Pharmacogenetic Application in Personalized Cancer Treatment

Hendy Kristyanto*, Ahmad R. Utomo**

* Faculty of Medicine University of Indonesia. Jl. Salemba 6 Jakarta Pusat 10430, Indonesia.
** Stem Cell and Cancer Institute, Jakarta.

Correspondence mail to: hendykris@gmail.com

ABSTRACT

Pharmacogenetic is broadly understood as study or clinical testing of genetic variations that contribute to differing response to drugs. In cancer treatment, applications of pharmacogenetic cover three areas: avoidance of adverse drug reaction (ADR), selection of treatment options, and prediction of cancer recurrence. Patients with genetic variations in UGT1A1 and DPYD genes are hypersensitive to Irinotecan and 5-Fluorouracil (5FU) respectively. Therefore, the chance for the patients to suffer from ADR from using those drugs can be predicted a priori by simple genetic tests. Secondly, the efficacy of targeted therapy drugs such as Cetuximab and Erlotinib, or non-targeted agents such as temozolomide and nitrosourea has been influenced by the presence of certain genetic or epigenetic markers in tumors. Lastly, microarray analysis to evaluate 70-gene expression profile in breast cancer samples has been shown in recent studies to predict probability of breast cancer recurrence. Patients whose tumors have been determined to have low probability score based on the gene expression profile may omit chemotherapy altogether, avoiding unnecessary therapeutic side effects. In summary, pharmacogenetic tests help patients, their caregivers, and doctors in deciding the best treatment options with favorable chance of success, as well as saving overall treatment costs.

Key words: predictive biomarker, prognostic biomarker, cancer, personalized chemotherapy.

INTRODUCTION

Cancer in general has excellent prognosis when detected early. Unfortunately, most cancer patients entered clinical wards suffering from advance stage of the disease with limited treatment options. Upon completion of Human Genome Project in 2003, understanding of cancer genetic heterogeneity is merely an academic exercise and has not influenced or changed current management of cancer treatment significantly in this post-genomic era. Recently, realization of the rich variety of cancer genetics as well as the diverse genetic background of cancer patients themselves has been pointed toward improving personalized medicine.

While practicing medicine and caring for patients have always been ‘personal’ in nature, oncologists may now incorporate the information of patients and their cancer’s unique genetic signature to improve the service of personalized cancer treatment. Data generated from Human Genome Project has accelerated the science of cancer pharmacogenetic, which is regarded as study or clinical testing of genetic variations that contribute to differing response to anti-cancer treatment drugs. There are efforts to predict a patient’s individual vulnerability to certain chemotherapy toxicity, to choose more effective antineoplastic regimen, or, even, to avoid the useless agents for patients with good prognosis. These efforts are based on the study of pharmacogenetics which aims to identify genetic markers underlying the response to drugs. This genetic understanding may challenge current protocol of choosing and dosing chemotherapeutic agents.

We summed up three areas where genetic studies related to cancer treatment have made contribution already to improve cancer treatment: avoidance of adverse drug reaction (ADR), selection of treatment options, and prediction of recurrence probability.
AVOIDANCE OF ADVERSE DRUG REACTION (ADR)

Routine cancer management using chemotherapy, whether as definitive or adjuvant therapy, has improved patient’s absolute survival when compared with non-chemotherapy control. However, chemotherapeutic agents have a low therapeutic index. Thus, the challenge to administer such drugs is the variability in drug disposition and clearance which accounts for the wide inter-patient response to conventional doses of antineoplastic agents.

It is common that patients receiving chemotherapy have to experience adverse drug reactions (ADR). In the US, ADR ranks between the 4th to 6th causes of death. ADRs often result in life threatening conditions or significant disability which requires hospital admission or prolongation of existing hospital stay. The main causes of chemotherapy induced hospitalization are fever or infection, neutropenia, thrombocytopenia, dehydration or electrolyte disorders, diarrhea, anemia, constitutional symptoms, deep venous thrombosis, pulmonary embolus, and malnutrition. Chemotherapy-induced pulmonary toxicity is also an important cause of respiratory failure. These adverse reactions have lowered quality of life tremendously.

Currently, body surface area has been used to decide normal total blood volume and renal function of an individual. It has also been a recommendation that chemotherapists use dose per body surface area to administer anticancer drug in order to lower the chance of toxicity. However, this does not correlate with the drugs’ clearance and metabolism, leaving patients at risk of developing severe toxicity.

There are efforts to predict a patient’s individual vulnerability to certain chemotherapy toxicity, to choose more effective antineoplastic regimen, or, even, to avoid the useless agents for patients with good prognosis. These efforts are based on the study of pharmacogenetics which aims to identify genetic markers underlying the response to drugs. This genetic understanding may challenge current protocol of choosing and dosing chemotherapeutic agents.

Irinotecan ADR

Irinotecan therapy might be prescribed for at least 15% of individuals with new colorectal cancer while other 70-80% of patients who are present with resectable tumor will undergo surgery followed with adjuvant therapy for high-risk cases. While combination of 5-Fluorouracil (5-FU) and leucovorin is the first choice regime in chemotherapy, other combination chemotherapy regimens involving the use of irinotecan and other drugs seem to be superior in improving the median survival. Thus, irinotecan and other drugs are increasingly prescribed for first line colorectal cancer chemotherapy.

Irinotecan is a prodrug that is activated by a process called glucuronidation. Glucuronidation reactions are important detoxification processes for endogenous as well as exogenous compounds such as bile acids, hormones, steroids, drugs, environmental toxicants, and carcinogens. An enzyme called carboxylesterase breaks down irinotecan prodrug to generate active metabolite SN-38 whose cytotoxicity is 100-1,000 times higher than the parental drug. SN-38 is further catalyzed into an inactive glucuronide derivative, SN-38G, by several hepatic and extrahepatic uridine diphosphate glucuronosyltransferase (UGT) enzymes, which is predominantly UGT1A1.

A reduced level of functional UGT1A1, called Gilbert’s syndrome or *28 allele, is associated with a higher risk for adverse reactions of irinotecan due to relatively high level of and/or prolonged exposure to the highly cytotoxic active form of the drug. Thus, inherited condition presents mild, fluctuating hyperbilirubinemia. The principal cause is reduction in hepatic bilirubin glucuronidating activity to about 30% of normal levels. Reduced expression of UGT1A1 is primarily caused by an insertion of two extra bases (TA) in TATAA element of the 5’ promoter region creating an A(TA),TAA element rather than the normal A(TA),TA. Alternatively, patients may be heterozygous for missense mutations in the UGT1A1 gene.

Prominent adverse reactions of irinotecan are severe neutropenia and diarrhea. Patients homozygous for the *28 allele are 3.5 times more likely to develop severe neutropenia compared to individuals with wild-genotype. Irinotecan toxicity is more common in patients with Gilbert’s syndrome carrying the UGT1A1*28 allele combined with reduced function UGT1A7 N129K/R131K and UGT1A7-57T/G SNP. Thus, the UGT1A1/UGT1A7 SNP combination haplotype appears to be a superior risk predictor than Gilbert’s syndrome alone.

Besides, UGT1A1, a highly polymorphic hepatic UGT1A9 is also involved in SN-38 detoxification due to its high affinity to this drug metabolite. An intronic polymorphic variant (I399C>T) in the UGT1A9 gene was found to be associated with increased glucuronidation of UGT1A1 and UGT1A9 substrates. Healthy Asian populations have a 44% frequency of this polymorphism. This frequency is similar to Caucasian population. The I399C>T polymorphic variant had approximately 2-fold lower systemic exposure to cytotoxic SN-38 compared to the reference population. In addition to UGTs, several
genes play a role in the irinotecan pathway, including carboxylesterases, drug transporters, topoisomerase I, and CYP3A4/5. Polymorphisms in these genes should be studied as well to gain a better understanding of this complex pathway.

Since the enzyme that inactivates SN-38 also regulates bilirubin metabolism, baseline serum of bilirubin is assumed to have property of inexpensive marker for UGT1A1 function. However, recent study showed that baseline serum bilirubin does not reliably predict overall irinotecan-related toxicity or efficacy.13 Thus, potential applications of pharmacogenetic information are needed to optimize irinotecan dosing and tailor therapy to individual patients.

5-FU ADR
Pharmacogenetic approach may also be applied in predicting 5-Fluouracil (5-FU) induced toxicity. 5-FU is the backbone of treatment for colorectal cancer and an important regimen for upper gastrointestinal tract carcinomas, and breast carcinomas. It inhibits thymidylate synthase, a rate-limiting enzyme in pyrimidine nucleotide synthesis. 5-FU is usually administered with leucovorin, a reduced folate, which stabilizes the binding of fluorouracil with thymidylate synthase.

5-FU is primarily degraded by dihydropyrimidine dehydrogenase (DPD). Partial loss of the enzyme due to a heterozygous G->A transition at the 5’ slicing donor consensus sequence in intron 14 leading to exon 14 skipping (IVS14+1 G->A, DPYD*2A) may be partly responsible for 5-FU induced toxicity. Caucasians have such a mutation with a frequency of 1-2% in the general population.12

Myocardial infarction, sudden death, unstable angina, hypertension, and pulmonary edema are the most common cardiovascular side effects of 5-FU. These side effects may be due to vasospasm. P456L (1358C>T) mutation as a novel DPYD variant may become a predictive marker for 5-FU related cardiotoxicity.13 In rare cases, patients who bear more than one polymorphism associated with vulnerability in pharmacogenetic syndromes, may suffer fatigue. A patient who had heterozygosity for DPD IVS14+1 G-> A mutation and UGT1A1 (TA)6/7 heterozygosity showed a rapidly deteriorating condition and died after receiving combination of 5-FU and irinotecan.12

SELECTION OF TREATMENT OPTION
Identification of biomarkers that serve as predictive factors has shown promises in improving efficacy of cancer treatment in a subset of cancer patients harboring specific genetic changes. For instance, amplification of Her2 gene in breast cancer and Bcr-Abl gene translocation in chronic myeloid leukemia have been indicated for Trastuzumab (Herceptin) and Imatinib mesylate (Gleevec) since late 1990s.

KRAS Genetic Mutation and Anti-EGFR Therapy
Epithelial growth factor receptor (EGFR) is a transmembrane receptor which binds selectively to six different ligands. Upon ligand binding to a single-chain EGFR, the receptor forms a dimer with other receptor. Subsequently, the dimerized receptors are generating intracellular signals through activation of tyrosine kinase activity located in the cytoplasmic domain of the receptors. Tyrosine autophosphorylation of EGFR causes a cascade of intracellular signaling events leading to cancer cell proliferation, apoptotic blockage, invasion and metastasis, and angiogenesis induction. The majority of human epithelial cancers are marked by functional activation of growth factors and EGFR family. Four EGFR antagonists are currently available for the treatment of four metastatic epithelial cancers, i.e. non-small cell lung cancer, squamous-cell carcinoma of the head and neck, colorectal cancer, and pancreatic cancer.14

EGFR antagonists are usually used in combination with other cytotoxic regimens. An underlying principle of combining cancer chemotherapy is that drugs that function through separate cytotoxic mechanisms and have different dose-limiting adverse effects can be administered together at full doses, with a superior outcome.15 CRYSTAL trial showed that cetuximab-FOLFIRI (fluorouracil, leucovorin, and irinotecan) regimen is superior to FOLFIRI regimen alone in prolonging progression-free survival in EGFR positive metastatic colorectal cancer (MCRC) (HR, 0.85; 95CI, 0.72-0.99; p=0.048).16 However, this benefit of cetuximab was limited to the patients with KRAS wild-type tumors.

Another randomized, phase III trial done in Canada aimed to examine the effect of cetuximab on survival among advance MCRC patients who had failed previously. Treatment with cetuximab improves overall and progression-free survival and preserves the quality of life in these patients compared to those treated with best supportive care alone (HR, 0.55; 95CI, 0.41-0.74; p<0.001 and HR, 0.40; 95CI, 0.30-0.54; p<0.001 respectively).17 Noticeably, favorable results were seen especially in patients with wild-type KRAS tumors; while mutations in KRAS gene had no influence on survival among patients treated with only best supportive care. Since treatment with cetuximab is relatively
expensive and leads to some adverse reactions, it would be most cost-effective if given to patients with the highest chance of response. Two previous trials showed that KRAS mutation is highly predictive of cetuximab resistance in metastatic colorectal cancer. On the basis of these results, the European Medicines Agency and Food and Drug Administration (FDA) have approved cetuximab and panitumumab only for patients with wild-type KRAS tumors.18,19

RAS proteins belong to Guanosine-5'-T Phosphatase (GTPase) superfamily, which includes KRAS, NRAS, and HRAS. They transduce stimuli from surface growth factor receptors. KRAS encodes a small G protein that relays ligand-dependent receptor activation to intracellular pathways of EGFR signaling cascade. Mutation at key sites within the gene, commonly at codons 12 and 13, triggers constitutive activation of KRAS-associated signaling. These mutations permit stimuli-independent activation leading to evasion of apoptosis and excessive growth. Mutations in the KRAS gene are found in approximately 15 to 30% of patients with non-small cell lung cancer (NSCLC) and 40 to 45% of patients with MCRC, and their presence typically correlates with a worse prognosis.14

Besides being a predictive marker for efficacy of cetuximab in MCRC, KRAS mutations may also serve as markers to predict efficacy of EGFR tyrosine kinase inhibitors (TKI), such as erlotinib and gefitinib in NSCLC.20 Though some mutations in KRAS are associated with cigarette smoking, KRAS tumor status cannot be easily predicted on the basis of smoking history. Mutations of genes other than KRAS gene, such as phosphatase and tensin homologue (PTEN), B-type Raf kinase (BRAF), and phosphatidylinositol 3-kinase (PI3K) were also associated with shorter survival of MCRC patients receiving EGFR antagonists.17 Thus, these mutations may serve as additional biomarkers to predict resistance of EGFR antagonists.

**MGMT Epigenetic Inactivation and Temozolomide**

Aberrant insertions of a methyl group at the fifth carbon of cytosines within the dinucleotide CpG islands located at the promoter region of specific genes have a great impact on acquisition of resistance to anticancer drugs.21 DNA methylation is an epigenetic process affecting gene expression and chromatin organization. Epigenetics is defined as heritable changes in gene expression that are not due to alteration in the DNA sequence.22 The most extensive evidence that DNA methylation markers can predict chemotherapy response is provided by MGMT promoter methylation in glioma. O6-methylguanine-DNA methyltransferase (MGMT) plays a crucial role in defense against alkylation agents.23 Alkylation agents-induced DNA damage is subject to repair by MGMT. The function of MGMT hypermethylation is best studied in brain tumor-glioma.

Glioblastoma multiforme is the most common primary tumor of the brain. The annual incidence of malignant gliomas is approximately 5 cases per 100,000 people.24 Despite multimodal aggressive treatment, the median survival time after diagnosis is less than 12 months. However, a small portion of glioblastoma patients survive for more than 36 months, referred to as long-term survivors. It is found that MGMT promoter hypermethylation is significantly more common in the long-term survivor group than in unselected patient group (p=0.0002).25

Attempts to give adjuvant chemotherapy to aggressive surgical resection and radiotherapy in treating glioblastoma have been insignificant in prolonging survival. Furthermore, there were few adverse events associated with the additional treatment.26 However, the results of multicenter phase III trial showed that the addition of temozolomide to radiotherapy for newly diagnosed glioblastoma resulted in a clinically and statistically significant survival benefit with minimal additional toxicity.27 In another study, temozolomide’s efficacy is associated with MGMT promoter methylation status of the patients.24 The difference between overall survival of unmethylated MGMT promoter group receiving temozolomide plus radiotherapy and those receiving radiotherapy alone was statistically not significant (HR, 0.69; 95CI, 0.47-1.02, p=0.06). On the other hand, the overall survival of methylated MGMT promoter group receiving temozolomide plus radiotherapy was significantly better than those who received radiotherapy alone (HR, 0.51; 95CI, 0.31-0.75, p=0.007).28 Thus, temozolomide may be beneficial to glioma patients with methylated MGMT promoter, but not to those with unmethylated one.

**Other Potential Predictive Biomarkers**

There are evidences to suggest that BRCA1, TP53, and TOP2A could be useful predictive markers of response to different types of chemotherapy agents.29-32 Tumors with BRCA1 mutation were highly sensitive to anthracycline-based chemotherapy, whereas wild-type BRCA1 promotes an increase in sensitivity to antimicrotubule agents.29 This gene seems to play a role in the development of breast cancer, ovarian cancer, prostate cancer, and NSCLC. Thus, the predictive capacity of BRCA1 may also be applied to these cancers’ treatment.

Another study showed that TP53 gene mutation,
especially the one affecting loop domain L2/L3 of p53 protein, may decrease the response rate of 5FU-mitomycin in treating locally advanced breast cancer, as compared to wild-type gene (p=0.006). Moreover, since anthracyclines inhibit topoisomerase II-α that is encoded in TOP2A gene, TOP2A-amplified tumors may have a significantly recurrence-free survival with anthracycline-based chemotherapy than patients with a normal status. However, DiLeo et al (2007), showed that patients with wild-type TP53 and TOP2A positive tumor in advanced breast cancer derived more benefit from anthracyclines as compared to those with one or none of the two factors (OR, 5.06; 95CI, 1.19-21.41; p=0.03). This finding still needs to be confirmed on a large number of patients.

Other study on pharmacogenetic biomarker is the influence of microsatellite instability (MSI) or microsatellite stability (MSS) on the efficacy of 5-FU in treating colorectal cancer. In stage II-III colorectal cancer, patients with MSI had a better 5-year global survival rate compared to MSS patients who did not receive adjuvant chemotherapy (HR, 0.31; 95CI, 0.14-0.72; p=0.004). In patients receiving adjuvant chemotherapy, the status of MSI did not correlate to increased 5-year survival rate (HR, 1.07; 95CI, 0.62-1.86; p=0.80). Thus, 5-FU base chemotherapy will benefit patients with MSS but may not benefit patients with MSI. These promising predictive markers in cancer chemotherapy are summed up in Table 1.

### Table 1. Summary of promising predictive markers in cancer chemotherapy

<table>
<thead>
<tr>
<th>Predictive Marker(s)</th>
<th>Predicted Result(s)</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UGT1A1*28/UGT1A7 SNP</td>
<td>Severe neutropenia after administration of irinotecan</td>
<td>9</td>
</tr>
<tr>
<td>DPYD*2A</td>
<td>5FU-induced toxicity</td>
<td>12</td>
</tr>
<tr>
<td>P456L (1358 C&gt;T) variant of DPYD</td>
<td>5FU-induced cardiotoxicity</td>
<td>13</td>
</tr>
<tr>
<td>KRAS mutation</td>
<td>Resistance to cetuximab in metastatic CRC patient</td>
<td>16,17</td>
</tr>
<tr>
<td></td>
<td>Resistance to erlotinib and gefitinib in NSCLC patient</td>
<td>20</td>
</tr>
<tr>
<td>MGMT promoter</td>
<td>High sensitivity to alkylating drugs in glioblastoma</td>
<td>28</td>
</tr>
<tr>
<td>hypermethylation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRCA1 mutation</td>
<td>High sensitivity to anthracycline-based chemotherapy</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>Resistance to anti microtubule agents</td>
<td></td>
</tr>
<tr>
<td>TP53 gene mutation</td>
<td>Resistance to 5FU-mitomycin</td>
<td>30</td>
</tr>
<tr>
<td>Wild type TP53-TOP2A (+)</td>
<td>High sensitivity to anthracyclines</td>
<td>32</td>
</tr>
<tr>
<td>Microsatellite</td>
<td>Resistance to 5FU in metastatic CRC patient</td>
<td>34</td>
</tr>
</tbody>
</table>

### PREDICTION OF RECURRENCE PROBABILITY

Cancer develops as a result of multiple genetic and epigenetic defects. Thus, individuals with the same type of tumors may have dissimilar genetic defects. This variability of genetic defects explains why patients who seem to have similar cancers respond differently to identical anticancer drugs, and thus shows difficulty in providing effective treatments for cancer. In addition to predictive biomarkers that have been explained previously, prognostic biomarkers are also crucial in determining which patients need chemotherapy. Prognostic biomarkers predict clinical outcomes of a patient independently of anticancer drugs treatment. Anticancer drugs as adjuvant therapies are often given to patients after undergoing tumor resection. It is not always clear who actually, among these patients, need adjuvant therapy. Those who have got their tumor cured by surgery but receive chemotherapy will experience unnecessary adverse reactions as a result of toxic therapy as well as financial burden to cover these expensive drugs. In contrast, patients with propensity to develop recurrent tumor after surgery and do not receive appropriate chemotherapy may suffer devastating consequences. However, the distinction between those patients who need adjuvant therapy and those who do not is often unclear. Thus, prognostic biomarkers are urgently needed to enable clinicians to determine the likelihood of recurrence.

The introduction of DNA-microarray technology has made it possible to assess the expression of tens of thousands of genes in a single experiment. Patterns of groups of genes that are associated with specific tumor traits, called signature, can be identified by analyzing systematically the gene-expression patterns of tumor samples. From as many as 250 candidate signature genes for breast cancer prognosis, it has been developed from two to 70-gene signature microarray to determine breast cancer prognosis. The 70-gene expression predictor discriminates between a good and a poor outcome in patients with early-stage breast cancer. The signature of poor prognosis demonstrates overexpression of genes regulating the cell cycle, invasion, metastasis, and angiogenesis. 70-gene expression profiling is one of the most well validated prognostic tools. It showed 77 to 81 percent agreement in outcome classification. Thus, patients with good prognosis are saved from having to undergo chemotherapy and are able to avoid unnecessary side effects. While awaiting the data of the prospective results of MINDACT (Microarray In Node-negative Disease may Avoid ChemoTherapy) trial, FDA has already approved the use of this 70-gene test also known as Mammaprint to test the probability of breast
from population to population. Genetic polymorphisms whose frequency might differ necessitates the epidemiologic studies of associated the most relevant signature genes in a population both efficacy and prognosis. The challenge of compiling microarray technology will be the best way in predicting both efficacy and prognosis. The challenge of compiling the most relevant signature genes in a population necessitates the epidemiologic studies of associated genetic polymorphisms whose frequency might differ from population to population.

REFERENCES


