Published in final edited form as:
Pharmacogenomics. 2012 August ; 13(11): 1257–1269. doi:10.2217/pgs.12.102.

The clinically relevant pharmacogenomic changes in acute myelogenous leukemia

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Abstract

Acute myelogenous leukemia (AML) is an extremely heterogeneous neoplasm with several clinical, pathological, genetic and molecular subtypes. Combinations of various doses and schedules of cytarabine and different anthracyclines have been the mainstay of treatment for all forms of AMLs in adult patients. Although this combination, with the addition of an occasional third agent, remains effective for treatment of some young-adult patients with *de novo* AML, the prognosis of AML secondary to myelodysplastic syndromes or myeloproliferative neoplasms, treatment-related AML, relapsed or refractory AML, and AML that occurs in older populations remains grim. Taken into account the heterogeneity of AML, one size does not and should not be tried to fit all. In this article, the authors review currently understood, applicable and relevant findings related to cytarabine and anthracycline drug-metabolizing enzymes and drug transporters in adult patients with AML. To provide a prime-time example of clinical applicability of pharmacogenomics in distinguishing a subset of patients with AML who might be better responders to farnesyltransferase inhibitors, the authors also reviewed findings related to a two-gene transcript signature consisting of high *RASGRP1* and low APTX, the ratio of which appears to positively predict clinical response in AML patients treated with farnesyltransferase inhibitors.

Keywords

acute myelogenous leukemia; AML; anthracycline; APTX; ara-C; cytarabine; farnesyltransferase; pharmacogenomic; RASGRP1

Acute myelogenous leukemia (AML) is one of the most heterogeneous malignancies with several clinical, pathological and genetic subtypes. For example, from a clinical standpoint, AML in younger adults is probably a different disease to AML that occurs in the older population. AML, secondary to other bone marrow disorders such as myelodysplatic syndromes or myeloproliferative neoplasms (MPN), behaves differently compared to *de novo* AML. Previously untreated AML in general has a better prognosis than relapsed or refractory AML.

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Financial & competing interests disclosure The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pnding, or royalties. No writing assistance was utilized in the production of this manuscript.

Karyotype or cytogenetic abnormalities represent the strongest pretreatment predictor of the rate of complete remission (CR), response duration and overall survival (OS) in adult patients with AML. On the basis of cytogenetics, AML is classified into three categories: favorable, with approximately 65–70% likelihood of cure with chemotherapy alone; intermediate, with 30–40% chance of long-term survival; and unfavorable, with less than 5–10% long-term survival without allogeneic stem cell transplantation. Intermediate-risk AML includes approximately 60% of patients and itself is comprised of a heterogeneous group with diverse structural and numerical chromosomal alterations. Cytogenetically normal AML belongs to the intermediate-risk category; however, discovery of several specific gene mutations such as *FLT-3*[1,2], *NPM1* [3], *CEBPA* [4], *DMNT3A* [5], *IDH1/2*[6,7], *KIT*[8], *WT-1* [9] and others, in patients with normal cytogenetic AML has provided further and sometimes independent prognostic insight. Nevertheless, different combinations of these genetic alterations in an individual person sometimes are too uncommon to be reliably allocated a prognostic value.

In the last four decades, combination of cytarabine (ara-C) and various doses of different anthracyclines has been the mainstay of treatment for all forms of AMLs in adult patients. Although this combination chemotherapy regimen, with addition of an occasional third agent, remains effective for treatment of some AML patients, it is far from ideal. Taken into consideration the heterogeneity of AML, one size does not and should not be tried to fit all.

Pharmacogenomics deals with the impact of genetic dissimilarity on pharmacokinetics, including absorption, distribution, metabolism and excretion of drugs as well as pharmacodynamics including efficacy and toxicity of drugs. Variations in drug-metabolizing enzymes, drug transporters and drug targets are the most practical aspects of pharmacogenomics.

In this article, the authors review currently understood, applicable and relevant pharmacogenomic findings in adult patients with AML that may suggest better strategies for the use of current chemotherapeutic agents. This includes choice of drugs, their dose intensities and schedules of their administration. The authors will also report on the clinical relevance of recent pharmacogenomic discoveries regarding new targets or new drug modifiers that may distinguish a subset of patients with AML who might be ‘better responders’ to novel agents under development for AML therapy. To provide a prime time example of clinical applicability of pharmacogenomics in distinguishing a subset of patients with AML who might be better responders to farnesyltransferase inhibitors, the authors specifically reviewed findings related to a two-gene transcript signature consisting of high *RASGRP1* and low *APTX*, the ratio of which appears to positively predict clinical response in AML patients treated with farnesyltransferase inhibitors.

This review does not intend to review epidemiology, etiology, pathophysiology, diagnosis and management of AML. Further information on these aspects of AML are reviewed elsewhere [10–12].

Genes involved in metabolic pathways & transporters of drugs used for AML treatment

Ara-C

Ara-C was synthesized in 1959 [13] and since then it has been the most effective and universally used chemotherapeutic agent in the treatment of AML. Structurally, its arabinose sugar moiety is epimeric at the 2′-position with ribose. This difference, after conversion to the ara-C triphosphate (ara-CTP) nucleotide, causes it to prevent the transformation of cytidylate to 2′-deoxycytidylate [14]. Other mechanisms of action of ara-C include

induction of miscoding after incorporation into DNA and RNA [15], and inhibition of DNA-dependent DNA polymerase [16]. Figure 1 illustrates the transporter and enzymes involved in ara-C metabolism.

After entering the cells via hENT1, ara-C is phosphorylated in a stepwise fashion at the 5′ position of arabinoside. Phosphorylation is mediated initially by DCK to convert ara-C to ara-C monophosphate (ara-CMP), then by deoxycytidylate kinase to ara-C diphosphate and finally by nucleoside diphosphate kinase to the active metabolite, ara-CTP. Intracellular concentration of ara-CTP is directly correlated with the therapeutic effect of ara-C. Inactivation of ara-C can occur through ara-CMP dephosphorylation by 5′-nucleotidase back to ara-C. Deaminase enzymes can convert and inactivate ara-C and ara-CMP to ara-uracil (ara-U) and ara-U monophosphate, respectively.

The most important parameters in sensitivity or resistance to ara-C include: conversion to nucleotides by kinases; inactivation via de amination by deaminases; half-life of active form ara-CTP; and the magnitude of its incorporation into DNA.

Ara-C transporter: hENT1

Although at plasma concentrations greater than 10 μM, which is achieved with high-dose ara-C (2–3 g/m^2 daily), the drug diffuses freely into the cell [17], at concentrations less than 1 μM, which is achieved with 100–200 mg/m^2 daily, ara-C influx into the cells is strongly correlated with the number of nucleoside transporters per leukemic blast [18,19].

hENT1, localized on chromosome 6, is the transporter for ara-C and other nucleoside analogues such as gemcitabine and cladribine. Significant differences in the mRNA expression levels of hENT1 have been noted in patients with AML. AML patients with hENT1 deficiency at diagnosis had significantly shorter disease-free survival (DFS) and OS [20]. Hence, because ara-C is commonly used for treatment of adult AML, the expression level of its transporter, hENT1, can be considered as a predictive biomarker rather than a prognostic biomarker for patients with AML. The cytotoxicity of a single concentration of ara-C was closely correlated with the cell surface abundance of nucleoside transporter sites in a flow cytometry assay [21]. In a study to investigate variants of hENT1 in ethnically diverse DNA samples from 247 individuals, two nonsynonymous changes were identified in the coding region of hENT1 with no contributory effect on its transportation function. It has been suggested that variability in hENT1 expression is mediated by transcriptional regulation. Recently, it has been shown that activation or overexpression of the transcription factor PPARα resulted in higher hENT1 transport activity [22]. In the future, identification of genetic variations that affect hENT1 expression or interaction with transcription factors such as HIF-α [23] may provide practical insight for selection of nucleoside analogues in the treatment of AML and other malignancies such as pancreatic cancer.

Ara-C kinases: DCK, CMPK1 & NDPK

DCK catalyzes the rate limiting first phosphorylation step in activation of many nucleoside analogues including ara-C. The enzyme attaches one phosphate group to carbon at 5′ position of the arabinose sugar moiety. The gene for DCK is located on chromosome 4. A greater than 30-fold variation in DCK mRNA expression was reported among AML cells, [24,25] and it appears that higher mRNA level correlates with longer event-free survival [20]. In a study involving approximately 120 Chinese patients with AML, two regulatory SNPs created two major haplotypes with lower mRNA level and poorer 2-year event-free survival associated with one compound genotype (-360CC/-201CC) [26]. Interestingly, no coding SNPs were detected in this population. These regulatory SNPs were reported with

much less frequency in European patients [27]. Several other genetic polymorphisms related to DCK have been discovered with yet-to-be proven clinical relevance and significance [28].

CMPK1 or deoxycytidylate kinase catalyzes the second phosphorylation step, converting ara-CMP to ara-C diphosphate. The final phosphorylation step is catalyzed by nucleoside diphosphate kinase to generate the active ara-CTP. Major genetic polymorphisms in these two enzymes with clinical application have not been identified.

An increased sensitivity to ara-C in AML cell lines was reported when the intracellular level of deoxy cytidine 5′-triphosphate (dCTP) is diminished. The enzyme CTP synthetase catalyzes the conversion of UTP to CTP. Inhibition of this enzyme depletes cellular dCTP pool and promotes phosphorylation of ara-C by diminishing inhibitory feedback of dCTP on DCK [29]. In addition to feedback inhibition of DCK and reduced activation of ara-C [30], dCTP influences the ara-C treatment outcome by competing with ara-CTP for incorporation into DNA [31] and by allosterically activating ara-C deactivating the enzyme CDA [32]. Higher level of dCTP results in more resistant blasts to ara-C[32].

Another example that underscores the consequences of change in intracellular dCTP pool on ara-C, therapeutic effect deals with enzyme ribonucleotide reductase (RR). This enzyme is composed of a dimerized large (RRM1) and small (RRM2) subunits. The variation in the expression of an individual or both subunit genes can influence response to ara-C [33]. RR catalyzes the reduction of ribonucleotides to deoxy ribonucleotides for DNA synthesis [33]. RR regulates dCTP and other deoxyribonucleotide levels inside the cells, hence its activity is directly associated with ara-C sensitivity or resistance. Inhibition of RR by novel agents can culminate in accumulation of ara-CTP. Combination of RR inhibitor 3-aminopyridine-2-carboxaldehyde thiosemicarbazone (Triapine) with ara-C and gemcitabine resulted in accumulation of metabolites of both agents in non-small-cell lung cancer cell lines [34]. We have tested the combination of triapine followed by the adenosine analogue fludarabine in adults with refractory acute leukemias and aggressive MPN in a Phase I clinical trial and demonstrated that this combination is active in refractory leukemias and warrants continuing study for patients with aggressive MPN [35].

Ara-CMP dephosphorylation: 5′-nucleotidase

5′-nucleotidases are enzymes with catalytic dephosphorylation function of deoxyribo- and ribo-nucleoside phosphates. Seven different members in 5′-nucleotidase family have been identified. NT5C2 reverses the function of CDK by removing 5′ phosphate from ara-CMP[36]. Increased expression of cytosolic 5′-nucleotidase II has been correlated with resistance to nucleoside-based chemotherapies including ara-C, gemcitabine and cladribine [37] and a lower rate of OS in approximately 100 patients with AML who were treated with ara-C [38].

Recently, sequencing the gene for cytosolic 5′-nucleotidase II identified 41 genetic variants (one insertion–deletion and 40 SNPs), including three nonsynonymous SNPs. Twenty-five of these SNPs were new [39]. Future studies to identify the clinical relevance of these genetic polymorphisms of cytosolic 5′-nucleotidase II are required.

Ara-C & ara-CMP deaminases

CDA inactivates ara-C by removing the amine group from its cytosine and converting it to ara-U. In patients with AML, elevated levels of CDA have been directly correlated with relapse and lower levels of CDA with prolonged remission [40,41]. In one study, CDA activity was the most sensitive parameter to predict adequate blast cell clearance [40]. Activity of CDA in previously untreated AML and in patients with CR is significantly lower than in refractory AML blasts and in persistent blast after induction chemotherapy,

respectively [41]. Genetic evaluation of CDA in African–Americans, Caucasians [42] and Japanese [43] resulted in identification of new nonsynonymous coding polymorphisms, which need further evaluation to demonstrate clinical significance [44]. For example, A79C in the *CDA* gene is a common polymorphism, which changes a lysine residue to glutamine resulting in reduced enzyme activity. *CDA* A79C genotypes were found in approximately 450 children with AML. It was reported that postinduction treatment-related mortality was significantly higher in children with the CC geno type [45]. Nevertheless, the actual impact of the A79C polymorphism on CDA activity and clinical outcome with nucleosidic analogues even tually remains controversial [46–48], and so are the other SNPs commonly described in the *CDA* gene (e.g., C437T and G208A, with apparently a strong influence of ethnicity for the latter one). Hence, single genotype-based studies should be interpreted cautiously and that broader strategies are probably necessary to better picture the role CDA plays.

DCTD deaminates ara-CMP to ara-UMP. The clinical effects of genetic polymorphisms in this enzyme are uncertain and demand further evaluations [49,50].

Anthracyclines

Anthracycline antibiotics (Figure 2) are another most important class of chemotherapeutic agents that have been widely used in the treatment of AML since the 1960s. In the 1950s, daunorubicin was isolated from soil-based bacteria by two independent research groups from Italy and France [51]. Minor modification in the structure of an anthracycline results in alteration of biological activity including potency of the compound. Idarubicin is the 4-demethoxy analogue of daunorubicin with five- to six-times higher antitumor potency. The 4-hydroxy analogue of daunorubicin, carminomycin, has been evaluated in Russia. Hydroxylation of the carbon 14 atom in daunorubicin results in another active chemotherapy, doxorubicin or adriamycin. Changing the spatial orientation of the hydroxyl group at the 4′ position of the sugar molecule of doxorubicin generates another anthracycline named epirubicin with presumed faster elimination from the body.

The major proposed mechanisms of action of anthracyclines include DNA intercalation, prevention of DNA replication by stabilization and inhibition of enzyme topoisomerase II, and production of reactive oxygen species generated by redox cycling of quinone moiety of the molecule [52–55].

Anthracyclines transporters: ATP-binding cassettes

One of the most clinically relevant pharmacogenomic aspects of anthracyclines is variation in drug transporters. The ATP-binding cassettes (ABCs) are membrane proteins that transport different types of molecules across the cellular membrane (influx, efflux or both) [56]. Forty nine ABC proteins are encoded by the human genome and classified into seven subfamilies with several of them not yet fully characterized [57,58]. The role of ABC efflux transporters in resistance to antineoplastic drug has been investigated in the last three decades[59,60]. Three ABC proteins appear to be responsible for most of multidrug resistance (MDR) in human; P-gp/ABCB1/MDR1, MRP1/ABCC1 and BCRP/ABCG2/ABCP/MXR[61]. Anthracyclines are among the chemotherapeutic agents that interact with these three ABC transporters [62]. Polymorphisms in the ABC drug transporters have been investigated extensively to better understand the significant variability in response to chemotherapies including anthracyclines.

The function and expression of P-gp (ABCB1) is related to complete remission rate and drug resistance in AML [63]. Both ABCB1 expression and functional drug efflux increase with patient age, from 17% in patients less than 35 years old to 39% in patients aged 50

years or older [63]. Expression, function and genetic polymorphism of P-gp were studied in 817 AML samples. It was shown that the genetic polymorphism 3435TT (which results in unstable mRNA) had a significant effect on P-gp expression. However, this was only observed in 40% of cases in which mRNA and protein were detectable. Low white blood cell count, secondary AML and poor risk cytogenetics had a much greater impact on prognosis than genetic polymorphisms of P-gp expression in AML blasts [64].

P-gp can be modulated with different class of molecules including immunosuppressive agents (cyclosporine A [PSC-833] and tacrolimus [FK506]), calcium channel blockers (verapamil and nifedipine), tyrosine kinase inhibitors (imatinib and gefitinib), H_2-receptor ant-agonists (cimetidine), and statins (lovastatin and simvastatin) [62]. On the basis of preclinical and preliminary studies that demonstrated an increased plasma concentration of an anthracycline when combined with cyclosporine A [65,66], several clinical trials tested the hypothesis of improvement in clinical benefit when combining P-gp modulators with different chemotherapy regimens. Unfortunately, these trials did not demonstrate significant clinical benefit including prolonged OS [67–72]. In some cases the combination of P-gp modulator with chemotherapy compared to chemotherapy alone resulted in worse toxicity profiles [67]. There have been several proposed explanations for the observed results including less frequent expression of the ABC transporters on AML blasts compared to normal hematopoietic stem/progenitor cells, abrogation of drug efflux completely by ABC modulators in normal cells but not in AML blasts, and different methods used to define MDR [73].

MRP1 (ABCC1) is expressed in approximately 10% of patients with AML with inverse correlation with age and with less significant relationship with chemotherapy response [63]. Neither ABCB1 nor ABCC1 expression were correlated with leukemia free survival or OS [63,74].

In a study aimed to investigate the correlation between the expression of other ABCC transporters including ABCC4 (MRP4), ABCC5 (MRP5) and ABCC11 (MRP8) on AML blasts with clinical outcomes, the researcher analyzed such expressions on blast samples from 50 patients with AML [75]. The results indicated that high expression of ABCC11 was inversely correlated with OS probability in 4 years ($p = 0.03$).

BCRP (ABCG2) was first discovered by Doyle and Ross at the University of Maryland in human MCF-7 breast cancer cell line that demonstrated an ATP-dependent reduction in the cellular levels of anthracyclines without overexpression of already indentified MDR transporters [76]. Compared to P-gp and MRP1, BCRP has a greater efflux transporting ability for mitoxantrone [77,78]. In a study involving blast cells from 20 AML patients, BCRP mRNA expression varied significantly (>1000-fold) among the samples with almost no expression in 50% of the samples and high expression in 33% [79]. The finding of high expression of BCRP in a subset of AML patients suggested further investigation of BCRP presence on AML blasts and its correlation with clinical outcome such as survival advantage. DNA from pretreatment bone marrow or blood samples from 261 adult patients with AML, who received ara-C and anthracycline-based chemotherapy was genotyped for eight nonsynonymous SNPs in the *ABCB1*, *ABCC1* and *ABCG2* genes. Heterozygous (AG) or homozygous (AA) variant genotypes for rs2231137 in the *ABCG2* gene, compared to the wild-type (GG) genotype were associated with significantly improved survival, and increased toxicity [80]. In a study of 85 samples from patients with AML, P-gp, MRP3 and BCRP activities were inversely correlated with CR rate, DFS and OS. When samples expressed one or none of P-gp, MRP3 or BCRP proteins, patients had a better prognosis compared to the patients expressing two or three of these transporters [81].

Apart from the three major transporters, one study has demonstrated that low levels of ABCA3 transporter expression on AML blasts were statistically significantly associated with improved progression-free survival and OS compared to high ABCA3 levels [82].

Carcinogene & chemotherapy neutralizing enzymes: glutathione *S*-transferases

Glutathione *S*-transferase (GST) enzymes are one of the major cellular detoxifiers [83]. These dimeric isoenzymes catalyze reactions between the reduced form of glutathione, which carries sulfhydryl (-SH) group, and many endogenous or exogenous compounds [84] including epoxides, unsaturated aldehydes, peroxidized lipids[85], polyaromatic hydrocarbons [86–89], alkylating agent chemotherapies [90,91], and anthracyclines [92,93]. Generation of high levels of reactive oxygen species post anthracycline treatment induces GST to nullify cellular oxidative stress. GST family in mammalians comprises at least six classes, including alpha (α), mu (μ), omega (ω), pi (π), theta (τ) and zeta (ζ) [94]. *GSTM1* and *GSTT1* polymorphisms have been detected in their population distribution and associated with the development of many solid tumors [95] and hematologic malignancies including AML[96–98]. Interestingly, *GSTM1* and *GSTT1* null genotypes were not correlated with higher rates of treatment-related AML in children with acute lymphoblastic leukemia after chemotherapy [99].

In a study of 106 patients with AML, homozygous deletion resulting in null genotypes at *GSTM1*, *GSTT1* or both were reported in 42, 28 and 18% of patients, respectively. The presence of at least one GST deletion appeared to be an independent negative prognostic risk factor for response to induction chemotherapy and OS. Compared with patients with an intact GST gene, AML patients with deletions of *GSTM1* or *GSTT1* or both achieved lower CR rate after induction therapy (60 vs 80%) and had shorter OS (8 vs 15 months) [100]. Concordant with these results, a study of 153 patients with *de novo* AML reported a lower probability of DFS in the presence of GST deletions [101].

On the contrary, a study of 200 AML patients older than 56 years treated with ara-C and dauno rubicin as induction therapy in the SWOG clinical trials reported opposite results [102]. In this study no statistically significant associations between treatment outcomes including CR rate, OS or toxicity and any GST genotypes were detected. The authors attributed the absence of correlation between GST polymorphisms and clinical outcomes to the older age of patients enrolled in this study, which could have trumped the potential effects of polymorphisms. Another study aimed to investigate the prognostic role of poly morphisms in three GST genes (*GSTM1/T1/P1*) in approximately 250 patients of the German Austrian AML Study Group confirmed the latter results[103]. In this study, *GSTM1* and *GSTT1* geno-types had no significant impact on response to induction therapy, relapse-free survival or OS. However, *GSTP1*105* Val allele was associated with quicker neutrophil and platelet recovery, and longer relapse-free survival and OS.

These conflicting results warrant further investigations perhaps in prospective randomized clinical trials with prespecified primary and secondary end points including correlative laboratory measurements.

After reviewing the above polymorphisms in the enzymes and transporters involved in ara-C and anthracycline metabolism, here the authors will review the implication of pharmacogenomics in modern drug discovery by providing an example that underscores the power of testing for genetic signature to distinguish patients with AML who may demonstrate a more profound response to a novel class of chemotherapeutic agent.

Pharmacogenomics contribution to personalized medicine: design of clinical trials incorporating farnesyltransferase inhibitors in the treatment of AML

Farnesyltransferase inhibitors (FTIs) are selective inhibitors of FTase, which catalyzes the transfer of a 15-carbon farnesyl moiety to different acceptors [104,105]. Some of the polypeptides to which farnesyl is transferred include centromeric proteins promoting completion of mitosis, and guanosine triphosphate-binding polypeptides of the Ras, Rho and Rheb families[106,107]. Tipifarnib, an oral methylquinolinone FTI, showed activity in refractory AML [108,109] and in newly diagnosed, unfavorable-risk elderly adults with AML [110,111]. A Phase III study compared single agent tipifarnib with best supportive care, including hydroxyurea, in approximately 450 patients older than 70 years with newly diagnosed AML who were not candidates for conventional chemotherapy [111]. The results showed no statistically significant survival advantage in patients treated with tipifarnib.

In primary AML cells obtained from patients, tipifarnib inhibited signaling downstream of the farnesylated Rheb and synergistically enhanced etoposide-induced antiproliferative effects *in vitro* [112]. These results prompted the design and execution of a multicenter Phase I clinical trial of tipifarnib (300–600 mg twice daily for 14 or 21 days) plus oral etoposide (100–200 mg daily on days 1–3 and 8–10 for each cycle) in 84 adults older than 70 years who were not candidates for conventional cytotoxic induction chemotherapy. A CR rate of 25% across multiple dose levels of both drugs, with a median OS of 22 months for patients in CR was reported [112].

In order to select patients, particularly elderly AML patients intolerable of conventional intensive chemotherapeutic regimens, who are most likely to benefit from tipifarnib-containing regimens, serial studies of gene-expression profiling of AML cell lines and primary AML marrow blasts were performed [113–115]. Studies in the newly diagnosed patients with AML revealed a two-gene transcript signature consisting of high *RASGRP1* (which encodes the Ras-activating guanine nucleotide exchange factor RASGRP1) and low *APTX* (which encodes the DNA excision repair protein aprataxin), the ratio of which positively predicted clinical response [115].

RASGRP1 is a member of a family of proteins characterized by a Ras guanine nucleotide exchange factor domain. The protein as a diacylglycerol-regulated nucleotide exchange factor regulates activation of Ras via the exchange of bound guanosine diphosphate for guanosine triphosphate. RASGRP1 also regulates T-cells and B-cells development and differentiation as well as activates the Erk/MAP kinase pathway [116,117]. Mutations in *APTX* gene as a single-stranded DNA repair protein have been associated with ataxia-ocular apraxia [118].

Retrospectively, the two-gene signature correlation with clinical response in elderly patients with AML treated with tipifarnib and etoposide was confirmed [119]. Analysis of bone marrow aspirates of patients who were treated with tipifarnib and etoposide in the clinical trial validated the two-gene signature as a reproducible and reliable predictor of response to tipifarnib. This suggests the feasibility of a prospective trial to discriminate those patients with AML who are likely to respond to tipifarnib compared with those who are not.

The authors showed that AML patients treated with the combination of tipifarnib and etoposide with a *RASGRP1*/*APTX* ratio of 5.2 or greater had a CR rate of 78% compared with patients with a ratio of less than 5.2, who had a CR rate of 13%. Based on these results, negative and positive predictive values were 87 and 78%, respectively [119]. The negative predictive value in studies that used tipifarnib as single agent was approximately 95% [115].

To determine the specificity of the two-gene signature for tipifarnib responsiveness versus general chemotherapy sensitivity, bone marrow blasts from 41 adults with newly diagnosed AML undergoing intensive multiagent induction chemotherapy, including ara-C, anthra cyclines and etoposide were examined. The results demonstrated no association between the two-gene ratio and clinical outcome for the patients who were not treated with tipifarnib, hence, no significant value in predicting clinical response to chemotherapeutic regimens that did not contain tipifarnib [119].

The two-gene signature and correlation with response to tipifarnib just make sense with respect to the proposed mechanism of action of FTIs, which requires post-translational farnesylation of Ras proteins for Ras-dependent signal transduction signals [106,107,120]. It has been demonstrated that RASGRP1 can act as a guanine nucleotide exchange factor, which activates H-Ras and N-Ras and is associated with decreased chemotherapy sensitivity in leukemia cells [121].

Another conclusion that can be drawn from these results is correlation between low *APTX* expression and overall tipifarnib responsiveness. It can be speculated that when AML cells with low *APTX* expression are treated with tipifarnib, they have a decreased capability to undergo DNA excision repair. The evidence for the hypothesis that *APTX* downregulation confers a diminished ability to repair tipifarnib associated DNA damage was provided in the Phase I trial of tipifarnib and etoposide, [112] where DNA damage and apoptosis in bone marrow blasts were seen a week after initiation of tipifarnib.

Conclusion

Although, currently in 2012, outside clinical trials, it is not a routine practice to guide treatment plans for adult patients with AML based on pretreatment results of genetic tests deemed to identify polymorphisms in the enzymes and transporters, the use of pharmacogenomics to guide such optimal treatment of adult patients with AML appears to be ready for prime time. Continuing and future efforts are warranted to harmonize laboratories, to standardize assays and to apply these standardized assays prospectively in well-designed clinical trials. This concept is already exploited in clinical practice in childhood acute lymphoblastic leukemia, where polymorphisms in drug-metabolizing enzymes determine specific drug administration and dosing [122–125]. The current NCI 8977 trial, a Phase II trial of tipifarnib in previously untreated older adults with AML and baseline presence of a specific two-gene expression signature ratio, is a vanguard in ability to select AML patients based on their gene signatures [201].

Future perspective

In this article, we aimed to show that pharmacogenomic changes in drug-metabolizing enzymes, drug transporters and drug targets may have important clinical implications in the treatment of patients with AML. We are aware of the fact that the majority of these findings were identified retrospectively and on the basis of ‘responder analysis’. Responder analysis is subject to significant levels of biases. For this reason, to test the validity, reliability and clinical benefit of different pharmacogenomic changes, future trials of AML need to test prespecified hypotheses and prospectively incorporate them in randomized clinical trials. This is the only way that we can determine whether or not these changes are ready for prime time.

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■of interest

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■ Website

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Executive summary

■ Cytarabine and anthracyclines are the most commonly used chemotherapies for the treatment of acute myelogenous leukemia (AML). Several clinical trials have enrolled patients with AML to find the optimal dose and schedule of administration of these agents. The heterogeneous nature of AML explains, to a great extent, significant differences in response rate, response duration and survival of patients treated with combination of cytarabine and anthracycline. Another important factor in this equation of response to chemotherapies is interpatient variability irrespective of the AML type.

■ Genetic polymorphisms in enzymes responsible for intracellular metabolism of cytarabine (ara-C) and anthracyclines may be important factors in determination of response to chemotherapy. DCK, 5′-nucleotidase, CDA, cytidine-5′-triphosphate synthetase, ribonucleotide reductase and glutathione *S*-transferase are enzymes that are directly or indirectly affect response to ara-C and anthracyclines, and their pharmacogenomic changes have been suggested to carry clinical relevance.

■ Polymorphisms in drug transporters are other determinants of sensitivity or resistance to ara-C and anthracyclines. hENT1 for ara-C and P-gp, MRP1 and BCRP for anthracyclines are important drug transporters, polymorphisms of which appear to be relevant in the clinic. Modulation of P-gp with different class of agents has been tested in several prospective randomized clinical trials. To date, these trials have not demonstrated significant clinical benefit including prolonged overall survival by such modulation.

■ It appears that farnesyltransferase inhibitors have reasonable antileukemia activity. However, not all patients with AML respond favorably to these class of drugs. AML patients treated with the combination of tipifarnib and etoposide with a *RASGRP1*/*APTX* ratio of 5.2 or greater had a significantly higher CR rate compared with patients with a ratio of less than 5.2. Negative and positive predictive values of response for this two-gene signature were 87 and 78%, respectively. This example underscores the potential for pharmacogenomics to play an important role in selection of most likely beneficial chemotherapy combination for patients with particular genetic characteristics in the future.

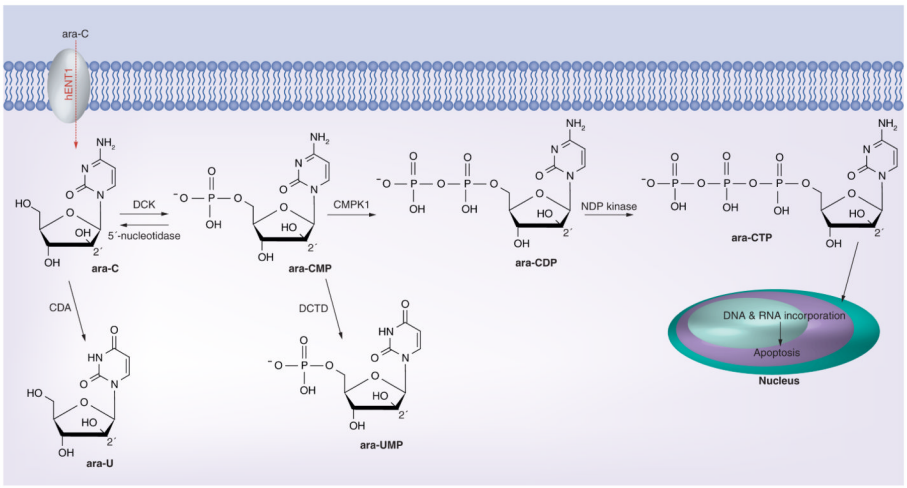

Figure 1.

Ara-C metabolism. Ara-C: Cytarabine; Ara-CDP: Ara-C diphosphate; Ara-CMP: Ara-C monophosphate; Ara-CTP: Ara-C triphosphate; Ara-U: Ara-uracil; Ara-UMP: Ara-U monophosphate.

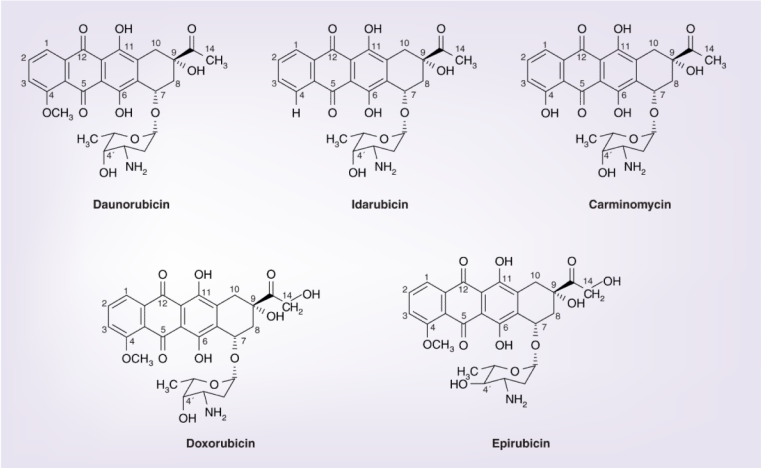

Figure 2.
Chemical structures of anthracyclines.