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Implementation of pharmacogenomic biomarkers in precision treatment

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Doctoral (Ph.D.) thesis

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I. ABBREVIATIONS

AIPC	androgen-independent prostate cancer
CR	clinical response
CRPC	castration resistant prostate cancer
CTX	cyclophosphamide
D	dosing
DDR	DNA damage repair
DNA	deoxyribonucleic acid
DLT	dose limiting toxicity
EGFR	epidermis growth factor receptor
EMA	European Medicines Agency
EAU	European Association of Urology
ESMO	European Society for Medical Oncology
EU	European Union
FDA	Food and Drug Administration
HR	homologous recombination
HRD	homologous recombination deficiency
HRDS	homologous recombination deficiency score
HRPC	hormone resistant prostate cancer
i.v.	intravenous
NCT	ClinicalTrials.gov identifier number
mCP	metastatic prostate cancer
mCRPC	metastatic castration resistant prostate cancer
pt/pts	number of patient/patients
OGYEI	National Institute of Pharmacy and Nutrition, Hungary
ORR	objective response rate
OR	overall response
OS	overall survival
pCR	pathologic complete response
PARP	poly ADP-ribose polymerase
PARPi	poly ADP-ribose polymerase inhibitor
PFS	progression free survival
PGx	pharmacogenomics
PM	precision medicine
pRR	pathologic response rate
PFS	progression free survival
PSA	prostate specific antigen
PC	prostate cancer
RR	response rate
rPF	radiographic progression-free survival
RECIST	response evaluation criteria in solid tumors
SNP	single nucleotide polymorphism
SmPC	Summary of Product Characteristics
TOX	toxicity
US	United States of America

II. INTRODUCTION

Pharmacogenomics and precision medicine

Pharmacogenomics (PGx) is a precision medicine (PM) tool to maximize treatment effectiveness while limit the drug toxicity by differentiating responders from non-responders to medications, based on an individual's genetic constitution [1]. PM stands for the accuracy of the diagnosis and the precision with which a diagnosis is made [2]. This concept is augmented by personalized medicine that covers a therapeutic approach to optimize individual therapy in contrast to population based medical decision making and contrary to the use of evidence-based treatment strategies for groups of patients [2, 3]. An adequate tool of both PM and personalized medicine is PGx, that uses clinical testing of genetic variation to assess response to drugs [2].

Owing to recent genetic research, our knowledge of the variability of drug response has advanced significantly in the last decade. It is well known, that genetic factors can modulate pharmacokinetic processes (e.g. absorption, distribution, metabolism and excretion) and pharmacodynamics (e.g. drug response and toxicity) as well [4]. Remarkable minorities of patients carry genetic polymorphisms that affect their response to various drugs, and adverse drug reactions remain a considerable impairment to public health, having a substantial impact on rates of morbidity, death, and on health-care costs [5, 6]. Since more than half of the drugs, most commonly involved in adverse drug reactions, are metabolized by polymorphic enzymes, the possible influence of genetic polymorphisms are worth considering [7]. Several valuable polymorphisms have been already identified and for some of them diagnostic tests are available. Taking into account the patients' genetic status, physicians could anticipate their response to definite drugs, leading to improved efficacy, less adverse drug reactions, and a superior cost-benefit ratio [8].

Despite the scientific results, regulators often encounter challenges by translating data from PGx studies into clinically important and useful product information. Subsequently, scientific evidence is hardly justified as inclusion or exclusion criteria or as any recommendation related to PGx data in drug labels. While the quantity of PGx information grows constantly, translating the complex and from time to time contradictory research results into clinical action requires information updated as soon as new findings evolve [9]. However, regulators made several measures to include PGx information into product descriptions. As a result, PGx has become

an integral part of drug development and pharmacovigilance, as reflected by the incorporation of PGx data in EU product information [10]. Likewise has FDA modified the drug labels or the Summary of Product Characteristics (SmPC) in response to emerging PGx findings [11]. Still, the validation of PGx biomarkers for both the molecular genetic mechanism and clinical effect is demanding [4].

Fortunately, healthcare professionals have a general positive mindset and interest towards PGx tests. However, unambitious own experience and moderate knowledge about interpretation and application of PGx results cause uncertainty in clinicians [12]. Moreover, the lack of clear guidelines translating genetic variation into actionable recommendations [13] and the insufficiency of evidence-based implementation systems discourage medical practitioners of PGx testing. Other barriers in clinical implementation of PGx results are reimbursement challenges and the complexity of the computational approaches [14]. Further obstacles of PGx utilization in clinical setting is the diversity of PGx assays. Thus, standardization of minimal test requirements; standardization of interpretation of variant effects; increase of data availability on cost benefit; improvement and standardization of analyses to promote reimbursement; development of comprehensive cost-effectiveness model as opposed to models for individual drug–gene pairs are needed to extend PGx biomarker use in everyday medical work [15-17].

It is presumable that regulations for drugs and diagnostics are similar between countries, since the same scientific data generated in an increasingly globally harmonized framework have to be evaluated by similar regulatory authorities [18]. Even so, the implementation of international regulatory harmonization of the PGx information in official drug labelling shows wide range of geographical diversity [19]. The European Medicines Agency (EMA) and the United States (US) Food and Drug Administration (FDA) evaluate jointly all phases of drug development to ensure appropriate PGx strategies. EMA is responsible for the centralized marketing authorization applications mainly in the European Union (EU), in Hungary as well. Once granted by the European Commission, the centralized marketing authorization is valid in all EU Member States. In Hungary, several drugs have previously undergone the independent Hungarian national marketing authorization process; therefore, the update of PGx information noted in drug labels might be doubtful. The number of drugs with PGx information in drug labels in EU broadens firmly and it will be a crucial task for the future to refine the legislation on how PGx information should be utilized for drug therapy improvement [10].

Although PGx tests have begun to affect the way medicine is practiced, it is recommended by US FD drug labels in only few clinical fields, mostly for the treatment of certain cancer types [8, 10, 11]. The clinical use of PGx data in oncology has become prevalent, with the vast majority of actionable information consisting of somatic mutations from tumor sequencing. However, a number of oncology drugs have actionable germline PGx information in their drug label as well [20]. Actionable PGx information means that the label includes data about modification of efficacy, dosage, metabolism or toxicity due to gene/protein/chromosomal variation or phenotypes; or the drug is contraindicated in a particular cohort of patients with particular genetic background [21].

Targeted therapies in oncology have undoubtedly set the stage for PM, but the drug–biomarker–disease network is more complex than it might seem at initial glance. For example, for two thirds of FDA approved anticancer drugs the requirement for predictive biomarker testing was established on clinical improvement limited only to biomarker-positive patients in 2015 [22]. Thus, evaluation of PGx data need a careful balance because of the risk of restricting drug indication to the wrong population. Another confusing question is how to evaluate if the targeted therapy was tested in clinical trials with a single biomarker (which is the drug target) and in only one disease, whereas other drugs have been tested with more biomarkers (which are not the drug's target) and in several diseases [23].

Prostate cancer

Prostate cancer (PC) is the second most common cancer in men and one among the leading causes of death among Western males [24]. Despite its prevalence, mortality and extensive scientific research the treatment of metastatic prostate cancer (mPC) is still highly challenging [25, 26]. Docetaxel chemotherapy was approved 15 years ago to treat metastatic castration resistant prostate cancer (mCRPC) and stayed the standard management for this disease stage [25]. Other drugs have since been developed, some of them are administered in combination with docetaxel, but docetaxel remained the first choice chemotherapeutic agent in mCRPC treatment [27]. However, the majority of patients develop eventually resistance and are not responding to any current therapies on long run. It is clear, that new clinical targets and therapies have to evolve for better and more personalized treatment options in aggressive and castration resistant prostate cancer (CRPC).

Every cell type has its individual molecular signature and traceable characteristics such as levels or activities of genes, proteins, or other molecular features; therefore, biomarkers can enhance the molecular definition of cancer [28]. Specifically, cancer biomarkers are biomolecules used for assessment of cancer development risk in a specific tissue or, alternatively, for estimation of cancer progression risk or potential therapy response [29]. Since PC has a high heritability [30], inherited biomarkers of genomic signature can be the foremost tool to guide treatment.

Treatment-associated inherited (germline) genomic biomarkers are principally static, can be easily detected and are powerful predictors of drug response, resistance and toxicity. Biomarkers, including somatic genomic alterations, structural variants (e.g. gene fusions, gene rearrangements), splice variants, miRNAs, and differential gene expression and methylation markers have also been shown to influence docetaxel treatment in PC [31]. However, we have to highlight that in the official docetaxel drug labels mandated by FDA [11] and EMA [32] there are no PGx biomarkers declared to guide PC treatment.

As reported by a study of biopsies from CRPC metastases the following common, potentially actionable or prognostic genomic alterations have been identified: *ERG* gene fusion (40%–50%), *AR* gene point mutation or amplification (50%–60%), *TP53* mutation or deletion (40%–50%), *PTEN* deletion (40%–50%), *RB1* deletion (20%), and alterations in DNA repair genes (20%). The analyses of circulating tumor DNA in CRPC patients showed intra-patient molecular heterogeneity and the possibility to follow dynamic changes during the course of therapeutic response and the appearance of resistance. It has to be underlined, that bi-allelic loss of DNA repair genes (e.g. *BRCA1/2, ATM*) correlated with CR during the poly ADP-ribose polymerase (PARP) inhibitor, olaparib treatment [33]. According to its new molecular mechanism, PARP inhibitors (PARPi) seem to open a new chapter in targeted management of mCRPC and became the focus of recent clinical investigations.

PARP enzymes are participating in base excision repair (the repair of DNA single-strand breaks) and alternative end joining (repair of DNA double-strand breaks) [34, 35]. DNA damage repair (DDR) mutations tend to make cancer cells more reliant on PARP than normal cells with full DNA repair capacity [36]. It has been proven that *BRCA1* or *BRCA2* DDR gene defects sensitize cells to PARP inhibition, which leads to the persistence of DNA lesions normally reversed by homologous recombination repair (HRR), and consequently results in chromosomal instability, cell cycle arrest and subsequent apoptosis [43,44]. This mechanism makes PARP a tempting target for cancer therapy.

PARPi treatment efficacy is highly dependent on the DDR gene mutations of PC patients, hence genetic biomarker based patient selection will be required for precision oncology in PC. The reasoning for using PARPi in PC treatment is the considerable genetic defects of DDR genes in mCRPC [37-39]. The incidence of inherited DDR mutations among men with mPC was found significantly higher (11.8%), than the incidence among men with localized PC (4.6%) and in the general population (2.7%) according to a multicentric study [40]. In addition, due to further investigations almost 23% of mCRPC patients have somatic DDR gene defects as well. Of these, *BRCA2, BRCA1*, and *ATM* account for 19.3% overall, and they were considerably more frequent in mCRPC patients compared to those with primary PC. Other possibly significant DDR gene mutations were found in *CDK12*, *FANCA*, *RAD51B* and *RAD51C* genes [42]. However, *BRCA1* and *BRCA2* mutations were identified to be the most frequent DDR gene mutations in patients with mCRPC [41].

Treatment of mCRPC is still an unsolved problem with significant personal and populational burden, and the need to target each case in a personalized manner is increasing. Personalized treatment approach is expected to improve patient response when applying targeted treatment for their specific disease [45].

Basic issues of the thesis

Although the scientific background of PGx biomarkers broadens gradually, the clinical application pursues far behind. In my thesis, first I assessed the applicability of the major PGx biomarker information resource of practicing clinicians - the drug labels – and investigated the potential role of PGx in clinical decision-making. The conclusion of my first study is that the most dominant clinical field of PGx biomarker implementation is oncology. This led me to study more extensively cancer, specifically PC, one of the leading causes of male death. Therefore, evaluation of PGx biomarkers in standard docetaxel chemotherapy of mCRPC was my second step to estimate the translational potential of PGx biomarkers in practice. As new therapies for mCRPC are around the corner, I investigated potential candidate PGx biomarkers of PARPi treatment of PC as third step. Finally, I highlighted the innovative technique of "liquid biopsy" for medical practitioners in my local language, in Hungarian, to pinpoint a future method for biomarker detection and precision medicine in cancer management.

III. AIMS OF THE THESIS

This thesis aimed to examine how PGx biomarkers are applied in clinical practice in context of drug labels and what are the current and future perspectives of PGx in the specific field of PC. The following **research questions** have been formulated:

1. **What are the PGx biomarker information differences between drug labels in the United States and Hungary?**

   1.a What is the current status of PGx biomarker information present in Hungarian and US drug labels in 2019?

   1.b Can we observe any dynamic change in perspective of PGx biomarkers in Hungarian and US drug labels?

   1.c Can we highlight any differences in the level of action of PGx biomarkers between Hungary and US according to drug labels?

   1.d What are the obstacles of PGx implementation into medical practice based on the information present in Hungarian drug labels?

   1.e. What recommendations can be made to enhance the uptake of PGx implementation by medical practitioners?

2. **Do PGx biomarkers modulate docetaxel treatment of PC?**

   2.a Which germline genomic biomarkers play a potential role in docetaxel monotherapy and docetaxel combination treatment of PC based on research studies?

   2.b What types of genomic biomarkers are incorporated in docetaxel clinical trials for PC?

   2.c Are PGx biomarkers included in treatment guidelines of PC?

   2.d What are the challenges and possible solutions of moving PGx biomarkers into clinical setting of PC treatment?

3. **Which candidate genetic biomarkers are identified in PARPi clinical trials of PC?**

    3.a Are PGx biomarkers applicable for future patient selection for targeted PARPi therapy in PC?

    3.b Do genomic biomarkers predict endpoints in PC clinical trials?

    3.c According to preliminary results of PC clinical trials which gene mutations affect these endpoints?

    3.d What are our future recommendations to improve PGx biomarker transition into medical practice?

4. **What are the future perspectives of detection and analysis of circulating cell-free DNA in cancer patients' blood?**

IV. OUTLINE OF THE THESIS

Paper 1 evaluated the US FDA information on available PGx biomarkers in drug labelling in comparison to Hungarian SmPCs of the same active substance in 2019. PGx information on the level of action was compared in the two countries. Equal data collection performed in spring 2017 enabled to provide an overview about the dynamic change of the implementation of PGx information in Hungarian drug labels. This research study highlights *available Hungarian resources for PGx biomarker implementation in medical practice,* and pinpoints potential needs to enhance it. This paper answered the research question 1.

Paper 2 investigated research studies for germline genomic biomarkers affecting individual differences in docetaxel monotherapy and combination treatment of PC published between 2006 and 2018. In addition, clinical trials for docetaxel treatment in PC incorporating a range of genomic signatures have been identified both from ClinicalTrials.gov and from EU Clinical Trials Register database. The PC treatment guidelines of the European Association of Urology (EAU) and European Society for Medical Oncology (ESMO) [46, 47] were reviewed for recommendations on pharmacogenetic testing in connection with docetaxel treatment of PC. *Synthesis of knowledge about clinical translational potential of identified germline genomic biomarkers in docetaxel treatment of PC* has been done. This paper answered the research questions 2.

Paper 3 presented the results of a study where the publicly available database www.clinicaltrials.gov was mined for the registered clinical trials to *identify candidate genetic biomarkers in PARP inhibitor clinical trials for possible application in precision treatment selection of PC patients*. This paper answered research questions 3.

In **Paper 4,** we discussed the *potential role and future perspective of "liquid biopsy" in cancer patient management and treatme*nt in comparison to classic tissue biopsy. The paper was published *in Hungarian* in order to enhance medical practitioner knowledge on their local language. This paper answered research question 4.

The **Novel findings** section of the academic dissertation lists the results of the PhD candidate.

The **Summary of new observations and future perspective** section gives a recapitulative overview of the thesis, recommendations for clinical practice, research and regulatory agencies.

V. PAPERS

Paper 1.

Pharmacogenomic biomarker information differences between drug labels in the United States and Hungary: implementation from medical practitioner view

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ARTICLE

Pharmacogenomic biomarker information differences between drug labels in the United States and Hungary: implementation from medical practitioner view

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Abstract

Pharmacogenomic biomarker availability of Hungarian Summaries of Product Characteristics (SmPC) was assembled and compared with the information in US Food and Drug Administration (FDA) drug labels of the same active substance (July 2019). The level of action of these biomarkers was assessed from The Pharmacogenomics Knowledgebase database. From the identified 264 FDA approved drugs with pharmacogenomic biomarkers in drug label, 195 are available in Hungary. From them, 165 drugs include pharmacogenomic data disposing 222 biomarkers. Most of them are metabolizing enzymes (46%) and pharmacological targets (41%). The most frequent therapeutic area is oncology (37%), followed by infectious diseases (12%) and psychiatry (9%) ($p < 0.00001$). Most common biomarkers in Hungarian SmPCs are *CYP2D6, CYP2C19*, estrogen and progesterone hormone receptor (ESR, PGS). Importantly, US labels present more specific pharmacogenomic subheadings, the level of action has a different prominence, and offer more applicable dose modifications than Hungarians (5% vs 3%). However, Hungarian SmPCs are at 9 oncology drugs stricter than FDA, testing is obligatory before treatment. Out of the biomarkers available in US drug labels, 62 are missing completely from Hungarian SmPCs ($p < 0.00001$). Most of these belong to oncology (42%) and in case of 11% of missing biomarkers testing is required before treatment. In conclusion, more factual, clear, clinically relevant pharmacogenomic information in Hungarian SmPCs would reinforce implementation of pharmacogenetics. Underpinning future perspective is to support regulatory stakeholders to enhance inclusion of pharmacogenomic biomarkers into Hungarian drug labels and consequently enhance personalized medicine in Hungary.

Introduction

Pharmacogenomics (PGx) is one of the precision medicine (PM) tools to be applied to maximize treatment effectiveness, while limit the drug toxicity by differentiating responders from nonresponders to medications, based on an individual's genetic constitution [1]. Pharmacogenomic information may be provided in drug labeling to inform healthcare providers about the impact of genotype on response to a drug through description of relevant genomic markers, functional effects of genomic variants, dosing recommendations based on genotype, and other applicable genomic information [2]. This can

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describe variability in clinical response and drug exposure, risk of adverse events, genotype-specific dosing, mechanisms of drug action, polymorphic drug target and disposition genes or trial design features [3].

Information on PGx biomarkers and laboratory testing provides the resource for practicing medical doctors to apply personalized medicine in clinic [4]. In order to implement PGx in clinical setting, practicing doctors need to have both information on PGx biomarkers or guidelines implementing the use of biomarkers, and available laboratory tests as input, and handy implementation tools to be able to generate output in clinics.

The drug labeling for some, but not all, of the products includes specific actions to be taken based on the PGx biomarker information. This information can appear in different sections of the labeling depending on the actions [3].

One would expect regulations for drugs and diagnostics not to differ significantly between countries, given that regulatory authorities evaluate the same scientific data generated in an increasingly globally harmonized context [5]. Despite international regulatory harmonization, implementation of the pharmacogenomic information in official drug labeling shows wide range of geographical variety [6]. The US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) work jointly and in multiple ways on scientific evaluation of drugs to ensure that pharmacogenomic strategies are applied appropriately in all phases of drug development. EMA is responsible for the centralized marketing authorization applications in the European Union and some additional countries. Once granted by the European Commission, the centralized marketing authorization is valid in all European Union Member States, in Hungary as well. However, several drugs have undergone the Hungarian national marketing authorization process previously, therefore the PGx information might be not updated.

The ultimate aim and rationale of this study is to:

(1) Provide an evaluation of current status of PGx biomarker information present in Hungarian drug labels.
(2) Summarize the potential needs of medical practitioners, healthcare providers.
(3) Identify the gaps of PGx implementation and potential solutions.

Materials and methods

All data presented in this work have been collected in July 2019. Consequently, the US FDA information on available pharmacogenomic biomarkers in drug labeling represents the most up-to-date current content as of 26 March 2019 (https://www.fda.gov). The Hungarian Summaries of Product Characteristics (SmPCs) of the same active substance were assessed from the National Institute of Pharmacy and Nutrition database of Hungary (www.ogyei.gov.hu/gyogyszeradatbazis/). PGx information on the level of action was collected on PharmGKb® (www.pharmgkb.org) and compared with the same information from the Hungarian SmPCs. Identical data collection was performed in 2017 spring, providing the opportunity to have an overview about the dynamic change of the implementation of PGx information in Hungarian drug labels.

Biomarkers in our investigation include but are not limited to germline or somatic gene variants (polymorphisms, mutations), functional deficiencies with a genetic etiology, gene expression differences, and chromosomal abnormalities; specific protein biomarkers that are used to select treatments for patients are also included.

The investigation does not include nonhuman genetic biomarkers (e.g., microbial variants that influence sensitivity to antibiotics), biomarkers that are used solely for diagnostic purposes (e.g., for genetic diseases) unless they are linked to drug activity or used to identify a specific subset of patients in whom prescribing information differs, or biomarkers that are related to a drug other than the referenced drug (e.g., influences the effect of the referenced drug as a perpetrator of an interaction with another drug).

For drugs that are available in multiple dosage forms, salts, or combinations, a single-representative product is listed. In the case of combination products, the single agent associated with the biomarker is listed unless the agent is only approved as a combination product, in which case all agents are listed.

We assessed PGx level of action categories according to PharmGKb® [7] of the doctor targeted section of Hungarian drug label as (1) testing required, (2) testing recommended, (3) actionable with dosing info, (4) actionable, and (5) informative.

In order to measure the statistical differences, two-sided p values were calculated using Pearson's chi-squared test or Fisher's exact test. A p value < 0.05 was considered to indicate a statistically significant result. Statistical analyses were performed applying Microsoft® Excel® for Mac® 2011 and IBM® SPSS® Statistics Version25 for Mac (SPSS Inc., Chicago, IL, USA).

Results

We identified 264 drugs in the US FDA Table of Pharmacogenomic Biomarkers in Drug Labeling after excluding duplicate active ingredients. Out of these 264 active ingredients we were able to identify 195 (74%) through the

website of the National Institute of Pharmacy and Nutrition in Hungary being available in Hungary (Table 1). Among the 195 drugs, 145 (75%) have PGx information included in the Hungarian product summary. Important to note that while taking a point-in-time snapshot, the number of drugs with PGx information in the drug label has elevated in the US with 57% vs in Hungary with 46% in last 26 months. PGx information is partially present in drug label of 20 (10%), completely missing from drug label of 30 (15%) available active ingredients in Hungary compared with US FDA (Table 1, italic and bold, respectively). These drugs without PGx biomarker information in their label belong to diverse therapeutic areas (23% oncology, 23% anesthesiology, 20% infectious diseases, 7% cardiology, 7% inborn error, 7% rheumatology, 3% dermatology, 3% hematology, 3% psychiatry, and 3% pulmonology). The 69 drugs not available in Hungary are listed in Supplementary Table 1. The distribution of therapeutic areas of drugs with PGx information in their labeling is presented on Fig. 1. The most frequent therapeutic area is oncology (37%), followed by infectious diseases (12%), psychiatry (9%), and neurology (8%) (χ^2 $p < 0.00001$).

As one drug's PGx can be affected by more than one specific biomarker, the identified 165 drugs with PGx data (including drugs with partially present data) dispose 222 biomarkers in the Hungarian SmPCs summarized in Table 2. In the Hungarian SmPCs, we identified information either on metabolizing enzymes ($n = 102$, 46%), pharmacological targets ($n = 90$, 41%), or other features ($n = 30$, 13%).

The most common biomarkers in Hungarian SmPCs are the *CYP2D6* ($n = 40$, 18%), the *CYP2C19* ($n = 18$, 8%), the estrogen and progesterone hormone receptors (ESR, PGR, $n = 15$, 6%), the *ERBB2* ($n = 12$, 5%), and the *G6PD* ($n = 10$, 4%). We also observed that none of the SmPCs containing PGx biomarker data has any PGx evidence specifically for Hungarian population, neither on clinical endpoints nor on pharmacokinetics.

Pharmacogenomic biomarkers influence the drug treatment on several different ways, thus one biomarker can have more than one impact. According to the Hungarian product summary, the aim of pharmacogenomic biomarker use can be the following: effects efficacy ($n = 84$), indicates toxicity ($n = 67$), belongs to the inclusion criteria ($n = 67$), belongs to the exclusion criteria ($n = 24$) because of elevated toxicity risk or effect dosage ($n = 18$). Moreover, 53 biomarkers (24% of all) are involved in drug–drug interaction management as dose modification or elevated toxicity risk is connected to the presence of enzyme inhibitor/inductor irrespective of the pharmacogenomic background. Highly importantly, eight biomarkers (4 %) are factual in point of dosing and formulate exact algorithm to manage gene–drug interaction.

Out of the biomarkers available in US drug labels, 62 (22%) are missing from the Hungarian SmPCs ($p < 0.00001$, Fisher's exact test). Our dynamic update shows that the percentage of missing PGx data in Hungarian drug labels has doubled in last 26 months as a result of accelerated PGx biomarker implementation in US FDA drug labeling. Most of the missing pharmacogenomic biomarkers belong to the therapeutic area of oncology (42%), followed by anesthesiology (18%), infectious diseases (13%); hematology (8%); cardiology, dermatology, gastroenterology, inborn errors of metabolism, psychiatry, pulmonology, rheumatology represent minor proportions (<4% each).

In order to be able to compare the level of action of PGx biomarkers between Hungary and the United States, we extracted the information from the Hungarian SmPCs for US FDA approved drugs available in Hungary and compared with the level of action available on The Pharmacogenomics Knowledgebase (www.pharmgkb.org) (Table 3). Testing is required at 72 biomarkers (25 %) in Hungary, from which 66 (92%) belong to field of oncology. In United States, in case of 79 (28%) biomarkers is testing obligatory before treatment. Four (1%) biomarkers in Hungarian drug labels are ranked into testing recommended category, six (2%) biomarkers in the United States. PGx information is actionable at 95 (34%) biomarkers in Hungary, compared with 108 (38%) in the United States. Out of the actionable biomarkers, 14 (5%) biomarkers dispose exact dosing adjustment in PharmGKB recommendation, but only eight (3%) of them are ranked into the same category in Hungary. The six (3%) remaining biomarkers predispose only actionable PGx data without dosing info in Hungarian drug inserts. Fifty-one (18%) biomarkers have informative PGx data in Hungarian drug label; however, in the United States 77 (27%) biomarkers are counted into this category ($p = 0.009$). Even from FDA US biomarkers 14 (5%) are missing from PharmGKB, which shows generally a rather delayed implementation of PGx information. This is the case for 62 (22%) biomarkers for Hungarian SmPC's ($p < 0.00001$).

Talking about the PGx level of action, out of the 62 missing biomarkers from Hungarian SmPC's 7 (11%) belong to testing required category, 27 (44%) belong to actionable PGx category and 21 (29%) belong to informative PGx category according to PharmGKB.

In order to implement PGx in everyday medical practice, we need to translate PGx biomarker information into drug level. It practically means that partially missing biomarkers in Hungarian SmPCs belong to 20, completely missing biomarkers to 30 drugs shown in Table 1. Notably, after checking the level of action, in case of 7 from these 50 drugs biomarker testing is required before treatment according to PharmGKB. It is of utmost importance that six from these seven drugs belong to oncology medication and

Table 1 Drugs in the Hungarian National Institute of Pharmacy and Nutrition database with complete ($n = 145$), with partial ($n = 20$ italic), and without ($n = 30$ bold) pharmacogenomic information in their Summary of Product Characteristics[a]

Abacavir	Diazepam	Lenalidomide	Ponatinib
Abemaciclib	Dinutuximab	Lesinurad	Prasugrel
Afatinib	Docetaxel	Letrozole	Pyrazinamide
Alectinib	Dolutegravir	**Lidocaine**	Prilocain
Amifampridine	Donepezil	*Lorlatinib*	Propafenone
Amitriptyline	Drospirenone	Lumacaftor	Propranolol
Anastrozole	Duloxetine	**Lusutrombopag**	Quinidine
Aripiprazole	Durvalumab	**Mepivacaine**	Quinine Sulfate
Arsenic Trioxide	Efavirenz	*Mercaptopurine*	Rabeprazole
Articaine	Elbasvir	Methylene Blue	**Raloxifene**
Atomoxetine	Eliglustat	*Metoclopramide*	Raltegravir
Avatrombopag	Elosulfase	Metoprolol	*Rasburicase*
Avelumab	Eltrombopag	*Midostaurin*	Ribociclib
Azathioprine	Encorafenib	Migalastat	**Rifampin**
Binimetinib	*Eribulin*	Mirabegron	Risperidone
Blinatumomab	Erlotinib	Mivacurium	Rituximab
Bosutinib	Escitalopram	Mycophenolic Acid	**Rivaroxaban**
Brentuximab	Esomeprazole	Nebivolol	**Ropivacaine**
Vedotin	Ethinyl Estradiol	Neratinib	Rosuvastatin
Brexpiprazole	Everolimus	Nilotinib	Rucaparib
Brigatinib	*Exemestane*	Niraparib	**Sevoflurane**
Brivaracetam	Fesoterodine	Nitrofurantoin	**Sodium**
Busulfan	Fluorouracil	*Nivolumab*	**Phenylbutyrate**
Cabozantinib	Fluoxetine	Nusinersen	**Sofosbuvir**
Capecitabine	**Flurbiprofen**	Obinutuzumab	Sulfadiazine
Carbamazepine	**Flutamide**	Olaparib	*Sulfamethoxazole*
Carglumic Acid	Fluvoxamine	Olaratumab	*Sulfasalazine*
Cariprazine	**Formoterol**	*Ombitasvir*	Talazoparib
Carvedilol	Fulvestrant	Paritaprev	**Tamoxifen**
Ceftriaxone	Galantamine	**Ombitasvir**	Tamsulosin
Celecoxib	Gefitinib	Omeprazole	Tetrabenazine
Ceritinib	Glimepiride	Ondansetron	**Tetracain**
Cerliponase Alfa	**Goserelin**	Osimertinib	Tezacaftor
Cetuximab	Grazoprevir	Ospemifene	Ticagrelor
Chloroquine	Ibrutinib	Oxcarbazepine	Toremifene
Cisplatin	Imatinib	**Oxymetazoline**	Tramadol
Citalopram	**Imipramine**	Palbociclib	*Trametinib*
Clobazam	Indacaterol	Palonosetron	Trastuzumab
Clomipramine	Inotersen	*Panitumumab*	**Tretinoin**
Clopidogrel	Inotuzumab	Pantoprazole	*Trimethoprim*
Clozapine	Ozogamicin	**Parathyroid**	Umeclidinium
Cobimetinib	*Ipilimumab*	**Hormone**	**Ustekinumab**
Codeine	Irinotecan	Paroxetine	Valproic Acid
Crizotinib	Isoflurane	Patisiran	Vemurafenib
Dabrafenib	Isoniazid	Pazopanib	*Venetoclax*
Daclatasvir	**Isosorbide**	**Peginterferon**	Velpatasvir
Dacomitinib	**Mononitrate**	**Alfa-2b**	Venlafaxine
Darifenacin	Ivacaftor	*Pembrolizumab*	**Vincristine**
Dasabuvir	Lacosamide	Pertuzumab	Voriconazole
Dasatinib	Lansoprazole	Phenytoin	Vortioxetine
Dexlansoprazole	Lapatinib	**Piroxicam**	Voxilaprevir
Dextromethorphan	**Ledipasvir**		Warfarin

The table represents the status of 2019 July

[a]Out of 264 FDA listed drugs with pharmacogenomic biomarkers in drug labeling, 195 are marketed in Hungary

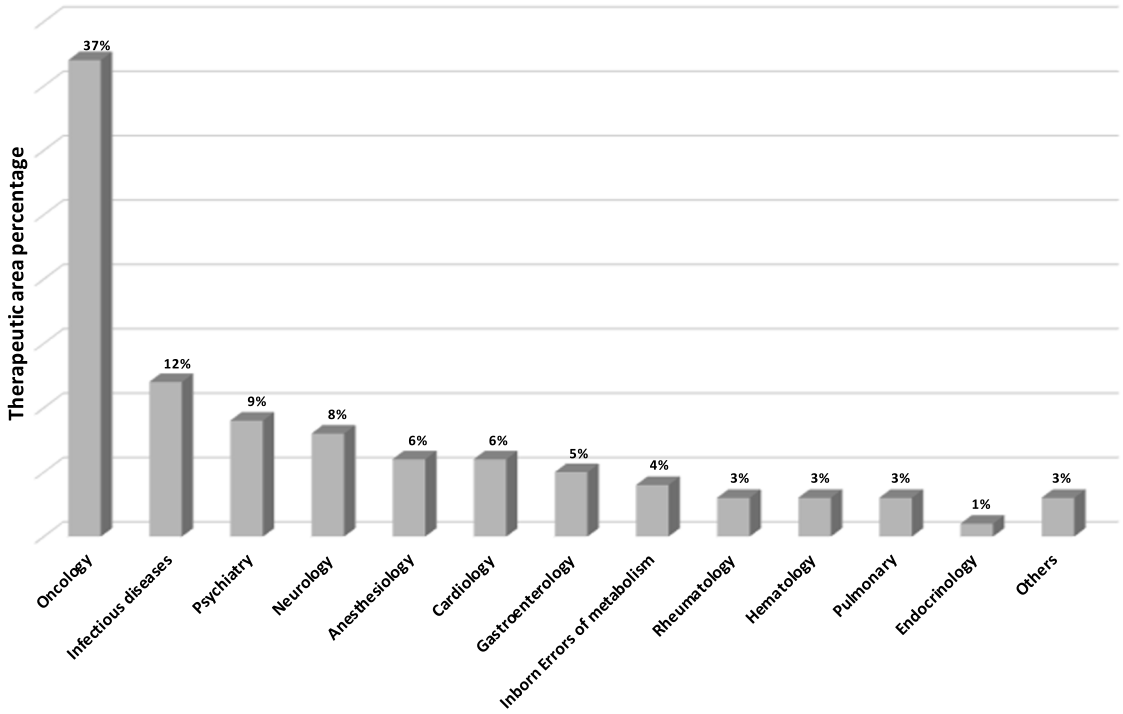

Fig. 1 Therapeutic areas of drugs with pharmacogenomic information in their labeling in Hungary

therefore define cancer treatment. On the other hand, in case of nine oncology drugs, the Hungarian SmPCs are even stricter than the FDA recommendation and genetic testing is required before treatment.

Hungarian SmPCs mention information on lab test availability at 76 biomarkers (34%). However, the product summary does not ever refer on an exact laboratory in Hungarian drug label. The information on lab test availability is based on clinics internal regulation and doctor's daily routine either on commercial test or on academic setting.

Discussion

PM strategies and PGx are becoming more prevalent in research and clinical practice and are integral part of drug development. Therefore, including appropriate pharmacogenomic information and accurate description in drug labels intend to support medical professionals and patients is critical [2, 8].

Territorial differences in drug label content of PGx biomarker information depending on responsible approval agencies do exist. For example, it is well known that cytochrome P450 pharmacogenetic information included in US FDA drug labels present significantly more specific pharmacogenetic information than analogous EU SmPCs [9].

Therefore, comparing labeling of medicines in Hungary versus the United States may identify gaps to solve. While investigating similarities and differences of PGx information in the United States and Hungarian drug label content, we identified that US labels presented significantly more specific pharmacogenetic subheadings than analogous Hungarian SmPCs. As 62 PGx biomarkers are missing completely from Hungarian SmPCs, Hungarian drug labels may need to be supplemented in future with the pharmacogenetic biomarker information in case of these active substances.

Our study demonstrates that the most frequent therapeutic area with pharmacogenomic information in the drug label is oncology both in the United States and in Hungary. This is in line with the EMA statement that PGx information are preferentially present in drug labels having antineoplastic properties [10]. In the field of oncology, pharmacogenetic biomarkers represent a complex combination of germline and somatic variants [11]. Importantly, somatic mutations in tumor cell are increasingly implicated biomarkers in targeted therapy, applied in treatment selection, and are also often associated with treatment efficacy [12]. This is well represented in Hungarian drug labels since the main aim of pharmacogenomic biomarker use is to tailor treatment efficacy. On the other hand, hereditary variants affect pharmacokinetics and pharmacodynamics, and are more often considered to address adverse drug reactions. Tumor sequencing for somatic mutation detection is applied

Table 2 Pharmacogenomic biomarkers in Hungarian Summaries of Product Characteristics of 165 drugs

Biomarker		Frequency ($n = 222$)	Percentage (%)
Metabolizing enzyme ($n = 102$)	CYP2D6	40	18.00
	CYP2C19	18	8.01
	G6PD	10	4.05
	UGT1A1	7	3.02
	CYP2C9	6	2.07
	CYP2B6	3	1.04
	DPYD	3	1.04
	NAT1	2	0.09
	TPMT	2	0.09
	BCHE	1	0.05
	CYP1A2	1	0.05
	CYP3A5	1	0.05
	GALNS	1	0.05
	GLA	1	0.05
	HPRT1	1	0.05
	NAGS	1	0.05
	NAT2	1	0.05
	SLCO1B1	1	0.05
	Urea cycle disorder	1	0.05
	VKORC1	1	0.05
Target ($n = 90$)	ESR, PGR	15	6.07
	ERBB2	12	5.05
	BCR-ABL1	8	3.06
	BRAF	8	3.06
	EGFR	6	2.07
	ALK	5	2.03
	Del 5q/17p/11q	5	2.03
	RAS	5	2.03
	BRCA	4	1.80
	CD274	4	1.80
	CFTR	2	0.09
	KIT	2	0.09
	MS4A1	2	0.09
	TTR	2	0.05
	FIP1L1-P	1	0.05
	FLT3	1	0.05
	PDGFRA	1	0.05
	PDGFRB	1	0.05
	PML-RARA	1	0.05
	RET	1	0.05
	ROS1	1	0.05
	SMN2	1	0.05
	TNFRSF8	1	0.05
	TP53	1	0.05
Other ($n = 30$)	HLA-B	5	2.03
	IFNL3	5	2.03
	F5	2	0.09
	HLA-A	2	0.09
	PROC	2	0.09
	PROS1	2	0.09
	SERPINC1	2	0.09
	Nonspecific (congenital methemoglobinemia)	1	0.05
	CYB5R	1	0.05
	F2	1	0.05
	HLA-DQA1	1	0.05
	IGH	1	0.05
	MYCN	1	0.05
	NUDT15	1	0.05
	POLG	1	0.05
	RYR1	1	0.05
	TPP1	1	0.05

The table represents the status of 2019 July

Table 3 Comparison of the level of action of pharmacogenomic information acquired from Hungarian SmPCs and the PharmGKB annotation of US FDA pharmacogenomic biomarkers ($n = 284$)

Pharmacogenomic level of action	Hungarian SmPC, *n* (%)	US FDA on PharmGKB, *n* (%)	*p* value*
Testing required	72 (25)	79 (28)	0.506
Testing recommended	4 (1)	6 (2)	0.523
Actionable	95 (34)	108 (38)	0.255
Informative	51 (18)	77 (27)	**0.009**
Missing	62 (22)	14 (5)	**<0.00001**

Based on 2019 July status

*χ^2 test; statistically significant difference is marked with bold, $p < 0.05$;

in Hungarian institutions, and produces matched germline information. However, targeted tumor genome sequencing, to provide precision treatment decisions for patients, more relevantly reflects the local practices. Most commonly tested biomarkers in oncology in Hungary are pharmacological targets, where molecular diagnostics is required for patient selection and personalized genotype-directed therapy. For example, EGFR/KRAS/ALK in non-small cell lung carcinoma, or BRAF, NRAS in melanoma, in agreement with the ESMO guidelines [13, 14]. In addition, BRCA1/2 are tested in breast and ovarian cancers, but it is not obligatory. In other tumors there is less consensus.

According to our results, US labels scored the level of action of PGx information on the same overall quality than the analogous Hungarian SmPCs, but the prominence is different. Hungarian SmPCs are stricter regarding oncological drugs than US labels. Rigor towards genetic testing before oncology drug treatment in Hungary may be caused by the high cost of these target molecules, therefore confirmation of efficacy is rather obligatory before treatment. However, the proportion of requirement or recommendation for PGx testing is higher in oncology than in other therapeutic areas in the United States [15]. Of note, FDA offers more applicable information about dose modifications than Hungarian SmPCs. FDA has recognized genetic differences in drug metabolism where clinically relevant drug–drug interactions or gene–drug interactions trigger dose adjustment or use of alternative drugs [16].

Considering differences in gene expression and physiological maturation between pediatric and adult populations, extrapolation of adult pharmacogenetic information in FDA approved pediatric drug labels is not always appropriate [17, 18]. Ontogeny-associated treatment response differences are specifically important in pediatric oncology drugs [18]. Nonetheless, pharmacogenomic biomarker information is commonly based on adult studies both in Hungarian SmPCs and FDA drug labels.

Classification of PGx biomarkers (e.g. metabolizing enzymes, pharmacological targets, and others) is not available in Hungarian data resources. Categorization of biomarkers need to be implemented in Hungarian SmPC's, in order to clarify PGx information and consequently enhance genetic biomarker testing in daily medical routine.

Pharmacogenetics-related drug-labeling updates do not always result in uniform clinical uptake of pharmacogenetic testing. Lack of simultaneous implementation of newly approved drugs linked to companion diagnostic biomarkers into the clinical practice has several reasons. Potential factors leading to heterogeneity in clinical uptake of pharmacogenetic testing include the strength of supportive evidence (1), which may originate from low contribution of known genetic variant to outcome or incomplete understanding of genetic variation effect; the consequences of a targeted adverse event or treatment failure (2); the availability of alternative agents or dosing strategies (3); the predictive utility of testing (4); test cost-effectiveness, accessibility, and turnaround time (5); reimbursement issues (6); professional society positions (7); or simple general resistance to use of genetic tests (8) [19, 20]. For example, information on lab test availability is unattached to Hungarian drug label and must have different source in the everyday medical work. The crucial solution can be establishment of the Europe-wide database for PGx laboratory test availability. Tough, a limited set of PGx biomarker test is available in Hungary, provided by three university laboratories (Pécs, Budapest, and Debrecen). All available obligatory tests are reimbursed by the Hungarian State Insurance if the genotyping has been done in noncommercial laboratory. The genotyping approach, the laboratory contacted depend on personal practice of the specific doctors. Also, implementation platforms delivering ready-to-apply genetic results in clinic are missing. In order to take advantage of PGx biomarkers in clinical practice integration with other personalized medicine approaches is also needed. On the other hand, preemptive pharmacogenomic testing of actionable genetic markers predicting systemic exposure can be the most future oriented approach to use PGx biomarkers in practice. All of these will unequivocally enhance the rate of uptake of PGx information by medical practitioners.

Acceleration is seen in implementation of PGx info both in the United States and Hungary, though the regulatory dynamics is different. In case regulatory agencies enhance the inclusion of PGx biomarker information in Hungarian drug labels less technical barriers hinder the implementation of PM. The laboratory and professional requirements for all FDA biomarker testing are certainly available in Hungary. Although, pharmacogenomic knowledge of healthcare professionals and the corresponding medical education in PGx [21], as one of the key factors in implementation, need to be improved as well [22].

Hungarian drug labels do not contain any PGx evidence for Hungarian population neither on clinical endpoints nor on pharmacokinetics. Regulatory approval and submission of new drug application are based on international clinical trial's outcome in Hungary. However, this can be due to the low number of inhabitants in Hungary (ten Million) and the population's genetic heterogeneity. More focus may be given to the investigation of dose and regimens for special populations before applying for marketing authorization. Consequently, regulators could review dose–exposure–response data with more certainty and better define dose recommendations in the label [23]. For unlicensed drugs we suggest representing PGx information in the SmPCs before marketing authorization such as for drugs under renewal or variation process.

Limitations of the study include the followings. The field of PGx is rapidly advancing, therefore drug labeling is not static. Updating PGx information is a dynamic process and new markers are constantly being added. This is shown by 57% elevation of FDA drugs with PGx biomarkers in their labeling in last 26 months, compared with 46% in Hungary. However, the timelines used by the Hungarian authorities to update SmPCs according to FDA drug labels are hard to predict.

In this study, FDA listed drugs ($n = 264$) with pharmacogenomic biomarkers in drug labeling were compared with drugs in the Hungarian National Institute of Pharmacy and Nutrition database with potential pharmacogenomic information in their SmPCs. Some active ingredients in Hungarian SmPCs may exist with pharmacogenomic information, although not mentioned by the FDA. These drugs remained hidden in our study.

According to a previous study, pharmacogenetic information is included in patient-targeted sections for a minority of drug labels [24]. Our research focused on drug labels' doctor targeted section, but rather superficial content of patient information leaflet was ignored.

Original active agents were investigated in the study. Differences between original and generic drug's label were neglected.

This study was performed in support for regulatory decisions. In order to minimize the drug-associated risks in the general Hungarian population and reduce uncertainties about application of PGx biomarkers for medical practitioners.

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literature assembly, manuscript writing, final approval of the manuscript. IS: additions to the study plan, interpretation of results, manuscript writing, final approval of manuscript. GT: pharmacological evaluation of the results, help in interpretation, final approval of the manuscript. LJS: help in data acquisition and statistical analyses, final approval of the manuscript. AS: interpretation of results, manuscript writing, final approval of the manuscript. SB: interpretation of results, final approval of the manuscript. CS: concept and design, study plan preparation, tables and figures correction, interpretation of results, manuscript writing and correcting, final approval of manuscript, manuscript submission, correspondence.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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Paper 2.

Pharmacogenomic Biomarkers in Docetaxel Treatment of Prostate Cancer: From Discovery to Implementation

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Review

Pharmacogenomic Biomarkers in Docetaxel Treatment of Prostate Cancer: From Discovery to Implementation

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Abstract: Prostate cancer is the fifth leading cause of male cancer death worldwide. Although docetaxel chemotherapy has been used for more than fifteen years to treat metastatic castration resistant prostate cancer, the high inter-individual variability of treatment efficacy and toxicity is still not well understood. Since prostate cancer has a high heritability, inherited biomarkers of the genomic signature may be appropriate tools to guide treatment. In this review, we provide an extensive overview and discuss the current state of the art of pharmacogenomic biomarkers modulating docetaxel treatment of prostate cancer. This includes (1) research studies with a focus on germline genomic biomarkers, (2) clinical trials including a range of genetic signatures, and (3) their implementation in treatment guidelines. Based on this work, we suggest that one of the most promising approaches to improve clinical predictive capacity of pharmacogenomic biomarkers in docetaxel treatment of prostate cancer is the use of compound, multigene pharmacogenomic panels defined by specific clinical outcome measures. In conclusion, we discuss the challenges of integrating prostate cancer pharmacogenomic biomarkers into the clinic and the strategies that can be employed to allow a more comprehensive, evidence-based approach to facilitate their clinical integration. Expanding the integration of pharmacogenetic markers in prostate cancer treatment procedures will enhance precision medicine and ultimately improve patient outcomes.

Keywords: castration resistant prostate cancer; docetaxel; pharmacogenomic biomarker; personalised treatment

1. Introduction

Prostate cancer (PC) remains the second most common cancer in men, and one of the leading causes of death among Western males [1]. This is due to the fact that treatment of metastatic prostate cancer (mPC) is becoming increasingly challenging [2,3]. Docetaxel chemotherapy was approved 15 years ago to treat metastatic castration-resistant prostate cancer (mCRPC), and is now standard care for this stage of disease [2]. Although other drugs have since been developed, some of which are administered in combination regimens with docetaxel, docetaxel remains the main choice of chemotherapeutic agent [4].

Significant progress has been made in genetic biomarker-based treatment of several cancer types [5,6]; however, personalized treatment of PC is lagging behind. Also, it is increasingly evident that the wide variability in treatment response, toxicity, and disease progression between PC patients is due to the genetic heterogeneity of the disease. Therefore, underlying genetic variations are potentially eligible biomarkers for targeted therapy, or to predict drug response and adverse side effects [7]. Treatment-associated, germline genomic biomarkers have several advantages: they are static, can be easily determined, and are robust predictors of drug response/resistance and toxicity. Biomarkers, including somatic genomic alterations, structural variants (e.g., gene fusions, gene rearrangements), splice variants, miRNAs, and differential gene expression, and methylation markers have also been shown to modulate docetaxel treatment of PC [8].

The focus of this review is to discuss the current state-of-the-art pharmacogenomic biomarkers modulating docetaxel treatment of PC. The review includes research studies focusing on germline genomic biomarkers, clinical trials designed to incorporate all type of biomarkers, and finally, the implementation of biomarkers in treatment guidelines.

2. Docetaxel in Prostate Cancer Treatment

Docetaxel is a taxane, a chemotherapeutic agent that produces antitumour activity. It has been previously approved for the treatment of breast cancer and non-small-cell lung cancer, and was approved by the United States Food and Drug Administration on May 19, 2004 for use in combination with prednisone for the treatment of metastatic, androgen-independent prostate cancer (AIPC)/hormone-refractory prostate cancer (HRPC) [9,10]. Docetaxel is a semi-synthetic, second-generation taxane derived from a compound found in the European yew tree (*Taxus baccata*). Docetaxel displays potent and broad antineoplastic properties. It binds to and stabilizes tubulin, thereby inhibiting microtubule disassembly, which results in cell-cycle arrest at the G2/M phase and cell death. This agent also inhibits pro-angiogenic factors, such as vascular endothelial growth factor (VEGF), and displays immunomodulatory and pro-inflammatory properties by inducing various mediators of the inflammatory response. Docetaxel has been studied for use as a radiation-sensitizing agent as well [11].

The pharmacodynamics and pharmacokinetics of docetaxel are extremely complex and have been the subject of intensive investigation. Docetaxel is metabolized both by CYP3A4 and CYP3A5 [12]. Docetaxel is the substrate for the ATP-binding, cassette multidrug transporters ABCB1, ABCG2, ABCC1 and ABCC2. However, SLCO1B3 was identified as the most efficient influx transporter for docetaxel [13].

Unfortunately, most patients develop resistance to docetaxel. Mechanisms of resistance to chemotherapy include tubulin alterations, increased expression of multidrug resistance genes, *TMPRSS2–ERG* fusion genes, kinesins, cytokines, components of other signaling pathways, and epithelial–mesenchymal transition [14].

It is important to note that docetaxel has no PC treatment-guiding pharmacogenomic biomarker included on the drug label, based on the information available from the U.S. Food and Drug Administration (FDA) [15] and the European Medicines Agency (EMA) [16].

3. Germline Genomic Biomarkers in Research Studies for Prostate Cancer Treatment with Docetaxel

Clinical research studies have investigated the genomic biomarkers of docetaxel monotherapy; however, combination therapies with distinct mechanisms of action represent a more effective strategy. Combination therapies are thought to exert cancer-killing functions through either concomitant targeting of multiple pro-cancer factors or more effective inhibition of a single pathway [17]. The exact mechanisms by which these combinations can overcome drug resistance have yet to be fully understood [17].

Studies of germline genomic biomarkers affecting individual differences in docetaxel monotherapy (I) and combination treatment (II) of PC published between 2006 and 2018 are summarized in chronological order in Table 1.

3.1. Docetaxel Monotherapy

Tran et al. [18] studied the pharmacokinetics of docetaxel and concluded that *CYP3A4* (rs2740574) and *CYP3A5* (rs776746) polymorphisms are associated with enhanced docetaxel clearance. Therefore, patients carrying the *CYP3A4*1B* allele may be underexposed to the treatment. Furthermore, *GSTP1*A/*B* (rs1695) and *MDR1* 3435TT (rs1045642) carriers are linked to excessive hematologic febrile neutropenia toxicity [18]. A second study has also suggested that variants in *ABCC2* (rs12762549) and *SLCO1B3* (rs11045585) may predict the risk of leukopenia/neutropenia induced by docetaxel chemotherapy [19]. However, in a study of 64 U.S. cancer patients who received a single cycle of 75 mg/m^2 of docetaxel monotherapy, the *ABCC2* variant rs12762549 showed a trend towards reduced docetaxel clearance, but no association with neutropenia was observed [20].

A case report of a 55-year-old male treated with docetaxel after a radical prostatectomy has suggested that the *CYP1B1* gene may play a role in modulating docetaxel activity [21]. The rs1056836 and rs1800440 *CYP1B1* missense variants were linked to better overall survival (OS) of the patient, who remained disease free until publication of the article (two years). The *CYP1B1* isoforms of Leu432 and Ser453 are characterized by inferior catalytic activity, and while docetaxel is not metabolized by *CYP1B1*, its low activity may favorably influence docetaxel sensitivity by impaired estrogen metabolite production, which in turn could interfere with binding of the drug to tubulin [21].

Sobek and colleagues studied variants of the ABCG2 transporter protein, which effluxes folate, dihydrotestosterone, and chemotherapeutic drugs, among other molecules, out of cells [22]. In in vitro experiments using HEK293 cells (as exogenous *ABCG2* expression in PC cell lines led to selective disadvantage), the rs2231142 (Q141K) variant was observed to efflux less folate. This variant makes the cells more sensitive to docetaxel treatment compared to the wild-type ABCG2. Based on these findings, the authors conclude that the Q141K variant predisposes the cells to less efficient docetaxel efflux, leading to increased intracellular docetaxel levels and thus increased docetaxel sensitivity. The effect of decreased folate efflux was also observed in PC patients carrying the Q141K variant; serum folate levels were significantly lower compared to patients carrying wild-type ABCG2. The authors suggested that increased intra-tumoral folate levels enhance cancer cell proliferation, which may explain why patients with the Q141K variant had a significantly shorter time to prostate-specific antigen (PSA) recurrence after a prostatectomy. The authors concluded that PC patients with the Q141K variant may have a better response to docetaxel, and they may respond differently to treatments that aim to inhibit the efflux of chemotherapeutic agents [22].

3.2. Docetaxel Combination Therapies

3.2.1. Docetaxel and Vinorelbine or Estramustine Phosphate

The first investigation of combination therapies was done in 2006. Here, the role of the *ABCG2* variant rs2231142 (421C>A; Q141K) in treatment response has been studied in HRPC patients treated with docetaxel and vinorelbine/estamustine phosphate [23]. There was a significant association between survival beyond 15 months and the *ABCG2* rs2231142 polymorphism. The increased survival seen in individuals with an *ABCG2* rs2231142 polymorphism may suggest a less functional drug efflux pump, leading to increased intracellular (intra-tumoral) docetaxel concentration and improved cytotoxic activity, lower transporter expression, and improved survival. This variant may therefore be an important predictor of response and survival in HRPC patients treated with docetaxel-based chemotherapy. The companion pharmacogenetic study assessed germ-line polymorphisms in genes known to play important roles in chemotherapy drug transport, metabolism, and mechanism of action. The effect of *ABCG2* polymorphisms on docetaxel pharmacokinetics is unknown [23].

3.2.2. Docetaxel and Estramustin, Thalidomide, and Prednisone

The role of *CYP1B1* variation in treatment response has also been investigated in AIPC patients receiving docetaxel-based combination therapies with estramustin, thalidomide, and prednisone [24]. Individuals carrying two copies of the *CYP1B1*3* (rs1056836) variant had a poor prognosis compared to individuals carrying at least one copy of the *CYP1B1*1* ancestral allele. The association between *CYP1B1*3* and response to therapy was not observed in comparable subjects receiving non-taxane-based therapy. The systemic clearance of docetaxel was also unrelated to *CYP1B1* genotype status, indicating that the association of *CYP1B1*3* with clinical response (CR) is not due to docetaxel metabolism. This pilot study provides evidence that *CYP1B1*3* may be an important marker for estimating docetaxel efficacy in patients with AIPC. This link is likely associated with *CYP1B1*3* genotype-dependent estrogen metabolism. Specifically, that CYP1B1-generated estrogen metabolites may bind to tubulin [25], and potentially could interfere with docetaxel-mediated tubulin stabilization. In addition, estrogen metabolites may also react with docetaxel and structurally alter the drug [24].

3.2.3. Docetaxel and Thalidomide

Docetaxel therapy in combination with thalidomide has led to several pharmacogenomic findings. Thalidomide is suggested to play a role in inflammation, immunomodulation, and anti-angiogenesis, and thus influences disease progression [26]. A study by Sissung et al. investigated the association of *ABCB1* 1236C>T (rs1128503), 2677 G>T/A (rs2032582), and 3435 C>T (rs1045642) polymorphisms and treatment efficacy, measured by survival after treatment or peripherial neuropathy in AIPC patients treated with docetaxel alone (n = 23) or docetaxel and thalidomide (n = 50) [27]. While the *ABCB1* 1236C-2677G-3435C ancestral haplotype was associated with improved OS in docetaxel treated patients, the *ABCB1* 2677T-3435T variant haplotype was significantly associated with shorter median OS in patients treated with both docetaxel and thalidomide. Among both treatment arms together, individuals carrying the 2677GG ancestral genotype had a significantly longer time to neuropathy. Finally, there was a strong trend toward patients carrying the 2677TT-3435TT diplotype having higher grades of neutropenia. Interestingly, none of the variants associated with OS or toxicity had a significant effect on docetaxel pharmacokinetics [27]. These results suggest that variant alleles associated with lowered *ABCB1* expression and altered function result in a clinical phenotype of reduced docetaxel efficacy and increased toxicity (TOX) in men with AIPC. It is possible that expression of *ABCB1* outside of the liver is responsible for these findings, as polymorphic *ABCB1* variants can modulate the exposure of *ABCB1* substrates in tumor cells where this gene is highly up-regulated. It is also notable that efficacy is decreased while TOX is increased in patients carrying variant alleles [27].

Additional genetic polymorphisms have been analysed for associations with clinical response (CR) and TOX in a study of CRPC patients receiving either docetaxel and thalidomide or docetaxel alone [28]. *PPAR-δ* variants rs6922548, rs2016520, rs1883322, rs3734254, and rs7769719, as well as the *SULT1C2* variant rs1402467 were all observed to be associated with CR. Several variants in the *CHST3* gene were linked to CR exclusively (rs4148943, rs4148947, rs12418, and rs730720), while others were liked to both CR and TOX (rs4148950, rs1871450, and rs4148945). Variants in *SPG7* (rs2292954, rs12960), *CYP2D6 (CYP2D6**19), *NAT2* (rs1799931), *ABCC6* (rs2238472), *ATP7A* (rs2227291), *CYP4B1* (rs4646487), and *SLC10A2* (rs2301159) were associated exclusively with TOX. These data revealed that polymorphisms in three genes (*PPAR-δ*, *SULT1C2*, and *CHST3*) were associated with clinical outcome measure of OS, whereas polymorphisms in eight genes (*SPG7, CHST3, CYP2D6, NAT2, ABCC6, ATP7A, CYP4B1*, and *SLC10A2*) were associated with TOX. Although all of these genes may be related to drug metabolism directly, and thus could be related to pharmacokinetics, they also participate in pathways that may affect drug action and could therefore be involved in pharmacodynamic interactions as well. Differences between the two treatment arms were seen exclusively in the *PPARδ* gene, where strong relationships with *PPARδ* single nucleotide polymorphisms (SNPs) were observed in only those patients who received both docetaxel and thalidomide, but not

docetaxel alone. This shows that allelic variation in *PPARδ* may influence the therapeutic efficacy of the anti-angiogenesis agent thalidomide [28].

As genetic variability in liver enzymes is often linked to interindividual variation in liver metabolism, Sissung et al. hypothesised that certain variants and genes in these pathways may be behind the risk and prognosis of CRPC [29]. Patients treated with docetaxel and thalidomide and who carried variants in *ABCB11* (rs7602171 GA/AA), *ABCB4* (rs2302387 CT), *ABCC5* (rs939339 AG), and *SLC5A6* (rs1395 GA/AA) had poor OS compared to those carrying only wild-type alleles, whereas the *GSTP1* rs1799811 CT genotype was associated with prolonged OS. Of considerable interest are several associations between CRPC prognosis and protein transporters that regulate bodily sterol and fatty acid deposition. In this small pilot study, there was suggestive evidence that SNPs in bile acid and fat catabolism genes may be related to CRPC OS. No evidence was found that any of the aforementioned SNPs were related to risk of developing CRPC [29].

3.2.4. Docetaxel and Prednisone

CYP1B1 variation has also been studied in relation to its role in modulating docetaxel treatment response when combined with prednisone [30]. Patients carrying the *CYP1B1*-432ValVal (rs1056836, corresponding to 4326GG) genotype experienced a significantly lower response rate, as well as shorter progression-free survival (PFS) and OS, and its prognostic significance for OS was confirmed. In contrast, no correlations were observed between both the *CYP1B1* C142G (rs10012) or *CYP1B1* A4390G (rs1800440) polymorphisms and clinical outcome in CRPC patients treated with docetaxel and prednisone. In summary, the *CYP1B1* 4326GG polymorphism was linked to docetaxel CR, and may represent a potential new marker for treatment optimization [30].

3.2.5. Docetaxel and Estramustine, Thalidomide, and Ketoconazole

To explore the role of variants in the estrogen pathway and treatment response in a clinical trial setting, CRPC patients treated with docetaxel monotherapy, or different combinations of docetaxel with estramustine, thalidomide, and ketoconazole were genotyped for polymorphisms in estrogen synthesis (*CYP19* rs700519) and estrogen target (*ERα* rs2234693, rs9340799) genes [31]. Patients carrying two copies of *ERα* polymorphisms had shorter progression-free survival (PFS) on docetaxel than other patients. When the analysis was limited to non-obese patients, the relationship between the *ERα* rs9340799 polymorphism and PFS improved. These results supported the hypothesis that reactive estrogen species cause genotoxicity, and may interfere with docetaxel-mediated tubulin polymerization, resulting in shortened survival in men with CRPC. The *CYP19* variant was moderately associated with the duration of survival after docetaxel therapy in patients who were greater than 70 years old. Both *ERα* polymorphisms were also associated with an increase in CRPC risk, and the association with *ERα* variant rs2234693 also improved in those men who were greater than 70 years old. This study demonstrates that estrogen-related genetic variation affects docetaxel CR, and that this relationship is dependent on age and body type in men with CRPC. Moreover, this study suggests that *ERα* polymorphisms confer the risk of developing CRPC, especially in men under 70 years of age [31].

3.2.6. Docetaxel, Prednisone, and Metronomic Cyclophosphamide

Since VEGF is thought to play an important role in angiogenesis and tumor proliferation, a study of the *VEGF* gene in mCRPC patients treated with a combination of docetaxel, prednisone, and metronomic cyclophosphamide was done [32]. The authors observed significantly longer PFS in patients carrying the *VEGF* rs1570360 AG/GG genotypes. Notably, the AA genotype was associated with reduced *VEGF* transcription, suggesting that tumors with the *VEGF* 21154 AG/GG genetic background may produce higher VEGF-A levels after the administration of standard chemotherapy. The authors suggest that *VEGF* and bFGF plasma levels at the end of the first cycle of chemotherapy and *VEGF* genotyping may be used to predict which patients will have greater PFS from this particular combination of therapies [32].

3.2.7. Docetaxel and Atrasentan

Finally, the role of variation in the α-1 acid glycoprotein *(AAG)* gene has been explored in PC patients receiving combination intravenous docetaxel and oral atrasentan therapy [33]. The results suggested that the *AAG* genetic polymorphism, rs250242, may explain some inter-patient variability in docetaxel pharmacokinetics. An evaluation of the pharmacokinetics of both drugs showed that the systemic clearance of docetaxel was increased by approximately 21% when given concomitantly with atrasentan; however, atrasentan pharmacokinetics did not appear to be influenced by docetaxel administration [33].

3.2.8. Docetaxel and Dexamethasone

A genome-wide association study of docetaxel treatment in combination with dexamethasone in hormone-refractory PC patients has shown that the rs875858 SNP in *VAC14* is significantly associated with increased neuropathy risk, irrespective of patient randomisation to bevacizumab or a placebo [34]. While not significant genome-wide, two additional *ATP8A2* SNPs, rs11017056 and rs1326116, showed a trend towards increased neuropathy risk. The authors recommend that *VAC14* should be prioritized for further validation to determine its role as a predictor of docetaxel-induced neuropathy and as a biomarker for treatment individualization.

Table 1. Research studies of germline biomarkers in docetaxel and combination treatment of prostate cancer.

Biomarker	Variant	Effect	Number of Samples/Study Method	Study Type	Country	Reference
I. Docetaxel Monotherapy						
CYP3A4 CYP3A5 GSTP1 MDR1	rs2740574 (c.−392G>A) rs776746(c.219−237A>G) rs1695 (A313G, Ile105Val) rs1045642 (C3435T, Ile1145Ile)	D (Clearance↑) D (Clearance↑) TOX TOX	58 patients initiating chemotherapy	Interventional	France	Tran et al. [18]
ABCC2 SLCO1B3	rs12762549 rs11045585	TOX TOX	84 patients: 28 patients with leukopenia/neutropenia vs. 56 with no TOX	Case–control	Japan	Kiyotani et al. [19]
CYP1B1	rs1056836 (C1294G, Leu432Val) rs1800440 (A1358G, Asn453Ser)	OS	55-year-old male with multifocal adenocarcinoma; 75 mg/m^2 docetaxel every three weeks for six cycles	Case report	Italy	Brandi et al. [21]
ABCC2 SLCO1B3	rs12762549 rs11045585	D (Clearance↓) No effect	64 patients received a single cycle of 75 mg/m^2 docetaxel	Interventional	United States	Lewis et al. [20]
ABCG2	rs2231142 (C421A, Q141K)	CR	HEK293 cells, 40 patients	In vitro, Validated in vivo	United States	Sobek et al. [22]
II. Docetaxel Combination Therapies						
Docetaxel and Vinorelbine, Estramustine Phosphate						
ABCG2	rs2231142 (C421A, Q141K)	OS	64 chemotherapy-naive patients with HRPC were randomized to (1) docetaxel (20 mg/m^2 i.v. days 1 and 8) + vinorelbine (25 mg/m^2 i.v. days 1 and 8) and (2) docetaxel (60–70 mg/m^2 i.v. day 1) + estramustine phosphate (280 mg oral 3x/day, days 1–5)	Interventional	United States	Hahn et al. [23]
Docetaxel and Estramustin, Thalidomide, Prednisone						
CYP1B1	rs1056836 (C4326G, Leu432Val)	OS	52 patients with AIPC: (1) docetaxel (n = 25, 1 h i.v.,30 mg/m^2); (2) docetaxel + estramustine + thalidomide (n = 20, 30 min i.v., 30 mg/m^2) docetaxel + prednisone (n = 7, 1 h i.v., 75 mg/m^2)	Observational retrospective	United States	Sissung et al. [24]

Table 1. *Cont.*

Biomarker	Variant	Effect	Number of Samples/Study Method	Study Type	Country	Reference
Docetaxel and Thalidomide						
ABCB1	rs1128503 (C1236T) rs2032582 (G2677T/A) rs1045642 (C3435T)	OS OS, TOX OS, TOX	AIPC patients; 50 patients with docetaxel + thalidomide; 23 patients with docetaxel;	Interventional	United States	Sissung et al. [27]
PPAR-δ	rs6922548 rs2016520 rs1883322 rs3734254 rs7769719 rs4148943 rs4148947	CR CR CR CR CR CR CR	74 CRPC patients: (1) CRPC patients (n = 25) with docetaxel (30 mg/m^2 weekly for three weeks, followed by a one-week rest); (2) patients (n = 49) with docetaxel (30 mg/m^2 weekly for three weeks followed by a one-week rest) + thalidomide (200 mg orally each day)	Interventional	United States	Deeken et al. [28]
CHST3	rs12418 rs730720 rs4148950 rs1871450 rs4148945	CR CR CR, TOX CR, TOX CR, TOX				
SULT1C2	rs1402467	CR				
SPG7	rs2292954 rs12960	TOX TOX				
CYP2D6	*19 (2539_2542delAACT)	TOX				
NAT2	rs1799931	TOX				
ABCC6	rs2238472	TOX				
ATP7A	rs2227291	TOX				
CYP4B1	rs4646487	TOX				
SLC10A2	rs2301159	TOX				
ABCB4	rs2302387	OS	74 CRPC patients: (1) patients (n = 49) with docetaxel (30 mg/m^2 weekly for three weeks followed by a one-week rest); (2) patients (n = 25) with docetaxel (same schedule) + thalidomide (200 mg orally each day)	Observational, retrospective	United States	Sissung et al. [29]
ABCB11	rs7602171	OS				
ABCC5	rs939336	OS				
GSTP1	rs1799811	OS				
SLC5A6	rs1395	OS				

Table 1. *Cont.*

Biomarker	Variant	Effect	Number of Samples/Study Method	Study Type	Country	Reference
Docetaxel and Prednisone						
CYP1B1	rs10012 (C142G, Arg48Gly)	No effect	60 CRPC patients: (1) docetaxel (1 h, 75 mg/m^2 on day 1) every 21 days, or (2) docetaxel (30 mg/m^2 weekly for five of every six weeks) + prednisone (10 mg os daily)	Interventional	Italy	Pastina et al. [30]
	rs1056836 (C4326G, Leu432Val)	CR, OS, PFS				
	rs1800440 (A4390G, Asn453Ser)	No effect				
Docetaxel and Estramustine, Thalidomide, Ketoconazole						
CYP19 (now CYP19A1)	rs700519 (c.C790T, R264C)	OS	111 CRPC patients: (1) n = 20 with estramustine, docetaxel, and thalidomide; (2) n = 21 with ketoconazole + docetaxel; (3) n = 50 with docetaxel + thalidomide; (4) n = 24 with docetaxel alone; 289 healthy controls	Observational, retrospective	United States	Sissung et al. [31]
ERα (now ESR1)	rs2234693	OS				
	rs9340799	OS				
Docetaxel and Prednisone and Metronomic CTX						
VEGF-A	rs699947 (A22578C)	PFS	41 mCRPC patients on day 1 received docetaxel (60 mg/m^2 intravenously every three weeks, up to 12 cycles) + prednisone (10 mg/day, from day 2 continuously) + celecoxib 200 mg orally 2×/day	Interventional	Italy	Derosa et al. [32]
	rs1570360 (A21154G)	PFS				
	rs2010963 (C2634G)	PFS				
	rs3025039 (C1936T)	PFS				
Docetaxel and Atrasentan						
AAG	rs250242 (A4069G)	Clearance↑. No info about dosage effect.	21 PC patients; docetaxel (60–75 mg/m^2, every 3 weeks, i.v.) + atrasentan (10 mg/day starting on day 3 of cycle 1, given continuously, oral)	Interventional	United States	Younis et al. [33]
Docetaxel and Dexamethasone						
ATP8A2	rs11017056	TOX	623 mCRPC Caucasian patients randomized into two arms; drugs were administered to both arms (arm 1 and arm 2): docetaxel (75 mg/m^2 i.v., 1 h on day 1 of each 21-day cycle) + dexamethasone (8 mg oral, 12, 3, 1 h prior to docetaxel i.v.) + prednisone (5 mg oral 2×/day); (arm 1) adding bevacizumab (15 mg/kg i.v. on day 1 of each cycle), and (arm 2) adding placebo (i.v. on day 1 of each cycle)	Interventional	United States	Hertz et al. [34]
VAC14	rs1326116	TOX				
	rs875858	TOX				

SNP: single nucleotide polymorphism; mCRPC: metastatic castration resistant prostate cancer; PC: prostate cancer; HRPC: hormone resistant prostate cancer; AIPC: androgen-independent prostate cancer; i.v.: intravenous; D: dosing; TOX: toxicity; OS: overall survival; CR : clinical response; PFS: progression free survival; CTX: cyclophosphamide.

4. Clinical Trials of Docetaxel Treatment in Prostate Cancer Incorporating Genomic Signature

Clinical trials have been identified both from ClinicalTrials.gov [35] and from the European Union (EU) Clinical Trials Register database [36]. Only trials that included patients with PC, docetaxel as the administered treatment, and evidence of incorporation of genomic signature analyses were included in this review.

ClinicalTrials.gov and the EU Clinical Trials Register use different terminology for describing the status of a trial. On ClinicalTrials.gov, the status can be "completed", "terminated", "withdrawn", "recruiting", and "active", as well as "not recruiting", "not yet recruiting" or "unknown". "Terminated" trials have stopped early, but participants have been recruited and they have received intervention, whereas "withdrawn" trials have stopped before the recruitment of participants. "Active" and "not recruiting" trials have recruited participants who are currently receiving intervention or are going through examinations, whereas "not yet recruiting" trials have not recruited any participants. Therefore, we collectively refer to the "recruiting", "active"/"not recruiting", and "not yet recruiting" trials as ongoing trials. In the EU Clinical Trials Register, the status of a trial can be "completed", "prematurely ended", or "ongoing".

4.1. Biomarkers in ClinicalTrials.gov

Overall, 132 trials were found from ClinicalTrials.gov with the search algorithm described above. After removing duplicate results and irrelevant trials, the number of the remaining and analysed trials was 24.

Of note, there were fewer "completed" or "terminated" trials (Table 2) than "ongoing" clinical trials (Table 3) [37], indicating the intense translational interest in this field. The reasons for trial terminations were withdrawal of funding (NCT00503984) or low participant enrollment (NCT01253642). Four trials had been withdrawn before recruitment of patients, and two trials had unknown status (Supplementary Table S1).

Table 2. Completed or terminated clinical trials for docetaxel treatment of prostate cancer (ClinicalTrials.gov).

National Clinical Trial Number	Study Period	Status	Intervention	Genomic Signature	Phase	Total Number of Participants	Study Type	Results
NCT00089609	Apr 2005–Jan 2018	Completed	docetaxel + thalidomide + prednisone + bevacizumab	Association of SNPs in CYP3A4, CYP3A5 (docetaxel), and CYP2C19 (thalidomide) with pharmacokinetics and efficacy	II	73	Interventional	Yes. Association of the SNPs and efficacy was not investigated.
NCT01308567	May 2011–May 2018	Completed	cabazitaxel + prednisone or docetaxel + prednisone	Pharmacogenomics of cabazitaxel	III	1170	Interventional	Yes. Results of pharmacogenomic studies were not published.
NCT00619996	Mar 2007–Jan 2009	Completed	sorafenib + docetaxel	Gene expression profiling on blood cells and tumor biopsy	II	43	Interventional	No.
NCT00503984	May 2007–Jun 2015	Terminated (withdrawal of funding)	azacitidine + docetaxel + growth factor support	GADD45A methylation and expression after azacitidine treatment in patients whose disease is progressing on docetaxel treatment	I, II	22	Interventional	Yes. Significant demethylation of GADD45A was observed. Azacitidine may reverse docetaxel resistance.
NCT01253642	Jul 2010–Sep 2017	Terminated (low enrollment)	phenelzine sulfate + docetaxel	Frequency of MAOA overexpression CRPC tumors that are progressing on docetaxel treatment. HIF-1alpha and MAOA expression in Circulating Tumor Cells (CTCs).	II	11	Interventional	Yes. MAOA was overexpressed in all examined tumors. HIF-1alpha and MAOA expression in CTCs was not analyzed.

Table 3. Ongoing clinical trials for docetaxel treatment in prostate cancer ("recruiting", "active"/"not recruiting", "not yet recruiting") (ClinicalTrials.gov).

National Clinical Trial Number	Status	Interventions	Genomic Signature	Phase	Participants (Estimated)	Study Type
NCT02975934	Recruiting	rucaparib or abiraterone + prednisone/enzalutamide/docetaxel + prednisone	Response in patients with evidence of a homologous recombination gene deficiency (*BRCA1/2* or *ATM*)	III	400	Interventional
NCT03442556	Recruiting	docetaxel + carboplatin + rucaparib	Response in patients with homologous recombination DNA repair deficiency (*BRCA1/2*, *ATM*, *PALB2* germline mutations)	II	20	Interventional
NCT02985021	Recruiting	docetaxel + carboplatin	Response in patients with germline or somatic inactivation of DNA repair pathway genes (*BRCA1*, *BRCA2*, *ATM*)	II	35	Interventional
NCT03517969	Recruiting	docetaxel + carboplatin or carboplatin + ATR[1] kinase inhibitor VX-970	Response in tumors with homologous recombination deficiency	II	130	Interventional
NCT02598895	Recruiting	docetaxel + carboplatin	Response in tumors with mutation of DNA repair pathway genes (*BRCA1*, *BRCA2*, *ATM*)	NA	14	Interventional
NCT03070886	Recruiting	ADT[2] + external beam radiotherapy + docetaxel or ADT + external beam radiotherapy	Response in genomically defined sub-groups of patients	II, III	612	Interventional
NCT02649855	Recruiting	docetaxel + PROSTVAC (vaccine)	Evaluate drug metabolism and transporters	II	74	Interventional
NCT03358563	Recruiting	ADT + docetaxel + Radical prostatectomy	Evaluation of genomic signatures and gene expression after treatment. Evaluation of biomarkers in tumor cells in circulation, as well a bone marrow before and after treatment.	Early I	30	Interventional
NCT03218826	Recruiting	docetaxel + AZD8186	Dose escalation and anti-tumor activity of AZD8186 when given together with docetaxel in patients' solid tumors with *PTEN* or *PIK3CB* mutations. Evaluation of co-mutated genes and their association with treatment response or resistance.	I	58	Interventional

Table 3. *Cont.*

National Clinical Trial Number	Status	Interventions	Genomic Signature	Phase	Participants (Estimated)	Study Type
NCT02362620	Active, not recruiting	docetaxel or cabazitaxel	Exploration of prognostic biomarkers (overall survival). Evaluation of the prognostic value of *TMPRSS2-ERG* re-arrengement, *PTEN* loss, and *AR* splicing variants. Association of somatic and germline mutations and the outcomes of the patients.	NA	402	Observational (prospective)
NCT03700099	Not yet recruiting	docetaxel + enzalutamide	Association of the *AR* gene alteration, *AR-V7* status, and PSA response.	II	30	Interventional
NCT03356444	Not yet recruiting	abiraterone + prednisone or docetaxel + prednisone	Exploration of some of the genes related to the treatment efficacy	II	140	Interventional
NCT03816904	Not yet recruiting	docetaxel or paclitaxel	Determination of the number of CAG triplets in the *KCNN3/SK3* gene associated with neuropathy	NA	250	Observational (prospective)

[1] ATR, ataxia telangiectasia and rad3-related; [2] ADT, androgen deprivation therapy.

The majority of trials were interventional, with only two being observational. In the group of interventional trials, the phase of the study was defined for 15 trials, most of which were in phase II [38] (Tables 2 and 3). In the majority of interventional trials, docetaxel was explored in different settings of combination treatments. In the observational studies, docetaxel was compared to cabazitaxel and paclitaxel (Table 3), novel antineoplastic agents that interfere with microtubule function, leading to altered mitosis and cellular death [39].

The genomic biomarkers evaluated in the trials were not always precisely defined, indicating only that the target of the investigation was a gene expression profile or genes related to treatment efficacy, but not specifying further. Furthermore, the genetic analyses were inexact in many cases. Here, we summarize the "completed" or "terminated" clinical trials with output measures and the "ongoing" trials with possible future results, with special focus on the trials where the genomic profiling is specified.

Results have been published on two "completed" and two "terminated" trials (Table 2). However, the results of the completed trials did not include genomic results. In one of these trials (NCT00089609), the intervention treatment included docetaxel, prednisone, thalidomide, and bevacizumab, and the studied genes were *CYP3A4* and *CYP3A5* for docetaxel metabolism and *CYP2C19* for thalidomide metabolism. The exact genetic variants studied and their association with efficacy were not described in the results. The other "completed" trial (NCT01308567) with results aimed to investigate the pharmacogenomics of cabazitaxel, but not docetaxel; however, docetaxel was included in the intervention.

The genetic results of the two "terminated" trials seem to be more impactful. The aim of one of these, NCT00503984, was to determine whether azacitidine could reverse docetaxel resistance in mCRPC patients by decreasing methylation of the proapoptotic *GADD45A* gene [40]. The authors had previously observed that methylation of *GADD45A* in DU145 PC cells increases during docetaxel treatment and contributes to docetaxel resistance [41]. In addition, they found that azacitidine treatment decreases the methylation of *GADD45A* and restores docetaxel sensitivity in resistant PC cells. In the clinical trial, changes in *GADD45A* methylation were examined in buffy-coat DNA of patients. After azacitidine treatment, methylation significantly decreased in ten patients, increased in four patients, and in one patient could not be assessed due to a lacking sample (Phase I, 15 patients). Six of the ten patients with decreased methylation also had a concomitant decrease in the PSA level, while none of the four patients with increased methylation had a PSA response. However, the difference was not statistically significant ($p = 0.085$). The authors concluded that the addition of azacytidine could be beneficial in mCRPC patients after initial docetaxel treatment failure [40]. With regards to the second "terminated" trial (NCT01253642), only the frequency of *MAOA* (monoamine oxidase A) overexpression in tumors that have progressed during docetaxel treatment was reported. *MAOA* overexpression was observed in all investigated progressing tumors.

The focus of several ongoing clinical trials (Table 3) is treatment response to docetaxel treatment in combination with emerging new medications in tumors harbouring inactive mutations in homologous recombination (HR) genes, including *BRCA1*, *BRCA2*, and *ATM*. Five recruiting trials plan to study the effect of these genes on treatment response, where treatments including a poly-ADP ribose polymerase (PARP) inhibitor (rucaparib), a nonsteroidal antiandrogen (enzalutamide), or a chemotherapy drug (carboplatin), combined with or compared to docetaxel.

A promising recruiting trial, NCT03218826, plans to evaluate the effect of docetaxel combined with AZD8186, a novel potent small molecule, which targets the lipid kinase PI3Kβ signaling and inhibits the growth of *PTEN*-deficient prostate tumors [42].

The effect of androgen receptor (*AR*) gene alterations and splice variants on treatment response are going to be evaluated in two trials. The impact of these alterations on PSA response will be evaluated in docetaxel treatment combined with enzalutamide (NCT03700099), and on patient prognosis related to docetaxel versus cabazitaxel treatment (NCT02362620), in addition to the effect of *TMPRSS2-ERG* rearrangement and *PTEN* loss.

Only one trial (NCT03816904) plans to focus on the adverse effects of docetaxel. The aim of this trial is to investigate the association between the number of CAG triplets in the *KCNN3* gene (which codes for the SK3 calcium channel) and taxane neuropathy in patients who are receiving either docetaxel or paclitaxel. This trial is a prospective observational trial, and plans to follow patients with different types of cancer, including PC patients.

4.2. Biomarkers in the EU Clinical Trials Register

In addition to the ClinicalTrials.gov database, clinical trials for docetaxel chemotherapy with pharmacogenetic aspects were searched for in the EU Clinical Trials Register [36]. A total of 76 trials were found, and after removing duplicate and irrelevant search results, only four trials remained.

Of the four trials, one was "completed", one was "terminated", and two were "ongoing" (Table 4). Results have been published for the completed and the terminated trials, but no pharmacogenetic aspects were presented, and only one trial (EudraCT 2006-004478-29) specified which genes (*CYP2B6*, *CYP2C19*, *CYP2C9*, and *CYP3A5*) they planned to investigate. In two of the trials, descriptions of the genetic biomarker investigations were included in a sub-study (EudraCT 2013-000809-23) or in a separate study planned to be conducted later based on samples collected during the actual trial (EudraCT 2008-000701-11); however, the specific biomarkers to be studied were not provided.

Table 4. Clinical trials for docetaxel treatment in prostate cancer in EU Clinical Trials Register.

Eudra Clinical Trial Number	Intervention	Genomic Signature	Results	Phase/Status	Study Type/Participants	Comparison with ClinicalTrials.gov
2008-000701-11	dasatinib + docetaxel + prednisone OR placebo + docetaxel + prednisone	Samples collected for future pharmacogenomic studies	Yes. Nothing on pharmacogenomics	III/Completed	Interventional/1930	Listed on ClinicalTrials.gov Pharmacogenomic aspect was not mentioned on ClinicalTrials.gov (NCT00744497).
2007-000323-17	docetaxel + ADT (leuprolide + bicalutamide) OR ADT alone	Evaluation of gene expression profiles, genetic changes, and quantitative methylation of different genes, and their ability to predict the treatment outcome of high-risk prostate cancer subjects	Yes. Nothing on pharmacogenomics	III/Terminated	Interventional/413	Trial was listed on ClinicalTrials.gov. Pharmacogenomic aspect was mentioned in the original but not in the current secondary outcome measures on ClinicalTrials.gov (NCT00514917).
2013-000809-23	masitinib + docetaxel + prednisone OR placebo + docetaxel + prednisone	In a sub-study: relationship between genomic data and overall survival	No	III/Ongoing	Interventional/581	Trial was listed on ClinicalTrials.gov Pharmacogenomic aspect was not mentioned on ClinicalTrials.gov (NCT03761225).
2006-004478-29	docetaxel + prednisone + ciclophosphamide + celecoxib	Evaluation of the most frequent genetic polymorphisms of CYP2B6, CYP2C19, CYP2C9, and CYP3A5 and their association with the observed response	No	II/Ongoing	Interventional/45	Not found on ClinicalTrials.gov

Interestingly, three of the four trials were found retrospectively on ClinicalTrials.gov, but none of them was found with the search algorithm used there. The reason for this is that the pharmacogenomic aspects were not mentioned on ClinicalTrials.gov, but they were included to the EU register, albeit briefly. Notably, in one of these trials the original secondary outcome measures on ClinicalTrials.gov included the evaluation of genetic biomarkers, but this outcome measure had later been deleted from the trial description. This change had not been updated in the EU Clinical Trials Register.

5. Pharmacogenomic Biomarkers in Prostate Cancer Treatment Guidelines

The European Association of Urology (EAU) [43,44] and European Society for Medical Oncology (ESMO) [45] PC treatment guidelines were reviewed for any recommendations on pharmacogenetic testing before or during docetaxel treatment. In general, the ESMO guideline states that there are no predictive biomarkers to guide treatment decisions, even though there are some known prognostic biomarkers. On the other hand, the EAU guideline discusses multiple diagnostic or prognostic genetic biomarkers and their use in the clinic. These guidelines suggest that the first future application of pre-emptive genetic testing commence and involve homologous recombination deficiency genes, since these patients might benefit from treatment with PARP inhibitors [43]. However, no definite recommendation has been made.

6. Biomarkers with Translational Potential in Docetaxel Treatment of Prostate Cancer

Predictive pharmacogenomic biomarkers of the highest importance, with clinical implementational potential, are the ones affecting clinical response. Based on research studies on germline genomic biomarkers, we can conclude that variants in *CYP1B1*, *ABCG2*, *CHST3*, *PPAR-δ*, and *SULT1C2* genes have a documented impact on better clinical response to docetaxel treatment in PC (Table 5). Pre-emptive genotyping of pharmacogenomic biomarkers affecting docetaxel clearance would be of especially great value for evidence-based dose decisions. Specifically, *CYP3A4*, *CYP3A5*, *AAG* gene variants are known to enhance, while the *ABCC2* variant is reported to reduce docetaxel clearance in PC treatment. This may cause an elevated or reduced docetaxel dose, respectively. Docetaxel toxicity in PC treatment may be avoided by testing for polymorphisms of the following biomarker genes: *CHST3*, *MDR1/ABCB1*, *ABCC2*, *ABCC6*, *ATP7A*, *ATP8A2*, *CYP2D6*, *CYP4B1*, *GSTP1*, *NAT2*, *SLC10A2*, *SLCO1B3*, *SPG7*, and *VAC14*.

Table 5. Germline genomic biomarkers in docetaxel treatment of prostate cancer with clinical translational potential.

Biomarker	Predicitive			Prognostic	
	Clinical Response (↑)	Toxicity	Dosing (Clearance)	Overall Survival (↑)	Progression Free Survival (↑)
CYP1B1 (rs1056836)	X			XXX	X
ABCG2 (rs2231142)	X			X	
CHST3 (rs4148950)	X	X			
CHST3 (rs1871450)	X	X			
CHST3 (rs4148945)	X	X			
MDR1/ABCB1 (rs1045642)		XX		X	
MDR1/ABCB1 (rs2032582)		X		X	
ABCC2 (rs12762549)		X	X (reduced)		
CHST3 (rs4148947)	X				
CHST3 (rs12418)	X				
CHST3 (rs730720)	X				
CHST3 (rs4148943)	X				
PPAR-δ (rs6922548)	X				
PPAR-δ (rs2016520)	X				
PPAR-δ (rs1883322)	X				
PPAR-δ (rs3734254)	X				
PPAR-δ (rs7769719)	X				
SULT1C2 (rs1402467)	X				
ABCC6 (rs2238472)		X			
ATP7A (rs2227291)		X			
ATP8A2 (rs11017056)		X			
ATP8A2 (rs1326116)		X			
CYP2D6*19		X			
CYP4B1 (rs4646487)		X			
GSTP1 (rs1695)		X			
NAT2 (rs1799931)		X			
SLC10A2 (rs2301159)		X			
SLCO1B3 (rs11045585)		X			
SPG7 (rs2292954)		X			
SPG7 (rs12960)		X			
VAC14 (rs875858)		X			
AAG (rs250242)			(enhanced)		
CYP3A4 (rs2740574)			X (enhanced)		
CYP3A5 (rs776746)			X (enhanced)		
ABCB4 (rs2302387)				X	
ABCB11 (rs7602171)				X	
ABCC5 (rs939336)				X	

Table 5. *Cont.*

Biomarker	Predicitive			Prognostic	
	Clinical Response (↑)	Toxicity	Dosing (Clearance)	Overall Survival (↑)	Progression Free Survival (↑)
CYP1B1 (rs1800440)				X	
CYP19A1 (rs700519)				X	
ERα/ESR1 (rs2234693)				X	
ERα/ESR1 (rs9340799)				X	
GSTP1 (rs1799811)				X	
MDR1/ABCB1 (rs1128503)				X	
SLC5A6 (rs1395)				X	
VEGF-A (rs699947)					X
VEGF-A (rs1570360)					X
VEGF-A (rs2010963)					X
VEGF-A (rs3025039)					X

Prognostic biomarkers have a high importance from clinical and patient perspective. Better overall survival is influenced by *CYP1B1, ABCG2, MDR1, ABCB4, ABCB11, ABCC5, CYP19A1, ERα/ESR1, GSTP1* and *SLC5A6* genes. Importantly, favorable progression-free survival is related to *CYP1B1* and *VEGF-A* polymorphisms.

In summary, the most important germline pharmacogenetic biomarker originating from the research studies is *CYP1B1* rs1056836, indicating both clinical response, overall and progression-free survival. In addition, on the same way *ABCG2* rs2231142 indicates a better clinical response and overall survival. *CHST3* variants (rs4148950, rs1871450, rs4148945) indicate better clinical response and toxicity. *MDR1/ABCB1* (rs1045642, rs2032582) variants play an important role in better overall survival and toxicity, while the *ABCC2* rs12762549 variant in reduced clearance/dosing and toxicity.

Only one single clinical trial gives a hint on the use of an azacytidine demethylating agent, which can be beneficial in mCRPC patients who have increased *GADD45A* gene methylation after initial docetaxel treatment failure.

Although genetic testing is not recommended yet, these prognostic and predictive germline genomic biomarkers may have the best translational value.

7. Challenges, Conclusions, and Outlook

The results of the research summarized above justify the increasing number of studies aimed at identifying the associations between the genetic signatures of PC patients and docetaxel drug response, resistance, and toxicity.

However, only a minority of the significant pharmacogenetic candidates have been taken forward for clinical validation. To overcome the challenge of moving biomarkers into a clinical setting, prospective study designs, larger discovery cohorts, and subsequent clinical validation in good quality randomized trials are urgently needed.

Another challenge is how to define the best approach for biomarker selection, with enough evidence to transition them to the clinic. The hurdles include the inherent low frequency of many of these markers, the lengthy validation process through trials, and legislative and economic issues.

The predictive capacity of pharmacogenomic biomarkers for specific clinical outcome measures can be improved via composing expanded multigene pharmacogenomic panels defined by drug efficacy, drug toxicity, clinical response, or survival. Integrating these clinical effect-based pharmacogenomic panels into future research studies and clinical trials would allow a more comprehensive, evidence-based approach to determine the significance and importance of genetic testing. Furthermore, with appropriate consent and pretesting education [46], incorporating biomarker assessment provides the opportunity to not only assess cancer risk, but facilitate clinical trial eligibility and treatment selection [47]. In addition, the use of germline genomic biomarkers in cancer treatment is considered to be a less invasive approach compared to biopsy-originated somatic biomarkers.

Technological requirements for the clinical implementation of biomarker assessment are now readily available. However, it is important to ensure that continued pharmacogenetic education is provided to clinical oncologists, and that the benefit of using genetic polymorphisms as predictive biomarkers in routine and clinical research is stressed.

In summary, considerable progress has been made in the discovery of clinically applicable pharmacogenomic signatures of docetaxel treatment in PC. However, a more collaborative approach between stakeholders and studies with specific clinical output measures are needed to pave the way towards the routine use of pharmacogenomic biomarkers in personalised treatment of PC.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4425/10/8/599/s1, Table S1: Withdrawn trials and trials with unknown status for docetaxel treatment in prostate cancer (ClinicalTrials.gov).

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Paper 3.

Precision treatment of prostate cancer: will genetic biomarker guided PARP inhibitors introduce a game-change?

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Under review

Precision treatment of prostate cancer: will genetic biomarker guided PARP inhibitors introduce a game-change?

Submitted to Pharmacogenomics- Under review

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ABSTRACT

Precision therapy for a subgroup of genetically defined metastatic castration resistant prostate cancer patients may be reality in near future. Poly (ADP-ribose) polymerase (PARP) inhibitor clinical trials for prostate cancer investigate both germline and somatic genomic alterations of a number of DNA damage repair genes in increasing tendency: *BRCA1, BRCA2, ATM, ATR, BARD1, BRIP1, CDK12, CHEK1, CHEK2, ERCC3, FAM175A, FANCA, FANCD2, FANCL, GEN1, HDAC2, MLH1, MLH3, MRE11, MSH2, MSH6, NBN, PALB2, PMS2, PIK3CA, PPP2R2A, PTEN, RAD51, RAD51B, RAD51C, RAD51D* and *RAD54L*. Clinical trials with preliminary results indicate *BRCA2* and *BRCA1,* but also *ATM,* additionally *BRIP1, FANCA, CDK12* as predictive genomic biomarkers affecting clinical endpoints, and applicable for genome guided patient selection in breakthrough therapy designated PARP inhibitor treatment.

KEYWORDS:

genomic biomarkers, PARP inhibitors, castration resistant prostate cancer, precision treatment

Introduction: management of metastatic castration resistant prostate cancer

Early detection of prostate cancer (PC) in localized or regional stage is well known to contribute to better survival [1]. If detected in advanced stage it is conventionally treated with androgen deprivation therapy (ADT), chemotherapy, androgen receptor (AR) signaling inhibitors, bone-directed therapy, radiation, or a combination of these treatments [2,3]. However, durable and complete response following first-line treatment in patients with advanced PC is uncommon and significant proportion of PC patients develop castration resistant disease. Currently there is no treatment exists for castration resistant prostate cancer (CRPC), thus new solutions are needed.

Poly (ADP-ribose) polymerase (PARP) enzymes are involved in base excision repair (the repair of DNA single-strand breaks) and alternative end joining (repair of DNA double-strand breaks) [4,5]. Cancer cells with DNA damage repair (DDR) mutated genes are often more reliant on a subset of repair pathways, therefore more dependent on PARP than are normal cells with full DNA repair capacity [6]. This makes PARP an attractive target for cancer therapy.

The therapeutic rationale for application of PARP inhibitors in PC treatment is based on substantial genomic alterations of DDR genes in metastatic castration resistant prostate cancer (mCRPC) [7-9]. A recent study found that the incidence of inherited DNA-repair gene alterations in metastatic prostate cancer (mPC) to be significantly higher (11.8%) than in both men with localized prostate cancer (4.6%) and in the general population (2.7%) [10]. Discovery of genomic landscape of mCRPC showed that approximately 23% of patients harbor somatic DNA repair pathway aberrations [11]. Of these, *BRCA2*, *BRCA1*, and *ATM* account for 19.3% overall and they were substantially more frequent in mCRPC compared to those in primary PC. In addition, mutational events were noted in several other DDR genes like *CDK12, FANCA, RAD51B* and *RAD51C [11]*. But *BRCA1/2* mutations were found to be the most common DNA-repair gene defects in patients with mCRPC [12].

It has been proven that *BRCA1* or *BRCA2* DNA repair defect causing mutations sensitize cells to PARP inhibition, which leads to the persistence of DNA lesions normally reversed by homologous recombination repair (HRR), and consequently results in chromosomal instability, cell cycle arrest and subsequent apoptosis [13,14]. This called synthetic lethality. Although, the genetic concept was proposed nearly a century ago, its exploitation in clinic is challenging because of the arising resistance to PARP inhibition or finding the optimal drug combination [15].

The applicability of PARP inhibitor (PARPi) treatment in PC is highly dependent on the DDR gene mutations of the patients, thus genetic biomarker based patient selection will be required for precision oncology in PC.

Identification of candidate genetic biomarkers in PARP inhibitor clinical trials for prostate cancer

The objective of this work was to evaluate PARPi clinical trials in PC for the followings:

a) involvement of genetic biomarkers applicable for future patient selection
b) genes, gene panels used to identify molecularly defined PC patient subpopulations
c) genomic biomarkers predicted endpoints

The time point of this study was November 2019. The publicly available database www.clinicaltrials.gov was mined for the registered clinical trials using the terms "metastatic castration resistant prostate cancer", "prostate cancer" , "poly (ADP-ribose) polymerase inhibitor", "PARP inhibitor", "gene" "drug name". After removing the duplicates and irrelevant trials, remained 28 trials, among them 9 trials had preliminary results. All 28 trials were interventional trials. 5 trials were already in phase III, but most of the trials were in phase II (n=22) and 1 in phase I. In the trials with interim results olaparib is the most investigated drug (n=5 trials), followed by niraparib, rucaparib, talazoparib and veliparib which all have been explored in 1-1 trials. This is consistent with the ongoing trials without interim results, where also olaparib is the most popular (n=9); 5 trials dealt with rucaparib, 3 dealt with niraparib and 2 with talazoparib.

It is of note, in several instances it was not clear from the trial description whether it includes germline or somatic alterations, although trial descriptions have been explored in details.

PARPI studies with preliminary results in prostate cancer

Several ongoing clinical trials investigated the association of DDR mutation status and PARPi efficacy in PC. The interim results of 9 ongoing PARPi trials in CRPC are summarized in **Table 1.** Primary outcome was most commonly PSA response rate, survival, radiographic progression free survival**.**

The most dominantly investigated PARPi in PC is oliparib. The first clinical trial of **olaparib** in mCRPC patients was conducted by AstraZeneca (NCT01682772, TOPARP). According to the results, radiographic progression-free survival (rPFS) was significantly longer in the DDR positive group 9.8 vs. 2.7 months ($p<0.001$). From all 7 patients with *BRCA2* loss all 5 with measurable disease had a radiologic partial response. 4 of the 5 patients with deleterious *ATM* mutations had a response to olaparib. All 7 patients (14%) with *BRCA2* loss had PSA levels that fell by 50% or more from baseline; overall survival (OS) was prolonged in the biomarker-positive group (somatic mutations of *BRCA1, BRCA2, ATM, CHEK2, FANCL*) 13.8 months vs. 7.5 months in the biomarker-negative group ($p = 0.05$). Patients with DDR mutation had a significantly higher response rate (RR) ($p<0.001$): 14 of 16 DDR mutation positive patients (88%) had a response to olaparib. Conversely, only 2 of 33 biomarker-negative patients (6%) were classified as having a response (sensitivity, 88%; specificity, 94%)[16].

In NCT03047135 trial **olaparib** as single agent in mCRPC resulted at 15% PSA50 response. All of the 3 men had *BRCA2* mutations; 2 had complete PSA response. 20% (4 other men) had minor PSA response in the trial. Median PSA progression-free survival was greater in men with *BRCA2/ATM* mutations (9 vs. 4 month, $p= 0.02$)[17].

Further investigations were conducted in **combination therapies with olaparib**. Interim results of NCT01972217 in combination with **abiraterone and prednisone** showed that rPFS was significantly longer in the olaparib group (13.8 months vs 8.2 months, $p=0.034$). However, data suggest that the drug combination might have resulted in rPFS benefit for patients regardless of HRR mutation status; since overall response, confirmed PSA response, and circulating tumor cell conversion rates were similar in both treatment groups[18].

In NCT03810105 trial **olaparib in combination with durvalumab** activity has been seen in patients with alterations in DDR genes (somatic and germline *BRCA1, BRCA2, ATM, BARD1, BRIP1, CHEK2, CDK12, FANCA, RAD51C, RAD51D, PALB2*) with a median rPFS of 16.1 months. 9 of 17 (53%) patients had a radiographic and/or PSA response. Patients with alterations in DDR genes were more likely to respond. In this study 2/3 of responders had DDR gene alterations[19].

The measure primary outcome of phase III ProFound Study (NCT02987543) with **olaparib versus enzalutamide or abiraterone acetate plus prednisone** in men with mCRPC was radiographic progression free survival in subjects with *BRCA1, BRCA2,* or *ATM* qualifying gene mutations. Olaparib had a favorable trend for OS (18.5 vs15.11 months, $p= 0.01$), and improved rPFS (7.39 vs 3.55 months $p<0.0001$), however significant association with DDR status was not confirmed yet[20].

Niraparib's efficacy, safety and pharmacokinetics in men with mCRPC associated with germline or somatic *BRCA1, BRCA2, ATM, BRIP1, CHEK2, FANCA, PALB2* or *HDAC2* mutations was evaluated in GALAHAD study (NCT02854436). Composite RR was defined as an objective response by the RECIST 1.1 (Response Evaluation Criteria In Solid Tumors) for measurable disease, circulating

tumor cell conversion to < 5 CTC per 7.5 mL of blood or PSA decline of ≥50% (PSA50). Niraparib monotherapy in mCRPC patients showed that composite and objective RRs in patients with biallelic *BRCA1/2* were 65% and 38%, respectively. 3/8 patients (38%) with measurable visceral metastases showed objective response[21].

The purpose of TRITON-2 (NCT02952534) study is to determine how patients with mCRPC and evidence of a homologous recombination gene deficiency respond to treatment with **rucaparib**. Patients must have a deleterious mutation in *BRCA1/2* or *ATM*, or molecular evidence of other homologous recombination deficiency (*BARD1, BRIP1, CDK12, CHEK2, FANCA, NBN, PALB2, RAD51, RAD51B, RAD51C, RAD51D, RAD54L*) for recruitment. Confirmed PSA response was observed in 51.1% (23/45) of patients with *BRCA1/2* alteration, in 1 patient with *CDK12* alteration, in 1 patient with *BRIP1* alteration, and in 1 patient with *FANCA* alteration. Of patients with a *BRCA1/2* alteration and measurable disease at baseline, 44.0% (11/25) had a confirmed radiographic response. A confirmed objective response by investigator assessment was also observed in 1 patient with a *BRIP1* alteration and 1 patient with a *FANCA* alteration[22].

In TALAPRO-2 (NCT03395197) trial **talazoparib was in combination with enzalutamide and compared to enzalutamide alone** as a frontline therapy for mCRPC patients. In the 1 mg and 0.5 mg talazoparib cohorts 92% and 100% of patients had a 50% decline from baseline in PSA level, respectively. The trial pre-stratified patients in DDR-mutated and DDR wild-type cohorts. The association between PFS, OS and DDR mutations has to be evaluated in the future[23].

Veliparib was investigated in NCT01576172 clinical trial. Patients with DDR gene mutation had significantly higher PSA RR (90% vs 56.7%, p = 0.007) and PSA decline ≥ 90% (75% vs 25%, p = 0.001); higher measurable disease RR (87.5% vs 38.6%, p = 0.001) and longer median PFS (14.5 vs 8.1 months, p = 0.025)[24].

Ongoing PARPi trials in prostate cancer

Nineteen currently ongoing clinical trials aim to investigate the role of PARPi in PC (**Table 2**.). Most of the ongoing studies are combination therapies: 7 from 9 olaparib trials, 3 from 3 niraparib trials, 3 from 5 rucaparib trials, 1 from 2 talazoparib trials. Primary outcome was most commonly progression free survival followed by RR, disease-free state and dose limiting toxicity.

NCT03317392 is the only ongoing phase I study investigates maximum tolerated dose of **oliparib** and radium Ra 223 dichloride in relation to rPFS in mCRPC patients using Oncopanel testing.

IMANOL trial (NCT03434158) investigates PSA progression free survival, PSA RR and number of adverse events in CRPC patients with deleterious mutations treated with **olaparib monotherapy**.

NCT03263650 investigates de effect of **olaparib** in aggressive type PC with genomic alterations in DDR pathway genes induced and/or selected by carboplatin and cabazitaxel chemotherapy.

Exploratory objective of NCT03570476 and NCT03432897 (BrUOG 337) trials are to evaluate whether **neoadjuvant olaparib** can reduce locally advanced PC with defects in DNA repair genes with inherited or somatic pathogenic variants prior to radical prostatectomy.

BRCAAway study (NCT03012321) evaluates the progression free survival of **olaparib**, abiraterone/prednisone or the combination abiraterone/prednisone and olaparib in mCRPC patients with canonical DNA repair defects in *BRCA1, BRCA2,* or *ATM*. Secondary outcome is to evaluate if noncanonical DNA repair defects have clinical relevance to PARP inhibition alone.

In NCT03516812 trial CRPC patients are treated with **olaparib** and testosterone. Primary outcomes are PSA decline of at least 50% below baseline and the incidence of adverse events according to National Cancer Institute Common Terminology Criteria for Adverse Events. 50% of enrolled subjects have unspecified homozygous deletions, deleterious mutations, or both in one or more of the DDR genes; the other 50% of patients must have an intact DDR pathway.

NCT02893917 evaluates association of not-specified homologous recombination deoxyribonucleic acid repair deficiency analyzed by BROCA-HR test with the clinical activity of the combination of **olaparib** and cediranib or olaparib monotherapy, as measured by radiographic progression free survival in mCRPC patients.

In phase III PROpel study (NCT03732820) **olaparib** plus abiraterone as first-line therapy in men with mCRPC and with germline or somatic mutations in *BRCA1, BRCA2,* or *ATM* and 12 other HRR genes will be investigated. Primary outcome is radiological progression free survival, secondary outcomes are time to first subsequent anticancer therapy or death, time to pain progression, overall survival.

Niraparib will be investigated in patients with high-risk, clinically localized PC before surgery (NCT04030559), to confirm the association of DDR mutations with pathologic RR.

NCT04037254 study will analyse the side effects and best dose of **niraparib t**o see how well it works in combination with standard of care radiation therapy and hormonal therapy (ADT) in treating patients with high risk, clinically localized PC. Plasma samples will be assessed for baseline and post-therapy alterations in a targeted gene panel and for reversion mutations in DNA repair genes as early biomarkers of treatment resistance.

The MAGNITUDE study (NCT03748641) will investigate mCRPC patients treated with **niraparib**, abiraterone acetate and prednisone versus abiraterone acetate and prednisone. During the pre-screening phase, participants will be evaluated for DDR and then will be assigned to one of the 2 cohorts based on their biomarker status. Primary endpoint is rPFS in both cohorts.

Rucaparib treatment response versus treatment with abiraterone acetate, enzalutamide, or docetaxel will be determined in TRITON3 trial (NCT02975934) in mCRPC patients with evidence of a deleterious mutation in *BRCA1/2* or *ATM*.

NCT04171700 and ROAR study (NCT03533946) will investigate both germline and somatic mutations during **rucaparib** treatment of PC.

TRIUMPH study (NCT03413995) is a trial of **rucaparib** in patients with metastatic hormone-sensitive PC harboring germline DNA repair gene mutations.

NCT03442556 trial will investigate how well docetaxel with carboplatin followed by **rucaparib camsylate** works in treating patients with mCRPC and somatic *BRCA2, BRCA1, ATM* or *PALB2* mutation.

Talazoparib alone (TALAPRO-1, NCT03148795) or in combination with avelumab (NCT03330405) are studied in CRPC patients with somatic DDR mutations analyzed either by FoundationOne CDx™ NGS gene panel or specified as somatic *BRCA1, BRCA2, ATM* mutations. Outcome measures are dose limiting toxicity, overall response, PSA, CA-125 tumor marker, time and duration to treatment response, progression-free survival, PSA response and overall survival.

Summary of candidate genomic biomarkers of PARP inhibitor sensitivity identified from clinical trials

Germline DDR mutations were found to have an effect on mCRPC outcomes that may be affected by the first line of treatment used [25]. In the prospective multicenter cohort study, the prevalence of germline DDR mutations were screened in 107 genes, and 16.2% of patients were found to be carriers (3.3% *BRCA2*, 1.9% *ATM*, 0.96% *BRCA1*, no *PALB2*). Cause-specific survival (CSS) was halved in germline *BRCA2* carriers, thus they were identified as an independent prognostic factor for CCS [25]. In another study, the mutations in *BRCA2* (5.3%), *CHEK2* (1.9%), *ATM* (1.6%), *BRCA1* (0.9%), *PALB2* (0.4%) and *RAD51D* (0.4%) genes were significantly enriched in patients with mPC compared to the general population, which suggests that they are more likely to develop mPC and may potentially benefit from PARPi therapy [10].

In trials with results, all report on DDR gene panel testing including different number of genes, except the study by Agarwal et al, where the studied genes were not specified. In terms of the type of DDR mutation, 4 trials reported on somatic mutations [16],[17], [20], [24], 4 trials on somatic or germline [18], [19], [21], [22]. There were no trials indicating the testing of solely germline mutations. Only a single trial used DDR mutation status for patient stratification [23].

From the 19 ongoing PARPi trials for PC in 6 trials (NCT03317392, NCT03263650, NCT02893917, NCT04037254, NCT03748641, NCT03148795) the exact DDR genes were not specified. In the trials specifying the tested DDR genes, 1 trial does not report the somatic or germline origin of the tested genomic alterations (NCT02975934); 6 trials report on germline or somatic genomic alterations (NCT03434158, NCT03012321, NCT03570476, NCT03732820, NCT04171700, NCT03533946), 2 trials used tumor tissue or cell-free DNA from peripheral blood as origin of genomic sample (NCT03516812, NCT03432897). Few trials test clearly only for germline (NCT03413995) or only for somatic genomic alterations (NCT03442556, NCT03330405, NCT04030559).

Candidate genomic biomarkers investigated in clinical trials to guide patient selection and precision treatment of PARPi treatment in PC are summarized in **Figure 1.** We found that 34 genes are under investigation to clear their role in patient selection for PARPi sensitivity. From them 31 are DNA repair genes or genes that interact with DNA repair pathways: *BRCA1, BRCA2, ATM, ATR, BARD1, BRIP1, CDK12, CHEK1, CHEK2, ERCC3, FAM175A, FANCA, FANCD2, FANCL, GEN1, HDAC2, MLH1, MLH3, MRE11, MSH2, MSH6, NBN, PALB2, PMS2, PIK3CA, PPP2R2A, PTEN, RAD51, RAD51B, RAD51C, RAD51D* and *RAD54L*. Further 3 genes (*AR, ETS fusion, TP53*) were included to earlier studies with preliminary results, which are not directly involved in DNA repair mechanisms.

Importantly, ongoing clinical trials involve preferentially higher number and more diverse set of genomic biomarkers (n=113) than studies with preliminary results (n=80). They expected to answer additional outcomes compared to trials with results, such as disease-free state and dose limiting toxicity, and imply increasing future chance for innovative molecularly targeted treatment in PC. Increasing inclusion tendency to the clinical trials can be observed for *BRCA1, BRCA2, ATM, PALB2, CHEK2, FANCA, FANCL, HDAC2* and *PPP2R2A.* In addition, a set of new DNA repair pathway genes gained interest as genomic biomarkers in ongoing PARPi clinical trials for PC: *GEN1, MRE11, RAD51, ERCC3, FAM175A, MLH1, MSH2, MSH6, PMS2, ATR, FANCD2, MLH3* (in decreasing order of inclusion frequency). On the other hand, *RAD51B, PIK3CA, PTEN, AR, ETS* fusion and *TP53* have been excluded from the ongoing trials, which reflects the more selective focus on DNA

repair genes. The popularity of *RAD* genes (*RAD51C, RAD51D, RAD54L)* and *BARD1* seems also to decrease in ongoing trials, and *BRIP1, CDK12, CHEK1* are at the same inclusion frequency.

Table 3. summarizes the DNA repair genes proved to have an effect on PC patient's endpoints in PARPi clinical trials with preliminary results. Therefore, these DDR genes may be credible for possible future clinical application. Clear emphasis is given to *BRCA1* and *BRCA2* gene loss, biallelic mutation, mutations or any alteration in affecting PSA RR, radiographic response, longer PSA progression-free survival and overall survival of PC patients according to clinical trials with preliminary results [16,17,21,22]. Testing for *ATM* gene mutations in *BRCA*-negative PC patients seem to be worthwhile in order to qualify for the PARPi treatment, and it can effect longer PSA progression-free survival and radiographic partial response according to our investigation [22]. Of note, studies showed that *ATM* mutations have higher enrichment in PC populations (1.9% [25], 1.6% [10]) than for example *BRCA1* mutations (0.96% [25], 0.9% [10]). In a research study, *ATM* mutated mCRPC patients were shown to experience inferior outcomes to PARPi therapy compared to those harboring *BRCA1/2* mutations, suggesting that alternative therapies should be explored for patients with *ATM* mutations [26]. *FANCA, BRIP1* and *CDK12* are both reported to affect the PSA response in clinical trials with preliminary results; *FANCA* and *BRIP1* are also affecting radiographic response in clinical trial, although only in a single patient [22]. *FANCA* is a DDR gene involved in inter-strand DNA cross-link repair, and it found to be altered in 1.3% of tumor samples in an extensive research cohort of more than 3K PC patients [27]. *BRIP1* (*BRCA1* interacting protein) is a DNA repair gene that contributes to the DNA repair function of *BRCA1 [28]*. Since, *CDK12* has been reported to control the expression of DDR genes [29], loss of function of *CDK12* appears to preferentially affect genes that have prominent roles in DNA repair [30]. The role of *FANCA*, *BRIP1* and *CDK12* in PC risk and PARPi treatment need to be further investigated.

Based on first clinical trial results we can conclude, that plausible candidate genomic biomarkers affecting clinical endpoints of PC patients, therefore eligible for targeted patient selection for PARPi treatment beyond the *BRCA* genes are the *ATM, BRIP1, FANCA, CDK12*. Application of these genes may enable the use of PARP inhibitors in *BRCA* wild type PC. Even more, PC patients without known DDR gene mutation have shown a 6% response rate to olaparib treatment [16].

PC has a broad spectrum of clinical presentation, thus it is of utmost importance that PRAP inhibitors are being trialed in several type and stage of the disease. Patients in studies with preliminary results include mCRPC, castration sensitive biochemically recurrent PC, castration sensitive biochemically recurrent non-mCRPC. Ongoing studies will test the applicability of PARP inhibitors in even wider spectrum of the disease, involving mCRPC, CRPC, metastatic PC, unresectable and locally advanced, locally advanced PC, high-risk clinically localized PC, localized PC, aggressive type PC, relapsed PC and even PC.

Today there are several PARP inhibitors battling for FDA approval in PC, and olaparib was believed to have a lead. The FDA has even granted its breakthrough therapy designation (BTD) for several PARPi (e.g. olaparib, niraparib) in *BRCA1/2* gene-mutated mCRPC patients who received prior taxane chemotherapy and AR-targeted therapy. The focus of the most of the ongoing trials is still on olaparib, followed by rucaparib, niraparib, talazoparib.

In summary, in this work we pinpointed the role of genome guided patient selection in PARPi treatment of PC and identified the set of clinically most actionable genetic biomarkers in order to

reinforce precision cancer treatment in PC. Important to note, that PARP inhibitors known to increase cytotoxicity by inhibiting DNA repair of normal healthy cells as well, which is a main disadvantage of the drugs under development.

FUTURE PERSPECTIVE

The future of treatment for PC may take us beyond androgen deprivation to combination therapies with PARP inhibition, such as combination with CYP17 inhibitor abiraterone, AR antagonist enzalutamide, the taxane cabazitaxel or the alpha-emitter radium-223.

The breakthrough is expected to come in DDR mutated mCRPC, however, none of the PARP inhibitors do have FDA approval for PC yet. Based upon preliminary results of clinical trials in PC, not only *BRCA1/2* but other DDR genes deleterious mutations are under investigation that could be associated with PARPi sensitivity. Genomic alterations especially in *ATM* gene as second line predictive biomarker of response to PARPi may be included to indications of use. The list of predictive biomarkers can be expanded by other DNA repair genes in future, like *BRIP1, FANCA, CDK12*, which have shown to affect sensitivity to PARP inhibitors. Even building a homologous recombination deficiency score (HRDS) is a future possibility.

From practical point of view, it has to be clearly indicated in the clinical trials whether they examine germline or somatic DDR mutations. Patient selection for PARPi therapy should be based on corresponding cleared and FDA/EMA approved companion diagnostic test for DDR genomic alterations present in drug label. In clinical application, liquid biopsy-based test would be most feasible to detect both germline and/or somatic DNA repair defects in circulating tumor DNA from whole blood, which will facilitate patient selection for PARPi treatment. Decreasing costs of next generation sequencing and available interpretation tools are foreseen to encourage the implementation of precision medicine in PC patients. Of note, long-term patient follow up is needed to evaluate efficacy and safety profiles of PARPi therapy in PC patients.

Limitations of clinical trials are clearly the small sample size of patients, therefore large cohorts and multicentric trials are needed to accelerate drug development and personalize clinical decision making by using biomarkers for drug sensitivity and response.

EXECUTIVE SUMMARY

- Application of DNA damage repair genes as predictive biomarkers in patient selection aids to design biomarker-driven targeted PARPi therapy in prostate cancer.
- Clinical trials with preliminary results showed that *BRCA2, BRCA1, ATM, BRIP1, FANCA* and *CDK12* mutations affect endpoints like PSA response rate, radiographic response, PSA progression-free survival and overall survival in CRPC.
- Based on these results, *BRCA2, BRCA1, ATM, BRIP1, FANCA* and *CDK12* mutations are candidate genomic biomarkers for PARPi sensitivity in CRPC.
- Beyond these mutations, ongoing trials explore the role of *ATR, BARD1, CHEK1, CHEK2, ERCC3, FAM175A, FANCD2, FANCL, GEN1, HDAC2, MLH1, MLH3, MRE11, MSH2, MSH6, NBN, PALB2, PMS2, PIK3CA, PPP2R2A, PTEN, RAD51, RAD51B, RAD51C, RAD51D* and *RAD54L* mutations in additional endpoints also as disease-free state and dose limiting toxicity of PC patients.
- Most frequently investigated PARPi in prostate cancer is olaparib followed by rucaparib, niraparib, talazoparib and veliparib.
- Validation of existing biomarkers have to be done for a wide range of prostate cancer subtypes, e.g. primary PC, locally advanced PC, aggressive type PC, CRPC, mCRPC.
- In clinic, a liquid biopsy-based tests would be most feasible to detect DNA repair defects in circulating tumor DNA from whole blood.
- Long-term follow up is needed due to the cytotoxic adverse events affecting normal healthy cells.
- PARP inhibitors show promise for a subset of mCRPC patients, and with the number of actionable genes/genomic alterations available, more trials have to be conducted to build available therapies.

Table 1. PARPi clinical trials with preliminary results in prostate cancer

PARP inhibitor (Manufacturer)	NCT identifier, STUDY NAME, Phase	Patient population (Number of Patients)	Treatment/ Dosage	Endpoints			DNA Repair Genes	Utility of DNA repair genes	Reference
				PSA response rate	Survival	Radiographic Progression Free Survival			
Olaparib @Lynparza (AstraZeneca)	NCT01682772 TOPARP II.	mCRPC, after one or two regimens of chemotherapy (49)	olaparib 400 mg daily	All 7 pts (14%) with BRCA2 loss had PSA levels that fell by 50% or more from baseline	OS was prolonged in the biomarker-positive group vs in the biomarker-negative group, 13.8 months vs. 7.5 months; p = 0.05	rPFS was significantly longer in the DDR positive group 9.8 vs. 2.7 months; p<0.001. From 7 pts with BRCA2 loss 5 pts had measurable disease and had a radiologic partial response. 4 of the 5 pts with deleterious ATM mutations had a response.	Somatic BRCA1, BRCA2 (14%), ATM (10%), CHEK2, FANCL. Alltogether,16 pts (33%) had somatic DDR gene mutation	PSA RR, OS, rPFS	Mateo et al 2015 [16]
	NCT03047135 II.	mCRPC (20)	olaparib 300 mg daily	2 (10%) men with BRCA2 mutations had complete PSA responses; 1 (5%) men with BRCA2 mutation had PSA50 response; 4 other men (20%) had minor PSA responses. Median PSA progression-free survival was greater in men with BRCA2/ATM mutations vs. without (9 vs. 4 months; p= 0.02)	No data	No data	Somatic BRCA1, BRCA2, ATM	PSA RR, PFS	Antonarakis et al 2019 [17]
	NCT01972217 II.	mCRPC (71+71=142)	arm A: oral olaparib 300 mg, abiraterone 1000 mg, prednisolone 5 mg; arm B: placebo, abiraterone 1000 mg, prednisolone 5 mg	Overall response, confirmed PSA response, circulating tumour cell conversion rates were similar in both treatment groups		rPFS was significantly longer in the olaparib group: 13.8 months vs 8.2 months, p=0.034. Data suggest that the drug combination might have resulted in rPFS benefit regardless of HRR mutation status.	Germline or somatic: BRCA1, BRCA2, ATM, BARD1, BRIP1, CDK12, CHEK1, CHEK2, FANCL, PALB2, RAD51B, RAD51C, RAD51D, RAD54L. HRR mutation status was not used as a stratification factor at randomisation.	-	Clarke et al 2018 [18]
	NCT03810105 II.	castration sensitive biochemically recurrent PC, castration sensitive biochemically recurrent non-mCRPC (17)	olaparib 600 mg daily, durvalumab 1500 mg i.v. every 28 days	9 of 17 pts (53%) had a radiographic and/or PSA response. Pts with alterations in DDR genes were more likely to respond. 2/3 of responders had DDR gene alterations.			Germline or somatic BRCA1, BRCA2, ATM, BARD1, BRIP1, CHEK2, CDK12, FANCA, PALB2, RAD51C, RAD51D	PSA RR, rPFS	Karzai et al. 2018.[19]

	NCT02987543 ProFound Study III.	mCRPC (245+142 = 387)	arm A: olaparib 300 mg; arm B: enzalutamide 160 mg OR abiraterone acetate plus 1.000 mg with prednisone 5 mg	No data	Olaparib favourable trend for OS:18.5 vs 15.11 months, p= 0.01	olaparib improved rPFS according to RECIST, Media 7.39 months vs 3.55 months, p<0.0001	Cohort A: somatic BRCA1, BRCA2, ATM. Cohort B: somatic BARD1, BRIP1, CDK12, CHEK1, CHEK2, FANCL, PALB2, PPP2R2A, RAD51B, RAD51C, RAD51D, RAD54L	-	Hussain et al 2019 [20]
Niraparib @Zejula Tesaro	NCT02854436 GALAHAD II.	mCRPC (123; 39 pts with biallelic DDR gene mutation; 23 BRCA1/2)	niraparib 300 mg	Composite RR was defined as an objective response by RECIST 1.1 for measurable disease, circulating tumor cell conversion to < 5/7.5 mL blood or PSA decline of ≥50% (PSA50). Composite and objective RRs were 65% and 38% in pts with biallelic BRCA1/2, respectively. 3/8 pts (38% [2/5 BRCA1/2 and 1/3 non-BRCA]) with measurable visceral metastases showed objective response.	Among the 20 biallelic responders, the duration of treatment has exceeded 4 months in 13 pts and 6 months in 8 pts; 14 pts remain on treatment.		Germline or somatic BRCA1, BRCA2, ATM, BRIP1, CHEK2, FANCA, HDAC2, PALB2	-	Smith et al 2019 [21]
Rucaparib @Rubraca Clovis Oncology	NCT02952534 TRITON2 II.	mCRPC (85)	rucaparib 1200 mg	Among pts with a BRCA1/2 alteration, 51.1% (23/45) had a confirmed PSA response. A confirmed PSA response was also observed in 1 pt with a CDK12 alteration, 1 pt with a BRIP1 alteration, and 1 pt with a FANCA alteration.	Median treatment duration in the overall population was 3.7 months (range, 0.5–12.9 months). Median treatment duration in pts with a BRCA1/2 alteration was 4.4 months (range, 0.5–12.0 months).	Of pts with a BRCA1/2 alteration and measurable disease at baseline, 44.0% (11/25) had a confirmed radiographic response. A confirmed radiographic response by 1 pt with a BRIP1 alteration and 1 pt with a FANCA alteration.	Germline or somatic BRCA1, BRCA2, ATM, BARD1, BRIP1, CDK12, CHEK2, FANCA, NBN, PALB2, RAD51, RAD51B, RAD51C, RAD51D, RAD54L	-	Abida et al 2018 [22]
Talazoparib @Talzenna Pfizer	NCT03395197 TALAPRO-2 III.	mCRPC (19)	talazoparib 0.5 OR 1 mg plus enzalutamide 160	92% and 100% of pts had a 50% decline from baseline in PSA in the 1 mg and 0.5 mg cohorts.	No data	No data	unspecified DDR mutations	-	Agarwal et al 2019 [23]

Veliparib (AbbVie)	NCT01576172 II.	mCRPC (72+76 = 148)	arm A: abirterone acetate 1 mg, prednisone 10 mg; arm B: arm A plus veliparib 600 mg	Pts with DDR gene mutation had significantly higher PSA RR: 90% vs 56.7%; p = 0.007, PSA decline ≥ 90%; 75% vs 25%; p = 0.001.	Pts with DDR gene mutation had measurable disease RR: 87.5% vs 38.6%; p = 0.001.	Pts with DDR gene mutation had longer median PFS: 14.5 vs 8.1 months; p = 0.025. Median PFS was longer in pts with normal PTEN: 13.5 v 6.7 months; p = 0.02, normal TP53: 13.5 vs 7.7 months; p = 0.01, and normal PIK3CA: 13.8 vs 8.3 months; p = 0.03.	Somatic BRCA1, BRCA2, ATM, FANCA, PALB2, RAD51B, RAD51C, TP53, PTEN, PIK3CA	RR, PFS, OS	Hussain et al 2018 [24]

Table 2. Ongoing PARPi trials in prostate cancer

PARP inhibitor (Manufacturer)	NCT number	Phase	Population	Treatment	Primary Outcome	DNA Damage Repair Genes
Olaparib @Lynparza (AstraZeneca)	NCT03317392	I.	mCRPC	olaparib with radium Ra 223 dichloride	maximum tolerated dose of olaparib and radium Ra 223 dichloride, rPFS	Not specified; Oncopanel testing
	NCT03434158 (IMANOL)	II.	mCRPC	olaparib	rPFS	Germline or somatic BRCA1, BRCA2, ATM, CHEK2, FANCL, MLH1, MRE11, MSH2, MSH6, PMS2, PALB2, RAD51C
	NCT03263650	II.	aggressive type PC	olaparib, when given after treatment with cabazitaxel, carboplatin and prednisone	PFS	Genomic alterations in DDR pathway genes induced and/or selected by carboplatin and cabazitaxel chemotherapy
	NCT03432897 (BrUOG 337)	II.	locally advanced PC	olaparib prior to radical prostatectomy	PSA RR	Tumor tissue or cell-free DNA from peripheral blood BRCA1, BRCA2, ATM, BARD1, BRIP1, CDK12, CHEK1, CHEK2, FANCL, HDAC2, PALB2, PPP2R2A, RAD51B, RAD51C, RAD51D, RAD54L
	NCT03570476	II.	localized PC	olaparib prior to radical prostatectomy	pCR rate, incidence of adverse events	Germline or somatic BRCA1, BRCA2, ATM, FANCA, PALB2
	NCT03012321 (BRCAAway)	II.	mCRPC	abiraterone/prednisone or olaparib, or abiraterone/prednisone/olaparib	PFS	Germline or somatic ATR, BRIP1, CDK12, CHEK2, ERCC3, FAM175A, FANCA, GEN1, HDAC2, MLH3, MRE11, MSH2, MSH6, NBN, PALB2, PMS2, RAD51 defects will be assigned to Arm IV with single agent olaparib
	NCT03516812	II.	CRPC	olaparib with testosterone	PSA decline of at least 50% below baseline. Incidence of adverse events according to National Cancer Institute Common Terminology Criteria for Adverse Events	Tumor tissue or cell-free DNA from peripheral blood: 50% of enrolled subjects have unspecified homozygous deletions, deleterious mutations, or both in one or more of the DDR genes; the other 50% of pts must have an intact DDR pathway
	NCT02893917	II.	mCRPC	olaparib with or without cediranib	rPFS	Not specified; HRD positive status analyzed by BROCA-HR test.
	NCT03732820 (PROpel)	III.	mCRPC	olaparib or abiraterone	rPFS	Germline or somatic BRCA1, BRCA2, ATM, HRR
Niraparib @Zejula (Tesaro)	NCT04030559	II	high-risk, clinically localized PC	niraparib before surgery	pRR	Somatic BRCA1, BRCA2, ATM, BRIP1, CDK12, CHEK1/2 FANCA, FANCD2, FANCL, GEN1, NBN, PALB2, RAD51, RAD51C
	NCT04037254	II.	PC	niraparib with standard combination radiation therapy and androgen deprivation therapy	disease-free state	Unspecified DDR mutations
	NCT03748641 (MAGNITUDE)	III.	mCRPC	niraparib with abiraterone acetate and prednisone versus abiraterone acetate and prednisone	rPFS	Unspecified DDR mutations
Rucaparib @Rubraca (Clovis Oncology)	NCT04171700	II.	unresectable, locally advanced or metastatic solid tumor and relapsed/progressive PC	rucaparib	Best OR rate as assessed by the investigator by RECIST	Germline or somatic BRCA1, BRCA2, BARD1, BRIP1, FANCA, NBN, PALB2, RAD51C, RAD51D

	NCT03413995 (TRIUMPH)	II.	metastatic PC	rucaparib	PSA RR	Germline BRCA1, BRCA2, ATM, CHEK2, GEN1, PALB2, RAD51D
	NCT03442556	II.	mCRPC	docetaxel with carboplatin followed by rucaparib	rPFS	Somatic BRCA1, BRCA2, ATM, PALB
	NCT03533946 (ROAR)	II.	mCRPC	rucaparib	50% reduction in PSA levels	Germline or somatic BRCA1, BRCA2, ATM, ATR, BARD1, BRIP1, CDK12, CHEK1, CHEK2, ERCC3, FAM175A, FANCA, FANCL, GEN1, HDAC2, MLH1, MRE11, NBN, PALB2, PPP2R2A, RAD51, RAD54L
	NCT02975934 (TRITON3)	III.	mCRPC	rucaparib or abiraterone acetate or enzalutamide or docetaxel	rPFS	BRCA1, BRCA2, ATM
Talazoparib @Talzenna (Pfizer)	NCT03330405	IB/II.	CRPC	talazoparib with avelumab	DLT, OR	Somatic BRCA1, BRCA2, ATM
	NCT03148795 (TALAPRO-1)	II.	mCRPC	talazoparib	ORR	Not specified; somatic DDR mutations analyzed by FoundationOne CDx™ NGS gene panel

Table 3. DNA repair genes affected endpoint of PARPi treated prostate cancer patients – preliminary results

Genetic alteration	**Affected endpoint**	**Rate**	**Reference**
BRCA2 loss	PSA50 response	7 from 7 patients (100%)	Mateo et al 2015 [16]
	radiographic partial response	5 from 7 patients (71%)	
BRCA1/2 biallelic mutation	composite PSA RR	65%	Smith et al 2019[21]
BRCA1/2 biallelic mutation	objective PSA RR	3 from 8 patients (38%)	
BRCA1/2 biallelic mutation	survival increase	13 from 20 patients (65%)	
BRCA2 mutation	PSA50 response/Complete PSA response	3 from 3 (100%) / 2 from 3 patients (67%)	Antonarakis et al 2019 [17]
BRCA1/2 alteration	PSA response	23 from 45 patients (51%)	Abida et al 2018 [22]
BRCA1/2 alteration	survival (measured by median treatment duration)	4.4 (BRCA1/2) vs 3.7 (overall)	
BRCA1/2 alteration	radiographic response	11 from 25 patients (44%)	
BRCA2/ATM mutations	longer PSA progression-free survival	-	Antonarakis et al 2019 [17]
ATM mutation	radiographic partial response	4 from 5 patients (80%)	Mateo et al 2015 [16]
BRIP1 alteration	PSA response	1 patient	Abida et al 2018 [22]
BRIP1 alteration	radiographic response	1 patient	
FANCA alteration	PSA response	1 patient	
FANCA alteration	radiographic response	1 patient	
CDK12 alteration	PSA response	1 patient	

Figure 1. Genomic biomarkers in PARPi clinical trials to guide precision treatment of prostate cancer

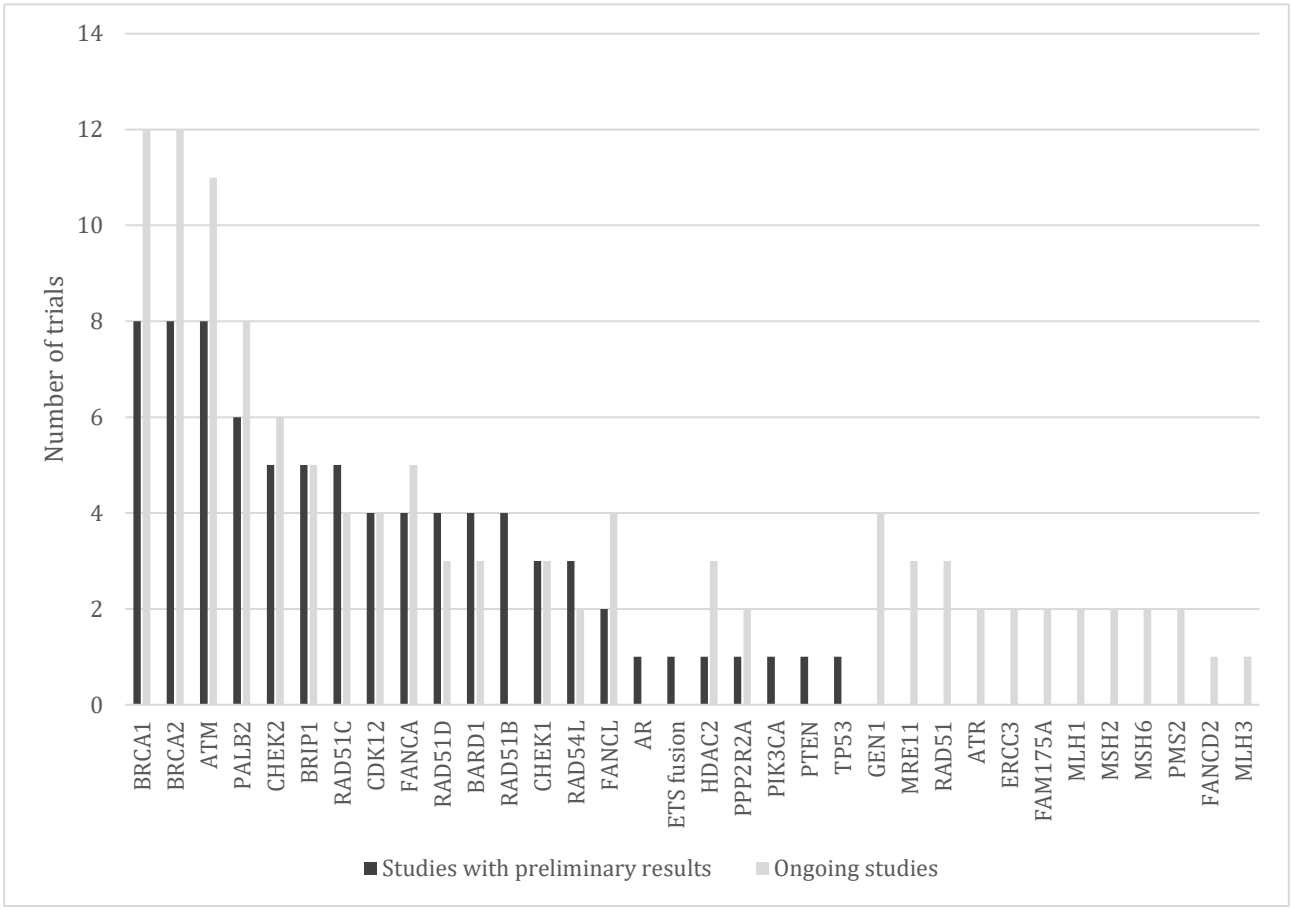

TABLE LEGENDS

DNA damage repair (DDR)
dose limiting toxicity (DLT)
homologous recombination deficiency (HRD)
homologous recombination deficiency score (HRDS)
ClinicalTrials.gov identifier number (NCT identifier)
number of patient/patients (pt/pts)
objective response rate (ORR)
overall response (OR)
pathologic response rate (pRR)
pathologic complete response (pCR)
progression free survival (PFS)
prostate specific antigen (PSA)
prostate cancer (PC)
response rate (RR)
radiographic progression-free survival (rPFS)
response evaluation criteria in solid tumors (RECIST)
single nucleotide polymorphism (SNP)

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DISCLOSURES

The authors have declared no conflict of interest.

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*Annotations: *of interest; **of considerable interest;*

*4. **Satoh et al: This study was the first and laid down the basis of PARP enzymes in DNA repair.*
*7. **Castro et al: Role of germline BRCA mutations in advanced prostate cancer*
*10.** Robinson et al: High priority study on genomics of advance prostate cancer.*
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*15. *Mateo et al: First study pinpointing that BRCA2 and ATM genes affect endpoints of olaparib treated prostate cancer patients.*
*31. *FDA: Indicate breakthrough therapy designation for PARP inhibitors by FDA*

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Paper 4.

"Liquid Biopsy" in the service of clinical oncology: a dream or an emerging reality?

Orvosi Hetilap. 2019, 60. évfolyam, 7. szám, 279.

Várnai R, Sipeky C. „Folyékony biopszia” a klinikai onkológia szolgálatában: álom vagy küszöbönálló valóság? Orvosi Hetilap. 2019, 60. évfolyam, 7. szám, 279.

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Translated from Hungarian to English

The original version is available as attachment of the PhD thesis (see page 91.)

Letter to the editor

"Liquid Biopsy" in the service of clinical oncology: a dream or an emerging reality?

The presence of cell-free DNA circulating in the blood has been of great interest to researchers for decades. Examination of fetal DNA from maternal blood is already a routine procedure, but circulating DNA analysis can be used in other areas of medicine too. Circulating cell-free DNA fragments enter the bloodstream through cells undergoing apoptosis and necrosis; for example, their levels may increase during cancer.

In case of targeted anticancer therapy or therapy resistance, the basis of the drug change is the genetic analysis of the histological sample obtained during biopsy. Unfortunately, besides the obvious disadvantages of biopsy (invasive, complications, costly, requiring prior appointment), the biopsy sample does not provide information on intra-tumoral and inter-metastatic heterogeneity. In contrast, taking a blood sample, a "liquid biopsy", is a minimally invasive procedure that can be performed at any time during treatment and can provide information about all tumor cells present in the body.

Despite the low levels of circulating tumor DNA in early-stage cancers, due to new molecular genetic testing methods (BEAMing, PAP, Digital PCR, TAM-Seq) small amounts of circulating tumor DNA fragments and rare genetic variants can also be detected. The average sensitivity of these new procedures is less than 0.01%, whereas the "liquid biopsy" sensitivity for stage IV tumors is close to 100%.

One of several possible uses of "liquid biopsy" is to *monitor tumor progression*. The half-life of circulating tumor DNA is only 2 hours, so changes in tumor size can be promptly

detected. In melanoma, ovarian, breast, and colon tumors, the amount of circulating tumor DNA increases steeply during tumor progression, whereas after successful medical or surgical treatment, the amount of circulating tumor DNA decreases.

Another potential application of "liquid biopsy" is the *detection of residual tumor* after surgical intervention for curative purposes. Studies have shown that all patients with postoperatively detectable circulating tumor DNA have relapsed, whereas patients with immeasurably low circulating tumor DNA have remained cancer free for 5 years.

"Liquid biopsy" could be used also for *early detection of acquired resistance* during chemotherapy, allowing for drug modification prior to the onset of clinical resistance. During imatinib treatment of Philadelphia chromosome positive myeloid leukemia; during gefitinib and erlotinib treatment of lung and colon cancers, the development of secondary resistance is not uncommon. The appearance of a *KRAS* mutation in circulating cell-free DNA in patients undergoing anti-EGFR treatment may predict radiologically detectable relapse months earlier.

In order to support clinical decision-making and establishment of therapeutic protocols with information acquired from circulating tumor DNA, standardization of studies, reduction of DNA analysis costs and appropriate collaboration with bioinformaticians are essential. Thereby, liquid biopsy may become an effective method of clinical oncology in the near future.

Abbreviations:

DNA = deoxyribonucleic acid; EGFR = epidermis growth factor receptor

Literature on which the paper is based on:

Crowley E, Di Nicolantonio F, Loupakis F, et al. Liquid biopsy: Monitoring cancer genetics in the blood. Nat Rev Clin Oncol. 2013; 10: 472-484.

Diaz LA Jr, Bardelli A. Liquid biopsies: genotyping circulating tumor DNA. J Clin Oncol. 2014; 32: 579-586.

VI. NOVEL FINDINGS

The aims of this academic dissertation were to examine how PGx biomarkers are applied in clinical practice in context of drug labels, and what are the current opportunities (docetaxel) and future perspectives (PARPi) of biomarker based precision treatment of PC.

Novel findings of my academic dissertation are summarized in this chapter.

Novel findings of Paper 1.

Pharmacogenomic biomarker information differences between drug labels in the United States and Hungary: implementation from medical practitioner view

- 264 drugs were identified in the US FDA Table of Pharmacogenomic Biomarkers in Drug Labeling. Out of these 264 active ingredients we were able to identify 195 (74%) through the website of the National Institute of Pharmacy and Nutrition in Hungary being available in Hungary.
- Among the 195 drugs 145 (75%) have PGx information included in the Hungarian SmPC. PGx information was partially present in drug label of 20 (10%), completely missing from drug label of 30 (15%) available active ingredients in Hungary compared to US FDA. These drugs without PGx biomarker information in their label belong to diverse therapeutic areas like 23% to oncology, 23% to anesthesiology, 20% to infectious diseases, 7% to cardiology, 7% to inborn error, 7% to rheumatology, 3% to dermatology, 3% to hematology, 3% to psychiatry, 3% to pulmonology.
- The identified 195 drugs with PGx data dispose 222 biomarkers in the Hungarian SmPCs. In the Hungarian SmPCs we identified information either on metabolizing enzymes (n=102, 46%), pharmacological targets (n=90, 41%) or other features (n=30, 13%).
- The most common biomarkers in Hungarian SmPCs are the *CYP2D6* (n=40, 18%), the *CYP2C19* (n=18, 8%), the estrogen and progesterone hormone receptors (*ESR, PGR*, n=15, 6%), the *ERBB2* (n=12, 5%) and the *G6PD* (n=10, 4%).

- We also observed that none of the SmPCs containing PGx biomarker data has any PGx evidence specifically for Hungarian population neither on clinical endpoints nor on pharmacokinetics.
- According to the Hungarian product summary, the aim of PGx biomarker use can be the following: effects efficacy (n=84), indicates toxicity (n=67), belongs to the inclusion criteria (n=67), belongs to the exclusion criteria (n=24) because of elevated toxicity risk or effects dosage (n=18). Moreover, 53 biomarkers (24% of all) are involved in drug-drug interaction management as dose modification or elevated toxicity risk was connected to the presence of enzyme inhibitor/inductor irrespective of the PGx background. Highly importantly, 8 biomarkers (4 %) are factual in point of dosing and formulate exact algorithm to manage gene-drug interaction.
- Out of the biomarkers available in US drug labels, 62 (22%) are missing from the Hungarian SmPCs. Most of the missing PGx biomarkers belong to the therapeutic area of oncology (42%), followed by anesthesiology (18%), infectious diseases (13%), hematology (8%); cardiology, dermatology, gastroenterology, inborn errors of metabolism, psychiatry, pulmonology, rheumatology represent minor proportions (less than 4% each).
- The level of action of PGx biomarkers between Hungary and US was compared. Testing is required at 72 biomarkers (25 %) in Hungary, from which 66 (92%) belong to field of oncology. In US, in case of 79 (28%) biomarkers testing is obligatory before treatment. 4 (1%) biomarkers in Hungarian drug labels are ranked into testing recommended category, 6 (2%) biomarkers in US. PGx information is actionable at 95 (34%) biomarkers in Hungary, compared to 108 (38%) in US. Out of the actionable biomarkers in US, 14 (5%) biomarkers dispose exact dosing adjustment in PharmGKB recommendation, but only 8 (3%) of them are ranked into the same category in Hungary. The 6 (3%) remaining biomarkers predispose only actionable PGx data without dosing info in Hungarian drug inserts. 51 (18%) biomarkers have informative PGx data in Hungarian drug label, however in the US 77 (27%) biomarkers are counted into this category (p=0.009). Even from US FDA biomarkers 14 (5%) are missing from PharmGKB, which shows generally a rather delayed implementation of PGx information. This was the case for 62 (22%) biomarkers for Hungarian SmPC's.

- Talking about the PGx level of action, out of the 62 missing biomarkers from Hungarian SmPC's 7 (11%) belong to testing required category, 27 (44%) belong to actionable PGx category and 21 (29%) belong to informative PGx category according to PharmGKB.
- The partially missing biomarkers in Hungarian SmPCs belong to 20 drugs, completely missing biomarkers to 30 drugs. Notably, after checking the level of action, in case of 7 from these 50 drugs biomarker testing is required before treatment according to PharmGKB. It is of utmost importance, that 6 from these 7 drugs belong to oncology medication and therefore define cancer treatment. On the other hand, in case of 9 oncology drugs the Hungarian SmPCs are even stricter than the FDA recommendation and genetic testing is required before treatment.

Dynamic update:

- The number of drugs with PGx information in the drug label has elevated in US with 57% vs in Hungary with 46% in last 26 months (May 2017 - July 2019).
- The percentage of missing PGx data in Hungarian drug labels has doubled compared to US in last 26 months because of accelerated PGx biomarker implementation in US FDA drug labeling.

Recommendation:

- None of the Hungarian product summaries did ever refer on an exact laboratory for biomarker testing. The information on lab test availability is based on clinics internal regulation and doctor's daily routine either on commercial test or on academic setting. More information for clinicians is needed about lab availability and test methodology.
- More factual, clear, clinically relevant PGx information in Hungarian SmPCs would reinforce implementation of pharmacogenetics.

Novel findings of Paper 2.

Pharmacogenomic Biomarkers in Docetaxel Treatment of Prostate Cancer: From Discovery to Implementation

Identified germline genomic biomarkers affecting individual treatment differences in docetaxel mono- and combination therapy of PC published between 2006 and 2018 are the following:

- *AAG, ABCB1, ABCB4, ABCB11, ABCC2, ABCC5, ABCC6, ABCG2, ATP7A, ATP8A2, CHST3, CYP1B1, CYP2D6, CYP3A4, CYP3A5, CYP4B1, CYP19A1, ESR1, GSTP1, MDR1, NAT2, PPAR-δ, SLCO1B3, SLC5A6, SLC10A2, SPG7, SULT1C2, VAC14* and *VEGF-A.*

Clinical translational potential of germline genomic biomarkers in docetaxel treatment of PC according to publications between 2006 and 2018 are the followings:

- CR was influenced by *CYP1B1* (rs1056836), *ABCG2* (rs2231142), *CHST3* (rs4148950, rs1871450, rs4148945).
- Toxicity risk was increased by *CHST3* (rs4148950, rs1871450, rs4148945), *MDR1/ABCB1* (rs1045642, rs2032582) and *ABCC2* (rs12762549).
- Dosing was reduced by *ABCC2* (rs12762549).
- OS was improved by *CYP1B1* (rs1056836), *ABCG2* (rs2231142) and *MDR1/ABCB1* (rs1045642, rs2032582).
- PFS was enhanced by *CYP1B1* (rs1056836).

Results of main relevant clinical trials of docetaxel treatment in PC incorporating genomic signatures are the following:

- The aim of NCT00503984 was to determine whether azacitidine could reverse docetaxel resistance in mCRPC patients by decreasing methylation of the proapoptotic *GADD45A* gene. With regards to the second *terminated trial* (NCT01253642), only the frequency of *MAOA* (monoamine oxidase A) overexpression in tumors that have progressed during

docetaxel treatment was reported. *MAOA* overexpression was observed in all investigated progressing tumors.

- The focus of several *ongoing clinical trials* was treatment response to docetaxel treatment in combination with emerging new medications in tumors harboring inactivating mutations in HR genes, including *BRCA1*, *BRCA2* and *ATM*.

Implementation of biomarkers in treatment guidelines:

- There are no predictive biomarkers to guide treatment decisions in PC according to EAU and ESMO guidelines, even though there are some known prognostic biomarkers. On the other hand, the EAU guideline discussed multiple diagnostic or prognostic genetic biomarkers and their use in the clinic.
- Guidelines suggest that the first future application of pre-emptive genetic testing commences and involves the HRD genes, since these patients might benefit from treatment with PARP inhibitors, but no definite recommendation has been made yet.

Novel findings of Paper 3.

Precision treatment of prostate cancer: will genetic biomarker guided PARP inhibitors introduce a game-change?

- Application of DNA damage repair genes as predictive biomarkers in patient selection aids to design biomarker-driven targeted PARPi therapy in PC.
- Clinical trials with preliminary results showed that *BRCA2, BRCA1, ATM, BRIP1, FANCA* and *CDK12* mutations affect endpoints like PSA RR, radiographic response, PSA PFS and OS in CRPC.
- Beyond these mutations, ongoing trials explore the role of *ATR, BARD1, CHEK1, CHEK2, ERCC3, FAM175A, FANCD2, FANCL, GEN1, HDAC2, MLH1, MLH3, MRE11, MSH2, MSH6, NBN, PALB2, PMS2, PIK3CA, PPP2R2A, PTEN, RAD51, RAD51B, RAD51C, RAD51D* and *RAD54L* mutations in additional endpoints as disease-free state and dose limiting toxicity of PC patients.
- Most frequently investigated PARPi in PC was olaparib followed by rucaparib, niraparib, talazoparib and veliparib.

Novel findings of Paper 4.

"Liquid Biopsy" in the service of clinical oncology: A dream or an emerging reality?

- Circulating tumor DNA analysis could be used for cancer treatment in *monitoring tumor progression,* in *detection of residual tumor* after surgical intervention and in *early detection of acquired resistance* during chemotherapy.
- Standardized circulating tumor DNA studies have to be evaluated and the results included in therapeutic protocols in order to support clinical decision-making.
- The reduction of DNA analysis costs and improved collaboration with bioinformaticians are crucial during adaption of “liquid biopsy” results for clinical implementation.

VII. SUMMARY OF NEW OBSEVATIONS AND FUTURE PERSPECTIVES

Summary highlights of new observations and future perspectives of my academic thesis are the followings.

Summary of PGx biomarker information found in US FDA and Hungarian drug labels:

1. US drug labels displayed significantly more specific PGx subtitles than similar Hungarian SmPCs. Oncology is the most common therapeutic area with PGx information in the drug label both in US and in Hungary. Regarding oncological drugs, Hungarian SmPCs are stricter in genetic testing requirement than US labels.
2. Principal objective of PGx biomarker use in Hungarian drug labels is the improvement of treatment efficacy. In Hungary, the most frequently tested biomarkers in oncology are pharmacological targets where molecular diagnostics is required for patient selection and genotype-directed precision therapy.
3. US FDA offers more relevant data about dose modifications than Hungarian drug labels.
4. PGx biomarker information is usually based on adult studies both in Hungarian and in US SmPCs; pediatric patient groups are rarity.
5. Hungarian drug labels do not clearly categorize the PGx biomarker into metabolizing enzymes, pharmacological targets and others. However, classification of biomarkers has to be included in Hungarian SmPC's, in order to provide clear PGx information and enable consequent implementation of genetic biomarkers in clinical setting.
6. Europe-wide database for PGx laboratory test availability would enhance clinical implementation. In Hungary PGx biomarker tests are provided by three university laboratories (Pécs, Budapest, Debrecen) and by industrial participant in limited sets. Laboratories are selected upon personal practice of the specific doctors now in Hungary. Ready-to-apply implementation platforms could enhance clinical output.
7. Forthcoming perspective is to encourage regulatory stakeholders to improve inclusion of PGx biomarkers into Hungarian drug labels and consequently strengthen PM in Hungary.

Summary of PGx biomarkers in docetaxel treatment of PC:

1. More and more research studies propose to determine the association between genetic makeup of PC patients and docetaxel drug response, resistance and toxicity. Nevertheless, only a few considerable PGx candidates moved forward to clinical validation.
2. To push biomarkers in direction of clinical implementation, prospective study designs, larger discovery cohorts and consecutive clinical validation in good quality randomized trials are needed.
3. Following genes seem to have translational potential in CR, toxicity, dosing, OS, PFS during docetaxel treatment of PC according to our results:
    a) *CYP1B1* gene encodes a member of the cytochrome P450 superfamily of enzymes that catalyze many reactions involved in drug metabolism. The *CYP1B1* rs1056836 gene variant seems to influence CR, OS, PFS during docetaxel treatment of PC.
    b) *ABCB1*, also known as multi-drug resistance protein 1 (*MDRP1*), is one of members in the superfamily of human adenosine triphosphate (ATP)-binding cassette (ABC) transporters that encode transporter and channel proteins that function as drug efflux pumps for xenobiotics compounds with broad substrate specificity and are involved in multidrug resistance. It is liable for decreased drug accumulation in multidrug-resistant cells and generally mediates the expansion of resistance to anticancer drugs. *MDR1/ABCB1* (rs1045642 and rs2032582) influences OS in docetaxel treatment of PC.
    c) *ABCG2* encodes an ATP-binding cassette (ABC) transporter. *ABCG2* rs2231142 gene variant affects CR and OS during docetaxel treatment of PC according to findings.
    d) *ABCC2* encodes another member of the superfamily of ABC transporters. These proteins are member of the MRP subfamily, and are involved in multi-drug resistance. Our result synthesis show, that *ABCC2* rs12762549 gene variant is associated with dose reduction and increased toxicity risk.
    e) *CHST3* gene encodes an enzyme which catalyzes the sulfation of chondroitin, a proteoglycan found in the extracellular matrix and most cells which is involved

in cell migration and differentiation [48,49]. *CHST3* (rs4148950, rs1871450 and rs4148945) influences CR and toxicity risk according the results.

Summary of genomic biomarkers guiding PARPi treatment in PC:

1. Next to *BRCA1/2,* deleterious mutations of other DDR genes could be associated with PARPi response according to preliminary results of clinical trials in PC. Especially *ATM* gene alterations may appear as second line predictive biomarkers of PARPi sensitivity.
2. Based on these results, *BRCA2, BRCA1, ATM, BRIP1, FANCA* and *CDK12* mutations are candidate genomic biomarkers for PARPi sensitivity in CRPC.
3. Constructing a homologous recombination deficiency score is an eventual opportunity.
4. PARPis offer potential for a subgroup of DDR mutated mCRPC patients. More trials have to be directed to amplify available therapies with the number of actionable genes and genomic alterations available. Long-term follow up is essential according to the cytotoxic adverse effects of PARPis influencing normal healthy cells.
5. Validation of existing biomarkers have to be done for all PC subtypes, e.g. primary PC, locally advanced PC, aggressive type PC, CRPC, mCRPC.

Summary of liquid biopsy perspectives:

1. Liquid biopsy is predicted to become a precision treatment tool in cancer patient management in the near future. Liquid biopsy based tests would be most feasible to detect DNA repair defects in circulating tumor DNA from whole blood in clinical setting.
2. Expanded multigene PGx panels defined by drug efficacy, drug toxicity, CR or survival would improve the predictive capacity of PGx biomarkers.
3. Continued PGx education is needed for clinical oncologists about the benefits of using genetic polymorphisms as predictive biomarkers in clinical routine and research.
4. Practicing medical doctors have to be informed about PGx biomarkers included in treatment guidelines, about available laboratory tests and about implementation tools to carry out PGx in clinical setting.

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IX. PUBLICATION LIST

Scientometrics

(as of February 2020)

Number of publications: 24

Indexed in PubMed: 9 (2 review, 7 research articles)

Cumulative impact factor: 20,457

Impact factor related to the thesis: 7,398

Impact factor of a submitted paper under review related to the thesis: 2,265

Total citations: 38

H-index: 3

i10-index: 1

First author: 15 articles

Co-author: 9 articles

Q1 article: The Pharmacogenomics Journal

ORCID: https://orcid.org/0000-0001-8440-3955

Google Scholar: https://scholar.google.com/citations?view_op=list_works&hl=en&user=fYcDXLwAAAAJ

Articles related to the thesis

1. **Varnai R**, Szabo I, Tarlos G, Szentpeteri LJ, Sik A, Balogh S, Sipeky C. Pharmacogenomic biomarker information differences between drug labels in the United States and Hungary: implementation from medical practitioner view. **Pharmacogenomics J**. 2019 Dec 2. **IF:3.503**
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1. **Várnai R,** Szentpéteri JL, Szabó I, Balogh S, Sipeky Cs. Precíziós orvoslás lehetősége farmakogenetiaki biomarkerek alkalmazásával Magyarországon. Családorvos Kutatók Országos Szervezetének XIX. Kongresszusa, Győr, 2020. február 27-29.
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4. **Várnai R,** Szentpéteri JL, Szabó I, Balogh S, Sipeky Cs. Elérhető farmakogenetikai vizsgálatok az alapellátásban Magyarországon. Családorvos Kutatók Országos Szervezetének XVIII. Kongresszusa, Debrecen, 2019. február 28 - március 2.

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Award

- **Best article in topic of general medicine 2020.** Családorvos Kutatók Országos Szervezete. **Varnai R**, Szabo I, Tarlos G, Szentpeteri LJ, Sik A, Balogh S, Sipeky C. Pharmacogenomic biomarker information differences between drug labels in the United States and Hungary: implementation from medical practitioner view. Pharmacogenomics J. 2019 Dec 2.

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Additional conference proceedings

(oral presentation, unless otherwise indicated)

1. **Várnai Réka**, Végh Mária. A Churg-Strauss szindrómáról egy beteg kapcsán. Esetbemutatás. Családorvos Kutatók Országos Szervezetének X. Kongresszusa, 2011. febr. 26.
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12. **Várnai Réka**, Végh Mária, Nagy Lajos. Gyógyszermellékhatások és interakciók szerepe a heveny gasztroduodenális vérzések kialakulásában. Családorvos Kutatók Országos Szervezetének VI. Kongresszusa. Hajdúszoboszló, 2007. február 23 – 24.

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XI. ATTACHMENT

„Folyékony biopszia” a klinikai onkológia szolgálatában: álom vagy küszöbönálló valóság?

A vérben keringő sejtmentes DNS jelenléte évtizedek óta élénken foglalkoztatja a kutatókat. A magzati örökítőanyag anyai vérből történő vizsgálata már rutineljárásnak számít, de a keringő DNS elemzése az orvoslás további területein is felhasználható. A keringő sejtmentes DNS-fragmentumok az apoptózison és nekrózison áteső sejtek révén jutnak a véráramba; mennyiségük például daganatos megbetegedés során emelkedhet.

Célzott daganatellenes kezelés, illetve terápiarezisztencia esetén a gyógyszerváltás alapja a biopsziás mintavétel során nyert szövettani minta genetikai vizsgálata. A biopszia egyértelmű hátrányai (invazív, szövődmények, költséges, előjegyzést igényel) mellett a biopsziás minta sajnos nem ad információt az intratumorális és intermetasztatikus heterogenitásról. A vérvétel, azaz a „folyékony biopszia” ezzel szemben minimálinvazív beavatkozás, a kezelés során bármikor kivitelezhető, és a testben jelen lévő összes daganatsejtről információt nyújthat.

Annak ellenére, hogy a korai stádiumú daganatos betegségeknél a keringő tumor-DNS mennyisége alacsony, az új molekuláris genetikai vizsgálati módszereknek köszönhetően (next-generation sequencing, BEAMing, PAP, Digital PCR, TAM-Seq) kis mennyiségű keringő tumor-DNS-fragmentumok, illetve ritka genetikai variánsok is kimutathatóvá váltak. Ezen új eljárások átlagos szenzitivitása 0,01% alatt található, míg IV-es stádiumú daganatok esetében a „folyékony biopszia” szenzitivitása már közel 100%.

A „folyékony biopszia” több lehetséges felhasználási területe közül az egyik a *tumor progressziójának* nyomon követése. A keringő tumor-DNS féléletideje mindössze 2 óra, így a daganat méretében bekövetkező változások hamar észlelhetők. Melanoma, petefészek-, emlő- és vastagbéldaganatok esetében a keringő tumor-DNS mennyisége meredeken emelkedik tumorprogressziókor, míg a sikeres gyógyszeres vagy sebészeti kezelést követően a keringő tumor-DNS mennyisége lecsökken.

A „folyékony biopszia” további lehetséges felhasználási területe a *residualis daganat felismerése* kuratív célú sebészeti beavatkozást követően. Vizsgálatok szerint vastagbéldaganat során a posztoperatíve kimutatható mennyiségű keringő tumor-DNS-sel rendelkező összes betegnél relapszus következett be, míg mérhetetlenül alacsony keringő tumor-DNS esetén a betegek 5 éven keresztül daganatmentesek maradtak.

A „folyékony biopszia” használható a kemoterápia során fellépő *szerzett rezisztencia korai felismerésére,* lehetővé téve a klinikai rezisztencia kialakulása előtti gyógyszermódosítást. Philadelphia-kromoszóma-pozitív myeloid leukaemia kezelésére használt imatinib-, tüdő-, illetve vastagbéldaganatok során alkalmazott gefitinib-, erlotinibkezelés során nem ritka a másodlagos rezisztencia kialakulása. KRAS-mutáció megjelenése a keringő sejtmentes-DNS-ben anti-EGFR-kezelés alatt álló betegeknél a radiológiailag kimutatható relapsust hónapokkal korábban előre jelezheti.

Ahhoz, hogy a keringő tumor-DNS-ből nyerhető információ támogassa a klinikai döntéshozatalt a terápiás protokollokon keresztül, elengedhetetlen a vizsgálatok standardizálása, a DNS-analízis költségének csökkenése, továbbá megfelelő bioinformatikusi együttműködés. Így válhat a folyékony biopszia a klinikai onkológia hatékony módszerévé a közeljövőben.

Rövidítések

DNS = dezoxiribonukleinsav; EGFR = epidermalis growth factor receptor

Irodalom

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7. sz. melléklet

DOKTORI ÉRTEKEZÉS BENYÚJTÁSA ÉS NYILATKOZAT A DOLGOZAT EREDETISÉGÉRŐL

Alulírott

név: Dr. Várnai Réka

születési név: Dr. Várnai Réka

anyja neve: Rigó Róza Mária

születési hely, idő: Pécs, 1982.07.04.

Implementation of pharmacogenomic biomarkers in precision treatment

című doktori értekezésemet a mai napon benyújtom a(z) PTE ETK

Egészségtudományi Doktori Iskola Doktori Iskola

Kardiovaszkuláris egészségtudomány / Kardio-cerebrovaszkuláris betegségek ellátása és primer, szekunder prevenciója Programjához/témacsoportjához

Témavezető(k) neve: Prof. Dr. Balogh Sándor, Dr. Sipeky Csilla

Egyúttal nyilatkozom, hogy jelen eljárás során benyújtott doktori értekezésemet
- korábban más doktori iskolába (sem hazai, sem külföldi egyetemen) nem nyújtottam be,
- fokozatszerzési eljárásra jelentkezésemet két éven belül nem utasították el,
- az elmúlt két esztendőben nem volt sikertelen doktori eljárásom,
- öt éven belül doktori fokozatom visszavonására nem került sor,
- értekezésem önálló munka, más szellemi alkotását sajátomként nem mutattam be, az irodalmi hivatkozások egyértelműek és teljesek, az értekezés elkészítésénél hamis vagy hamisított adatokat nem használtam.

Dátum: 2020. 04. 01.

.....
doktorjelölt aláírása

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