Pharmacogenetics and pharmacogenomics in oncology therapeutic antibody development

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Pharmacogenetics and pharmacogenomics are keys to the success of personalized medicine, prescribing drugs based on a patient’s individual genetic and biological profile. In this review, we will focus on the application of pharmacogenetics and pharmacogenomics in developing monoclonal antibody (MAb) therapeutics in oncology. The significance of pharmacogenomics in MAb therapeutics is highlighted by the association between polymorphisms in Fc receptors and clinical response to anti-CD20 MAb rituximab (Rituxan®) or anti-ganglioside GD2 MAb 3F8, as well as the potential link between polymorphisms in HER2 and cardiac toxicity in patients treated with the anti-HER2 MAb trastuzumab (Herceptin®). The dependence on gene copy number or expression levels of HER2 and epidermal growth factor receptor (EGFR) for therapeutic efficacy of trastuzumab and cetuximab (Erbitux®), respectively, supports the importance of selecting suitable patient populations based on their pharmacogenetic profile. In addition, a better understanding of target mutation status and biological consequences will benefit MAb development and may guide clinical development and use of these innovative therapeutics. The application of pharmacogenetics and pharmacogenomics in developing MAb therapeutics will be largely dependent on the discovery of novel surrogate biomarkers and identification of disease- and therapeutics-relevant polymorphisms. Challenges and opportunities in biomarker discovery and validation, and in implementing clinical pharmacogenetics and pharmacogenomics in oncology MAb development and clinical practice will also be discussed.

INTRODUCTION
Pharmacogenetics and pharmacogenomics are multidisciplinary research efforts to study the relationship between genotype (i.e., polymorphisms and genetic mutations), gene expression profiles (the level of gene expression of all the genes in the genome), and phenotype, as expressed in variability between individuals in response to or toxicity from drugs. Pharmacogenetics typically refers to effects involving a limited number of genes, often involving drug metabolism, whereas pharmacogenomics involves the study of complex multigene patterns within the genome.

Genetic polymorphisms are variants in individual genomes and remain constant throughout a person’s lifetime. There are estimated 1.4 million single nucleotide polymorphisms (SNPs) identified in the human genome, and many of them contribute to variability in drug pharmacokinetic and pharmacodynamic processes (1). Genetic mutations are acquired changes in gene sequences and occur only in certain cells. Tumor cells may acquire mutations at an increased rate compared to host tissues (2). These genetic variants collectively affect drug transport and metabolism, cellular targets, signaling pathways, and cellular responses to treatment (3). Gene expression profiling can identify patterns of gene expression by microarray analysis of messenger RNA (mRNA), and these patterns can be characterized and classified by a variety of mathematical techniques (4–6).

The ultimate goal for pharmacogenetics and pharmacogenomics is the development of personalized medicine, defining the population diversity of polymorphisms, genetic mutations, and gene expression profiles of clinical interest, thereby facilitating prescription of drugs based on a patient’s individual genetic or biological profile, or the individual genetic profile of his/her tumor. In oncology, pharmacogenetics and pharmacogenomics have already been applied to predict cancer susceptibility, tumor progression and recurrence, patient survival, and response to and toxicity associated with traditional chemotherapy treatments (7–9). With increasing success of targeted anticancer agents including monoclonal antibody therapeutics, it is important to evaluate the impact of pharmacogenetics and pharmacogenomics on clinical efficacy and toxicity of these new therapies.

PHARMACOGENETICS AND PHARMACOGENOMICS IN ONCOLOGY THERAPEUTIC MONOCLONAL ANTIBODIES

Antibody Therapeutic Efficacy and FcR Polymorphism
Two key mechanisms-of-action underlying the cytotoxic activity of naked monoclonal antibodies (MAbs) are antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicty (CDC) (10). Tumor cell killing by ADCC is triggered by the binding of the Fc region of an antibody to cell surface immunoglobulin G γ (IgGγ) Fc receptors (FcγR) on immune effector cells, including macrophages, monocytes, dendritic cells, natural killer (NK) cells, and neutrophils. CDC is initiated by complement component C1q binding to the Fc region of an antibody, which is bound to the surface of a tumor cell. Subsequent target-cell lysis can occur in a cell-dependent or -independent manner (10). There are two main types of FcγRs, activating FcγRs such as the high-affinity receptor, CD64 (FcγRI), and the low-affinity receptors, CD32A (FcγRIIA) and CD16A (FcγRIIa), and inhibiting FcγRs CD32B (FcγRIIB) (10–12). Slightly different variations in FcγRs exist in different individuals. These genetic differences result in changes in the FcγR protein sequences. Such allotypic variants are collectively known as FcγR polymorphism, and its association with clinical response to MAb therapy has now been increasingly appreciated.

Anti-CD20 rituximab. The relationship between individual clinical outcomes and FcγR polymorphism has been most extensively studied for the IgG1 anti-CD20 MAb rituximab. In patients with non-Hodgkin’s lymphoma, clinical and molecular responses to rituximab were associated with the FcγRIIa genotype, determined by a SNP at residue 158 (13). This gene dimorphism in FCGRA3, the gene encoding FcγRIIB receptor, results in either a phenylalanine (F) or a valine (V) at residue 158 of FcγRIIa receptors (14,15). Patients with 158VV genotype showed better clinical responses to rituximab than those 158F carriers. This clinical finding is consistent with the fact that the 158 residue directly interacts with the lower hinge region of IgG1 (16), and the FcγRIIB receptor 158V allotype displays a higher affinity for human IgG1 MAb than the 158F allotype. A subsequent study investigated the contribution of two activating FcγR allotypes, FcγRIIa CD32 and FcγRIIa CD16, to clinical outcomes following rituximab treatment.
Drug Toxicity and Target Polymorphism

Genetic polymorphism not only influences antibody drug efficacy but may also contribute to drug toxicity. In a recent study of HER2 genetic polymorphism in breast cancer patients receiving the anti-HER2 MAb trastuzumab-based treatment, a potential link between the development of cardiotoxicity and HER2 polymorphism was observed (21). In this small size study with genotype information available for 56 patients, all 5 patients who developed cardiotoxicity after trastuzumab treatment carried the HER2-655Val/Ile (valine/isoleucine) phenotype, with no apparent link to anthracycline treatment. It is still preliminary to conclude that the HER2-655Val/Ile allele is predictive for cardiotoxicity associated with trastuzumab treatment, and the presence of this allotype and its association with cardiotoxicity need to be further analyzed in larger studies. If validated, this correlation between HER2 polymorphism and cardiotoxicity may provide a means of managing trastuzumab toxicity in breast cancer patients by avoiding treatment of a high risk subgroup.

There is also evidence suggesting that cardiotoxicity of trastuzumab is further enhanced if it is given together with anthracycline chemotherapy. Cardiotoxicity due to anthracycline chemotherapy is also affected by the genetic background of the host. The cardiotoxicity is mediated by iron, and genetic studies in mice reveal worsened cardiotoxicity when the HFE gene, defective in hereditary hemochromatosis, is knocked out (22).

Antibody Therapeutic Efficacy and Target Expression Level, Gene Amplification, and Mutation Status

For MAb therapeutics targeting cell surface proteins, the treatment responses depend on the presence and often the expression level of the target on tumor cells. The anti-HER2 MAb, trastuzumab (Heceptin®), represents the first MAb therapeutic developed for treating solid tumors. It was approved in 1998 along with a molecular diagnostic that could determine, based on the expression level of HER2, the target for Heceptin, whether a patient with breast cancer is among 30% of the population that would benefit from the drug (23). In contrast to this clear dependence on HER2 expression for trastuzumab treatment response, correlation between expression status of epidermal growth factor receptor (EGFR) and clinical efficacy of anti-EGFR therapies including cetuximab (Erbitux®) has yet to be well established (24,25). This lack of association is hard to explain biologically, since EGFR is the only known target for anti-EGFR antibodies, both cetuximab and panitumumab. Results from a recent study may furnish an alternative explanation (26). In this small and retrospective study, 8 of 9 or approximately 90% of responders assessable by fluorescence in situ hybridization (FISH) who showed objective response to anti-EGFR MAb therapy had an increased EGFR copy number assessed by FISH analysis. By contrast, only less than 5% of patients (1 of 21 nonresponders assessable by FISH) had EGFR gene amplification. Unlike small molecule EGFR inhibitors, such as gefitinib, of which clinical responses are affected by sequence variations in the EGFR (27), it was also demonstrated in the same study that clinical response to anti-EGFR MABs was not apparently affected by point mutation status in the EGFR itself or its immediate downstream effectors (26). The EGFR gene amplification status also determines the in vitro antiproliferation efficacy of cetuximab in various colorectal cancer cell lines that harbor different mutations (26,28). Therefore, it is plausible to propose that response to anti-EGFR MAb therapies may also be solely dependent and determined by EGFR gene amplification or expression level, as has been observed in anti-HER2 therapies. This dependence of clinical response on target gene amplification and increased protein expression (23,26,29,30), but not on activation mutations, also suggests that MAb therapeutics may work most efficiently against targets that are amplified rather than against those affected by point mutations.

Responses to therapeutic MABs can also be affected by variations in expression of other elements of the signaling pathway. PTEN, a tumor suppressor gene, is a tyrosine phosphatase that antagonizes the effects of PI3 kinase in the AKT signaling pathway, which is affected by erbB2 (Her2/Neu), the target of trastuzumab (31). Trastuzumab inhibits the binding of src kinase to erbB2, in turn reducing tyrosine phosphorylation of PTEN. This leads to increased membrane localization and phosphatase activity of PTEN, an effect which is seen in vitro in erbB2-expressing cell lines with trastuzumab treatment well before down-regulation of erbB2 and can be mimicked by src kinase inhibitors. In xenograft models, inhibition of PTEN with antisense oligonucleotides induced resistance to trastuzumab both as a single agent and in combination with paclitaxel. In a clinical study, 47 Her2/Neu-positive breast cancer patients had PTEN expression levels evaluated and clinical responses to trastuzumab in combination with paclitaxel noted. Those with low PTEN expression had an 11% response rate compared to 66% of those with high PTEN expression, a highly statistically significant result. In fact, PTEN expression levels by immunohistochemistry (IHC) were more predictive of clinical response than Her2 expression levels (31).

Use of Biomarkers to Predict MAB Therapeutic Responses

The use of pharmacogenetics and pharmacogenomics in oncology MAb development and clinical use will increase rapidly in the next decade and is expected to drive personalized medicine in clinical practice. Biomarkers may be prognostic, which is correlated with disease outcome regardless of therapy (32), or they may actually be predictive of the outcome after a specific therapy. The terms pharmacogenetics and pharmacogenomics apply only to the latter type of biomarker, and it is important to make this distinction before applying a biomarker. Because of the very large number of genes in the genome, studies designed to look across the genome at polymorphisms, mutations, or gene expression patterns almost always uncover possible associations with clinical outcome, but some of these may be false positives due to the play of chance. Thus, early studies really lead to hypotheses about biomarkers, which must be prospectively validated in independent data sets, ideally from large randomized clinical trials. When a biomarker involves a panel or weighted sum of genetic characteristics, both the choice of genes and the choice of weights must be validated (33–35). Panels of genes may allow classification of patients into clinically relevant subgroups, even if some of the genes are false positives, provided enough of the genes used are indeed relevant. However, if the goal is to understand a drug’s mechanism of action, it is important to test the relevance of putative biomarkers in direct molecular experiments.
Intratumoral mRNA expression levels of several components in the EGFR signaling pathway were determined in patients with colorectal cancer treated with cetuximab (36). Higher vascular endothelial growth factor (VEGF) expression levels are associated with resistance to cetuximab. The combination of low gene expression of Cox-2, EGFR, and interleukin 8 (IL-8) was significantly associated with overall survival. Patients with a lower EGFR mRNA had a longer overall survival compared with patients that had a higher mRNA amount. Patients with lower expression of Cox-2 had a significantly higher rate of skin reactions under cetuximab treatment. Patients developing acne-like skin rash tend to respond better to cetuximab treatment (24,25), suggesting potential surrogate biomarkers may also exist in skin for predicting responses. Although these preliminary results need to be validated in prospective and large size studies, they nevertheless may shed light into the molecular mechanism underlying cetuximab response and resistance. In another clinical study, changes in biomarker expression in response to GM-CSF and anti-GD2 MAb 3F8 combination treatment were found to be prognostic for treatment efficacy in pediatric patients with neuroblastoma (37). The expression status of two molecular markers, GD2 synthase and tyrosine hydroxylase in the bone marrow, were evaluated before and after treatment. If the expression status changed from being positive to negative after treatment, patients were more likely to survive progression-free. When these two biomarkers remained unchanged (i.e., remained positive before and after treatment), remission was shorter. Lastly, changes from being negative to positive after treatment were associated with early disease progression. These changes in expression status were evaluated after only two cycles of treatment, therefore providing an early assessment of clinical responses that was informative in trial decision making.

**CHALLENGES AND OPPORTUNITIES IN APPLYING CLINICAL PHARMACOGENETICS AND PHARMACOGENOMICS IN ONCOLOGY MAb THERAPIES**

There are accompanying challenges and issues emerging in both development and clinical application stages of MAb therapeutics. For example, in order to select patients to receive trastuzumab treatment, the HER2 expression status needs to be determined. However, there are at least two different methodologies being used for this purpose, IHC analysis of HER2 protein or FISH analysis of HER2 gene amplification. FISH analysis provides accurate assessment of HER2 gene amplification status (29,38). In contrast, the more convenient IHC methods, including Food and Drug (FDA)-approved HercepTest™ (DakoCytomation, Carpinteria, CA, USA), may not consistently detect HER2 alternation (38). IHC methods can be affected by the fixation method, method of antigen retrieval, the immunohistochemical reaction, amplification, and visualization methods, diagnostic thresholds, and presence or absence of internal controls (39). Thus, standardization of HER2 detection methods in clinical practice would be highly desirable.

Similarly, the lack of a clear correlation between tumor EGFR expression level and anti-EGFR therapy could potentially be attributed to the heterogeneity of EGFR overexpression in clinical tumor specimens (26) and the lack of a standard methodology for detecting EGFR expression and mutations in fixed cancer samples. An accurate assessment of EGFR may require not simply assessing general EGFR expression, but documenting the overall percentage of positive cancer cells, the percent of cancer cells with membranous staining and with cytoplasmic staining, and the intensity of this staining, as recommended by EGFR pharmDx™ kit (DakoCytomation). Alternative methodologies with higher sensitivity, such as intratumoral quantitative reverse transcription PCR (RT-PCR) (36), or increased consistency, such as FISH (26), may need to be introduced into clinical practice. It also needs to be further explored whether response to anti-EGFR MAbs, like EGFR inhibitors, may also depend on variables other than target gene amplification or expression level, such as the activation status of EGFR-dependent signaling pathways (40) or the mutational status of the EGFR protein (27).

Since in oncology many important biomarkers are limited to the tumor cells themselves, a significant challenge involves obtaining tumor tissue from patients with metastatic disease who may not have biopsy-accessible tissue. Techniques now exist to capture small numbers of tumor cells in the circulation of patients with metastatic cancer, and genetic analysis of these cells may contribute to biomarker development in oncology (41).

The most significant challenge to incorporate the use of pharmacogenetic and pharmacogenomic biomarkers into MAb therapeutic development and clinical practice is the unequivocal validation of these surrogate biomarkers. In its Pharmacogenomics Data Submissions guidance, the U.S. FDA classified biomarkers into two categories. “Valid biomarkers” are surrogate markers that have physiological, toxicological, pharmacological, or clinical significance, while “probable biomarkers” are those have not yet reached the status of valid biomarkers because their significance has yet to be confirmed in large prospective clinical studies. Most of these biomarkers were either discovered from preclinical studies or retrospective clinical studies with small sample sizes. Prospective clinical trials to validate these biomarkers are necessary to allow for a wide implementation of a rational and systematic individualization of cancer therapy. Statistical criteria have been established for validation of a biomarker (42–44). For a biomarker predictive of therapeutic outcome, not only must it correlate with the outcome of interest in a prospective randomized trial, but it must capture the variation in outcome due to the therapeutic effect of the drug of interest.

Despite these challenges, clinical pharmacogenetics and pharmacogenomics may be introduced into the process of MAb development and clinical practice. Implementation of biomarker discovery strategies in preclinical and in early phase clinical development, and validation in and incorporation into late phase clinical development may transform the current drug development process. Instead of testing therapeutic agents in subjects defined by clinicopathological classifications (i.e., tumor types), MAb therapeutics may be tested in patients with molecularly classified diseases (i.e., dependence of tumor progression on certain key pathways). The implementation of validated biomarkers in late phase clinical studies will limit clinical study populations to those patients who will most likely respond to the testing agents, thus reducing the cost and failure rate associated with this stage of development. In this paradigm, the MAb may be approved for clinical use together with a diagnostic test that may either be optional or required for identifying the patient population. Therefore, it is expected that incorporation of biomarkers into therapeutics development may segment the market after successful validation in clinical trials and final approval of these biomarkers accompanying the approval of the therapeutics. Even though molecular profiling-based approval will reduce the number of patients within a single indication, it may have the potential to increase market penetration by specifically targeting a subset of patients who will have the most likelihood of responding to and therefore benefiting from these molecularly targeted agents. In addition, validated surrogate biomarkers may also facilitate the identification of additional indications, therefore benefiting more patients and increasing the total market. For example, imatinib is currently used in gastrointestinal stromal tumors (GIST) based on the realization that it inhibits platelet-derived growth factor (PDGF) signaling pathway (45).

Likewise, pharmacogenomic and other biomarkers may also offer a better way for clinicians to personalize therapies for their patients in clinical practice. Instead of prescribing treatments to cancer patients based on the tissue or organ origin of cancer,
personalized treatment may be based on molecular profiling of biomarkers in tumor biopsies or even in samples obtained via noninvasive means. These biomarkers may also help oncologists to better refine treatment regimens, to closely monitor patient responses to treatment, and to manage or avoid side effects and drug toxicities. Collaboration between the pharmaceutical and biotechnology industries, academia, and government, as well as guidance and support from health authorities, will be required to make personalized medicine a reality.

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COMPETING INTERESTS STATEMENT
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REFERENCES

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