for MDR cancers. These modulators exhibit dual activity; in addition to inhibitory effect, they also bear their own anticancer properties. Due to this unique feature, these novel modulators are able to synergize with resistance victim drugs inside tumors. In turn, such effect allows for drug dose reduction and overcomes complications with systemic toxicity observed in studies with inhibitors lacking intrinsic anticancer properties. There were described promising properties of several SMTDs (nilotinib, lapatinib, etc.; Fig. 10) that are able to synergistically modulate MDR to victim cytostatics (paclitaxel, doxorubicin, topotecan, etc.) both *in vitro* and *in vivo* (Kathawala et al., 2015). Currently, few MDR-antagonizing combinations including those with erlotinib, lapatinib, nintedanib and sorafenib have been evaluated in clinical trials for the treatment of various cancer types (Wu and Fu, 2018).

Fig. 10. Schematic representation of action of novel dual-activity chemosensitizers.

Adopted from: (Kathawala et al., 2015)

3. AIM OF THE WORK

The submitted dissertation thesis consists of a set of original scientific works that were created in the period 2017–2021 during my postgraduate studies at the Department of Pharmacology and Toxicology. The primary aim was to study the mechanisms of pharmacokinetic resistance and the possibilities of their modulation. The sub-aims are as follows:

I. Investigation on the interactions of novel SMTDs towards ABC efflux transporters (ABCB1, ABCG2, ABCC1) and their utilization for overcoming MDR.

II. Study on the role of CYP3A4, CYP3A5 and CYP2C8 enzymes in the resistance to taxanes and vincristine.

III. Identification of the inhibitory profiles of SMTDs toward CYPs (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4 and CYP3A5): impact on the modulation of docetaxel resistance and pharmacokinetic drug-drug interactions.

IV. Implementation of primary non-small cell lung cancer (NSCLC) explants and their application for testing of drug combinations.

4. RESULTS AND CANDIDATE'S PARTICIPATIONS

This dissertation thesis is organized as an annotated set of five research published articles and three manuscripts, which were either submitted or prior submission. The candidate is the first author in two published articles and two manuscripts. All the publications/manuscripts have been accepted/submitted in/to international journals with an impact factor. The outlines of these publications (P) and the candidate's contribution is listed below.

P1.   Hofman J., Sorf A., **Vagiannis D.**, Sucha S., Novotna E., Kammerer S., Küpper J-H., Ceckova M., Staud F.: Interactions of Alectinib with Human ATP-Binding Cassette Drug Efflux Transporters and Cytochrome P450 Biotransformation Enzymes: Effect on Pharmacokinetic Multidrug Resistance. Drug Metab Dispos 2019; 47(7): 699-709, **IF$_{2019/2020}$ = 3.231, Q1.**

- *co-author, performance of part of the cell-based experiments, participation in data analysis and manuscript preparation*

P2.   Hofman J., Sorf A., **Vagiannis D.**, Sucha S., Kammerer S., Küpper J-H., Chen S., Guo L., Ceckova M., Staud F.: Brivanib Exhibits Potential for Pharmacokinetic Drug-Drug Interactions and the Modulation of Multidrug Resistance through the Inhibition of Human ABCG2 Drug Efflux Transporter and CYP450 Biotransformation Enzymes. Mol Pharmaceut 2019; 16(11): 4436-4450, **IF$_{2019/2020}$ = 4.321, Q1.**

- *co-author, performance of part of the cell-based experiments, participation in data analysis and manuscript preparation*

P3.   **Vagiannis D.**, Novotna E., Skarka A., Kammerer S., Küpper J-H., Chen S., Guo L., Staud F., Hofman J.: Ensartinib (X-396) Effectively Modulates Pharmacokinetic Resistance

Mediated by ABCB1 and ABCG2 Drug Efflux Transporters and CYP3A4 Biotransformation Enzyme. Cancers 2020; 12(4): E81, **IF$_{2019/2020}$ = 6.126, Q1.**

- *first author, participation in the design of experiments, performance of all experiments except for UHPLC-MS/MS analysis, molecular docking and induction studies in hepatocyte models, data analysis, preparation of manuscript, preparation of a revised version of the manuscript and answers to opponents*

P4. **Vagiannis D.**, Zhang Y., Novotna E., Morell A., Hofman J.: Entrectinib reverses cytostatic resistance through the inhibition of ABCB1 efflux transporter, but not the CYP3A4 drug-metabolizing enzyme. Biochem Pharmacol 2020; 178: 114061, **IF$_{2019/2020}$ = 4.960, Q1.**

- *first author, participation in the design of experiments, performance of all experiments except for molecular docking and induction studies, data analysis, preparation of manuscript, preparation of a revised version of the manuscript and answers to opponents*

P5. Hofman J**.**, **Vagiannis D.**, Chen S., Guo L.: The role of CYP3A4, CYP3A5 and CYP2C8 drug-metabolizing enzymes in the pharmacokinetic cytostatic resistance. Chem Biol Interact 2021, **IF$_{2019/2020}$ = 3.723, Q2.** *just accepted (March 2021)*

- *co-author, performance of part of the cell-based experiments, participation in data analysis, manuscript writing*

Submitted papers (SP)

SP1. **<u>Vagiannis D.</u>**, Budagaga Y., Morell A., Zhang Y., Novotná E., Skarka A., Kammerer S., Küpper JH., Hanke I., Rozkoš T., Hofman J.: Pharmacokinetic interactions of tepotinib with drug efflux transporters and biotransformation enzymes: the role in combating cytostatic resistance *in vitro* and *ex vivo*. Pharmacological Research, *submitted (March 2021)*

- *first author, participation in design of experiments, performance of majority of experiments, data analysis, preparation of manuscript*

SP2. **<u>Vagiannis D.</u>**, Zhang Y., Budagaga Y., Novotná E., Skarka A., Kammerer S., Küpper JH., Hofman J.: Alisertib shows negligible potential for perpetrating pharmacokinetic drug-drug interactions, but acts as dual-activity resistance modulator through the inhibition of ABCC1 transporter. Toxicology and Applied Pharmacology, *submitted (March 2021)*

- *first author, participation in design of experiments, performance of majority of experiments, data analysis, preparation of manuscript*

SP3. Sorf A., **<u>Vagiannis D.</u>**, Ahmed F., Hofman J., Ceckova M.: Dabrafenib inhibits ABCG2 and cytochrome P450 isoenzymes; potential implications for combination anticancer therapy. *prior to submission*

- *co-author, participation in the performance of CYP-oriented experiments and accumulation assays in MDCKII cells, revision of manuscript*

5. DESCRIPTION OF THE RESULTS

Aim I. Investigation on the interactions of novel SMTDs towards ABC efflux transporters (ABCB1, ABCG2, ABCC1) and their utilization for overcoming MDR.

A large part of our work consists of publications focused on studying of SMTDs' drug interactions with ABC drug efflux transporters, focusing on ABCB1, ABCG2, and ABCC1 transporters, which have been shown to participate in drug resistance *in vivo* (Szakács et al., 2006, Fletcher et al., 2010).

In studies **P1**, **P2**, **P3**, **P4**, **SP1**, **SP2**, and **SP3**, we described the pharmacokinetic interactions of alectinib, brivanib, ensartinib, entrectinib, tepotinib, alisertib, and dabrafenib with the drug transporters ABCB1, ABCG2, ABCC1. The tested drugs are under development or approved for the treatment of several tumor types, including non-small cell lung cancer (NSCLC), the deadliest type of cancer in both genders. Our results showed that brivanib inhibited all three tested transporters (ABCB1, ABCG2, ABCC1). The drugs alectinib, ensartinib, tepotinib and dabrafenib showed potent inhibition towards ABCB1 and ABCG2 transporters, while entrectinib inhibits only ABCB1 and alisertib selectively inhibits ABCC1 transporter. Our findings regarding alisertib are interesting with respect to the absence of specific inhibitors of ABCC1 in clinical practice (Kunická and Souček, 2014).

Moreover, we did not only describe the above-mentioned interactions, but also their ability to modulate transporter-mediated resistance to conventional MDR-victim cytostatics. For the quantitative analysis of these combination effects, we used the combination index method according to Chou-Talalay (Chou, 2006). Although brivanib was shown to inhibit ABCC1 efflux activity, only ABCB1 and ABCG2 inhibitions were demonstrated to result in effective MDR modulation for this drug. Alectinib, ensartinib, tepotinib and dabrafenib were found to effectively modulate ABCB1- and ABCG2-mediated resistance to daunorubicin and mitoxantrone, respectively. In addition, entrectinib synergistically modulated ABCB1-

mediated resistance to daunorubicin and alisertib antagonized ABCC1-based resistance to daunorubicin.

In **P3**, **SP1**, and **SP2** we also assessed the substrate affinity of tested SMTDs using transport experiments across a polarized monolayer of MDCKII cells. Ensartinib, tepotinib and alisertib were found to be substrates of the ABCB1 transporter, but not of ABCG2 or ABCC1. Although the tested drugs were designated as ABCB1 substrates, comparative proliferation studies in cell lines with or without transporter overexpression showed lack of ABCB1's effect on the anticancer capacity of tested drugs (probably due to relatively high lipophilicity). The same results were obtained for ACBG2 and ABCC1. We concluded that tested drugs are not victims of transporter-mediated MDR, which increases their value as possible dual-activity MDR modulators. Comparative studies were also performed for all other drugs mentioned in Aim I. with identical outcomes.

Finally, we tested possible effects of tested drugs on the expression of examined ABC drug efflux transporters in several systemic (LS174T, Caco-2) as well as tumoral (e.g. NCI-H1299, A549) models. Except for dabrafenib, which provoked ABCB1 and ABCC1 inductions, no significant changes of mRNA levels of target genes were observed in these studies. We concluded that the tested drugs (except for dabrafenib) shows negligible potential for perpetrating induction-based drug-drug interactions or strengthening of MDR phenotype of cancer cells.

Aim II. Study on the role of CYP3A4, CYP3A5 and CYP2C8 enzymes in the resistance to taxanes and vincristine.

The role of CYPs in cytostatic resistance has been addressed in only a few studies so far, most of which either provided indirect evidence or were affected by interference elements (such as the multienzymatic character of models). In our recent study (**P5**), we used HepG2

cells stably transduced with CYP3A4, CYP3A5, and CYP2C8 enzymes to study the effect of functional expression of these enzymes on the antitumor effects of paclitaxel, docetaxel, and vincristine. The cells were obtained from the collaborating National Center for Toxicological Research, which is part of the US FDA. The results of our viability and apoptotic measurements showed that only the CYP3A4 enzyme might participate in drug resistance to docetaxel and that specific inhibitor ketoconazole can modulate this resistance. The findings of this study served as an important basis for several other studies (namely **P2**, **P3**, **P4**, **SP1**, **SP2**, and **SP3**), which focused, among other issues, on the use of drug interactions of new targeted drugs to overcome docetaxel resistance (see Aim III).

Aim III. Identification of the inhibitory profiles of SMTDs toward CYPs (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4 and CYP3A5): impact on the modulation of docetaxel resistance and pharmacokinetic drug-drug interactions.

Apart from ABC drug efflux transporters, we also focused on CYP metabolizing enzymes. Thus, in **P1**, **P2**, **P3**, **P4**, **SP1**, **SP2**, and **SP3**, we evaluated novel drugs' interactions with eight CYP isoforms (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4, and CYP3A5) to describe their potential to act as: 1) dual-activity modulators of CYP3A4-mediated resistance towards docetaxel (see Aim II above) and 2) perpetrators of clinically relevant drug-drug interactions (Zhang et al., 2009b). Both ensartinib and tepotinib showed potent inhibition of CYP3A4 and CYP2C9. Brivanib strongly inhibited the CYP2B6, CYP2C19, CYP2C8, and CYP2C9 isoforms. Dabrafenib was found to inhibit the CYP3A4, CYP3A5, CYP2C8, and CYP2C9. Entrectinib showed potent inhibition towards CYP3A4, CYP3A5, CYP2C8, CYP2C9, CYP2C19 and CYP2D6. Alectinib and alisertib interacted with CYP isoforms with low to moderate affinity. Thus, we provided a complex overview on the potential of tested drugs to perpetrate inhibition-based drug-drug interactions.

Next to the transporter-oriented experiments, in works **P2**, **P3**, **P4**, **SP1**, **SP2**, and **SP3**, we also addressed the possibility of modulation of CYP3A4-mediated resistance to docetaxel. For this purpose, we have introduced suitable models (including HepG2 cells overexpressing CYP3A4 from colleagues from the USA), methods and analytical procedure for the analysis of the combination effects (sensitization effect). Ensartinib (**P3**) and dabrafenib (**SP3**) were demonstrated to synergistically overcome docetaxel resistance mediated by CYP3A4 activity. Other drugs either did not achieve a sufficient extent of inhibitory interaction at the cellular level or did not potentiate docetaxel effects in CYP3A4 overexpressing cells.

Furthermore, we performed CYP induction studies in **P1**, **P2**, **P3**, **P4**, **SP1**, and **SP2**. We showed that they did not significantly increase nor reduce the mRNA levels of *CYP1A2*, *CYP3A4*, and *CYP2B6*. Based on EMA guidelines (EMA, 2012) we concluded that any of these drugs have the potential to perpetrate induction-based drug interactions.

Aim IV. Implementation of primary non-small cell lung cancer (NSCLC) explant and their application for testing of drug combinations.

The establishment of *ex vivo* explants derived from NSCLC biopsies was a demanding, but important step for our research. *In vitro* models are far from the conditions which can be found in *in vivo* models. Primary explants have similar phenotype and behavior as the tumor cells, and therefore, have been highlighted as an important tool for preclinical studies (Miserocchi et al., 2017, Hamacher and Bauer, 2017, Friedman et al., 2015). To verify the real clinical potential of our *in vitro* results obtained with anti-NSCLC drugs, we thus decided to establish primary NSCLC cultures in cooperation with clinicians from University Hospital Hradec Králové. The establishment of this technique and its application for our research is presented in **SP1**. In primary NSCLC explants, protein expression levels of ABC drug efflux transporters (ABCB1, ABCG2, and ABCC1) were detected. Subsequently, we performed

combination studies and demonstrated that tepotinib is able to overcome transporter-mediated resistance to conventional cytostatics in explants with high expression of examined transporters. This outcome confirms our *in vitro* findings observed in transporter-overexpressing cell lines. Additionally, we conducted gene induction studies in explants, which showed that tepotinib does not potentially affect the MDR phenotype of NSCLC cells. Thus, our explant-based findings confirm the potential clinical value of tepotinib as a dual-activity modulator and emphasize the need for following rules of personalized medicine in the use of this therapeutic strategy.

6. SUMMARY, CONCLUSIONS AND PERSPECTIVES

Cancer is a complicated disease with high mortality rates. Tumor formation results in abnormal organ function, and if not treated, is often fatal. Although cancer chemotherapy has tremendously improved, the obstacle of drug resistance has not been overcome yet. Resistance is the result of several mechanisms, both pharmacokinetic and pharmacodynamic origin. Drug transporters and biotransformation enzymes play an essential role in the pharmacokinetic MDR phenomenon. Thus, the study of these mechanisms and the possibility of their modulation is the main aim of the presented dissertation thesis.

Our combination assays demonstrated the abilities of several targeted drugs to effectively antagonize ABC transporter-mediated resistance in a synergistic fashion. These synergistic outcomes are essential factors that determine the clinical success of numerous combination chemotherapy regimens. Synergism allows for drug dose reduction, thus increasing treatment safety while retaining sufficient clinical efficacy (Szakács et al., 2006, Mokhtari et al., 2017). Our results suggested several drug combinations that could be further tested *in vivo* and later in clinical conditions. Moreover, to reduce the risk of developing resistance, it is essential to adhere to the basic principles of anticancer pharmacotherapy, including the modulation of multiple drug transporters at once (Robey et al., 2018). As ABC transporters share overlapping substrate affinity, they can substitute one another in the case of inhibition of single transporter. Several SMTDs tested in our studies target multiple transporters, thus following this rule. Importantly, adherence to the principles of personalized medicine, such as measuring the level of expression and presence of single nucleotide polymorphisms in tumors, is a critical determinant for the successful application of the dual-activity modulators strategy.

A significant part of our research was focused on the investigation of MDR-modulatory properties of SMTDs, which are used for the treatment of NSCLC. Therefore, we developed *ex vivo* primary NSCLC explants derived directly from patient specimens to confirm our *in vitro*

findings and verify their clinical potential. Nevertheless, we would like to take a step further and verify our results in 3D-structured patient-derived *in vivo* NSCLC xenografts; in cooperation with Princess Margaret Cancer Centre at Toronto, Canada, once the epidemiological situation allows us. 3D-organoid xenografts represent the model with the highest possible preclinical quality. We believe that results obtained with these models will reveal the importance of pharmacokinetic MDR mechanisms and help develop the combination strategy that might find an application in clinical practice.

Apart from the dual-activity modulators, we believe that MDR could successfully be antagonized by nanomedicine and targeted distribution (Pucci et al., 2019, Kashkooli et al., 2020). These strategies show enormous potential considering the differential effect of the pharmacokinetic mechanisms, i.e. protective detoxification role in physiological tissues and resistance role in tumors. With these approaches, it would be possible to affect the pharmacokinetic resistance mechanisms (either at the level of activity or expression levels) selectively in the tumor environment.

While the role of ABC transporters in pharmacokinetic resistance has been described in detail, research on the involvement of biotransformation enzymes is far from transporters' situation. In our studies, we used the CYP3A4-overexpressing model and demonstrated the involvement of this enzyme in docetaxel resistance. Interestingly, phase I enzyme-mediated resistance seems to be significantly less powerful than the transporter-based one (based on IC$_{50}$ comparisons of MDR victim drugs in overexpressing/control cells). The relatively low level of resistance pattern was also observed in studies with aldo-keto reductase 1C3, a phase I enzyme playing a role in anthracycline resistance (Novotná et al., 2018a, Novotná et al., 2018b, Tavares et al., 2020, Morell et al., 2020). However, in the ensartinib and dabrafenib studies, we demonstrated that CYP3A4-mediated docetaxel resistance can be reversed along with the antagonism of transporter-based MDR. By targeting both efflux transporters and drug

metabolizing enzymes, drugs like ensartinib and dabrafenib have the potential to simultaneously affect multiple mechanisms of pharmacokinetic MDR, which significantly reduce the risk of avoiding reversal effect by using alternative transport or metabolic pathways.

As mentioned above, adherence to the concept of personalized medicine is a crucial aspect, which must be kept in mind in case of modulation of pharmacokinetic MDR. Thus, patients' selection for clinical studies should be based on the knowledge of their genetic background. This rule is not valid just for ABC transporters, but also for CYP3A4, which exhibits high interindividual variability in expression levels and the presence of single nucleotide polymorphisms (Wojnowski, 2004). Comparison of outcomes of modulating MDR in patients with low vs. high expression of MDR mechanisms will finally verify the clinical relevance and applicability of dual-activity modulators.

In conclusion, cancer and drug resistance are unlikely to be eradicated by mankind, but partial progress can be made to improve therapy in this area, thus helping many patients. We hope that in the future, we will be able to demonstrate the clinical usefulness of at least part of our results, thereby contributing to this progress.

7. LIST OF OTHER OUTPUTS OF THE CANDIDATE

7.1. Other papers of the author not related to the main topic of the study

Sucha S., Sorf A., Svoren M., **Vagiannis D.**, Ahmed F., Visek B., Ceckova M.: ABCB1 as beneficial target of midostaurin in acute myeloid leukemia. *prior to submission*

7.2. Oral presentations

Vagiannis D., Hofman J., Ceckova M., Staud F.. Pharmacokinetic interactons of novel anticancer drugs with ABC drug efflux transporters and Cytochrome P450. Oral presentation at: 8th Postgraduate and 6th Postdoc Conference, Faculty of Pharmacy in Hradec Kralove, Charles University. 24-25 January 2018

Vagiannis D., Hofman J., Staud F.. Interaction of ABC transporters and cytochrome P450 isoforms with the tyrosine kinase inhibitor ensartinib. Oral presentation at: 9th Postgraduate and 7th Postdoc Conference, Faculty of Pharmacy in Hradec Kralove, Charles University. 23-24 January 2019

Vagiannis D., Zhang Y., Budagaga Y., Skarka A., Staud F., Hofman J.. EMD1214063 reverses multidrug resistance by inhibiting the efflux function of ABCB1 and ABCG2 transporters. Oral presentation at: 10th Postgraduate and Postdoc Conference, Faculty of Pharmacy in Hradec Kralove, Charles University. 27-28 January 2020

Vagiannis D., Morell A., Zhang Y., Budagaga Y., Hanke I., Rozkoš T., Hofman J.. The establishment of *ex vivo* primary lung tumor models and their application for testing of drug combinations. Oral presentation at: 11th Postgraduate and Postdoc Conference, Faculty of Pharmacy in Hradec Kralove, Charles University. 22-23 January 2021

7.3. Poster presentations

Vagiannis D., Svoren M., Staud F., Ceckova M., Hofman J.. Protein kinase inhibitor alisertib inhibits ABCC1 drug efflux transporter as well as various CYP450 isoforms and modulates pharmacokinetic multidrug resistance. Poster presentation at Pharmacological days, Faculty of Medicine Charles University Hradec Kralove, 5-7 September 2018

Vagiannis D., Hofman J., Svoren M., Staud F., Ceckova M.. c-Met tyrosine kinase inhibitor tepotinib interacts with ABCB1 and ABCG2 drug efflux transporters as well as various CYP450 isoforms and attenuates pharmacokinetic multidrug resistance in vitro. Poster presentation at International Transmembrane Transporter Society Vienna, 18-21 September 2018

Vagiannis D., Svoren M., Staud F., Ceckova M., Hofman J.. Ensartinib inhibits ABC drug efflux transporters and biotransformation enzymes and modulates pharmacokinetic multidrug resistance *in vitro.* Poster presentation at 2018 NCRI Cancer Conference, Glasgow 4-6 November 2018

Vagiannis D., Skarka A., Staud F., Ceckova M., Hofman J.. c-Met tyrosine kinase inhibitor EMD1214063 is able to effective modulate transporter-mediated cytostatic resistance. Poster presentation at BioMedical Transporters 2019, Lucerne 4-8 August 2019

7.4. Grant projects

Principal investigator

- GAUK 1568218/C - Interactions of novel anticancer drugs with ABC drug efflux transporters and cytochromes P450; their role in multidrug resistance, Grant Agency of Charles University.

Co-investigator

- GAČR 16-26849S - Interactions of protein kinase inhibitors with drug transporters and biotransformation enzymes; role in overcoming resistance in anticancer therapy, Czech Science Foundation.

- GAČR 20-20414Y - Study on the role of novel targeted breast and lung anticancer drugs in the phenomenon of pharmacokinetic drug resistance.

- GAUK 334120/C - The role of pharmacokinetic interactions of new targeted drugs in the modulation of efficacy of cytotoxic drugs in non-small cell lung carcinoma, Grant Agency of Charles University.

- PRIMUS/20/MED/010 - Pharmacokinetic mechanisms of drug resistance in acute myeloid leukemia, their affecting and regulation, PRIMUS.

7.5. Diploma theses, in which candidate was a consultant.

- Author: PharmDr. Gabriella Burianová

  Title: Flow-cytometric analysis of inhibitory effect of novel targeted drugs on the activity of ABC drug efflux transporters. Defended in June 2020. Marked as excellent.

- Author: Júlia Jurčáková

  Title: The assessment of inhibitory effects of selected targeted anticancer drugs on the activity of ABC drug efflux transporters. Expected to defend in June 2021.

7.6. Teaching activities

- lecturer of Practical courses of Pharmacokinetics (summer semester 2018 and 2019)

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