Published in final edited form as:

Pharmacogenomics. 2009 October ; 10(10): 1657–1674. doi:10.2217/pgs.09.118.

Genetic factors influencing cytarabine therapy

Jatinder K Lamba

Department of Experimental and Clinical Pharmacology, University of Minnesota, Minneapolis, MN 55455, USA, Tel: +1 612 624 8651, Fax: +1 612 625 4294, lamba004@umn.edu

Abstract

The mainstay of acute myeloid leukemia chemotherapy is the nucleoside analog cytarabine (ara-C). Numerous studies suggest that the intracellular concentrations of the ara-C active metabolite, ara-CTP, vary widely among patients and, in turn, are associated with variability in clinical response to acute myeloid leukemia treatment. Thus, genetic variation in key genes in the ara-C metabolic pathway – specifically, deoxycytidine kinase (a rate-limiting activating enzyme), 5′ nucleotidase, cytidine deaminase and deoxycytidylate deaminase (all three are inactivating enzymes), human equilibrative nucleoside transporter (ara-C uptake transporter) and ribonucleotide reductase (RRM1 and RRM2 – enzymes regulating intracellular deoxycytidine triphosphate pools) – form the molecular basis of the interpatient variability observed in intracellular ara-CTP concentrations and response to ara-C. Understanding genetic variants in the key candidate genes involved in the metabolic activation of ara-C, as well as the pharmacodynamic targets of ara-C, will provide an opportunity to identify patients at an increased risk of adverse reactions or decreased likelihood of response, based upon their genetic profile, which in future could help in dose optimization to reduce drug toxicity without compromising efficacy. The pharmacogenetic studies on ara-C would also be equally applicable to other nucleoside analogs, such as gemcitabine, decitabine, clofarabine and so on, which are metabolized by the same pathway.

Keywords

cytarabine; cytidine deaminase; deoxycytidine kinase; nucleoside analog; pharmacogenetics; SNPs

Cytarabine (1-β-arabinofuranosylcytosine [ara-C]) a deoxycytidine nucleoside analog, has been the most effective chemotherapeutic agent used in the treatment of acute myeloid leukemia (AML) since the 1960s [1–3]. In addition to AML, ara-C is also used in the treatment of other hematological malignancies, such as acute lymphoblastic leukemia, chronic myelocytic leukemia, erythroleukemia and mantle-cell lymphoma. Despite the fact that front-line chemo-therapy regimens containing ara-C induce complete response in 65–80% of newly diagnosed AML patients, clinical outcome is suboptimal as most of the patients relapse with resistant disease and poor response to subsequent therapies. Thus, development of cellular resistance and inefficient response to first-line chemotherapy remains the greatest impediment to successful AML therapy.

© 2009 Future Medicine Ltd

Financial & competing interest disclosure

The author is supported by a NIH grant R01CA132946. The author has no other 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 apart from those disclosed.

No writing assistance was utilized in production of this manuscript.

The intracellular penetration of ara-C depends on its plasma concentration [4–6]. Standard low-dose (LD) ara-C (100–200 mg/m²) regimens achieve a steady state plasma concentration of 0.5–1 μ M, but high-dose (HD) ara-C (2–3 g/m²) regimens achieve plasma concentrations greater than 10 μ M. Although ara-C is a component of virtually all standard induction regimens for AML, the optimal dose remains to be determined. During recent years, increasing use of HD ara-C has significantly improved survival, particularly in the translocation (8:21) and inversion (16) AML subtypes. Unfortunately, 15–30% of patients still show no response to treatment and 30–80% of patients experience relapse. Although the adult AML patients who receive HD ara-C have a higher probability of 4 years relapse-free survival than those who receive LD ara-C, HD ara-C use has been associated with serious side effects, including fatal toxicities [7].

Metabolic fate of ara-C

At ara-C plasma concentrations of 0.5–1 μ M (achieved with standard LD ara-C regimens), expression levels of the uptake transporter human equilibrative nucleoside transporter (hENT1/SLC29A1) are crucial for ara-C uptake into the cell, but at higher plasma concentrations the simple diffusion rate of ara-C exceeds that of pump-mediated transport [8]. Inside the cell, ara-C phosphorylation by deoxycytidine kinase (DCK) is the rate-limiting step in its activation (Figure 1). The resulting ara-C monophosphate (ara-CMP) is then further phosphorylated by pyrimidine kinases to the active 5'-triphosphate derivative, ara-cytidine-5'-triphosphate (ara-CTP). Conversely, the enzyme 5'-nucleotidase (NT5C2) can dephosphorylate ara-CMP back to ara-C. Ara-C and ara-CMP can both be converted into the inactive forms, ara-U and ara-UMP, by the action of the enzymes cytidine deaminase (CDA) and deoxycytidylate deaminase (DCTD), respectively. DNA incorporation of ara-CTP in place of deoxycytidine triphosphate (dCTP) results in chain termination, blocking DNA and RNA synthesis and causing leukemic cell death, which, in turn, is associated with therapeutic response of ara-C [9–11]. Thus, the intracellular concentration of ara-CTP is related to the clinical efficacy of ara-C chemotherapy. Biochemical modulation of ara-CTP metabolism by other nucleoside analogs, such as fludarabine and clofarabine, has been shown to be feasible in clinical studies of adult AML [12] and pediatric acute leukemias [13]. The addition of a nucleoside analog as a biochemical modulator has been shown to increase ara-CTP accumulation in the circulating blast cells in both adult [14,15] and pediatric AML [13].

Mechanism of resistance to ara-C

In vitro studies have demonstrated that the intracellular concentrations of ara-CTP are higher in ara-C sensitive cells than in resistant cells [16]. Furthermore, leukemic cells from patients with chronic myelogenous leukemia (which is not responsive to ara-C) have only half the ara-CTP levels as compared with leukemic cells from patients with AML (which is responsive to ara-C) [17]. Intracellular ara-CTP levels are also positively correlated with clinical response after HD-ara-C treatment [18]. The primary mechanism underlying ara-C resistance, thus, appears to be the insufficient intracellular levels of the active triphosphate metabolite ara-CTP, which may be owing to:

- Inefficient cellular uptake of ara-C due to low levels and/or activity of the transporter, hENT1;
- Reduced levels of activating enzymes, primarily DCK;
- Increased levels of inactivating enzymes, such as NT5C2 and CDA;
- Increased cellular dCTP pools, that can compete with DNA incorporation of ara-CTP and also inhibit DCK activity through feedback inhibition [19].

The intracellular dCTP pools, in turn, are regulated by the enzyme ribonucleotide reductase (RR) (consisting of RR motif subunits RRM1 and RRM2).

Thus, the observed interpatient variation in clinical response and sensitivity to ara-C could be owing, in part, to the interpatient variation in expression and/or activity of proteins that affect ara-C uptake, activation and degradation (Figure 1). SNPs and small insertions or deletions in these genes could result in amino acid changes that could in turn influence the protein and/or activity of the respective genes. Furthermore, the mRNA expression of these candidate genes could be influenced by SNPs in their regulatory regions. Thus, genetic polymorphisms in genes within the metabolic pathway of ara-C could influence their expression and/or activity, which could in turn, influence the intracellular ara-CTP levels and its clinical efficacy. In addition, variation in the expression or activity of pharmacodynamic targets of ara-C could also contribute to the observed variation in response.

Studies addressing these candidate genes are discussed below and are also highlighted in Table 1.

Candidate genes in the metabolic pathway of ara-C

Human equilibrative nucleoside transporter

hENT1 (SLC29A1), an uptake transporter of the nucleoside analogs ara-C, gemcitabine, fludarabine and cladribine, is responsible for up to 80% of ara-C influx into human leukemic cells [20,21]. Thus, when given at the standard dose, ara-C intracellular concentration is critically dependent on uptake by hENT1. Considerable interpatient variation in the *hENT1* mRNA expression levels has been observed in AML patients; further *in vitro* cytotoxicity assays using diagnostic samples from AML patients indicated a significant inverse correlation of hENT1 expression with the lethal dose 50 (LC₅₀) values for ara-C and other nucleoside analogs [22,23]. When given ara-C in standard induction therapy, AML patients, whose blasts were negative for hENT1 expression, had a shorter disease-free survival (7.7 months vs 13 months) and an approximately eight-fold higher risk of early relapse than hENT1-positive patients [24]. Greater cellular sensitivity to ara-C of infant acute lymphoblastic leukemia (ALL), which has high incidence of mixed lineage leukemia as compared with ALL cells from older children, was attributed to high hENT1 mRNA expression in leukemic cells from patients with mixed lineage leukemia gene rearranged ALL [25]. Further hENT1 content measured by flow cytometry was also shown to be associated with sensitivity to ara-C in AML and ALL cells [26,27]. In pancreatic cancer patients treated with gemcitabine, high hENT1 expression was associated with longer overall survival, disease-free survival and time to disease progression [28], as well as with time to relapse in patients with gemcitabine-sensitive tumors [29]. Recently, in a large prospective randomized trial, in a cohort of pancreatic adenocarcinoma patients, hENT1 protein levels were significant determinants of patients receiving gemcitabine [30].

hENT1 is localized on chromosome 6q21.1–21.2 and its coding region was sequenced in Caucasians, African-Americans, Asians and other ethnic groups [31]. *hENT1* demonstrated exceptionally low mutability as compared with the other transporters. Within the coding region, four synonymous and two nonsynonymous changes (Ile216Thr, Glu391Lys) were identified [31]. Figure 2 depicts a multispecies alignment of the hENT1 protein sequence for the protein region harboring these nonsynonymous changes. As depicted in Figure 3, the amino acids in hENT1 are not as conserved as other proteins are. *In vitro*, functional assays demonstrated that these polymorphisms had no effect on hENT1-mediated uptake of nucleosides and nucleoside analogs [31]. This indicated that the coding variants might not contribute to the interindividual variation of nucleoside analogs.

As no functional coding SNPs are known to modulate the function of hENT1 and because expression of hENT1 is reported to affect sensitivity to ara-C, transcriptional regulation of hENT1 is the most likely contributor to the observed variability in hENT1 expression. Although the *hENT1* promoter has so far not been completely characterized, it is reported to have hypoxia inducible factor 1 (Hif-1) binding sites. Hif-1-mediated repression of hENT1 expression has been observed in hypoxia [32,33]. Thus, any genetic variation in the *hENT1* locus that disrupts or creates binding sites for Hif-1 and other transcription factors that are important for hENT1 expression could alter hENT1 expression.

Analysis of the 1.6 kb of proximal *hENT1* promoter upstream of the initiation codon, identified SNPs at -1345C>G, -1050G>A and -706G>C. TRANSFAC® analysis (Biobase International, Wolfenbuttel, Germany) predicted -1345C>G to result in the loss of the FTF/LHR-1 binding site, -706G>C to result in the loss of a barbiturate-inducible element and the gain of binding sites for MyT1 zinc finger transcription factor, AhR nuclear translocator and X-box binding protein. Further analysis identified haplotypes associated with *hENT1* expression [34]. Analysis of the *hENT1* gene in a Japanese population identified 30 novel polymorphisms, including two nonsynonymous Asp59Glu and Ala430Thr changes [35]. The functional significance of these nonsynonymous changes remains to be determined. Figure 3 summarizes the potentially functional SNPs identified in *hENT1*. The prognostic significance of hENT1 indicates that hENT1 expression or genetic variants that affect hENT1 expression, may provide a new tool for patient-risk stratification.

Deoxycytidine kinase

Deoxycytidine kinase (EC 2.7.1.74) is a pyrimidine salvage enzyme involved in the phosphorylation of deoxycytidine, deoxyadenosine and deoxyguanosine. It is a rate-limiting enzyme that catalyzes the first step in the activation of nucleoside analogs such as ara-C, gemcitabine, cladribine, fludarabine and clofarabine. Reduced or loss of DCK activity in cell lines resistant to ara-C has been demonstrated in multiple studies [36–38]. Transfection of the *DCK* gene in DCK-resistant cell lines had been demonstrated to restore *in vitro* sensitivity to ara-C [39,40]. The activity and expression of DCK varies widely in normal and malignant cells and tissues. There is a 50-fold variation in DCK mRNA expression in patient leukemic cells [41], a 35-fold variation in DCK mRNA in primary AML cells, a 36-fold variation in liver tissue and a 150-fold variation in human liver metastases of colorectal cancer origin [42]. Although a strong correlation between DCK protein levels and activity has been reported, no correlation between mRNA and enzymatic activities measured using multiple substrates (ara-C and cladribine) was observed in blood cells from chronic lymphocytic leukemia patients [43]. DCK mRNA expression is related positively to AML treatment outcome. When treated with ara-C, patients with higher DCK expression demonstrate longer event-free survival than patients with low DCK expression [44]. Furthermore, the pretreatment levels of DCK have also been related to gemcitabine sensitivity in solid tumors of different origins [45]. These studies provide evidence for the involvement of DCK in the activation of ara-C and other nucleosides, thereby making it a key candidate for pharmacogenetic studies.

The *DCK* gene is localized on chromosome 4q13.3-q21.1 and the coding region is composed of seven exons. The proximal (1.5 kb) *DCK* promoter and all the coding exons were sequenced in 24 normal and 24 leukemic Chinese patients, five regulatory SNPs (-125G>T, -201G>T, -289T>A, -360C>G and -740G>C) and one synonymous coding SNP were identified. Two regulatory SNPs (-360C>G and -201C>T) were in linkage disequilibrium and were associated with lower DCK mRNA expression, as well as reduced transcriptional activation in reporter assays [46]. Furthermore, the -360G/-201T haplotype was associated with a good clinical response when compared with the -360C/-201C haplotype. The -360C>G change resulted in the creation of activator protein 2 (AP2) sites and the -201C>T change abolished binding sites

for Sp1 and AP2. Joerger *et al.* reported six novel SNPs in *DCK*: -243G>T, -135G>C, 261G>A, 364C>T(Pro121Ser), 727A>C (Lys242Gln) and intron 6 T>A (predominantly found in Caucasians) [47]. Recently, we performed deep sequencing of the *DCK* proximal promoter and all seven coding exons in the International HapMap Project panels [201] with European (Centre d' Etude du Polymorphisme Humain [CEPH], Paris, France, panel, n = 90) and African (Yoruba people in Ibadan, Nigeria: [YRI] n = 90) ancestry. A total of 64 genetic polymorphisms, including three nonsynonymous coding changes (Ile24Val, Ala119Gly and Pro122Ser) were identified on the *DCK* locus [48]. All three coding changes were conserved in different species (Figure 2), implying that these codons might have functional significance. Functional studies using recombinant DCK proteins indicated the association of these polymorphisms with reduced DCK activity (using cladribine as a substrate), with the 24Val, 119Gly and 122Ser isoforms demonstrating 85, 66 and 43% activity as compared with the wild-type (WT) isoform. Lymphoblast cell lines harboring the coding changes were also associated with lower DCK activity. Furthermore, DCK 119Gly and 122Ser variant isoforms had significantly lower K_m ($p < 0.01$) and V_{max} ($p < 0.001$) as compared with the DCK WT protein [48]. Both Ala119Gly and Pro 122Ser changes occur in close proximity to ERS motif, which interacts with the substrate within the active site of DCK. Lymphoblast mRNA expression levels of DCK in these samples indicated ethnic differences, with significantly higher expression in African ancestry as compared with European ancestry. Using Haploview, it was observed that SNPs at the *DCK* locus were in strong linkage disequilibrium, resulting in four block/groups within the CEPH population and nine block/groups of SNPs within the YRI population. Interestingly, SNPs in block 1/group 1 demonstrated significant frequency differences in these ethnic panels (allele frequency of 0.05 and 0.77 in CEPH and YRI, respectively). These differences in allele frequencies could contribute to ethnic differences observed in phenotypes. SNPs from block 1/group 1 were also associated with DCK mRNA expression. For a block 1 representative SNP at 35708C>T (that occurs in LD with 13 intronic and one other 3'UTR SNP) in the 3'UTR of *DCK*, the 35708T allele was associated with significantly higher DCK mRNA expression in both European and African ancestry panels as compared with allele 35708C [48]. In addition, SNPs at -245G>C, 36113C>T and 29377C>T within the African ancestry panel were associated with DCK mRNA expression levels (Figure 4). An exploratory analysis in AML patients indicated association of the 35708C allele (3'UTR SNP) with lower leukemic blast cell intracellular levels of ara-CTP when compared with patients homozygous for the 35708T allele [48]. Resequencing of *DCK* in 240 DNA samples from African-American, Caucasian-American, Han Chinese-American and Mexican-American subjects identified 28 polymorphisms in *DCK* including the three nonsynonymous changes indicated earlier [49]. Functional analysis revealed lower activities for these isoforms (Val24: 70%; Gly119: 42% and Ser122: 66%) when compared with WT. Furthermore, the isoforms with multiple variants namely, Val24/Gly119/Ser122, Gly119/Ser122 and Val24/Gly119, demonstrated 105, 82 and 44% activity as compared with WT [49]. Substrate kinetics studies indicated no change in apparent K_m and V_{max} values except for Val24/Ser122 isoform. The differences in the results of these two studies might be due to the use of different model systems (recombinant bacterially expressed protein vs mammalian Cos-1 cells) or different substrates used (purine analog cladribine vs pyrimidine analog gemcitabine) to study substrate kinetics. More recently, analysis of the *DCK* gene in a Japanese population identified 29 polymorphisms including Pro122Ser coding change, and further analysis indicated considerable ethnic differences in *DCK* SNPs and haplotypes [50]. Figure 4 highlights selected *DCK* SNPs with potential functional or clinical implications.

Cytosolic 5' nucleotidase

The 5' nucleotidases (NT5C2/cN II; EC 3.1.3.5) are a group of enzymes with different substrate specificities that catalyze the dephosphorylation of common ribo- and deoxyribo-nucleoside phosphates, and thus maintain balanced nucleotide pools in cells [51]. To date,

seven human 5' nucleotidases have been isolated and characterized. NT5C2 or 'cytosolic 5' nucleotidase II' is ubiquitously expressed in human tissues. NT5C2 is an inosine monophosphate selective nucleotidase and its higher expression in multiple *in vivo* and *in vitro* studies, has been associated with the development of resistance to clinically important nucleoside analogs, such as ara-C, cladribine and gemcitabine [52]. NT5C2 activity opposes that of DCK by dephosphorylating ara-CMP, thereby preventing the production of the active triphosphate form, ara-CTP [37]. Galmarini *et al.* reported a higher expression of NT5C2 to be associated with a lower disease-free and overall survival in adult AML patients undergoing treatment with ara-C [24,53]. In non-small-cell lung cancer (NSCLC) patients treated with gemcitabine, a higher NT5C2 expression was related to lower overall survival [54]. Although NT5C2 is involved in the inactivation of monophosphate forms of nucleoside analogs, thereby contributing to the development of resistance, it may also reflect the proliferation status of the leukemic cell and may act as a marker of disease aggressiveness. Intracellular ara-CTP production as well as ara-C sensitivity has been shown to be significantly associated with the DCK:NT5C2 ratio in the HL-60 leukemic cell line and its ara-C-resistant variants [55].

There is no published report so far identifying and characterizing the functional significance of genetic polymorphisms in NT5C2. However, data from international HapMap project indicates the presence of approximately 100 polymorphisms, including two synonymous and two nonsynonymous coding polymorphisms (Ala3Thr and Arg136Gln). The functional significance of these coding polymorphisms requires evaluation. Multispecies alignment of NT5C2 SNPs across the coding SNPs is shown in Figure 2.

Cytidine deaminase & deoxycytidylate deaminase

Cytidine deaminase (EC 3.5.4.5) is the predominant inactivating enzyme in the ara-C metabolic pathway and is involved in irreversibly deaminating ara-C to ara-U. Elevated CDA expression has been associated with resistance to ara-C and other nucleoside analogs [56,57]. In AML patients, higher CDA levels have been associated with disease recurrence and lower CDA levels with longer duration of remission [58,59]. Ara-C resistance has been demonstrated in mammalian cells constitutively overexpressing human CDA [60]. CDA expression and/or activity has also been implicated in sensitivity to gemcitabine in multiple studies [61]. More recently, in a case study, extensive CDA activity has been implicated in a severe toxicity reaction to capecitabine, which is activated to 5-FU by carboxyl esterase, CDA and thymidine phosphorylase [62].

Deep sequencing of CDA in the Japanese [63], Caucasians and African-Americans [64] identified three nonsynonymous coding polymorphisms (A79C/Lys27Gln, G208A/Ala70Thr, T435C). All these coding changes were conserved in different species (Figure 2) implying that these codons might have functional significance. Functional studies using recombinant CDA demonstrated no influence on immunoreactive proteins but reduced activity for gemcitabine for the Gln27 CDA isoform [64]. The apparent Km of the Gln27 isoform was also significantly higher than that of the WT Lys27 isoform in the substrate kinetic study [64]. The A79C/Lys27Gln isoform has been associated with a reduced deamination of ara-C *in vitro* [65]; however, no change in CDA activity was observed between yeast transformants expressing CDA 27Lys or 27Gln variants for deamination of cytidine or ara-C. Furthermore, ara-C sensitivity of yeast transformants expressing Gln27 was also not different than the Lys27 isoform [63]. Of the children with AML treated on the Children's Cancer Group (CCG) 2941 and 2961 protocols, patients homozygous for the CDAGln27 polymorphism (considered the low activity allele) had increased risk of treatment-related mortality with ara-C-based therapy [66]. In contrast to results from the studies indicated above, Lys27Lys was associated with lower CDA gemcitabine deamination activity in samples from lung cancer patients [67], with lower toxicity, time to progression and overall survival in NSCLC [68]. The inconsistency in

the results for the functional significance of the Lys27Gln allele precludes its clinical evaluation.

The 208G>A polymorphism identified in the Japanese population demonstrated significantly lower CDA activity and increased sensitivity to ara-C [63], but was not present in Caucasians or African-Americans [64]. Within Japanese pancreatic cancer patients receiving gemcitabine, a patient homozygous for 208 G>A SNPs demonstrated a fivefold higher gemcitabine levels and showed severe hematologic and nonhematologic toxicities [69]. Recently, within a larger cohort of Japanese gemcitabine-naïve patients, the CDA haplotype with Ala70Thr (208G>A) SNP was associated with pharmacokinetics of gemcitabine [70], as well as with toxicity to gemcitabine [70], as well as with toxicity to gemcitabine [71]. However, it should be noted that the three studies above originated from the same group and G208A SNP has been identified within the Japanese ethnic group [63,69,70] with an allele frequency of approximately 0.012 [71]. Screening of 1.6 kb of the proximal promoter of *CDA* (upstream of exon 1) in Caucasians and African-Americans identified a haplotype defined by three SNPs (−92 A>G, −451C>T and −897C>A), which were associated with high levels of promoter and enzymatic activity [72]. Whether this haplotype is associated with ara-C chemosensitivity or clinical response to ara-C remains to be determined. Recently, analysis in AML patients receiving ara-C identified *CDA* SNP −451 as an independent prognostic parameter for survival [73]. Figure 5 highlights potentially significant SNPs at the *CDA* locus along with clinically significant observations.

The enzyme DCTD (EC 3.5.4.12) is involved in the deamination of ara-CMP to ara-UMP. However, its role in the pathogenesis of an ara-C-resistant phenotype is poorly defined. Although the expression of DCTD has not been demonstrated to be associated with ara-C sensitivity or clinical response [74], some studies have suggested a substantial role for DCTD in the metabolism of ara-CMP in T-lymphoblastic leukemia [75–77]. Recently, the screening of coding regions and the proximal promoter of *DCTD* in Caucasian and African ethnic groups identified a nonsynonymous SNP (Asn58Asp) with a significant loss of activity in *in vitro* assays [64].

Cytidine-5'-triphosphate synthetase

Cytidine-5'-triphosphate synthetase ([CTPS] EC 6.3.4.2) catalyzes the conversion of UTP to CTP and is a key enzyme in pyrimidine biosynthesis. Inhibition of CTPS with cyclopentenyl cytosine (CPEC) has been shown to deplete CTP/dCTP pools, as well as increase sensitivity to ara-C in neuroblastoma cells [78], human T-lymphoblastic cell lines and myeloid leukemia cell lines [79–81]. Low levels of cellular dCTP pools due to CTPS inhibition facilitates ara-C phosphorylation (by reducing feedback inhibition of DCK) as well as incorporation of ara-CTP into DNA. CPEC-mediated inhibition of CTPS increases activation of DCK and the cytotoxicity of neuroblastoma cell lines to gemcitabine [82].

Clustered mutations within the coding region of *CTPS* have been identified in ara-C-resistant strains of Chinese hamster ovary cells [83]; however, screening of the *CTPS* gene in 36 patients with recurrent and resistant leukemia failed to identify similar mutations [84]. Data from the HapMap project indicates at least 24 SNPs within the *CTPS* locus (two in the 5'UTR, one in 3'UTR and the rest intronic). In addition, the SNP database indicates two synonymous SNPs, Gln167Gln and Val500Val, occurring with the allele frequency of 0.28 and 0.25, respectively. Future research warrants systematic evaluation of *CTPS* SNPs.

Ribonucleotide reductase, RRM1 & RRM2 subunits

Ribonucleotide reductase catalyzes the reduction of ribonucleotides to their corresponding deoxyribonucleotides, which are the building blocks for DNA replication. Hence RR is a target of several anticancer agents, including nucleoside analogs and hydroxyurea (reviewed by Shao

et al. [85]). The RR holoenzyme consists of the dimerized large and small subunits, RRM1 and RRM2. RR regulates intracellular pools of dCTP, which in turn, have been implicated in the development of ara-C resistance. Several cell lines and AML blasts with high levels of dCTP have been found to be resistant to ara-C [77,85–87]. dCTP regulates ara-C metabolism at three levels:

- By feedback inhibition of DCK resulting in reduced activation of ara-C [88];
- By allosteric activation of the inactivating enzyme CDA [87];
- By competing with the active metabolite ara-CTP for incorporation into DNA [89].

Nucleoside analogs, such as ara-C, gemcitabine, fludarabine, cladribine and clofarabine, act as inhibitors of RR after intracellular conversion to their dNDP or dNTP forms. Therefore biochemical modulation of ara-C by nucleoside analogs such as fludarabine and cladribine, has been shown to be feasible in adult and pediatric leukemia patients as they stimulate ara-CTP accumulation by the inhibition of RR [12,90–93]. Recently, the use of Triapine® (Vion Pharmaceutical Inc., CT, USA) a new potent RR inhibitor, has been demonstrated to stimulate accumulation of active metabolites of both gemcitabine and ara-C, in NSCLC cell lines [94]. The therapeutic efficacy of RR inhibitors would thus be greatly influenced by any interpatient variability in the expression and/or activity of both *RRM1* and *RRM2* genes. Patients with low expression and/or activity of *RRM1* or *RRM2* might not derive much benefit from combination therapies with RR inhibitors as compared with patients with high expression.

RRM1 overexpression has been correlated with gemcitabine resistance in human pancreatic cancer [95], in NSCLC cell lines [96,97] and in mice [98]. Preliminary analysis of gene expression in leukemic blasts from AML patients treated with ara-C, demonstrated a significant inverse correlation between *RRM1* and *RRM2* gene expression levels at diagnosis and the 24 h post ara-C treatment blast ara-CTP levels [MITRA *ET AL.*, UNIVERSITY OF MINNESOTA. UNPUBLISHED DATA].

Chemosensitivity to gemcitabine in 62 human cancer cell lines of various origin have been associated with the 2464G>A SNP (a silent coding change Ala744Ala) in *RRM1* [99]. Recently, the *RRM1* promoter allelotype for SNPs –37A>C and –524C>T have been demonstrated to influence response rates to gemcitabine based chemotherapy [100]. Although *RRM1* 2464G>A does not result in an amino acid change, it has been shown to influence mRNA expression of *RRM1* [87]. An association of the *RRM1* haplotype for 2455A>G (Thr741Thr) and 2464G>A SNPs was linked with a lower frequency of chemotherapy-induced toxicity [101]. However, so far there has been no systematic study to explore the role of genetic variation in *RRM1/2* in the therapeutic efficacy of ara-C.

Additional candidate genes identified by genome-wide analysis

ATP-binding cassette transporter proteins (ABC drug transporters) have been implicated in drug resistance. Recently, it has been demonstrated that *ABCC10* (MRP7) confers resistance to cytarabine in HEK293 cells by reducing accumulation of cytarabine [102] and *ABCC11* (MRP8) has been demonstrated to have a differential expression in AML blasts as well as serve as a predictive marker for treatment outcome [103]. Within MRP8-transfected LLC-PK1 cells, reduced accumulation of ara-C as well as its metabolites has been observed [103]. Hence, these two ABC transporters are potential candidates for future pharmacogenetic studies.

In addition, genetic variations in the pharmacodynamic targets of ara-C (such as DNA polymerase α) and in genes of the apoptotic pathway could also contribute to the interpatient variation in clinical response to ara-C.

Cellular sensitivity to gemcitabine and ara-C in lymphoblastoid cells has been analyzed for association with genome-wide gene expression. Genes with the strongest association with IC₅₀ values were involved mainly in cell death, cancer, cell cycle and nucleic acid metabolism [104]. Some important genes include *FKBP5*, *NT5C3*, *ES2*, *GCAT*, *MYBBP1A*, *TLE4* and *ZNF278*. Additional functional validation demonstrated downregulation of *FKBP5* and *NT5C3* to be associated with altered sensitivity to both drugs [104].

Using lymphoblastoid cell lines that are part of the International HapMap project (European [CEU] or African [YRI] ancestry); Hartford *et al.* analyzed more than 2 million single SNPs (narrowed down further for SNPs that affected mRNA expression) for association with susceptibility to ara-C [105]. Four SNPs could explain 51% of the variability in sensitivity to ara-C among the CEU and five SNPs could explain 58% of the variation among the YRI. The new candidate genes identified were:

- GIT1, an intracellular scaffolding protein involved in diverse processes, including agonist-coupled receptor endocytosis and focal adhesion assembly, as well as with intra-cellular signaling cascade proteins, including those in the MAPK pathway, such as MEK1 and ERK1/2;
- RAD51-associated protein 1, implicated in homologous recombination;
- SLC25A37, member of the SLC25 solute carrier family [105].

Although both these studies used lymphoblastoid cell lines for screening ara-C (or gemcitabine and ara-C) cytotoxicity and used genome-wide gene-expression arrays to identify the candidate genes associated with ara-C /gemcitabine cytotoxicity, there was almost no overlap between the top genes identified in these two studies. Although there could be a multitude of reasons for this, ranging from different array platforms to different cell lines, in the study by Hartford *et al.*, one of the causes was suggested to be a narrowing of focus by concentrating on SNPs within genes that affect gene expression [105].

A few studies in the literature have tried to identify candidate genes associated with cellular resistance to ara-C. Two of the published studies have used ALL and/or CML cell lines to study the candidate genes in ara-C metabolic pathways. Takagaki *et al.* compared gene expression changes between CCRF-CEM (an ara-C sensitive ALL cell line) and K562 (an ara-C resistant CML cell line) and reported a downregulation of chaperone genes in the CCRF-CEM versus upregulation of hemoglobin genes in K562 cells [106]. In the ara-C-resistant K562, upregulation of *IGF-IR* was observed compared with the corresponding ara-C-sensitive parental cell line [107]. The major caveat in both these studies is that a very restricted set of genes were tested using either an in-house cDNA array or human cancer chip array.

Two additional reports, both from the same group, have used murine leukemia cell lines. They identified neurofibromin (NF1) deficiency as being associated with the development of resistance to ara-C [108]. NF1 is a negative regulator of RAS signaling. However, whether *NF1* or other key genes in the mTOR and MAPK pathways are predictive of ara-C sensitivity in human AML is not known. A more recent study used a mouse gene-expression array to characterize expression differences between ara-C-resistant and parental murine leukemia cell lines [109]. Out of 929 genes with significant expression differences, 24 overlapped with prognostically important genes in human studies. However, as this study was performed in murine cell lines, the results of this study need to be evaluated in humans. Another limitation of this and the other studies previously mentioned, is the use of cell lines that were made resistant by growing them in increasing concentrations of ara-C over a long period. Ara-C-resistant cell lines prepared by this method usually exhibit multiple new chromosomal changes following exposure to increased doses of ara-C and this could influence the observed differences in expression.

Conclusion & future perspective

Research on the role of pharmacogenomics in cytarabine response/resistance is still in its infancy. The key candidate genes in the activation pathway of ara-C, namely *DCK*, *NT5C2*, *CDA*, *DCTD*, *SLC29A1* and *RRM1/2*, are being explored by various investigators and have been shown to harbor polymorphisms of functional significance. Since sensitivity to cytarabine depends on intracellular ara-CTP levels, genetic variations in these candidate genes could predict its antileukemic effect and the clinical outcome. Association studies are required in larger patient populations to confirm the observed results and to identify other candidate genetic variants of relevance to ara-C chemotherapy. In addition, the heterogeneity of AML itself, multidrug treatment regimens, gene–gene and gene–environment interactions should be accounted for in order to completely decipher the observed inter-patient variation in clinical response.

Although studies to identify the pharmacodynamic targets of ara-C in sensitive versus resistant cell lines are ongoing, future studies should be targeted in patient samples in order to identify the candidate genes contributing to resistance. Genome-wide association studies have also recently identified a few previously unknown candidate genes. However, whether these genes contribute to ara-C sensitivity in patients requires further evaluation. Once confirmed, the impact of genetic variation in genes of pharmacodynamic significance could be tested for ara-C sensitivity.

Future studies should be targeted at validating and confirming the pharmacogenetic markers that will identify patients with an altered response to ara-C therapy. This will result in an identification of the panel of candidate genes and potentially significant SNPs and would eventually be used as a diagnostic tool to predict response to ara-C. Such a panel of SNPs would be equally, if not more, useful markers for response to other nucleoside analogs such as cladribine, fludarabine, clofarabine, CNDAC and gemcitabine, as they all share a common pathway of drug activation.

Executive summary

Candidate cytarabine pathway genes

- Cytarabine is the key agent used in the treatment of acute myeloid leukemia.
- Seven key candidate genes implicated in metabolic activation of cytarabine (ara-C) to ara-cytidine-5'-triphosphate (ara-CTP) are *DCK*, *NT5C2*, *CDA*, *DCTD*, *SLC29A1*, *RRM1* and *RRM2*.

Pharmacogenetics of cytarabine

- Genetic variation in these candidate genes could influence clinical outcomes.
- A number of coding and regulatory polymorphisms of functional and clinical significance have been identified in these candidate genes.

Genome-wide studies

- Genome-wide studies using lymphoblast cell lines derived from subjects with different ancestries have identified previously unknown candidate genes of relevance to ara-C sensitivity.
- Genome-wide gene-expression profiles in ara-C sensitive versus resistant cell lines performed in order to understand molecular mechanism of ara-C resistance have also identified candidate genes of pharmacodynamic significance.
- These genes should be analyzed in future pharmacogenetic studies.

Future perspective

- Future studies should be designed to comprehensively evaluate genetic variation in the key pharmacokinetics/pharmacodynamics pathway genes.
- Association studies in larger patient cohorts (cooperative group trials) should analyze the pharmacogenetic markers after accounting for disease heterogeneity, gene–gene and gene–drug interactions.
- Systematic identification and validation of SNPs within ara-C pathway genes will expedite the creation of a database of diagnostic SNPs that could initiate the clinical use of pharmacogenetics for tailoring therapy based on an individual patient's genetic makeup.
- The comprehensive SNP panel will also be applicable to other nucleoside analogs such as cladribine, clofarabine, fludarabine and gemcitabine and so on.

Acknowledgments

Amit Kumar Mitra's support with the bibliography and comments on this review are highly appreciated.

Bibliography

Papers of special note have been highlighted as:

▪ of interest

▪▪ of considerable interest

1. Wang JJ, Selawry OS, Vietti TJ, Bodey GP. Prolonged infusion of arabinosyl cytosine in childhood leukemia. *Cancer* 1970;25:6.
2. Creutzig U, Berthold F, Boos J, et al. Improved treatment results in children with AML: Results of study AML-BFM 93. *Klin. Padiatr* 2001;213:175–185. [PubMed: 11528551]
3. Krance RA, Hurwitz CA, Head DR, et al. Experience with 2-chlorodeoxyadenosine in previously untreated children with newly diagnosed acute myeloid leukemia and myelodysplastic diseases. *J. Clin. Oncol* 2001;19:2804–2811. [PubMed: 11387351]
4. Capizzi RL, Yang JL, Rathmell JP, et al. Dose-related pharmacologic effects of high-dose ara-C and its self-potential. *Semin. Oncol* 1985;12:65–74. [PubMed: 3892704]
5. Galmarini CM, Mackey JR, Dumontet C. Nucleoside analogues: mechanisms of drug resistance and reversal strategies. *Leukemia* 2001;15:875–890. [PubMed: 11417472]
6. Wiley JS, Taupin J, Jamieson GP, Snook M, Sawyer WH, Finch LR. Cytosine arabinoside transport and metabolism in acute leukemias and T cell lymphoblastic lymphoma. *J. Clin. Invest* 1985;75:632–642. [PubMed: 3871794]
7. Weick JK, Kopecky KJ, Appelbaum FR, et al. A randomized investigation of high-dose versus standard-dose cytosine arabinoside with daunorubicin in patients with previously untreated acute myeloid leukemia: a Southwest Oncology Group study. *Blood* 1996;88:2841–2851. [PubMed: 8874180]
8. Capizzi RL, Yang JL, Cheng E, et al. Alteration of the pharmacokinetics of high-dose ara-C by its metabolite, high ara-U in patients with acute leukemia. *J. Clin. Oncol* 1983;1:763–771. [PubMed: 6668493]
9. Kufe DW, Major PP, Egan EM, Beardsley GP. Correlation of cytotoxicity with incorporation of ara-C into DNA. *J. Biol. Chem* 1980;255:8997–8900. [PubMed: 7410404]
10. Major PP, Egan EM, Beardsley GP, Minden MD, Kufe DW. Lethality of human myeloblasts correlates with the incorporation of arabinofuranosylcytosine into DNA. *Proc. Natl Acad. Sci. USA* 1981;78:3235–3239. [PubMed: 6942429]

11. Raza A, Gezer S, Anderson J, et al. Relationship of [3H] Ara-C incorporation and response to therapy with high-dose Ara-C in AML patients: a Leukemia Intergroup study. *Exp. Hematol* 1992;20:1194–1200. [PubMed: 1426099]
12. Gandhi V, Estey E, Keating MJ, Plunkett W. Biochemical modulation of arabinosylcytosine for therapy of leukemias. *Leuk. Lymphoma* 1993;10(Suppl):109–114. [PubMed: 8481660]
13. Avramis VI, Wiersma S, Krailo MD, et al. Pharmacokinetic and pharmacodynamic studies of fludarabine and cytosine arabinoside administered as loading boluses followed by continuous infusions after a Phase I/II study in pediatric patients with relapsed leukemias. *The Children's Cancer Group. Clin. Cancer Res* 1998;4:45–52. [PubMed: 9516951]
14. Faderl S, Gandhi V, Keating MJ, Jeha S, Plunkett W, Kantarjian HM. The role of clofarabine in hematologic and solid malignancies – development of a next-generation nucleoside analog. *Cancer* 2005;103:1985–1995. [PubMed: 15803490]
15. Gandhi V, Plunkett W. Cell cycle-specific metabolism of arabinosyl nucleosides in K562 human leukemia cells. *Cancer Chemother. Pharmacol* 1992;31:11–17. [PubMed: 1458554]
16. Kufe DW, Munroe D, Herrick D, Egan E, Spriggs D. Effects of 1- β -d-arabinofuranosylcytosine incorporation on eukaryotic DNA template function. *Mol. Pharmacol* 1984;26:128–134. [PubMed: 6431261]
17. Chou TC, Arlin Z, Clarkson BD, Phillips FS. Metabolism of 1- β -d-arabinofuranosylcytosine in human leukemic cells. *Cancer Res* 1977;37:3561–3570. [PubMed: 332337]
18. Plunkett W, Iacoboni S, Estey E, Danhauser L, Liliemark JO, Keating MJ. Pharmacologically directed ara-C therapy for refractory leukemia. *Semin. Oncol* 1985;12:20–30. [PubMed: 4012338] • Demonstrates the relationship of intracellular ara-cytidine-5-prime-triphosphate (ara-CTP) levels with treatment response.
19. Momparler RL, Fischer GA. Mammalian deoxynucleoside kinase. I. Deoxycytidine kinase: purification, properties, and kinetic studies with cytosine arabinoside. *J. Biol. Chem* 1968;243:4298–4304. [PubMed: 5684726]
20. Clarke ML, Mackey JR, Baldwin SA, Young JD, Cass CE. The role of membrane transporters in cellular resistance to anticancer nucleoside drugs. *Cancer Treat. Res* 2002;112:27–47. [PubMed: 12481710]
21. Sundaram M, Yao SY, Ingram JC, et al. Topology of a human equilibrative, nitrobenzylthioinosine (NBMPR)-sensitive nucleoside transporter (hENT1) implicated in the cellular uptake of adenosine and anti-cancer drugs. *J. Biol. Chem* 2001;276:45270–45275. [PubMed: 11584005]
22. Hubeek I, Peters GJ, Broekhuizen AJ, et al. Immunocytochemical detection of deoxycytidine kinase in haematological malignancies and solid tumours. *J. Clin. Pathol* 2005;58:695–699. [PubMed: 15976334]
23. Hubeek I, Stam RW, Peters GJ, et al. The human equilibrative nucleoside transporter 1 mediates *in vitro* cytarabine sensitivity in childhood acute myeloid leukaemia. *Br. J. Cancer* 2005;93:1388–1394. [PubMed: 16333246] • Demonstrates the relationship between hENT1 mRNA and response to ara-C in pediatric acute myeloid leukemia.
24. Galmarini CM, Thomas X, Calvo F, et al. *In vivo* mechanisms of resistance to cytarabine in acute myeloid leukaemia. *Br. J. Haematol* 2002;117:860–868. [PubMed: 12060121]
25. Stam RW, den Boer ML, Meijerink JP, et al. Differential mRNA expression of ara-C-metabolizing enzymes explains ara-C sensitivity in *MLL* gene-rearranged infant acute lymphoblastic leukemia. *Blood* 2003;101:1270–1276. [PubMed: 12406912]
26. Gati WP, Paterson AR, Belch AR, et al. Es nucleoside transporter content of acute leukemia cells: role in cell sensitivity to cytarabine (araC). *Leuk. Lymphoma* 1998;32:45–54. [PubMed: 10037000]
27. Gati WP, Paterson AR, Larratt LM, Turner AR, Belch AR. Sensitivity of acute leukemia cells to cytarabine is a correlate of cellular es nucleoside transporter site content measured by flow cytometry with SAENTA-fluorescein. *Blood* 1997;90:346–353. [PubMed: 9207471]
28. Giovannetti E, Del TM, Mey V, et al. Transcription analysis of human equilibrative nucleoside transporter-1 predicts survival in pancreas cancer patients treated with gemcitabine. *Cancer Res* 2006;66:3928–3935. [PubMed: 16585222]

29. Michalski CW, Erkan M, Sauliunaite D, et al. *Ex vivo* chemosensitivity testing and gene expression profiling predict response towards adjuvant gemcitabine treatment in pancreatic cancer. *Br. J. Cancer* 2008;995:760–767. [PubMed: 18728667]
30. Farrell JJ, Elsaleh H, Garcia M, et al. Human equilibrative nucleoside transporter 1 levels predict response to gemcitabine in patients with pancreatic cancer. *Gastroenterology* 2009;136(1):187–195. [PubMed: 18992248]
31. Osato DH, Huang CC, Kawamoto M, et al. Functional characterization in yeast of genetic variants in the human equilibrative nucleoside transporter, ENT1. *Pharmacogenetics* 2003;13:297–301. [PubMed: 12724623] • First report on functional characterization of *hENT1* genetic variants.
32. Chaudary N, Naydenova Z, Shuralyova I, Coe IR. Hypoxia regulates the adenosine transporter, mENT1, in the murine cardiomyocyte cell line, HL-1. *Cardiovasc. Res* 2004;61:780–788. [PubMed: 14985075]
33. Eltzschig HK, Abdulla P, Hoffman E, et al. HIF-1-dependent repression of equilibrative nucleoside transporter (ENT) in hypoxia. *J. Exp. Med* 2005;202:1493–1505. [PubMed: 16330813]
34. Myers SN, Goyal RK, Roy JD, Fairfull LD, Wilson JW, Ferrell RE. Functional single nucleotide polymorphism haplotypes in the human equilibrative nucleoside transporter 1. *Pharmacogenet. Genomics* 2006;16:315–320. [PubMed: 16609362]
35. Kim SR, Saito Y, Maekawa K, et al. Thirty novel genetic variations in the *SLC29A1* gene encoding human equilibrative nucleoside transporter 1 (hENT1). *Drug Metab. Pharmacokinet* 2006;21:248–256. [PubMed: 16858130]
36. Bhalla K, Nayak R, Grant S. Isolation and characterization of a deoxycytidine kinase-deficient human promyelocytic leukemic cell line highly resistant to 1- β -D- arabinofuranosylcytosine. *Cancer Res* 1984;44:5029–5037. [PubMed: 6091869]
37. Dumontet C, Fabianowska-Majewska K, Mantincic D, et al. Common resistance mechanisms to deoxynucleoside analogues in variants of the human erythroleukaemic line K562. *Br. J. Haematol* 1999;106:78–85. [PubMed: 10444166]
38. Verhoef V, Sarup J, Fridland A. Identification of the mechanism of activation of 9- β -D- arabinofuranosyladenine in human lymphoid cells using mutants deficient in nucleoside kinases. *Cancer Res* 1981;41:4478–4483. [PubMed: 6272978]
39. Hapke DM, Stegmann AP, Mitchell BS. Retroviral transfer of deoxycytidine kinase into tumor cell lines enhances nucleoside toxicity. *Cancer Res* 1996;56:2343–2347. [PubMed: 8625309]
40. Stegmann AP, Honders WH, Willemze R, Ruiz van Haperen VW, Landegent JE. Transfection of wild-type deoxycytidine kinase (*dck*) cDNA into an AraC- and DAC-resistant rat leukemic cell line of clonal origin fully restores drug sensitivity. *Blood* 1995;85:1188–1194. [PubMed: 7532033]
41. Kakihara T, Fukuda T, Tanaka A, et al. Expression of deoxycytidine kinase (dCK) gene in leukemic cells in childhood: decreased expression of dCK gene in relapsed leukemia. *Leuk. Lymphoma* 1998;31:405–409. [PubMed: 9869205]
42. van der Wilt CL, Kroep JR, Loves WJ, et al. Expression of deoxycytidine kinase in leukaemic cells compared with solid tumour cell lines, liver metastases and normal liver. *Eur. J. Cancer* 2003;39:691–697. [PubMed: 12628850]
43. Lotfi K, Karlsson K, Fyrberg A, et al. The pattern of deoxycytidine- and deoxyguanosine kinase activity in relation to messenger RNA expression in blood cells from untreated patients with B-cell chronic lymphocytic leukemia. *Biochem. Pharmacol* 2006;71:882–890. [PubMed: 16436271]
44. Galmarini CM, Thomas X, Calvo F, et al. Potential mechanisms of resistance to cytarabine in AML patients. *Leuk. Res* 2002;26:621–629. [PubMed: 12008078]
45. Kroep JR, Loves WJ, van der Wilt CL, et al. Pretreatment deoxycytidine kinase levels predict *in vivo* gemcitabine sensitivity. *Mol. Cancer Ther* 2002;1:371–376. [PubMed: 12477049]
46. Shi JY, Shi ZZ, Zhang SJ, et al. Association between single nucleotide polymorphisms in deoxycytidine kinase and treatment response among acute myeloid leukaemia patients. *Pharmacogenetics* 2004;14:759–768. [PubMed: 15564883] •• First report on deoxycytidine kinase genetic variants in a Chinese population.
47. Joerger M, Bosch TM, Doodeman VD, Beijnen JH, Smits PH, Schellens JH. Novel deoxycytidine kinase gene polymorphisms: a population screening study in Caucasian healthy volunteers. *Eur. J. Clin. Pharmacol* 2006;62:681–684. [PubMed: 16799820]

48. Lamba JK, Crews K, Pounds S, et al. Pharmacogenetics of deoxycytidine kinase: identification and characterization of novel genetic variants. *J. Pharmacol. Exp. Ther* 2007;323:935–945. [PubMed: 17855478] ▪ First report on deoxycytidine kinase genetic variants and association with leukemic cell ara-CTP levels in pediatric acute myeloid leukemia patients.
49. Kocabas NA, Aksoy P, Pellemounter LL, et al. Gemcitabine pharmacogenomics: deoxycytidine kinase and cytidylate kinase gene resequencing and functional genomics. *Drug Metab. Dispos* 2008;36:1951–1959. [PubMed: 18556440]
50. Kim SR, Saito Y, Maekawa K, et al. Twenty novel genetic variations and haplotype structures of the *DCK* gene encoding human deoxycytidine kinase (dCK). *Drug Metab. Pharmacokinet* 2008;23:379–384. [PubMed: 18974616]
51. Bianchi V, Pontis E, Reichard P. Interrelations between substrate cycles and *de novo* synthesis of pyrimidine deoxyribonucleoside triphosphates in 3T6 cells. *Proc. Natl Acad. Sci. USA* 1986;83:986–990. [PubMed: 3456577]
52. Hunsucker SA, Mitchell BS, Spychala J. The 5'-nucleotidases as regulators of nucleotide and drug metabolism. *Pharmacol. Ther* 2005;107:1–30. [PubMed: 15963349]
53. Galmarini CM, Cros E, Thomas X, Jordheim L, Dumontet C. The prognostic value of cN-II and cN-III enzymes in adult acute myeloid leukemia. *Haematologica* 2005;90:1699–1701. [PubMed: 16330448]
54. Seve P, Mackey JR, Isaac S, et al. cN-II expression predicts survival in patients receiving gemcitabine for advanced non-small cell lung cancer. *Lung Cancer* 2005;49:363–370. [PubMed: 15923058] ▪ Demonstrates the association of cN-II (NT5C2) mRNA expression with gemcitabine response.
55. Yamauchi T, Negoro E, Kishi S, et al. Intracellular cytarabine triphosphate production correlates to deoxycytidine kinase/cytosolic 5'-nucleotidase II expression ratio in primary acute myeloid leukemia cells. *Biochem. Pharmacol* 2009;77:1780–1786. [PubMed: 19428333]
56. Grant S. Ara-C: cellular and molecular pharmacology. *Adv. Cancer Res* 1998;72:197–233. [PubMed: 9338077]
57. Maring JG, Groen HJ, Wachters FM, Uges DR, de Vries EG. Genetic factors influencing pyrimidine-antagonist chemotherapy. *Pharmacogenomics J* 2005;5:226–243. [PubMed: 16041392]
58. Jahns-Streubel G, Reuter C, uf der LU, et al. Activity of thymidine kinase and of polymerase α as well as activity and gene expression of deoxycytidine deaminase in leukemic blasts are correlated with clinical response in the setting of granulocyte-macrophage colony-stimulating factor-based priming before and during TAD-9 induction therapy in acute myeloid leukemia. *Blood* 1997;90:1968–1976. [PubMed: 9292531]
59. Schroder JK, Kirch C, Seeber S, Schutte J. Structural and functional analysis of the cytidine deaminase gene in patients with acute myeloid leukaemia. *Br. J. Haematol* 1998;103:1096–1103. [PubMed: 9886326]
60. Schroder JK, Seidelmann M, Kirch HC, Seeber S, Schutte J. Assessment of resistance induction to cytosine arabinoside following transfer and overexpression of the deoxycytidylate deaminase gene *in vitro*. *Leuk. Res* 1998;22:619–624. [PubMed: 9680112]
61. Giovannetti E, Mey V, Loni L, et al. Cytotoxic activity of gemcitabine and correlation with expression profile of drug-related genes in human lymphoid cells. *Pharmacol. Res* 2007;55:343–349. [PubMed: 17296311]
62. Mercier C, Dupuis C, Blesius A, et al. Early severe toxicities after capecitabine intake: possible implication of a cytidine deaminase extensive metabolizer profile. *Cancer Chemother. Pharmacol* 2009;63:1177–1180. [PubMed: 19107485]
63. Yue L, Saikawa Y, Ota K, et al. A functional single-nucleotide polymorphism in the human cytidine deaminase gene contributing to ara-C sensitivity. *Pharmacogenetics* 2003;13:29–38. [PubMed: 12544510] ▪ First study to report cytidine deaminase polymorphisms in a Japanese population.
64. Gilbert JA, Salavaggione OE, Ji Y, et al. Gemcitabine pharmacogenomics: cytidine deaminase and deoxycytidylate deaminase gene resequencing and functional genomics. *Clin. Cancer Res* 2006;12:1794–1803. [PubMed: 16551864] ▪ First report on sequencing and identification of cytidine deaminase and deoxycytidylate deaminase polymorphisms in multiple ethnic panels.

65. Kirch HC, Schroder J, Hoppe H, Esche H, Seeber S, Schutte J. Recombinant gene products of two natural variants of the human cytidine deaminase gene confer different deamination rates of cytarabine *in vitro*. *Exp. Hematol* 1998;26:421–425. [PubMed: 9590659]
66. Bhatla D, Gerbing RB, Alonzo TA, et al. Cytidine deaminase genotype and toxicity of cytosine arabinoside therapy in children with acute myeloid leukemia. *Br. J. Haematol* 2009;144:388–394. [PubMed: 19036079]
67. Giovannetti E, Laan AC, Vasile E, et al. Correlation between cytidine deaminase genotype and gemcitabine deamination in blood samples. *Nucleosides Nucleotides Nucleic Acids* 2008;27:720–725. [PubMed: 18600531]
68. Tibaldi C, Giovannetti E, Vasile E, et al. Correlation of *CDA*, *ERCC1*, and *XPB* polymorphisms with response and survival in gemcitabine/cisplatin-treated advanced non-small cell lung cancer patients. *Clin. Cancer Res* 2008;14:1797–1803. [PubMed: 18347182]
69. Yonemori K, Ueno H, Okusaka T, et al. Severe drug toxicity associated with a single-nucleotide polymorphism of the cytidine deaminase gene in a Japanese cancer patient treated with gemcitabine plus cisplatin. *Clin. Cancer Res* 2005;11:2620–2624. [PubMed: 15814642]
70. Sugiyama E, Kaniwa N, Kim SR, et al. Pharmacokinetics of gemcitabine in Japanese cancer patients: the impact of a cytidine deaminase polymorphism. *J. Clin. Oncol* 2007;25:32–42. [PubMed: 17194903]
71. Ueno H, Kaniwa N, Okusaka T, et al. Homozygous *CDA**3 is a major cause of life-threatening toxicities in gemcitabine-treated Japanese cancer patients. *Br. J. Cancer* 2009;100:870–873. [PubMed: 19293806]
72. Fitzgerald SM, Goyal RK, Osborne WR, Roy JD, Wilson JW, Ferrell RE. Identification of functional single nucleotide polymorphism haplotypes in the cytidine deaminase promoter. *Hum. Genet* 2006;119:276–283. [PubMed: 16446974]
73. Mahlknecht U, Dransfeld CL, Bulut N, et al. SNP analyses in cytarabine metabolizing enzymes in AML patients and their impact on treatment response and patient survival: identification of *CDA* SNP C-451T as an independent prognostic parameter for survival. *Leukemia*. 2009 (Epub ahead of print).
74. Chang H, Salma F, Yi QL, Patterson B, Brien B, Minden MD. Prognostic relevance of immunophenotyping in 379 patients with acute myeloid leukemia. *Leuk. Res* 2004;28:43–48. [PubMed: 14630079]
75. Capizzi RL, White JC, Powell BL, Perrino F. Effect of dose on the pharmacokinetic and pharmacodynamic effects of cytarabine. *Semin. Hematol* 1991;28:54–69. [PubMed: 1780754]
76. Fridland A, Verhoef V. Mechanism for ara-CTP catabolism in human leukemic cells and effect of deaminase inhibitors on this process. *Semin. Oncol* 1987;14:262–268. [PubMed: 3035721]
77. Liliemark JO, Plunkett W. Regulation of 1- β -d-arabinofuranosylcytosine 5'-triphosphate accumulation in human leukemia cells by deoxycytidine 5'-triphosphate. *Cancer Res* 1986;46:1079–1083. [PubMed: 3484676]
78. Bierau J, Van Gennip AH, Leen R, Helleman J, Caron HN, Van Kuilenburg AB. Cyclopentenyl cytosine primes SK-N-BE(2)c neuroblastoma cells for cytarabine toxicity. *Int. J. Cancer* 2003;103:387–392. [PubMed: 12471622]
79. Verschuur AC, Brinkman J, Van Gennip AH, et al. Cyclopentenyl cytosine induces apoptosis and increases cytarabine-induced apoptosis in a T-lymphoblastic leukemic cell-line. *Leuk. Res* 2001;25:891–900. [PubMed: 11532523]
80. Verschuur AC, Van Gennip AH, Leen R, Voute PA, Brinkman J, Van Kuilenburg AB. Cyclopentenyl cytosine increases the phosphorylation and incorporation into DNA of 1- β -d-arabinofuranosyl cytosine in a human T-lymphoblastic cell line. *Int. J. Cancer* 2002;98:616–623. [PubMed: 11920624]
81. Verschuur AC, van Gennip AH, Leen R, Voute PA, van Kuilenburg AB. Cyclopentenyl cytosine increases the phosphorylation and incorporation into dna of arabinofu-ranosyl cytosine in a myeloid leukemic cell-line. *Adv. Exp. Med. Biol* 2000;486:311–317. [PubMed: 11783507] • The role of CTPS in cellular response of myeloid leukemia cell line to ara-C was investigated.
82. Bierau J, van Gennip AH, Leen R, Meinsma R, Caron HN, van Kuilenburg AB. Cyclopentenyl cytosine-induced activation of deoxycytidine kinase increases gemcitabine anabolism and

- cytotoxicity in neuroblastoma. *Cancer Chemother. Pharmacol* 2006;57:105–113. [PubMed: 16133534]
83. Whelan J, Phear G, Yamauchi M, Meuth M. Clustered base substitutions in CTP synthetase conferring drug resistance in Chinese hamster ovary cells. *Nat. Genet* 1993;3:317–322. [PubMed: 7981751]
 84. Whelan J, Smith T, Phear G, Rohatiner A, Lister A, Meuth M. Resistance to cytosine arabinoside in acute leukemia: the significance of mutations in CTP synthetase. *Leukemia* 1994;8:264–265. [PubMed: 8309250]
 85. Shao J, Zhou B, Chu B, Yen Y. Ribonucleotide reductase inhibitors and future drug design. *Curr. Cancer Drug Targets* 2006;6:409–431. [PubMed: 16918309]
 86. Chabner BA, Hande KR, Drake JC. Ara-C metabolism: implications for drug resistance and drug interactions. *Bull. Cancer* 1979;66:89–92. [PubMed: 420952]
 87. Chiba P, Tihan T, Szekeres T, et al. Concordant changes of pyrimidine metabolism in blasts of two cases of acute myeloid leukemia after repeated treatment with ara-C *in vivo*. *Leukemia* 1990;4:761–765. [PubMed: 2232889]
 88. Tattersall MH, Ganeshaguru K, Hoffbrand AV. Mechanisms of resistance of human acute leukaemia cells to cytosine arabinoside. *Br. J. Haematol* 2006;27:39–46. [PubMed: 4368900]
 89. Meuth M. The molecular basis of mutations induced by deoxyribonucleoside triphosphate pool imbalances in mammalian cells. *Exp. Cell Res* 1989;181:305–316. [PubMed: 2647496]
 90. Avramis VI, Nandy P, Kwock R, et al. Increased p21/WAF-1 and p53 protein levels following sequential three drug combination regimen of fludarabine, cytarabine and docetaxel induces apoptosis in human leukemia cells. *Anticancer Res* 1998;18:2327–2338. [PubMed: 9703875]
 91. Gandhi V, Estey E, Du M, Keating MJ, Plunkett W. Minimum dose of fludarabine for the maximal modulation of 1- β -d-arabinofuranosylcytosine triphosphate in human leukemia blasts during therapy. *Clin. Cancer Res* 1997;3:1539–1545. [PubMed: 9815841]
 92. Gandhi V, Estey E, Du M, Nowak B, Keating MJ, Plunkett W. Modulation of the cellular metabolism of cytarabine and fludarabine by granulocyte-colony-stimulating factor during therapy of acute myelogenous leukemia. *Clin. Cancer Res* 1995;1:169–178. [PubMed: 9815970]
 93. Gandhi V, Kemena A, Keating MJ, Plunkett W. Fludarabine infusion potentiates arabinosylcytosine metabolism in lymphocytes of patients with chronic lymphocytic leukemia. *Cancer Res* 1992;52:897–903. [PubMed: 1737352]
 94. Sigmond J, Kamphuis JA, Laan AC, Hoebe EK, Bergman AM, Peters GJ. The synergistic interaction of gemcitabine and cytosine arabinoside with the ribonucleotide reductase inhibitor triapine is schedule dependent. *Biochem. Pharmacol* 2007;73:1548–1557. [PubMed: 17324380]
 95. Nakahira S, Nakamori S, Tsujie M, et al. Involvement of ribonucleotide reductase M1 subunit overexpression in gemcitabine resistance of human pancreatic cancer. *Int. J. Cancer* 2007;120:1355–1363. [PubMed: 17131328]
 96. Davidson JD, Ma L, Flagella M, Geeganage S, Gelbert LM, Slapak CA. An increase in the expression of ribonucleotide reductase large subunit 1 is associated with gemcitabine resistance in non-small cell lung cancer cell lines. *Cancer Res* 2004;64:3761–3766. [PubMed: 15172981]
 97. Rosell R, Danenberg KD, Alberola V, et al. Ribonucleotide reductase messenger RNA expression and survival in gemcitabine/cisplatin-treated advanced non-small cell lung cancer patients. *Clin. Cancer Res* 2004;10:1318–1325. [PubMed: 14977831]
 98. Bergman AM, Eijk PP, Ruiz vHV, et al. *In vivo* induction of resistance to gemcitabine results in increased expression of ribonucleotide reductase subunit M1 as the major determinant. *Cancer Res* 2005;65:9510–9516. [PubMed: 16230416]
 99. Kwon WS, Rha SY, Choi YH, et al. Ribonucleotide reductase M1 (*RRM1*) 2464G>A polymorphism shows an association with gemcitabine chemosensitivity in cancer cell lines. *Pharmacogenet. Genomics* 2006;16:429–438. [PubMed: 16708051]
 100. Kim SO, Jeong JY, Kim MR, et al. Efficacy of gemcitabine in patients with non-small cell lung cancer according to promoter polymorphisms of the ribonucleotide reductase M1 gene. *Clin. Cancer Res* 2008;14:3083–3088. [PubMed: 18483375]
 101. Rha SY, Jeung HC, Choi YH, et al. An association between *RRM1* haplotype and gemcitabine-induced neutropenia in breast cancer patients. *Oncologist* 2007;12:622–630. [PubMed: 17602053]

102. Hopper-Borge E, Xu X, Shen T, Shi Z, Chen ZS, Kruh GD. Human multidrug resistance protein 7 (ABCC10) is a resistance factor for nucleoside analogues and epothilone B. *Cancer Res* 2009;69:178–184. [PubMed: 19118001]
103. Guo Y, Kock K, Ritter CA, et al. Expression of ABCC-type nucleotide exporters in blasts of adult acute myeloid leukemia: relation to long-term survival. *Clin. Cancer Res* 2009;15:1762–1769. [PubMed: 19240178]
104. Li L, Fridley B, Kalari K, et al. Gemcitabine and cytosine arabinoside cytotoxicity: association with lymphoblastoid cell expression. *Cancer Res* 2008;68:7050–7058. [PubMed: 18757419] ▪ First genome-wide study to screen for association of gene expression with cellular cytotoxicity to gemcitabine and ara-C, and identified *NT5C3* and *FKBP5* as potential candidate genes.
105. Hartford CM, Duan S, Delaney SM, et al. Population-specific genetic variants important in susceptibility to cytarabine arabinoside cytotoxicity. *Blood* 2009;113:2145–2153. [PubMed: 19109566] ▪ Study comparing ara-C cellular cytotoxicity of HapMap cell lines with genome-wide genotype and gene expression data.
106. Takagaki K, Katsuma S, Horio T, et al. cDNA microarray analysis of altered gene expression in Ara-C-treated leukemia cells. *Biochem. Biophys. Res. Commun* 2003;309:351–358. [PubMed: 12951057]
107. Abe S, Funato T, Takahashi S, et al. Increased expression of insulin-like growth factor I is associated with Ara-C resistance in leukemia. *Tohoku J. Exp. Med* 2006;209:217–228. [PubMed: 16778368]
108. Yin B, Kogan SC, Dickins RA, Lowe SW, Largaespada DA. Trp53 loss during *in vitro* selection contributes to acquired Ara-C resistance in acute myeloid leukemia. *Exp. Hematol* 2006;34:631–641. [PubMed: 16647569]
109. Yin B, Tsai ML, Hasz DE, Rathe SK, Le Beau MM, Largaespada DA. A microarray study of altered gene expression after cytarabine resistance in acute myeloid leukemia. *Leukemia* 2007;21:1093–1097. [PubMed: 17301810]

Website

201. International HapMap Project. www.hapmap.org

Figure 1. Metabolic pathway of ara-C

Key candidate genes involved in the metabolic activation of ara-C are shown. DCK and hENT1 are favorable for therapeutic efficacy of ara-C and other nucleoside analogs and CDA, DCTD and NT5C2 are not.

Ara-C: Cytarabine; CDA: Cytidine deaminase; DCK: Deoxycytidine kinase; DCTD: Deoxycytidylate deaminase; NT5C2: 5' nucleotidase.

Figure 2. Multispecies alignment of protein sequences of key candidate genes in the metabolic pathway of ara-C

The nonsynonymous coding polymorphisms are indicated on top of the alignment.

Figure 3. Key functional SNPs studied in *SLC29A1*

The reference SNP numbers are provided where available and allele frequency is indicated in parenthesis beside the acronym for the respective ethnic group.

AA: African-American; AS: Asian; CA: Caucasian; EA: European-Americans; FTP: Fetoprotein transcription factor; JA: Japanese; LHR: Liver receptor homolog-1; ME: Mexican American; PA: Pacific Islander; TF: Transcription factor.

Figure 4. Key functional SNPs studied in *DCK*

The rs numbers are provided where available and allele frequency is indicated in parenthesis beside the acronym for the respective ethnic group.

A: HapMap YRI samples with African Ancestry; AA: African-American; AML: Acute myeloid leukemia; Ara-CTP: ara-cytidine-5'-triphosphate; CA: Caucasian-American; CH: Chinese; E: HapMap CEPH samples with European ancestry; HCA: Han Chinese-American; JA: Japanese; LD: Linkage disequilibrium; MA: Mexican-American; YRI: Yoruba people in Ibadan, Nigeria.

Figure 5. Key functional and clinically significant SNPs studied in CDA

AA: African-American; AML: Acute myeloid leukemia; CA: Caucasian-American; JA: Japanese; PK: Pharmacokinetics; TRM: Treatment-related mortality.

Table 1

Overview of potential candidate pathway genes of relevance for pharmacogenetic studies on ara-C and other nucleoside analogs.

Gene	Gene symbol	Polymorphisms of relevance	Patient data with respect to nucleoside analogs	Other data (primarily gene sequencing)	Ref.
<i>Candidate metabolic pathway genes</i>					
Deoxycytidine kinase	<i>DCK</i>	-360C>G, -201C>T, Ile24Val, Pro122Ser, Ala119Gly, 3' UTR SNPs 35708C>T, 36113C>T	Chinese AML patients receiving ara-C based therapy, Pediatric AML patients receiving ara-C based therapy	DNA samples from multiple ethnic panels	[41-45]
5' nucleotidase	<i>NT5C2</i>	Over 100 SNPs in the HapMap database including two Ala3Thr, Arg136Gln	Not available	Not available	[201]
Human equilibrative nucleoside transporter	<i>hENT1</i> <i>SLC29A1</i>	-1345C>G, -1050G>A and -706G>C haplotype of promoter SNPs, Asp59Glu, Ala430Thr	Not available	DNA samples from multiple ethnic panels	[27,30,31]
Cytidine deaminase	<i>CDA</i>	-92A>G, -451C>T, -897C>A, Ala70Thr; Lys27Gln, Ala208Thr	Lung cancer, NSCLC, pancreatic cancer, patients receiving gemcitabine based therapy; Childhood patients with acute leukemia	DNA samples from multiple ethnic panels	[56,57,60-67]
Deoxycytidylate deaminase	<i>DCTD</i>	Asp58Asn	Not available	DNA samples from multiple ethnic panels	[57]
Ribonucleotide reductase motif 1	<i>RRM1</i>	-37A>C, -524C>T, 2464G>A, 2455A>G	Breast cancer patients	-	[86-88]
Ribonucleotide reductase motif 2	<i>RRM2</i>	6 SNPs in HapMap data	Not available	Not available	[201]
<i>Candidate genes identified by genome-wide studies</i>					
G protein coupled receptor kinase-interacting protein 1	<i>GIT1</i>	increased expression associated with ara-C sensitivity and rs17808412 associated with expression and ara-C sensitivity	Not available	HapMap cell lines	[92]
RAD51 associated protein 1	<i>RAD51AP1</i>	Increased expression associated with ara-C sensitivity and rs10973320 associated with expression and ara-C sensitivity	Not available	HapMap cell lines	[92]
SLC25 solute carrier family	<i>SLC25A37</i>	Increased expression associated with ara-C sensitivity and rs2775139 associated with expression and ara-C sensitivity	Not available	HapMap cell lines	[92]
FK506 binding protein 5	<i>FKBP5</i>	Increased expression associated with greater ara-C/gemcitabine sensitivity	Not available	Lymphoblast cell lines from different ethnic groups	[91]
Nucleotidase	<i>NT5C3</i>	Increased expression associated with ara-C/gemcitabine reduced sensitivity	Not available	Lymphoblast cell lines from different ethnic groups	[91]
Sorcin	<i>Sri</i>	Upregulated in ara-C resistant cell lines	Not available	Murine leukemia cell lines	[96]
Amyloid b (A4) precursor protein	<i>APP</i>	Upregulated in ara-C resistant cell lines	Not available	Murine leukemia cell lines	[96]

Gene	Gene symbol	Polymorphisms of relevance	Patient data with respect to nucleoside analogs	Other data (primarily gene sequencing)	Ref.
Neurofibromin	<i>NF1</i>	Loss of NF1 increases ara-C resistant		Murine leukemia cell lines	[95]
IGR receptor I	<i>IGFRI</i>	Upregulated in ara-C resistant cell lines		Leukemia cell lines	[94]

AML: Acute myeloid leukemia; IGR: Insulin-like growth factor receptor; NSCLC: Non-small-cell lung cancer; UTR: Untranslated region.