MOLECULAR ONCOLOGY 6 (2012) 251–259

available at www.sciencedirect.com

SciVerse ScienceDirect

www.elsevier.com/locate/molonc

Review

Germline pharmacogenomics in oncology: Decoding the patient for targeting therapy

Peter H. O'Donnell[a,b,c,d,*], Mark J. Ratain[a,b,c,d]

[a]Section of Hematology/Oncology, Department of Medicine, The University of Chicago, 5841 S. Maryland Avenue, MC 2115, Chicago, IL 60637, USA
[b]Committee on Clinical Pharmacology and Pharmacogenomics, The University of Chicago, 5841 S. Maryland Avenue, MC 2115, Chicago, IL 60637, USA
[c]Comprehensive Cancer Center, The University of Chicago, 5841 S. Maryland Avenue, MC 2115, Chicago, IL 60637, USA
[d]Center for Personalized Therapeutics, The University of Chicago, 5841 S. Maryland Avenue, MC 2115, Chicago, IL 60637, USA

ARTICLE INFO

Article history:
Received 20 September 2011
Received in revised form
14 December 2011
Accepted 13 January 2012
Available online 21 January 2012

Keywords:
Pharmacogenomics
Targeted therapy
Oncology

ABSTRACT

Pharmacogenomics is the study of genetic factors determining drug response or toxicity. The use of pharmacogenomics is especially desirable in oncology because the therapeutic index of oncology drugs is often narrow, the need for favorable drug response is often acute, and the consequences of drug toxicity can be life-threatening. In this review, we examine the state of pharmacogenomics in oncology, focusing only on germline pharmacogenomic variants. We consider several critical points when assessing the quality of pharmacogenomic findings and their relevance to clinical use, and discuss potential confounding factors limiting interpretation and implementation. Several of the most extensively studied drug–gene pairs (irinotecan and *UGT1A1*; tamoxifen and *CYP2D6*; 5-fluorouracil and *DPYD*) are inspected in depth as illustrations of both the state of advancement—and the current limitations of—present knowledge. We argue that there will likely soon be a critical mass of important germline pharmacogenomic biomarkers in oncology which deserve clinical implementation to provide optimal, personalized oncologic care. We conclude with a vision of how routine clinical testing of such germline markers could one day change the paradigm for cancer care.

1. Introduction

Pharmacogenomics is the study of genetic factors determining response to, or toxicity from, drugs. While the field originally centered on the relationship between drugs and single genes (pharmacogenetics), pharmacogenomics now encompasses information from the entire genome including germline variation (single nucleotide polymorphisms [SNPs], gene copy number alterations) and acquired changes (tumor mutations) as they relate to drug response or toxicity (Wang et al.,

* *Corresponding author*. Section of Hematology/Oncology, Department of Medicine, The University of Chicago, 5841 S. Maryland Avenue, MC 2115, Chicago, IL 60637, USA. Tel.: +1 773 702 7564.
E-mail address: podonnel@medicine.bsd.uchicago.edu (P.H. O'Donnell).

2011; Watson and McLeod, 2011). In contrast to disease genetics, pharmacogenomics focuses specifically on predictive genetic markers of outcome from pharmacologic interventions.

The use of pharmacogenomic markers is perhaps especially desirable in the field of oncology, where the therapeutic index of drugs is often narrow, and the consequences of drug toxicity can be life-threatening. However, since adverse drug reactions are reported to be the fifth leading cause of death in the United States, the risks are not specific to oncology drugs (Davies et al., 2007). At the same time, it is likely that we have failed to capitalize on the increased benefit that could be achieved with some therapies if we knew which patients were most likely to respond, or which patients required alternative dosing. If we could better predict which individuals are at the greatest risk of suffering chemotherapy-related toxicities while simultaneously identifying those most likely to benefit, then the overall care of cancer patients could be greatly improved.

In this review, we will examine the state of pharmacogenomics in the field of oncology. We will specifically restrict our considerations to germline genetic discoveries related to oncologic therapeutics; a discussion of the growing number of "molecularly-targeted" drugs based upon tumor pharmacogenomics is beyond the scope of this current manuscript. To date, most germline oncology pharmacogenomic information has simply been cataloged, or, in a few instances, has led to FDA drug label changes. Therefore, we will also consider the barriers and means by which oncology pharmacogenomic information—which is increasing every day—can become more commonly integrated into the routine care of cancer patients. We will posit that use of such patient-specific information should soon become the standard of care, rather than the exception.

2. Major current pharmacogenomic findings in oncology

The number of germline oncology drug–gene pharmacogenomic pairs having high levels of evidentiary support is relatively small compared to other drugs. Perhaps the strongest examples are those for which the strength and scope of the data has resulted in FDA-mandated label changes so that prescribing clinicians are aware of well-characterized, pertinent germline pharmacogenomic information when prescribing (United States Food and Drug Administration, 2011). These highest level drug-variant pairs, along with several other of the most extensively studied oncology drug-variant pairs, are summarized in Table 1.

As can be seen from Table 1, most of the existing described relationships have focused on genetic predictors of oncology drug-related toxicity phenotypes, rather than disease outcome phenotypes, although accumulating data suggest that germline polymorphisms might also affect treatment outcomes (see references in Table 1; and selected others (Huang et al., 2011; Wu et al., 2010; Ziliak et al., 2011; Yang et al., 2009)). Of the drug–gene pairs in Table 1, the pharmacogenomic relationships between irinotecan and *UGT1A1* (for neutropenia) (Relling et al., 2011) have the most consistent, strong supporting evidence in favor of their routine use. For *UGT1A1* as an example, several prospective studies have demonstrated that patients with the high-risk genotypes (*UGT1A1*28* and *UGT1A1*6*) are significantly more likely to experience neutropenia, with two of these studies corroborating the relationship with pharmacokinetic supportive data (Innocenti et al., 2004; Minami et al., 2007). In the largest such study of 250 metastatic colorectal cancer patients, the odds ratio of risk of cycle 1 grade 3 or 4 neutropenia was ~9, although the relationship did not persist for subsequent cycles (Toffoli et al., 2006). A meta-analysis of published studies on *UGT1A1*-irinotecan (including 821 patients) also confirmed the association for patients homozygous for the *UGT1A1*28* allele who are receiving higher doses of irinotecan ($\geq$150 mg/m^2) (Hoskins et al., 2007). Including other risk alleles within *UGT1A* in a haplotype-based analysis may increase the predictive value of pharmacogenomic testing, since several other variants in these genes have now also been shown to alter enzymatic activity and impact irinotecan-related outcomes (Cecchin et al., 2009).

There has also been significant interest in the relationship between tamoxifen and *CYP2D6* (discussed further below). While the preponderance of the published data support the utility of *CYP2D6* testing for tamoxifen use, there has not been a recommended pharmacogenomic FDA label change for this drug, and recent data presented in abstract form have been contradictory (Goetz et al., 2009; Rae et al., 2010; Leyland-Jones et al., 2010). There is also a large body of growing evidence for many more oncology drug polymorphisms and various phenotypes. The best-performed studies of emerging pharmacogenomic associations now routinely include replication testing upfront, and these drug-variant pairs deserve further examination for how they might be considered in clinical utility investigations.

Despite the existence of well-performed studies and validation in many cases, some have still questioned whether any present germline oncology findings are currently clinically actionable without further prospective follow-up trials being performed (Coate et al., 2010). Certainly even the best-studied drug–gene relationships have recognized limitations in applicability which must be considered (Lee and McLeod, 2011). We believe that the clinical utility of each finding must be interpreted not only in light of the composite evidence describing a given relationship but also in the context of the clinical scenario in which the relative benefit versus risk must be considered. If a pharmacogenomic test could potentially mitigate risk without compromising efficacy, then we believe its practical value is high. We will discuss this topic further below. It is important to first consider interpretation of published pharmacogenomic findings as a starting point.

3. Limitations to pharmacogenomic data interpretability

As evidenced by the examples shown above, the most convincing drug-variant relationships are those identified

Table 1 – Summary of the most extensively studied germline pharmacogenomic relationships for oncology drugs.

Drug	Phenotype(s) evaluated	Genes	Variants	FDA label includes pharmacogenomic prescribing considerations?	Important considerations	Key references
Irinotecan	Neutropenia	*UGT1A1*	*28; plus others likely important	YES	1) Variants may only be predictive for patients receiving higher drug doses; 2) Unclear if other irinotecan toxicities (like diarrhea) are similarly governed; 3) Optimal strategy for treating *28/*28 patients is not defined	Innocenti et al., 2004; Minami et al., 2007; Toffoli et al., 2006; Hoskins et al., 2007; Cecchin et al., 2009; Innocenti and Ratain, 2006
6-mercaptopurine /thioguanine	Myelosuppression	*TPMT*	*1, *2, *3A, *3B, *3C, *4, plus others	YES	1) Complementary clinical laboratory tests are available to functionally assess TPMT activity	Relling et al., 2011
Tamoxifen	Disease recurrence	*CYP2D6*	Loss-of-function alleles: *3 (rs35742686); *4 (rs3892097); *5 (gene deletion); *6 (rs5030655); *7 (rs5030867)	NO	1) Some studies have been unable to reproduce the relationships;	Schroth et al., 2007, 2009; Nowell et al., 2005; Kiyotani et al., 2010; Jin et al., 2005; Ferraldeschi and Newman, 2010; Rae, 2011
			Decreased function alleles: *10 (rs1065852); *41 (rs28371725); *9 (rs5030656) Plus potentially others		2) Many studies have not included all of the known, main alleles; 3) Genotyping (consideration of gene duplication) may be technically difficult which could confound results	
5-fluorouracil /capecitabine	Neutropenia, stomatitis, diarrhea	*DPYD*	*DPYD*2A* (IVS14 + 1 G > A), plus others	YES, but genetic variants are not mentioned; only functional DPD deficiency is included as a consideration	1) Sensitivity of best-studied *DPYD* variant is only ~30% and has not been consistently reproducible; 2) Results with other *DPYD* variants, or with variants in other genes (*TYMS*, *MTHFR*), have been inconsistent	Yen and McLeod, 2007; van Kuilenburg, 2004
Rituximab/cetuximab /trastuzumab	Disease progression, response	*FcγRIIa*, *FcγRIIIa*	FcγRIIa-131H/R; FcγRIIIa-158 V/F	NO	1) Some conflicting data; positive data mostly from small studies	Bibeau et al., 2009; Musolino et al., 2008; Kim et al., 2006; Weng and Levy, 2003; Carlotti et al., 2007

false discovery is minimized either (optimally) by inclusion of a replication set, or by conservative adjustment for multiple comparisons (Chanock et al., 2007; van den Oord, 2008). Studies which are underpowered to adequately test less common variants—variants which in reality may be potentially important pharmacogenomic markers—can have (falsely) negative results and can confuse the ability to understand conflicting data from several studies on a given drug–gene pair. Inadequate consideration of the potentially numerous different alleles which may contribute to a given phenotype may also cause false negative results. This latter scenario may, in fact, be one of the causes of the recent conflicting data surrounding tamoxifen pharmacogenomics and *CYP2D6* (Higgins and Stearns, 2011; Ferraldeschi and Newman, 2010; Rae, 2011).

For that drug–gene pair, multiple studies have demonstrated that patients with poor metabolizer genotypes are more likely to have worse outcomes. This is due to suboptimal conversion (primarily via *CYP2D6*) of tamoxifen into the more potent, active antiestrogenic metabolites, endoxifen and 4-hydroxytamoxifen (Higgins and Stearns, 2010), a relationship which is supported by pharmacokinetic data showing that patients with these genotypes have lower levels of endoxifen (Borges et al., 2006). In one study, 206 tamoxifen-treated patients receiving the drug in the adjuvant setting were compared based upon genotype groups (Schroth et al., 2007) for disease-related outcomes. Patients with poor metabolizer *CYP2D6* genotypes were significantly more likely to experience recurrence of breast cancer, had shorter times to relapse, and worse event-free survival compared with patients having functional alleles (Schroth et al., 2007). Importantly, this study also examined genotypes for an identical control group of women not treated with adjuvant tamoxifen, and genotype had no bearing on disease-related outcomes. A 1325-patient international consortium study confirmed these findings (Schroth et al., 2009). A smaller prior study (also including a control group) had failed to demonstrate the association of three loss-of-function *CYP2D6* genotypes (*CYP2D6**3*, **4*, and **6*) with reduced tamoxifen-related survival benefit, but importantly, this study did not test for any of the other now known loss-of-function and reduced-function alleles (Nowell et al., 2005). The recent data presented only in abstract form (Goetz et al., 2009) from the International Tamoxifen Pharmacogenomics Consortium study on >2800 patients receiving adjuvant tamoxifen did not show an association with survival outcomes, however, a number of patients was excluded from the analysis because of incomplete genotypic or clinical data, including lack of information about concomitant medication use (Ferraldeschi and Newman, 2010). Two other, recent large prospective trials (both also only presented in abstract form thus far) which examined *CYP2D6* genotypes with outcomes in patients receiving tamoxifen also failed to show associations (Rae et al., 2010; Leyland-Jones et al., 2010). The apparent importance of considering co-administered drugs—including simply whether the antineoplastic drug being studied is being given as monotherapy or as part of a larger combination regimen—has been elegantly illustrated by Kiyotani et al. (2010). These authors showed that, for multiple studies (including theirs) where tamoxifen between *CYP2D6* genotype and disease outcomes. However, in their study and in seven of eight other prior published studies of patients receiving tamoxifen as monotherapy, the relationship between *CYP2D6* genotype and tamoxifen response was positive (Kiyotani et al., 2010).

This drug–gene example is instructive for three reasons. First, for genes where genotyping may be difficult or complex, inaccurate or incomplete genotyping can be a significant barrier to pharmacogenomic interpretation. *CYP2D6* is known to be frequently duplicated, which can confound interpretation of genotyping results if duplication is not well-characterized. Moreover, over 100 different alleles of *CYP2D6* have been reported (Higgins and Stearns, 2010; Bradford, 2002), with at least five of these variants well-characterized as loss-of-function alleles, and another three well-described as associated with decreased enzymatic function (Becquemont et al., 2011). None of the above studies comprehensively included all of the common functional variants. The lack of standardized inclusion of all of the various known functional variants in clinical studies may therefore be a source of inconsistency in reported response outcomes. Secondly, the presence of concomitant medications may be important when interrogating pharmacogenomic relationships. For tamoxifen, co-administered inhibitors of *CYP2D6* can functionally "cause" the poor metabolizer phenotype (Jin et al., 2005) and confound genetic influences. Or, as just mentioned, even the presence of drugs not known to be directly acting via the same pathway as the antineoplastic of interest (including other concomitant antineoplastics) may mask the "penetrance" of pharmacogenomic risk alleles. The reduced penetrance could be due to direct effects of the other drugs, plus potentially the reduced effect on the drug of interest, especially if there was dose reduction. This issue has now been suggested to be important for both the tamoxifen (Kiyotani et al., 2010) and irinotecan examples (Hoskins et al., 2007). Third, one of the common problems confounding oncology pharmacogenomics is that evaluated studies often lack a control group (the relatively well-performed study referenced above on tamoxifen—which did—is an exception). Especially when the phenotype of interest is progression-free survival or overall survival, without such a group, or without an intermediate phenotype which relates the ultimate outcome to drug response, it can be difficult to determine whether an associated variant is actually predictive of treatment effect (truly pharmacogenomic) rather than simply prognostic (i.e., a marker for disease severity). This consideration can be especially relevant when the gene(s) being studied could theoretically be related to not only drug response, but also disease propensity or severity (like, for example, genes in DNA repair pathways). All three of these are important points to consider when assessing the quality of pharmacogenomic findings and their relevance to clinical use, and their confounding nature has tempered clinical implementation of some results.

Separately, racial/ethnic differences in genetic variation must be considered. The example of dihydropyrimidine dehydrogenase (DPD) deficiency and 5-fluorouracil (5-FU) toxicity exemplifies this point. DPD catabolizes >80% of 5-FU into fluorinated β-alanine (Heggie et al., 1987). A causative link be-

1999; van Kuilenburg et al., 2000; Johnson et al., 1999). While clinical assays of enzymatic DPD activity are available, they are not always easy to obtain, and there has been a substantial effort to characterize causative genetic variants within the *DPYD* gene relating to the DPD deficient phenotype (Yen and McLeod, 2007; Van Kuilenburg et al., 1999). In fact, over 40 SNPs and deletion mutations have been identified within *DPYD*, but most have been shown to have no functional consequences on enzymatic activity (Yen and McLeod, 2007). The best-studied of these SNPs, the IVS14 + 1 G > A variant (*DPYD**2A), has been found in up to 40–50% of people with partial or complete DPD deficiency (van Kuilenburg, 2004). Yet a recent summary of the data on *DPYD**2A, including multiple studies of this variant alone or in combination with other common variants, showed a performance sensitivity (the percentage of actual patients with severe toxicity who were correctly identified by the allele) ranging between 6.3 and 83%, with a median sensitivity of 30% (Yen and McLeod, 2007). Even more importantly, despite the fact that the prevalence of functional enzymatic DPD deficiency is higher in African Americans (Mattison et al., 2006), the *DPYD**2A variant is not even present in African Americans (van Kuilenburg, 2004), making such testing of limited generalizability and utility. Direct to consumer genetic testing services like 23andMe fail to convey these nuances: 23andMe Inc (2011) advertises genetic testing for 5-FU sensitivity, but their testing consists only of genotyping of the *DPYD**2A variant, and it is not mentioned directly on their website that there is likely to be no relevant information about 5-FU susceptibility for certain ethnic groups like African Americans. This notwithstanding, the data on *DPYD* testing overall is insufficient to support routine pharmacogenomic testing for 5-FU, in our opinion.

Finally, even the results of well-performed studies which include replication may be of limited utility because of the opposite problem: it might be unclear how to assimilate a larger number of different variants—each of which might have a small (but real) impact on the phenotype of interest—into one coherent pharmacogenomic model, let alone a model which might also include clinical factors. The very novel finding of 102 different variants associated with treatment outcome in pediatric acute lymphoblastic leukemia—identified through a very well-conducted analysis of two independent cohorts—might beg that question (Yang et al., 2009). Even if a clinician could test for all these variants, how would he or she assimilate information about all the variants in composite when determining treatment options? These types of questions are becoming more relevant as pharmacogenomic discoveries increase and as the field moves into tackling the issues not of discovery, but of clinical implementation.

4. Clinical implementation of pharmacogenomics into oncology practice

In 2001, when the first draft sequence of the human genome was released (Lander et al., 2001; Venter et al., 2001), there was significant public expectation that this information would be quickly utilized to individualize medical care (Ratain and rather than germline variation as the keys to advancement in clinical care. The two disciplines—tumor versus germline variation—are of course very different. The former explains variability in disease, which can usually be associated with differences in natural history and/or etiology, and occasionally in treatment response. On the other hand, germline variation explains variability in the patient, which does affect both pharmacokinetics and pharmacodynamics, as well as potentially disease risk (even risk for specific mutations (Liu et al., 2011)). Some might argue that especially for the latter group of drug-related germline variants, the list of the most extensively studied within oncology (summarized in Table 1) and especially the list of those that has become routinely clinically tested remains relatively small.

Implementation into routine practice has been hindered by lack of knowledge about such information (on the part of both patients and physicians), uncertainty about how to order such tests, and reimbursement, and timeliness of results. We believe that we are now at a point where SNP genotyping has become so widely available and inexpensive that this should no longer be the barrier. Indeed, whole genome sequencing is itself likely to quickly surmount these same barriers in the very near future. And it is also likely that in the very near future, we will have a critical mass of information regarding germline pharmacogenomic biomarkers in oncology which deserve clinical implementation to provide optimal (personalized) oncologic care. Before discussing the ways to bring this goal to fruition, it is worthwhile to examine the question of whether prospective, randomized data need to be demonstrated for a drug-variant pair before clinical implementation can be considered.

5. Necessity of prospective validation?

Pharmacogenomic findings even from a well-performed single study require validation in a separate patient population to confirm that such results are reproducible (Chanock et al., 2007). Successful reproducibility in a separate cohort provides considerable confidence that the original findings were not false positives and were not misleading due to some unique phenotypic or measurement characteristics of the original population. Outside of the oncology realm, however, even two of the most prominent drugs with repeatedly reproducible pharmacogenomic information—warfarin and clopidogrel—have not yet seen widespread clinical implementation of genomic prescribing. It has been felt that prospective, randomized studies for each of these drugs (the ongoing Clarification of Optimal Anticoagulation through Genetics [COAG] trial for warfarin (French et al., 2010); and the proposed and funded Pharmacogenomics of Anti-Platelet Intervention [PAPI-2] study for clopidogrel (United States Department of Health and Human Services, 2011)) are needed to demonstrate the clear utility of the pharmacogenomic information. Skeptics of pharmacogenomics will argue that this type of prospective randomized validation (ideally double-blind) might be necessary for any pharmacogenomic discovery before it is clinically implemented, including those for oncology drugs. In contrast,

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.