Genetic tests for predicting the toxicity and efficacy of anticancer chemotherapy

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The standard anticancer therapy based on "one size fits all" modality has been determined to be ineffective or to be the cause of adverse drug reactions in many oncologic patients.

Most pharmacogenetic and pharmacogenomic studies so far have been focused on toxicity of anticancer drugs such as 6-mercaptopurine, thioguanine, irinotecan, methotrexate, 5- fluorouracil (5-FU). Variation in genes are known to influence not only toxicity, but also efficacy of chemotherapeutics such as platinum analogues, 5-FU and irinotecan.

The majority of current pharmacogenetic studies focus on single enzyme deficiencies as predictors of drug effects; however effects of most anticancer drugs are determined by the interplay of several gene products. These effects are polygenic in nature.

This review briefly describes genetic variations that may impact efficacy and toxicity of drugs used in cancer chemotherapy.

Key words: genetic variability, toxicity, efficacy, anticancer therapy

Oncology has now entered an era in which the knowledge of genetic variability is helpful for the optimal approach to patient care – preventive, diagnostic and therapeutic [1–13]. In few other areas of medicine is the study of the relationship between specific genetic variations and drug effects more striking than in oncology. Many patients are treated with high dosages of anticancer drugs that are near the upper limits of the therapeutic window. Chemotherapeutic drugs have relatively small range between the toxic and therapeutic dose. Even small increase in plasma concentrations of chemotherapeutic drugs may easily result in toxicity. The standard anticancer therapy based on "one size fits all" modality is widely known as either being ineffective or leading to adverse drug reactions.

Adverse drug reactions associated with chemotherapeutic agents are estimated to increase the overall drug costs by 15%. [14]. Acute and late toxicity due to anticancer chemotherapy may be life-threatening.

Anticancer agents show wide interindividual variability [15]. (Tab.1).

Interindividual variability in drug response may result from variety of factors such as age, sex, race, organ function, interactions among drugs and genetic variations between patients [16–18].

Awareness of interindividual variability based on interactions among drugs is extremely important, because it is more common in older cancer patients. Today, more than two-thirds of patients who develop cancer are over the age of 65 and interactions among drugs in this group of patients when treated with chemotherapeutic drugs and enzyme inhibitors, for example antimycotics, macrolide antibiotics, have higher occurence rates [19, 20].

Genetic variations between patients are another example of interindividual variability in response to a drug. Genetic polymorphism is defined as occurence of two or more alternative genotypes together in a population; each at a frequency greater than the one maintained by recurrent mutation alone. A locus is considered to be polymorphic if the rarer allele has a frequency more than 1% [21]. Genetic variations in response to a drug can be based on tandem repeats, insertions, deletions, microsatellites and single nucleotide polymorphisms (SNPs), which can alter the amino acid sequence of the encoded proteins or RNA splicing [14].

Pharmacogenetics is focused mainly on the inherited variability in genes involved in drug transport, degradation, activation, genes encoding target proteins (receptors, enzymes) and on genes indirectly influencing drug responses [14, 22–25].

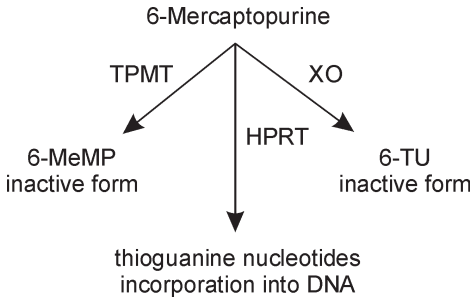

Fig. 1. Inactivation of 6-mercaptopurine.
HPRT – hypoxanthinphosphoribosyltransferase, TPMT – thiopurine methyltransferase, XO – xantinoxidase, 6-TU – 6-thiouric acid.

The term pharmacogenomics is being used to describe a broader strategy to identify the entire set of genes that are relevant to the pharmacological effects of a given drug [9, 26, 27].

Thus development of genetic tests for predicting the efficacy and/or toxicity of chemotherapy is one of the critical issues facing physicians and scientists.

In the context of cancer pharmacogenetic studies most research so far has focused on toxicity of anticancer drugs such as 6-mercaptopurine, thioguanine, irinotecan, methotrexate, 5- fluorouracil (5-FU) [22, 28].

However, not only toxicity, but also efficacy of chemotherapeutics – platinum analogues, 5-FU, irinotecan and other chemotherapeutics – can be influenced by variations in genes [29–31].

Until now, the Food and Drug Administration (FDA) has approved label changes for two anticancer drugs, 6-mercaptopurine (6-MP) and irinotecan, to include pharmacogenetic testing as a potential means to reduce the rate of severe toxic events. Comprehensive evaluation of the clinical benefits and cost effectiveness of screening strategies with pharmacogenetic tests have not been completed [32].

Tab. 1 Interindividual variability of anticancer drugs (modified according to Krynetski and Evans, 1998) [15].

Anticancer agent	Metabolic pathway	Interindividual variability	Polymorphism
5-fluorouracil	Inactivation by dihydropyrimidine dehydrogenase	10-fold	Inherited
Mercaptopurine Thioguanine Azathioprine	Inactivation by TPMT	>30 fold	Inherited
Busulfan	Inactivation by glutathione S-transferase	10-fold	?
Irinotecan	Inactivation by uridine diphosphate glucuronosyl-transferase	50-fold	Inherited
Cyclophosphamide	Activation by cytochrome P450	4-9 fold	Inherited

Many genetic variants linked to efficacy and toxicity of chemotherapeutics have been described in adulthood. These associations are still less defined in children. Incidence of malignancies during childhood is lower than in adults. Although treatment outcomes for pediatric oncologic patients have dramatically improved over the past four decades, the knowledge of genetic predictors of acute and late toxicity and efficacy of therapy may help to improve quality of their lives [1, 8, 33–36]. Pretreatment genetic testing for prediction risk of relapse and of individual response to anticancer therapy have been used in major pediatric centers in the attempt for dose optimization.

Responses to treatment can be altered by polymorphisms in single genes encoding metabolizing enzymes, such as thiopurine methyltransferase (TPMT) and glutathione S- transferases (GSTs) [37, 38]. However, most drug responses are determined by the interplay of several gene products – these effects are polygenic in nature, e.g. the folate/methotrexate metabolism pathway [22, 39].

Drug-related phenotypes can be analyzed using three approaches – single gene approach (analyses the favorite candidate gene), candidate-pathway-gene approach (analyses several functionally related candidate genes), genome-wide approach (analyses the whole genome) [9].

Polymorphisms in drug-metabolizing enzymes.

Thiopurine S-methyltransferase (TPMT). Antimetabolic agents, 6-mercaptopurine (6-MP) and 6-thioguanine (6-TG), are bioactivated in thiopurine nucleotides. Thiopurine methyltransferase (TPMT) methylates (inactivates) MP, reducing its bioavailability for conversion into cytotoxic thioguanine nucleotides (TGN). The alternative pathway of inactivation is the reaction mediated by xantinoxidase (XO), when the 6-thiouric acid (6-TU) is formed (Fig. 1). However, due to insufficient activity of xantinoxidase in the bone marrow, the inactivation of mentioned drugs is catalyzed mainly by TPMT.

The activity of TPMT enzyme shows population variability based on genetic polymorphism.

Krynetksi EY et al. characterized the first human TPMT variant allele [37]. Later other alleles were identified. The genetic polymorphism of TPMT comprises at least 22 alleles (TPMT* 1 – TPMT* 22) responsible for three distinct drug metabolic phenotypes termed normal to high, intermediate, and deficient methylators [40]. Relling et al. showed that TPMT heterozygotes are at higher risk of 6-MP dose-limiting haematopoietic toxicity [33].

Patients with genetic deficiency in TPMT accumulate TGN at toxic concentrations. Reduced TPMT activity is responsible for toxicity of standard doses of 6-MP resulting in severe or life-threatening myelosupression.

Until now, at least ten TPMT variants associated with low enzyme activity have been described. TPMT*2, TPMT*3A and TPMT*3C are the most common variants with low TPMT

activity phenotypes in Caucasians. The bioinactivation of 6-MP and 6-TG in patients with these variants is insufficient. Based on the findings up to 95% of cases of toxicity have carried one or more of the mentioned mutations.

Approximately 10% of patients are heterozygous for these inactivating alleles (wild/mutation genotype). These patients have intermediate TPMT enzyme activity and tolerate approximately 65% of 6-MP dosage. About 0.3% patients are homozygous for the variant TPMT alleles (m/m genotype). They have deficient TPMT activity and high risk for myelosupression after administration of standard doses of 6-MP and 6-TG and should receive 5-10% of the standard 6-MP dose [15, 41–45].

Reduced TPMT activity is also associated with increased risk of secondary malignancies, such as leukemia and brain tumors in children suffering from acute lymphoblastic leukemia (ALL) treated with 6-MP and combined with cranial irradiation or etoposid application [34]. On the other hand, ALL patients with reduced TPMT activity have higher response rate to 6-MP and better prognosis for being cured in comparison to ALL patients with wild-type alleles [36].

The interethnic variability has been reported as well. TPMT*3A variant is most common in Caucasians, TPMT*3C in Asians, Africans and Americans [25, 46].

TPMT status genotyping appears to be the most appropriate method for dose adjustment due to possible influence of blood transfusions on the direct measurement of TPMT activity in red blood cells. Relling et al. found that pharmacogenetic dosage individualization strategies can be used to mitigate toxicity without compromising efficacy [36].

Glutathione S-transferases. Glutathiones play a role in inactivations of many xenobiotics – alcylating agents (cyclophosphamide) topoisomerase II inhibitors, platinum agents and anthracyclines and in detoxification of endogenous products of reactive oxidation [9, 14].

Genotypes in glutathione S-transferases (GST) may predict treatment-related outcomes. De Michele et al. recently estimated the effect of genotype on disease-free survival (DFS) and overall survival (OS) in a cohort of node-positive breast cancer patients who received anthracycline-based adjuvant chemotherapy followed by high-dose multiagent chemotherapy with stem-cell rescue [47]. Patients who did not carry homozygous deletions in both GSTM1 and GSTT1 (and carried homozygous CYP3A4*1B and CYP3A5*3 variants) had a five-fold poorer DFS and a four-fold poorer OS in comparison to individuals who did not carry any CYP3A4*1B or CYP3A5*3 variants, but had deletions in both GSTT1 and GSTM1. Combined genotypes at GSTM1, GSTT1, CYP3A4 and CYP3A5 influence the probability of treatment failure after high-dose adjuvant chemotherapy for node-positive breast cancer.

Moreover, a SNP causes an isoleucine to valine substitution at codon 105 (I 105 V) in the GSTP1 gene. The valine allele is occurring at frequency of 33% in Caucasian population. It is associated with reduced GSTP1 activity compared to the isoleucine allele (Watson, 1998). Patients homozygous for the valin (low activity) allele had a median of 25 months

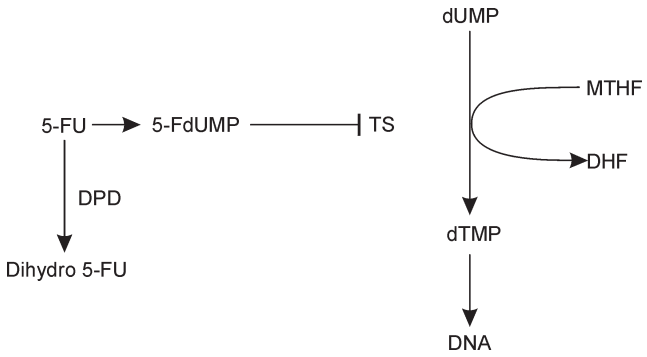

Fig. 2. Degradation of 5- fluorouracil. 5-FU – 5-fluorouracil; 5-FdUMP – 5-fluoro-2-deoxyuridine monophosphate; TS – thymidylate synthase; DPD – dihydropyrimidine dehydrogenase; dUMP – deoxyuridine monophosphate; dTMP – deoxythymidine monophosphate; MTHF – methylenetetrahydrofolate; DHF – dihydrofolate.

survival, compared to 8 months for patients homozygous for the isoleucine allele [48].

Additionally, GSTM1 non-null genotype is associated with reduced risk of ALL relaps in children [49].

Reactive oxygen species metabolizing genes GSTP, GSTT, GSTM, superoxide dismutase (SOD1), catalase are genes relevant also to the anthracycline pharmacodynamics. Anthracycline cytostatics, which are used for primary treatment in a variety of malignancies during childhood and in adult patients are associated with subclinical and clinical cardiotoxicity specifically the development of cardiomyopathy and congestive heart failure (CHF) [50, 51]. Pathogenesis of anthracycline cardiotoxicity is a complex process. Important role in this process is played by free-radical mediated action through quinine-semiquinine recycling and doxorubicin-iron recycling that increase oxidative stress and reduce concentrations of antioxidant enzymes [50]. Reactive oxygen species damage subcellular and cellular structures of the heart. Hypothesis postulated by Minotti et al. suggests that the early cardiac damage is mediated mostly by oxidative stress, while the more chronic type of toxicity is induced by anthracycline alcohol metabolites (for example doxorubicinol) synthetized by carbonyl reductases (CBRs) [52]. Therefore, genetic polymorphisms in genes encoding enzymes involved in oxidative stress pathways, and the metabolism of anthracyclines may have impact on the risk of anthracycline-related congestive heart failure among cancer survivors.

Results of study presented on Annual Meeting of ASCO in 2006 by Aplenc et al. confirmed that the GSTP +313A>G polymorphism was a significant risk factor of CHF after anthracyclines [53]. Study found also an association between CBR3 V244M polymorphism (in gene responsible for secondary alcohol formation) and the risk of CHF after treatment with anthracyclines.

Dihydropyrimidine dehydrogenase. Dihydropyrimidine dehydrogenase (DPD) represents the initial and rate-limiting enzyme in the catabolism of pyrimidine antimetabolic drug 5-

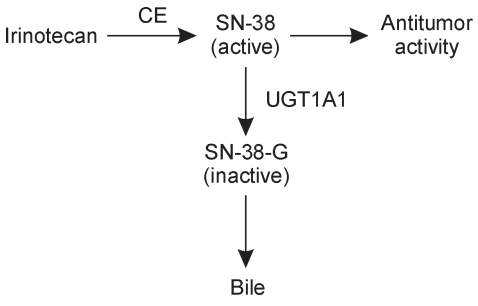

Fig.3. Irinotecan inactivation by UGT1A1. UGT1A1 – UGT-glucuronosyltransferase 1A1; CE – carboxylesterase.

fluorouracil (5-FU). The majority of 5-FU is degraded by DPD to 5,6-dihydro-5-fluorouracil in the liver (Fig.2) [29, 54, 55].

To date, 20 polymorphisms in the DPD gene have been described. The complete DPD deficiency occurs in 0.1% and partial deficiency in 3-5% of the population [26]. The DPD*2A alleles represent the most common polymorphism. It is caused by G>A transition at a GT splice donor site flanking exon 14 of the DPD gene. Decreased activity of DPD can lead to severe toxicity (myelosupression, neurotoxicity and gastrointestinal toxicity) and fatal outcome of 5-FU treatment [25]. Carriers of the DPD exon 14-skipping mutation have significantly higher risk of life-threatening myelosuppression upon 5-FU treatment, even when their allelic status is heterozygous. Based on these findings routine testing for the exon 14 skipping mutation and additional 5-FU pharmacokinetics for heterozygous patients prior to 5-FU treatment is an important step towards individually tailored therapy in cancer patients [56].

Dihydropyrimidine dehydrogenase (DPD) deficiency accounts for approximately 43% of grade 3–4 toxicity to 5-FU.

The molecular basis of 5-FU toxicity is not limited to DPD deficiency; since molecular defects in genes downstream of DPD can potentially impair also 5-FU catabolism. Reed et al. described molecular changes responsible for deficiency of dihydropyrimidinase (DHP) enzyme encoded by the DPYS gene and/or beta-ureidopropionase enzyme, encoded by the BUP-1 gene [57]. Genetic testing for molecular defects in DPYS and BUP-1 may predict patients at risk of developing 5-FU toxicity despite having normal DPD enzyme activity. Assessment of the integrity of the entire uracil catabolic pathway might be a crucial step in an effort to avoid toxicity in a significant group of patients receiving 5-FU or a related drug.

Uridine diphosphate glucuronosyltransferase 1A1 (UGT1A1). Uridine diphosphate (UDP) glucuronosyltransferase catalyzes the conjugation of lipophylic xenobiotics with glucuronic acid resulting in higher water solubility of xenobiotics thus aiding their excretion [58]. Such substance is also an important anticancer agent irinotecan.

Irinotecan is a semisynthetic analogue of camptothecin, which is activated by carboxylesterase to 7-ethyl-10-hydroxycamptothecin (SN-38) [59]. Irinotecan's main pharmacodynamic effect is based on the inhibition of topoisomerase-I by the mentioned active metabolite (Rothenberg et al., 1993). Irinotecan is inactivated via two metabolic pathways. The first one involves oxidation of irinotecan catalyzed by isoenzyme 3A4 of cytochrome P450. The irinotecan active metabolite, SN38, is also inactivated through glucuronidation mainly by uridine diphosphate glucuronosyltransferase (UGT) 1A1 (Fig. 3).

The activity of UGT1A1 shows population polymorphism. The genetic substrate of this variability is in a different number of repeated TA elements in the promoter region. Patients with seven TA repeats (UGT1A1*28) have lower UGT1A1 activity in comparison to those with wild type number of six [14, 60]. UGT1A1*28 alleles were found in 35% of Caucasians and African-Americans. On the other hand, the frequency of these alleles is much lower in Asians [61, 62]. The risk of asverse drug reactions is higher increased in patients with insufficient conjugation of the active metabolite SN-38. The ratio conjugated/non-conjugated bilirubin did not appear to be a valid marker for prediction of irinotecan toxicity. Therefore, the analysis of UGT1A1 gene may be useful in prediction of patients at risk for irinotecan toxicity. In contrast to other studies, the UGT1A1*28 was not associated with irinotecan toxicity according to Seymour et al. [63].

However, the extrahepatic UGT1A7 and the hepatic UGT1A9 are known to be also involved in SN38 glucuronidation. Therefore, polymorphic variants of these genes may also affect irinotecan toxicity.

Drug target polymorphism.

Thymidylate synthase. Thymidylate synthase (TS) is the critical enzyme in DNA synthesis. TS is the main target for 5-fluorouracil (5-FU). 5-FU is activated to 5-fluorouridine monophosphate that binds and inhibits TS [4]. TS polymorphism analysis may aid in prediction of high grade toxic events in patients treated with 5-FU. TS is not only the target for 5-FU, but also for folate based antimetabolites such as methotrexate. The overexpression of TS is associated with resistance to TS-targeted agents (5-FU and other TS inhibitors). The expression of TS is regulated by several polymorphic tandem repeats in the TS enhanced region (TSER). Higher number of tandem repeat copies is related to increased TS activity. The higher TS activity correlates with a lower sensitivity of tumor to 5-FU treatment [22]. Homozygotes for the TS promoter alleles TSER*3 (three tandem repeats) had significantly higher TS activity than those with TSER*2 (two tandem repeats). The clinical impact was a higher sensitivity for 5-FU treatment, but also higher toxicity in patients homozygous for TSER*2 [23]. TSER*2 and TSER*3 are present in all ethnic groups with higher number of repeats occurring only in Africans [25]. The TS genotyping may represent a useful tool for selection of responders to treatment with 5-FU and its analogues [64].

In patients treated for ALL, homozygotes TS 3/3 have higher TS activity and higher risk of ALL relaps, homozy-

gotes for TS 2/2 or heterozygotes for 2/3 have lower TS activity and are predisposed to toxicity of antileukemic drugs.

Methylenetetrahydrofolate reductase. 5,10-methylenetetrahydrofolate reductase (MTHFR) is an important enzyme in the folate metabolic pathway.

MTHFR reduces 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which is a methyl group donor for methylation of DNA and homocysteine. About 10% of Caucasian population have MTHFR variant C677T, (Ala>Val), which encodes the protein with 30% of the wild type activity. Homozygotes for SNPs C677T and A1298C have reduced activity of MTHFR in comparison to heterozygotes. Heterozygote (40%) population has approximately 60% enzyme activity. Another MTHFR variant with lower activity is A1298C (Glu>Ala). This variant has been linked with susceptibility to leukemia. The intracellular folate pool is influenced by decreased MTHFR activity. Therefore, the polymorphism can increase the risk of folate antimetabolites toxicity. Mucositis and slower recovery of platelet number after chemotherapy was described in C677T homozygotes in comparison to C677T heterozygotes [64]. MTHFR 677 TT genotype predicted toxicity in patients with acute lymphoblastic leukemia.

The full range of polymorphisms and intragene haplotypes in the human MTHFR gene remains unclear. Common genetic polymorphisms in the human MTHFR gene are associated with individual variation in the efficacy and toxicity of 5-fluorouracil and methotrexate (MTX).

Drug transporter polymorphisms.

MDR1 (P-glycoprotein, ABCB1). P-glycoprotein was initially described in multi-drug resistance of cancer cells. The cells characterized by overexpression of P-glycoprotein show resistance to many anticancer agents, such as anthracyclines, vinca alkaloids, taxanes, or epipodophyllotoxins. P-glycoprotein is acting as a pump that enables excretion of drugs from intracellular space. This protein is ATP-dependent membrane transporter involved in transmembrane transport of a large number of hydrophobic agents, including hormones, carcinogens and anticancer drugs, e.g. doxorubicin, paclitaxel [66, 67]. P-glycoprotein, an efflux transporter, plays an important role also in normal tissue cell (canalicular domain of hepatocytes, proximal tubules of kidney, brush border of the small intestine, colon, adrenal glands or capillary endothelium of the brain and testes). P-glycoprotein is responsible for active efflux of exogenic substances from intracellular space. P-glycoprotein is coded by MDR1 gene that shows genetic polymorphism. Even though, the resistance to anticancer drugs is a multifactorial process, the involment of P-glycoprotein was proven in this context in some studies [68–70]. MDR1 exon 26 TT genotype was found to be a predictor of toxicity of antileukemic therapy in children with ALL [9].

Reduced folate carrier. Reduced folate carrier (RFC; also known as SLC19A1) is the most important facilitating transport system for folates and antifolates in mammalian cells. The polymorphism of the gene for RFC (80 G/A genotype) has been indicated as one of the causes of resistance development to methotrexate. Children with the homozygous AA variant have worse prognosis compared to patients with GG variant. This fact could be explained by decreased cellular uptake of methotrexate [71, 72]. Kishi et al. (2003) observed no significant differences in MTX toxicity associated with RFC 80 G/A genotypes [11].

Vitamin D-receptor (VDR, Fok I start site, intron 8/exon9). Osteopenia and osteonecrosis are serious clinical late complications of treatment with glucocorticoids and methotrexate, especially in patients over the age of 10 [73, 74]. Genetic polymorphisms in the vitamin D receptor have been linked to regulation of bone mineral density (BMD). Polymorphisms in VDR gene were proven to be associated with the risk of osteopenia. Evaluation of the relation between selected VDR polymorhisms (Fok I, Bsm I. Apa I, Taq I, Cdx2 promoter), BMD and fractures were recently performed in 26 242 participants [75]. According to results of this metaanalysis Cdx2 polymorphism in VDR gene may be associated with vertebral fractures. Osteonecrosis occurs in 10-15% of ALL patients [73]. Association of osteonecrosis with polymorphisms in VDR gene (Fok I start site CC genotype) and TS 2/2 enhancer repeat genotype have been confirmed in recently published pediatric study [76]. Whether the C allele in VDR gene confirms greater direct or indirect sensitivity to toxicity of glucocorticoid is unclear.

Conclusions. Cancer pharmacogenetics had a great start, but still much research has to be done to identify both responders and nonresponders to anticancer therapy as well as patients who will profit from a "tailored therapy". Clinical use of cancer pharmacogenetics knowledge is restricted by several limitations.

Majority of current pharmacogenetic studies focus on single enzyme deficiencies as predictors of drug effects, however, effects of most anticancer drugs are determined by the interplay of several gene products. These effects are polygenic in nature, for example the folate metabolism is a complex process involving 29 genes. It is quite rare that a SNP results in significant changes in the ability to metabolize drugs [25, 77].

Moreover, compensation for the effect of polymorphism in one gene is possible in complex pathways. The concordance among genotype, gene expression and enzyme activity is highly variable. Proteomic approaches are expected to provide in depth insight into gene translation and post-translational modifications that alter drug responses. Cancer therapy genotyping technologies must be improved particularly in their availability and extensively validated before their application in individualization of anticancer therapy.

Interethnic differences in genetic polymorphisms among Caucasian, Asian and African populations should be also taken into consideration in application of personalized therapy with anticancer drugs. Gene expression in pharmacologic pathways differs in subtypes of malignancies (e.g. folate pathway genes in subtypes of acute lymphoblastic leukemias) [78].

Numerous clinical studies documented that genetic polymorphisms may influence drug response in other type of

therapy e.g. hypertension, asthma bronchiale, psychiatric disorders, hormone replacement therapy and treatment of pain [79–84].

Presently, the true promise of personalized medicine is too early to estimate, however, benefits of pharmacogenetics and pharmacogenomics are expected to be visible in the clinical practice within a decade.

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