REVIEW

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# Pharmacogenomics research: a potential strategy for drug development

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Adverse drug reactions (ADR) and drug ineffectiveness are the common in clinical practice. Recent studies have indicated their strong connection to the genetic feature of patients. To further illustrate this relationship, the discipline of Pharmacogenomics (PGx) was born. At present, *in vitro* cell models and *in vivo* transgenic animal models have a large potential to study the influence of human genetic mutations on drug response. Moreover, PGx guided clinical trials also provide benefits to drug development. With the drug targets introduced by PGx, great success has been achieved in targeted therapy (eg. gefitinib, cetuximab and ado-trastuzumab). Although the progress on PGx research is fascinating, the translation of PGx into drug development is unsatisfactory. To improve this situation, a rounded system that includes individuals, medical staff, academics associations, Government and Pharmaceutics-Biotechnology Industry should be established, as well as a connected pipeline consisting of policy guidance, PGx research, genotyping technology, preclinical and clinical studies.

## 1. Introduction

Pharmacogenomics (PGx) is the convergence of pharmacology and genomics, as well as the derivation of pharmacogenetics (Somogy 2008). The notion of PGx firstly came up in 1997, promoted by the progression of the Human Genome Project (HGP), and its ultimate goal was to clarify the influence of genetic features (variations in drug-metabolizing enzymes, receptors, transporters and targets), on drug response (O'Shaughnessy 2006). During the past decade, a mounting number of indicative genetic variations or biomarkers have sprouted up in candidate gene studies and the Genome Wide Association Study (GWAS). Some of these markers were associated with drug-related phenotypes, such as drug ADRs or ineffectiveness, while others cross-linked in the disease pathology and became the targets for drug design (Streetman 2007). At present, a consensus is being established over the utility and possibly the indispensable nature of PGx approaches as components in the pipeline of drug development (Crews et al. 2012; Harper and Topol 2012). Some pharmacologists even recommend that all commonly used medications should undergo PGx investigation (Akkari et al. 2009; Harper and Topol 2012). Although plausible application strategies of PGx have been suggested (Burt and Dhillon 2013), the translation of PGx in drug development, especially in the early phase of clinic trial, is still largely unknown and full of challenges (O'Donnell and Stadler 2012). Here, we review the current status of PGx research and the potential value of PGx in each phase of drug development (Fig. 1).

## 2. PGx organizations

Since the beginning of 21st century, many official and academic PGx organizations have been formed. The first agent - NIH Pharmacogenetics Research Network (PGRN) - was founded in 2005, to elucidate the correlation between genotypes and drug response, and to apply the knowledge of PGx into personalized therapeutic regimen (Giacomini et al. 2007). In the same year, the Pharmacogenetics and Pharmacogenomics Knowledge Base (PharmGKB) was established, providing investigators and clinicians with a large multitude of PGx information (Owen et al. 2007; Thorn et al. 2005). As a shared project by PGRN and PharmGKB, the Clinical Pharmacogenetics Implementation Consortium (CPIC) was founded in 2009, with goals to guide clinicians and laboratories how to use pharmacogenetic tests wisely in the clinic (Relling and Klein 2011). To promote training and research in PGx and Theranostics in Europe, and to produce quality-controlled information for applications in clinical practice and for patients, a society named European Society of Pharmacogenomics and Theranostics was established in France (Seist 2012). Meanwhile, drug organizations and government departments such as the U.S. Food and Drug administration (FDA), The Ministry of Health, Labour and Welfare (MHLW) and European Medicines Agency (EMA), have released several milestone guidance or reports, with the purpose to accelerate the translation and application of PGx (Table 1)

## 3. PGx on drug safety and efficacy

Drug safety and efficacy have long been the core issues in the drug development and clinical medication. ADRs are a key factor leading to failure of treatment and admission to hospital. In U.S., ADRs contribute to 6.7% of total hospital admission number (Lazarou et al. 1998), while in the UK, a survey showed that 545 out of 3695 hospital in-patients experience one or more ADRs (Pirmohamed et al. 2004). It was noteworthy that half

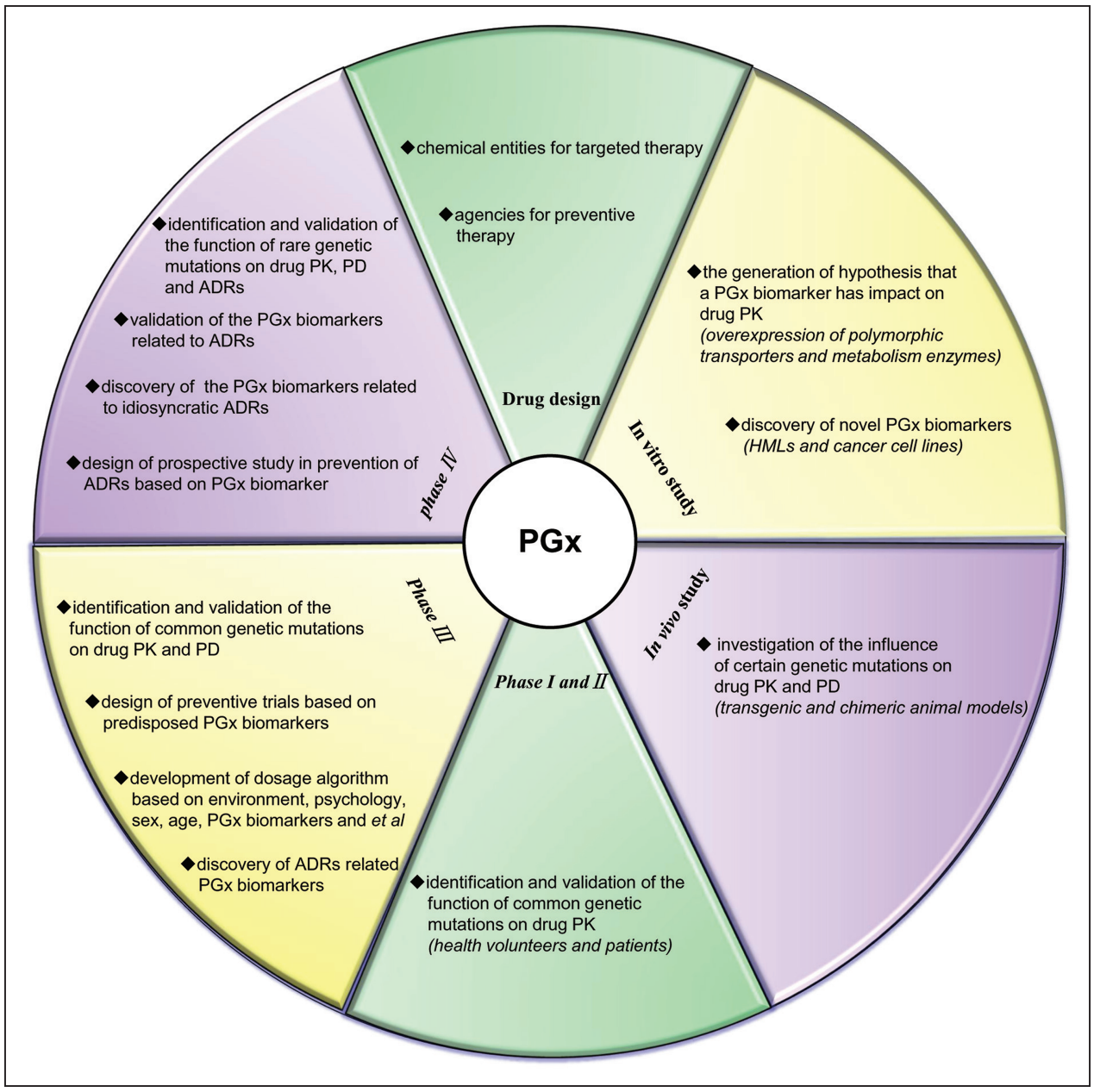


Fig. 1: Application of PGx research in each phase of drug development.

of these accidents were definitely or possible avoidable (Davies et al. 2009). As for the outpatient in U.S., it has been estimated that drug-related problems cost $77 to $177 billion annually (Ernst and Grizzle 2001; Johnson and Bootman 1995). In the past 20 years, more than 40 drugs were recalled by the FDA due to their severe adverse effects, causing more than 40 billion dollars loss to the pharmaceutical companies (Little 2005). However, current reports suggested that the side effects of at least ten of those recalled drugs (eg. sibutramine and cerivastatin) were closely associated with the genetic mutations of metabolizing enzymes and transporters, as well as drug targets (Zhang et al. 2012).

At present, PGx biomarkers are effective indicators for drug efficacy and toxicity. Take warfarin as an example. It is an economic anticoagulant for preventing thrombosis and thromboembolism. However, patients who take warfarin often cannot achieve therapeutic effects or develop the side effect of bleeding because of unpredictable plasma concentrations. Recent PGx studies confirm that the genotypes of CYP2C9 and VKORC1 can account for 30–35% of the variability in warfarin dosing (Eriksson and Wadelius 2012). According to these studies, FDA placed a warning label on warfarin stating that the CYP2C9 and VKORC1 genotypes may be useful in determining initial dosing ranges of warfarin. In another case, HLA-B*5701 and *1502 genotypes that predict the severe cutaneous adverse reactions (SCARs), were also listed as the official biomarkers of abacavir and carbamazepine, respectively. In the past decade, FDA has labeled a variety of drugs with PGx information (http://www.fda.gov/Drugs/). These genomic biomarkers in drug label can tell the effectiveness to medications, the precaution of adverse events, and even the optimization of drug dose, facilitating more accurate and less risky therapy for patients.

## 4. *In vitro* PGx studies

*In vitro* and *in vivo* PGx studies are extraordinarily important for the generation of hypotheses concerning drug response and PGx biomarkers. To date, many genetic biomarkers on drug metabolic enzymes and transporters are reported to affect drug

**Table 1: Guidance or reports on PGx implementation**

| Institution | Time | Title of Guidance | Content | Reference or Website |
|---|---|---|---|---|
| FDA | 2005 | Guidance for Industry: PGx Data Submissions | Guidance suggests the requirement of providing PGx data when submitting an application of a new drug | (Little 2005) |
| FDA | 2011 | Guidance for Industry: Clinical PGx-Premarketing Evaluation in Early Phase Clinical Studies | This guidance describes detailed requirements of clinical DNA sample collection and characteristics identification related to drug response. It also recommends that PGx studies be considered in early phase of clinical trials in evaluating how variations in the human genome could affect the clinical pharmacology and clinical drug responses. | http://www.policymed.com |
| FDA | 2013 | Guidance for Industry: Clinical PGx-Premarketing Evaluation in Early Phase Clinical Studies and Recommendations for labeling | This guidance is intended to assist the pharmaceutical industry and other investigators engaged in new drug development in evaluating how variations in the human genome, specifically DNA sequence variants, could affect a drug's pharmacokinetics (PK), pharmacodynamics (PD), efficacy, or safety. | http://www.policymed.com |
| PWC | 2009 | The new science of personalized medicine: Translating the promise into practice | Guidance suggests that PGx in drug development can dramatically reduce the time, cost, and sample size of the clinical trial as well as the risk of drug withdrawal from the market due to the safety issue. | http://www.pwc.com |
| MHLW | 2005 | Submission of Information to Regulatory Authorities for Preparation of Guidance for the Use of PGx in Clinical Trials | The notification encourage sponsors to voluntarily submit a list of information to the MHLW on planned, ongoing, and past PGx clinical trials. | (Ishiguro et al. 2008) |
| The Japanese Society of Clinical Pharmacology and Therapeutics | 2007 | Current Situations and Future Tasks for Utilization of PGx in Drug Evaluation | It summarize the current knowledge and usefulness of PGx and identified future tasks that should be taken into consideration in regulatory sciences to promote PGx utilization in dug development and clinical practices in Japan | (Ishiguro et al. 2008) |
| ICH | 2008 | International Conference on Harmonization (ICH) - Guidance for Industry: E15 Definitions for Genomic Biomarkers, PGx, Genomic Data and Sample Coding Categories | This guidance contains definitions of key terms in the discipline of PGx and pharmacogenetics, namely genomic biomarkers, PGx, pharmacogenetics,and genomic data and sample coding categories. | http://www.ich.org |
| Health Canada | 2008 | GUIDANCE DOCUMENT: Submission of PGx Information | The document applies to sponsors intending to submit PGx information, either in support of an application or submission for a drug, biologic, or medical device intended for human use. The document also applies to the submission of PGx information as part of ongoing post-market activities. | http://www.hc-sc.gc.ca |
| EMA | 2010 | Guideline on the use of pharmacogenetic methodologies in the pharmacokinetic evaluation of medicinal products | Guideline addresses the influence of PGx on drug pharmacokinetics and encompassing considerations and requirements for the design of pharmacogenetic investigations in drug development. | http://www.ema.europa.eu |

pharmacokinetics (PK). Therefore, it is necessary to characterize the influence of these biomarkers to newly designed chemical entities *in vitro* first. Moreover, it is easy to characterize the function of the rare mutations *in vitro*, but is difficult to achieve in human trials.

Human liver microsomes (HLM), once used to examine the hepatic metabolism of orally administered drugs, can presently assess the impact of PGx on phase I and II drug metabolism with predisposed genotypes (Hesse et al. 2004; Trdan Lusin et al. 2011; Xu et al. 2013). Recombinant CYP450 and *phase*

*II* metabolic enzymes with different genotypes are also efficient tools to study genetic effluence on drug metabolizing (Hiratsuka 2012). Similarly, transporter over-expressing cell lines with the non-synonymous polymorphisms can predict genetic effluence on drug absorption and excretion. Besides, drug-drug interactions can be easily investigated *in vitro* using the over-expressing model. Recently, researchers (Kido et al. 2011; Wittwer et al. 2013) used organic cation transporter (OCT) and multidrug and toxin extrusion transporter (MATE) over-expressed cell models and computer simulation to screen drugs inhibiting these transporters. Over 900 drugs in prescription drug library were assayed, 84 potential MATE1 inhibitors and 244 OCT2 inhibitors were found, and possibly, four and seven out of these two type inhibitors, respectively, can cause clinical drug-drug interactions (Kido et al. 2011; Wittwer et al. 2013). Although most of these studies can indicate a warning on the dangerous PGx biomarkers, how to translate these *in vitro* kinetic data into clinical application remains challenging.

Unlike the role of HLMs, recombinant enzymes and transporters in drug metabolism and disposition, Epstein-Barr-virus-transformed lymphoblastoid cell lines (LCLs) from different populations and numerous cancer cell lines are more efficient in the discovery of PGx biomarkers. LCLs cannot only represent the phenotypes of blood-based toxicity, but also identify relevant genetic variants predictive of tumor response or genes related to drug sensitivity. A genome-wide approach in 179 LCLs of different origin identified single nucleotide polymorphisms (SNPs) associated with platinum susceptibility, and two SNPs were significantly associated to platinum response, other SNPs were weakly associated with hematologic toxicities in a subsequent replication study of sixty head and neck cancer patients (Ziliak et al. 2011). Using the same approach, Brown et al. (2012) identified a subset of SNPs in the O(6)-methylguanine-DNA methyltransferase (MGMT) gene strongly associated with temozolomide response, as well as the expression of MGMT ($p < 10^{-26}$), providing insight into the overall mechanisms of the relationship between temozolomide response and MGMT. More recently, a large genome-wide association mapping of the cytotoxic response of 520 LCLs from European-Americans to 29 different anticancer drugs was performed. Eighteen new loci, as well as three previously implicated ones, were found associated with drug sensitivity (Brown et al. 2014). These studies suggest the utility of LCLs in identifying genes that have clinical importance in drug response and help interpret the underlying mechanisms of drug action.

Drug screens in the NCI60 cell line panel pioneered the approach of using cancer cell lines to associate drug sensitivity with PGx data (Shoemaker et al. 1988; Weinstein et al. 1997). To uncover new biomarkers related to cancer therapeutics, a panel of several hundred cancer cell lines was screened with 130 drugs. Besides of the classic oncogene targets, a new marked relationship between the harboring of EWS-FLI1 gene translocation and sensitivity of Ewing's sarcoma cells to PARP inhibitors, was revealed (Garnett et al. 2012). By linking drug activity to the functional complexity of cancer genomes, systematic PGx profiling in cancer cell lines provides a powerful biomarker discovery platform to guide rational cancer therapeutic strategies.

## 5. *In vivo* PGx studies

Existing *in vitro* systems (using human or rodent cells) and traditional *in vivo* testing in animal species have not always accurately predicted human PK or human-specific drug metabolism pathways for candidate medications. Pharmacogenetic hypotheses upon drug development should further investigated in animals with the concerned genetic biomarkers. An animal with the mimic genotype of a human being, is a natural approach for precisely predicting the interaction between genotype and drug outcome. For example, mu opioid C77G (P26R) polymorphism in rhesus monkeys displays a mimic function of the human opioid receptor, mu 1 (OPRM1) A118G genotype. Vallender et al. (2010) adopted these monkeys to compare naltrexone responsiveness to alcohol abuse between different genotypes. The results suggested that animals harboring the G/G genotype were more sensitive to the effects of naltrexone and showed greater reductions in alcohol consumption at lower naltrexone doses compared to the C/G or C/C genotypes. Nevertheless, this polymorphism resemblance is scarce, the genetic profile of mice, rats, dogs or other primates cannot represent humans. With respect of this disadvantage, transgenic humalized animals may be able to resolve the issue. Currently, researchers have inserted many human gene sequences with the desired polymorphisms into a mice genome, the mode of which further elucidate the physiological function of these gene polymorphisms (Table 2). It is easy to conceive that these genetic mouse models are also effective approaches to study the relation between gene polymorphisms and drug response.

In addition to genetic replacement, Hu et al. (2013) produced a chimeric mouse model by transplanting human liver cells into the liver of a herpes simplex virus type 1 thymidine transgene immunodeficient mouse strain (TK-NOG), in which the mouse liver cells can be ablated temporally by a exposure to a nontoxic dose of ganciclovir. CYP2C19 or CYP2C9 genotypes of the transplanted human hepatocytes, inserted in TK-NOG mice, were correlated with the rate of generation of human-predominant drug metabolites for S-mephenytoin and diclofenac in these chimeric mice, respectively. With these newly developed technologies, it is possible to investigate both rare and common genetic polymorphisms of drug metabolic enzymes, transporters and targets *in vitro*. Although limited to laboratory research presently, these technologies will attract more attention in drug development.

## 6. Considerations of PGx in traditional clinical trials

Because of the relatively small sample sizes in phase I trials, only common mutations in absorption, distribution, metabolism and excretion (ADME) with a hypothesis, can be introduced in this stage, whereas the mutations of target pathway need to be validated in a larger phase II study. Special statistic methods and software are needed to analyze and integrate the PGx data associated with drug PK and PD. Based on these analysis, investigators should decide whether or which mutations will be relevant to phase III, and whether to choose a dose-adjustment strategy according to subjects' genotypes. With their large sample sizes, phase III studies will further validate the relation between drug PK and PD and common variations, as well as the rare mutations hypothesized during the preclinical phases. It is questionable that how phase III studies should incorporate PGx guidelines. If preclinical and phase I / II studies can provide convincing proofs on the association between genomic biomarkers and drug response, it is better to choose a three-arms, randomized trial including a PGx dose adjustment arm, a non-PGx treatment arm, and a control arm. Moreover, the DNA sample from subjects must be properly kept for a retrospective study of other possible candidate gene mutations, and to discover the possible ADRs related variants. However, it is not powerful enough to find mutations related to the rarely happening ADRs, which is the task of *phase* IV. In addition to PGx biomarkers, factors such as environment, psychology, sex, age, physical and disease status, also should takes into consideration for tailoring personalized therapeutic regimens. The warfarin dosage algo-

**Table 2: Influence of human genetic polymorphisms on physiological functions using transgenic mice models**

| Gene | Transgenic mode | Mutation | Genotype | Influence on phenotype | Ref |
|---|---|---|---|---|---|
| OPRM1 | *in situ* partial replacement | Asn40Asp | Asn/Asn vs Asp/Asp | opioid-mediated $Ca^{2+}$ current inhibition and morphine-mediated antinociception | (Mahmoud et al. 2011) |
| Leptin receptor | *in situ* partial replacement | Gln 223 Arg | Gln/Gln vs Arg/Arg | more susceptibility to E. histolytica infection and changed gene expression of infammatory and immune pathway | (Mackey-Lawrence et al. 2013) |
| COMT | *in situ* replacement | Met 158 Val | Met/Met vs Val/val | 30% reduction in enzymatic activity reductions in spatial working memory | (Risbrough et al. 2014) |
| P53 | - | Pro72Arg | - | P72 showing increased response to liver toxins; R72 showing increased response to metabolic stress | (Leu et al. 2013) |
| UGT1A1 | *ex situ* replacement | *1/*28 | *28 vs *1 | elevated levels of total bilirubin and CNS injury | (Fujiwara et al. 2010) |
| BDNF | - | Val66Met | MET/MET vs MET/+ or wild type | more effective therapeutic intervention for anxiety disorders | (Li et al. 2010) |

rithm is a successful example, which can predict 50-70% of dosage variability (Lee and Klein 2013). Possibly in future, it is the collaboration of pharmacokineticist, pharmacogenomian, statistician, and clinician to discuss and design the personalized dosage algorithm, within a large-scale phase III study. Moreover, PGx also play a pivotal role in the post-marketing stage. Because of the exposure to a larger population, it is easy to further validate the rare mutations related to drug efficacy or common ADRs. For idiosyncratic ADRs, such as SCARs and drug-induced liver injury (DILI), retrospective GWAS or whole-genome studies are prone to discover their related genetic biomarkers. If evidential relevance can be given, prospective studies should be continued to incorporate PGx warning on drug labels.

## 7. Highlights of PGx guided clinical trials

At present, many drugs, especially in cancer treatment, are designed according to specific PGx biomarkers, such as human epidermal growth factor receptor 2 (HER2) and the epidermal growth factor receptor (EGFR). Because of the PGx selection for population with potential biomarkers, a smaller size of clinic study can achieve statistic power and drug effectiveness will be largely enhanced. For these drugs, patients with the predisposed biomarkers are enrolled in the trials, and various forms of design can be adopted according to the research aims and disease feature. As targeted to a newly clarified the echinoderm microtubule associated protein like 4- anaplastic lymphoma kinase (EML4-ALK) rearrangement, 2 multi-center, single-arm studies (study A and B) were designed, in the study of crizonib. Totally, 255 patients were enrolled, with objective response rate (ORR) of 50% in study A and 61% in study B. Under the FDA's accelerated approval program, the drug crizonib (Xalkori) was marketed since 30th, August, 2011, with just five years from first-in-human phase I studies to final approval. However, in the phase III study of ado-trastuzumab (T-DM1), the 2-arm, open-label clinical trial was designed in HER2-positive advanced or metastatic breast cancer patients, with one arm of 495 patients receiving T-MD1 *versus* another 496 receiving lapatinib plus capecitabine.

To our knowledge, a traditional clinic trial merely investigates a population with a certain disease. However, it is becoming clear that the genetic status plays an important role in the prediction of diseases (Panza et al. 2014). Therefore, preventive studies in populations with the disease biomarkers are available and of great promise. Instead of recruiting patients as subjects, a preventive clinical trial can enroll a sub population carrying disease biomarkers, which also means an early therapy is available for a population having a large probability of developing disease, rather than since the onset of symptoms. In a previous retrospective cohort study, it was found that the descendants of individuals with a presenilin 1 E280A mutation, also known as the Paisa mutation, were all prone to develop premature Alzheimer's disease in their forties (Acosta-Baena et al. 2011). With a promising effect in treatment of Alzheimer's disease, crenezumab, a monoclonal antibody targeting to multiple forms of aggregated Aβ protein, was investigated for the prevention of Alzheimer's disease. In this pioneer study, the efficacy and safety was evaluated between a crenezumab arm and a placebo arm of PSEN1 E280A carriers (Garber 2012). Another pathogenic mutation in breast cancer susceptibility genes (BRCA) 1 and 2 confer increased risks for breast and ovarian cancer (Narod et al. 2014). The estimated probability for breast cancer by age 70 is about 64% in BRCA1 and 69% in BRCA2, whereas the probability of developing ovarian cancer is about 37% and 25% for BRCA1 and BRCA2, respectively (Beristain et al. 2010). Although surgery is the main preventive strategy (Collins et al. 2013), medications targeted to mutated BRCA1/2 are an alternative approach that avoids physical trauma. In the secondary prevention of breast cancer, tamoxifen is a promising agent for mutated BRCA1/2 patients, based on a study of the association between tamoxifen treatment and contralateral breast cancer (CBC) risk among

**Table 3: Oncology targets and related drugs**

| Targets | Mutation | Drugs | Therapeutic area | Type |
|---|---|---|---|---|
| EGFR | Mutations in exon18,19,20 | Afatinib | NSCLC | SMI |
| | | Erlotinib | NSCLC | SMI |
| | | Gifitinib | NSCLC | SMI |
| | | Cetuximab | mCRC, head and neck cancer | mAb |
| | | Panitumumab | mCRC, head and neck cancer | mAb |
| HER2 | HER2 positive | Ado-Trastuzumab Emtansine | mBC | ADC |
| | | Lapatinib[a] | mBC | SMI |
| | | Pertuzumab | mBC | mAb |
| | | Trastuzumab | mBC | mAb |
| BRAF | pV600E | Dabrafenib | melanoma | SMI |
| | | Trametinib | melanoma | SMI |
| | | Vemurafenib | melanoma | SMI |
| EML4-*ALK* | EML4-ALK fusion | Crizotinib | NSCLC | SMI |
| CD20 | [b] | Ofatumumab | CLL | mAb |
| | | Rituximab | CLL | mAb |
| | | Ibritumomab Tiuxetan | CLL | mAb |
| | | Tositumomab | CLL | mAb |
| CD25 | [b] | Denileukin Diftitox | CTCL | ADC |
| CD30 | [b] | Brentuximab Vedotin | HL, sALCL | ADC |
| BCR/Abl1 (Philadelphia translocation) | BCR-Abl1 fusion | Ponatinib | CML, ALL | SMI |
| | | Nilotinib | CML | SMI |
| | | Dasatinib | CML, ALL | SMI |
| | | Bosutinib | CML | SMI |
| | | Imatinib[a] | CEL | SMI |
| PDGFR | [b] | Sorafenib[a] | HCC, RCC | SMI |
| | | Sunitinb[a] | Imatinib resisted GIST and RCC | SMI |
| Kit | Kit (CD117) positive | Imatinib[a] | GIST | SMI |
| VEGFR | [b] | Bevacizumab | mCRC | mAb |
| | | Sunitinb[a] | Imatinib resisted GIST and RCC | SMI |

Abbreviation: ADC, Antibody-drug conjugates; ALL, acute lymphoblastic leukemia; CEL, chronic eosinophilic leukemia; CLL, chronic lymphocytic leukemia; CML, chronic myeloid leukemia; CTCL, Cutaneous T-cell lymphoma; GIST, gastrointestinal stromal tumors; HCC, hepatocellular carcinoma; HL, Hodgkin lymphoma; mAb, mono-antibody; mBC, metastatic breast cancer; mCRC, metastatic colorectal carcinoma; NSCLC, non-small-cell lung carcinoma; RCC, renal cell carcinoma; sALCL, systemic anaplastic large cell lymphoma; SMI, some molecular inhibitor
Data were extracted from FDA official website
[a] Drugs that have muti-targets
[b] mutation status is not indicated

1,583 BRCA1 and 881 BRCA2 mutation carriers (Phillips et al. 2013). The adjusted hazard ratios of contralateral breast cancer, for BRCA1 and BRCA2 mutation carriers, were 0.38 and 0.33, respectively (Phillips et al. 2013).

## 8. Development of targeted therapy

Recently, targeted therapy has shown promising therapeutic outcome for patients, whose gene expression profiling or mutation status were known. In the past three years, a total of 14 new targeted drugs were licensed by the FDA for cancer therapy, which take up nearly 15% to the total number, with even more targeted drugs entering clinic trials. About 22% newly developed anti-tumor drugs have been accompanied by PGx information. Monoclonal antibodies, small molecules and antibody-drug conjugates are the three drug categories in targeted therapy. Monoclonal antibody deriving from human, mouse or chimeric, can combine with the abnormal member receptors and inhibit the downstream pathway, whereas chemosynthetic small molecules insert into active domain of proteins or enzymes and inhibit tumor progression. Conjugates are novel biochemical entities that consist of a chemosynthetic antitumor drug and the amino acid domain targeting to the specific biomarker in tumor surface. Eleven common oncology targets and their related drugs are listed in Table 3. Of these oncology targets, EGFR, HER2 and v-raf murine sarcoma viral oncogene homolog B1 (BRAF) are most well-known ones. The discovery of these targets benefits a huge number of patients, and also makes us re-recogenize cancer. EGFR subfamily is a group of four transmembrane receptors (EGFR/ErbB1, HER2/ErbB2, HER3/ErbB3 and HER4/ErbB4) with intracellular tyrosine kinase activity except for HER3 (Ciardiello and Tortora 2008). The abnormal expression or mutations of these receptors will lead to pathogenesis or aggressiveness of tumor, such as over-expression of HER2 in breast cancer, the deletions in exon 19 and L858R mutation of EGFR in NSCLC. In the targeted therapy of HER2-positive breast cancer, trastuzumab and pertuzumab specifically bind to the domain IV of the extracellular segment of the HER2 receptor and leads to cell cycle capture, while lapatinib is a small molecule with a dual inhibiting effect to both EGFR and HER2 (Burris 2004). In 2013, a new drug named ado-trastuzumab (T-MD1) was licensed by the FDA. T-DM1 is a new generation entity, which comprises three elements, the antibody drug conjugate (trastuzumab), a stable thioether linker, and the potent cytotoxic agent DM1 (derivative of maytansine) (Lewis Phillips et al. 2008). The *phase III* randomized trial EMILIA has shown that T-DM1 provided objective tumor responses and significantly improved progression free survival and overall survival compared to lapatinib and capacitabine combination therapy (Welslau et al. 2014).

As refered to NSCLC, the role of PGx is also pivotal. EGFR mutations, with a prevalence of 45% in Pacific Asian patients and 24% in Caucasian, is a superior predictor of response to EGFR tyrosinekinase inhibitor (TKI) therapy including gefinitib, erlotinib, lapatinib, as well as the newly approved

**Table 4: Benefits and challenges of PGx application in drug development**

| |
|---|
| 1. Benefits |
| i. Discover new drug targets and disease pathway for drug design |
| ii. Improve the process of drug development and clinical trial |
| iii. Reduce the size of clinical trial |
| iv. Improve the effectiveness, and reduce ADRs of drugs |
| v. Prevent the occurrence and progress of disease in population with pathogenic genomic biomarkers |
| 2. Challengens |
| i. Incorporating PGx evaluation throughout the preclinical and clinic trial would enhance the expenditure |
| ii. For certain medicine, genetic mutations may be the leading factors of drug response, but for others, factors such as environment, psychology, society and personal health status, can be dominate. |
| iii. Many details on how to implement the PGx in the pipeline of drug development are missing. It is still unclear which genomic biomarker on drug PK or PD should be investigated *in vivo*, how to translate the data of *in vivo* and *in vitro* study into human trial, and how to select the dose and size of trial when considering PGx into clinic trial. |
| iv. The biotechnology for genotyping or bioassay is relatively expensive, lack of timeliness in large-scale application, even with a poor repeatability and accuracy. |

molecular gilotrif. EML4-ALK rearrangement is a recently identified subset in NSCLC, with a prevalence rate of about 4%. EML4-ALK rearrangement codes a new fusion protein which continues promoting cell proliferation and leads to a bad prognosis (Rikova et al. 2007; Soda et al. 2007). In this subset, a dual inhibitor of ALK and c-Met receptor kinases (Christensen et al. 2007), crizotinib, shows a better effectiveness than other drugs. Apart from mutated EGFR and ALK positive genotype, recent PGx studies have identified many other PGx biomarkers, including c-ros oncogene 1 receptor tyrosine kinase (ROS1) rearrangements, ret proto-oncogene (RET) fusions, met proto-oncogene (MET) amplification, and activating mutations in BRAF, HER2, and KRAS, all of which are the potential therapeutic target for NSCLC treatment (Cardarella and Johnson 2013).

In 2002, the Sanger Institute firstly discovered that 60% of melanomas harbor the activating mutation of BRAF gene (Davies et al. 2002). The mutation happens within the kinase domain, and leads to a single substitution (V600E/K), which causes an elevated kinase activity and continually activates the downstream mitogen-activated protein kinase (MAPK) pathway. Subsequently, a specific inhibitor of mutated BRAF, vemurafinib, was found with a fascinating effect in treatment of melanomas (Gray-Schopfer et al. 2007; Yang et al. 2010). In 2013, a new BRAF inhibitor named dabrafenib was also licensed for treatment of melanomas with BRAF mutation (V600E/K), with a median PFS of 5.1 compared with 2.7 months for dacarbazine (Hauschild et al. 2012). Also in this year, trametinib, a MEK inhibitor, was approved for the treatment of metastatic melanoma carrying the BRAF mutation as a single agent after a promising *phase III* clinical trial. As targeted to different kinases, a combined investigation of trametinib and dabrafenib was executed. According to the result of this study, the FDA approved the combination of dabrafenib and trametinib for the treatment of patients with BRAF V600E/K-mutant metastatic melanoma on January 8, 2014.

## 9. Future perspectives

As PGx research have provided considerable benefits for drug development (Table 4), pharmaceutical industry has stepped into PGx studies (Raaijmakers et al. 2010). In the past 5 years, PGx information of more than 140 new drugs have been voluntarily submitted to the FDA. Although the progress on PGx studies seems fascinating, the current translational level in drug development is relatively low. A recent survey of clinic investigations from 1999 to 2012 found a total of 323 PGx guided studies, which represented only 0.23% of the total 138,416 studies registered during that period (Burt and Dhillon 2013). Out of the total, 258 (80%) were sponsored by academic institutions, but only 27% by industry and 7% were by industry–academic collaborations. Challenges such as the complexity of drug characteristics, increasing expenditure of the pharmaceutic industry and the lack of official standards, inevitably impede the PGx application (Table 4).

Effectiveness, accuracy and economy are core issues on whether PGx can guide drug development. It is urgent to find more accurate phenotypes or biomarkers that can link the drug effectiveness or toxicity with patients' genotype. More importantly, specialized statisticians and computer programs are needed to integrate and analyze the data gathered from both *in vitro* and *in vivo* PGx research, and to decide which PGx biomarker will have a notable influence on drug PK and PD and how to incorporate the PGx biomarker into the design of a clinical trial. The approach of genotyping is also a problem that constrains the progress of PGx. Currently, outpatients cannot be informed about their genotype timely and the result of genotyping is often unified among different testing agencies. Thus, quick and accurate detection methods, as well as official guide should be established. To conclude, only through a rounded system that includes both individual and academics associations, medical staff and scientific researchers, government and pharmaceutics-biotechnology industry, and a connected pipeline consisting of policy guidance, PGx research, genotyping technology, preclinical and clinical study, can PGx assign a more profound role in drug development (Raaijmakers et al. 2010).

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