Editorial
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Pharmacogenomics

Pharmacogenetics in the treatment of chronic lymphocytic leukemia: what does the future hold?

"…there is accumulating evidence that treatment outcome in chronic lymphocytic leukemia can be influenced by germline polymorphisms that affect drug disposition and/or pharmacodynamics, and that these effects may explain some of the variability in treatment outcome that cannot be explained by genotypic and phenotypic variation of the malignant clone."

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Chronic lymphocytic leukemia (CLL) is a commonly occurring leukemia in adults. It results from a clonal expansion of antigen-experienced mature B-cells and is characterized by a chronic relapsing course requiring multiple different treatment regimens and frequently culminating in therapy resistance. For a number of years the single most important clinical decision tool in the treatment of CLL has been the presence or absence of p53 protein defects caused by deletion and/or mutation of the *TP53* gene on chromosome 17p. These defects, which result in chemoresistance, are associated with the worst prognosis in CLL. *TP53* defects are found in approximately 10% of newly diagnosed patients and approximately 50% of patients with chemorefractory disease [1].

For those patients possessing a *TP53* deletion, many authorities recommend avoidance of chemotherapy-based treatments in favor of drugs that do not depend on p53 for their action, such as the anti-CD52 monoclonal antibody alemtuzumab and high-dose glucocorticoids, such as methylprednisolone, either alone or in combination. This approach illustrates the application of stratified medicine in CLL therapy.

For the majority of CLL patients who do not possess a *TP53* deletion, the front-line treatment regimen of choice is chemotherapy with fludarabine plus cyclophosphamide (FC) in combination with the anti-CD20 monoclonal antibody rituximab (FCR) [2]. However, FCR is appropriate only for those patients who are considered sufficiently fit to tolerate the toxicity of this drug combination. Patients without *TP53* defects and considered unfit for FCR are treated less intensively with chlorambucil or bendamustine, usually in combination with rituximab [3,4].

Irrespective of which drug regimen patients receive, the efficacy and toxicity of treatment varies widely between individual patients. For example, in the pivotal GCLLSG CLL8 trial that demonstrated the benefit of adding rituximab to FC chemotherapy, the complete response rate was 44%, with 56% of patients experiencing significant hematological toxicity [2]. Although some of this variation in sensitivity to FCR can be explained by genotypic and phenotypic variation within the malignant clone, it is possible that treatment outcome is also influenced by germline polymorphisms in genes that affect drug disposition and pharmacodynamics.

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Fludarabine is a purine analogue that undergoes intracellular phosphorylation to its active 5′ triphosphate form in order to exert its cytotoxic effects [5]. This phosphorylation step is primarily carried out by DCK [5]. A number of SNPs have been identified in the *DCK* gene, and some of these have been shown to influence the expression and/or activity of the corresponding protein, or have been associated with clinical outcome. For example, a synonymous SNP (p.A100A) in the *DCK* gene has been associated with altered toxicity in patients with follicular lymphoma following treatment with FCR [6]. Additionally, cellular influx and efflux of fludarabine has been demonstrated to be mediated by a number of different transporters, including hCNT2, hCNT3, hENT1, hENT2, ABCG2 and ABCC4. Polymorphisms within the genes encoding these transporters have been shown to confer altered function and therefore have the potential to influence transport and bioavailability of nucleoside analogues including fludarabine [7].

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The alkylating agent cyclophosphamide requires conversion to its active metabolite 4-hydroxycyclophophamide to exert its cytotoxic action. This activation involves a number of hepatic CYP450 isoforms, including CYP2B6, CYP2C9 and CYP3A4, with CYP2B6 thought to make the major contribution to this step [8]. The *CYP2B6* gene is highly polymorphic with over 100 known SNPs identified in humans, which have differential frequencies across ethnicities, and in some confer alterations in CYP2B6 expression and function [9]. In our recent publication, we demonstrated that the *CYP2B6*6* allelotype (SNPs 516G>T and 785A>G) was associated with inferior response and more adverse events among patients with CLL who received FC in the LRF CLL4 trial [10]. This was the first demonstration that host pharmacogenetics can have a significant effect on the efficacy and toxicity of CLL therapy.

Rituximab acts by eliminating $CD20^+$ B cells. Its cytotoxic effects are mediated by three different mechanisms: antibody-dependent cell-mediated cytotoxicity, complement-dependent cytotoxicity and induction of apoptosis as a direct result of antigen engagement [11]. Genetic variation in components of these pathways has the potential to influence the effects of rituximab. For example, antibody-dependent cell-mediated cytotoxicity requires the involvement of Fc gamma receptors on natural killer cells to induce death of the tumor cell, and polymorphisms in Fc gamma receptor genes have been linked to the clinical response to rituximab in different disease settings. In particular, a nonsynonymous SNP in the *FCGR2A* gene (p.H131R) has been shown to predict response to rituximab-containing regimens in patients with non-Hodgkin's lymphoma. However, investigation of this SNP and another in *FCGR3A* (p.F176V) in the REACH trial failed to show any impact of these SNPs on response or survival rates in patients with relapsed CLL who received FCR [12]. With regard to complement-dependent cytotoxicity, the antitumor activity of rituximab is abolished in mice deficient in C1q, which triggers activation of the complement cascade [13]. The *C1QA* gene contains several SNPs that have been shown to impact on rituximab action in a number of hematological malignancies. For example, in follicular lymphoma, the *C1QA* c.A276G polymorphism was shown to affect therapeutic response and response duration following treatment with single-agent rituximab [14]. The efficacy and toxicity of rituximab might also be affected by polymorphisms that have the potential to influence pharmacodynamics. For example, over 500 SNPs have been described in the gene encoding the rituximab target antigen, CD20. Although the frequency and function of these SNPs is largely unknown, one study demonstrated an association between a SNP in exon 2 and therapeutic response to rituximab, cyclophosphamide, hydroxyldaunorubicin (doxorubicin), oncovin (vincistine) and prednisone/prednisolone (R-CHOP) combination therapy in patients with diffuse large B-cell lymphoma [10].

Germline polymorphisms might also influence the efficacy and toxicity of chemotherapy drugs used in patients considered unfit for FCR. In particular, glutathione-*S*-transferases (GSTs) add glutathione to electrophilic compounds such as chorambucil, leading to their detoxification, and overexpression of GSTP1 is believed to be an important mechanism in tumor cell resistance to alkylating agents. *GSTP1* is known to be polymorphic, and variants of this enzyme have been shown to catalyze glutathione conjugation of chlorambucil with different levels of efficiency [15].

Bendamustine is an alkylating agent that is extensively metabolized via hydrolytic and conjugative pathways to form metabolites with low cytotoxic activity. Major metabolites have low cytotoxic activity whereas two active minor metabolites, γ-hydroxybendamustine (M3) and *N*-desmethylbendamustine (M4) are primarily formed via CYP1A2 [16]. The concentrations of the active metabolites are low compared with the parent compound, suggesting that the majority of

the cytotoxic activity of is due to bendamustine itself. There are wide interindividual differences in CYP1A2 expression and activity in humans, probably as a result of both genetic and environmental factors. Indeed, more than 15 variant *CYP1A2* alleles and many more subvariants have been identified, and many of these have been associated with altered drug clearance or response as well as disease susceptibility [17].

Polymorphisms also have the potential to affect the pharmacodynamics of therapeutic glucocorticoids such as methylprednisolone. Indeed, small-scale studies in healthy volunteers have demonstrated a potential association between a nonsynonymous SNP of the histamine *HNMT* gene (p.T105I) and variable suppression of cortisol secretion [18]. It is plausible that the altered glucocorticoid pharmacodynamics associated with this genetic polymorphism could translate into a clinically significant effect in CLL patients receiving methylprednisolone.

The therapeutic landscape in CLL is about to be transformed owing to the advent of highly effective small-molecule inhibitors, such as ibrutinib [19] and idelalisib [20], which target components of the B-cell receptor signaling pathway. Although these drugs are characterized by their high efficacy and low toxicity, not all patients respond, and some have to discontinue treatment owing to adverse drug reactions. As with chemotherapeutic agents and monoclonal antibodies, the efficacy and toxicity of small-molecule inhibitors has the potential to be influenced by germline polymorphisms that influence drug disposition and/or pharmacodynamics.

In summary, there is accumulating evidence that treatment outcome in CLL can be influenced by germline polymorphisms that affect drug disposition and/or pharmacodynamics, and that these effects may explain some of the variability in treatment outcome that cannot be explained by genotypic and phenotypic variation of the malignant clone. Further investigation for associations between polymorphisms and treatment outcome could, with proper validation, provide clinicians with pharmacogenomic tools to allow greater patient stratification and ultimately more effective treatment.

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