Comment on Miraki-Moud et al, page 4060

Arginine addiction in AML

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In this issue of Blood, Miraki-Moud et al demonstrate that the majority of acute myeloid leukemias (AMLs) have low expression of argininosuccinate synthetase-1 (ASS1), rendering AML cell lines and primary AML blasts dependent on exogenous arginine and sensitized to arginine deprivation.1

Pathogenic processes associated with the etiology of cancer sometimes involve reprogramming of the expression of metabolic enzymes that creates a metabolic dependency such as reliance on glucose (the Warburg effect)2 or on glutamine.3 Recognition of metabolic requirements that are specific to a malignancy have pointed the way to the development of selective therapeutic approaches. Early understanding of tumor-specific requirements for normal metabolites stimulated the creation of metabolic mimics such as the antifolates and 5-fluorouracil. Directly diminishing the availability of metabolites that tumors are unable to synthesize was achieved in acute lymphoblastic leukemia by administration of 1-asparaginase, which degrades asparagine made available through the diet or normal tissues.

More recently, the understanding that multiple tumors require arginine because of the deficiency in the expression of the urea cycle enzyme ASS1 has provided the rationale to pursue the latter approach.4 6 This enzyme uses citrulline, aspartate, and adenosine triphosphate to form argininosuccinate, which is converted to arginine by argininosuccinate lyase (ASL) (see figure). Cells lacking either enzyme are dependent on salvaging arginine directly from the environment. Autotrophy for arginine has been demonstrated in cell lines derived from many tumor types including melanoma, hepatocellular carcinoma, renal cell carcinoma, glioblastoma, and prostate cancer. This has been linked to deficiency of ASS1 expression in primary tumors of the same types from which the deficient cell lines were derived. This is in contrast to normal tissues, in which the enzyme is expressed ubiquitously.

The authors determined that 28 of 30 AML bone marrow trephines were negative for immunohistochemistry staining of ASS1. Immunoblotting of lysates from 19 AML samples indicated that only 2 strongly expressed ASS1. This is in contrast with strong expression in samples from 2 patients with acute promyelocytic leukemia and 10 samples from lymphoma patients who lacked marrow involvement. Real-time polymerase chain reaction analyses of primary AML samples were consistent with these results. The finding that 7 of 7 samples from AML patients expressed normal levels of ASL, the final enzyme in the pathway, indicated low ASS1 as the cause of the lesion in arginine production in AML. Additional studies demonstrated that the ASS1 promoter was methylated in 21 of 28 samples. A similar finding had previously been reported for lymphoma cell lines,7 suggesting a common epigenetic mechanism of ASS1 silencing. Tumor cell lines that lack the ability to synthesize arginine initiate autophagy and/or apoptosis on arginine deprivation.8 Experimental investigations and clinical trials have depleted arginine from medium or plasma using enzymes that catabolize arginine. Miraki-Moud et al use pegylated arginine deiminase (ADI-PEG 20), a pegylated formulation of a mycoplasma-derived arginine deiminase, which converts arginine to citrulline. Treatment of 38 primary AML blast samples in vitro with ADI-PEG 20 depleted arginine from the blasts and the medium. One-half (19 of 38) of these samples had greater caspase cleavage and annexin positivity than controls, suggesting apoptosis as a mechanism of cell death, although there was heterogeneity with regard to the extent of these effects.

Xenografts of 6 samples of primary AML blasts with poor prognostic cytogenetic characteristics were created in immune-deficient mice and served as separate experimental groups to investigate the in vivo effects of arginine depletion on AML blasts. Weekly intraperitoneal injections of ADI-PEG 20 depleted plasma arginine with a corresponding increase in citrulline. This

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5. Tijchon E, Havinga J, van Leeuwen FN, Scheijen B. Melanoma, hepatocellular carcinoma, renal cell carcinoma, glioblastoma, and prostate cancer. This has been linked to deficiency of ASS1 expression in primary tumors of the same types from which the deficient cell lines were derived. This is in contrast to normal tissues, in which the enzyme is expressed ubiquitously. The authors determined that 28 of 30 AML bone marrow trephines were negative for immunohistochemistry staining of ASS1. Immunoblotting of lysates from 19 AML samples indicated that only 2 strongly expressed ASS1. This is in contrast with strong expression in samples from 2 patients with acute promyelocytic leukemia and 10 samples from lymphoma patients who lacked marrow involvement. Real-time polymerase chain reaction analyses of primary AML samples were consistent with these results. The finding that 7 of 7 samples from AML patients expressed normal levels of ASL, the final enzyme in the pathway, indicated low ASS1 as the cause of the lesion in arginine production in AML. Additional studies demonstrated that the ASS1 promoter was methylated in 21 of 28 samples. A similar finding had previously been reported for lymphoma cell lines,7 suggesting a common epigenetic mechanism of ASS1 silencing. Tumor cell lines that lack the ability to synthesize arginine initiate autophagy and/or apoptosis on arginine deprivation.8 Experimental investigations and clinical trials have depleted arginine from medium or plasma using enzymes that catabolize arginine. Miraki-Moud et al use pegylated arginine deiminase (ADI-PEG 20), a pegylated formulation of a mycoplasma-derived arginine deiminase, which converts arginine to citrulline. Treatment of 38 primary AML blast samples in vitro with ADI-PEG 20 depleted arginine from the blasts and the medium. One-half (19 of 38) of these samples had greater caspase cleavage and annexin positivity than controls, suggesting apoptosis as a mechanism of cell death, although there was heterogeneity with regard to the extent of these effects.

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was associated with significant decreases in AML cells in the bone marrows of 3 of the 6 groups. Examination of marrow sections showed that the mouse cells expressed ASS1, whereas the human AML blasts did not, supporting the conclusion that differential ASS1 expression is the basis for selectivity. In support of this point was the finding that sorted healthy human marrow cell populations with phenotypes of hematopoietic stem cells (CD34+CD38-) or progenitors (CD34+CD38-) were enriched in ASS1 mRNA expression relative to lymphocytes or cells with other expression patterns (CD34-CD3-CD19-). In particular, ASS1 expression was greater than that of the AML blast samples sensitive to ADI-PEG 20 treatment in vitro.

Administration of subcutaneous cytarabine for 10 days alone, which approximated a low-dose 20-mg twice daily regimen, decreased tumor presence in bone marrow after 4 weeks in 4 of 6 xenograft groups. Combination treatment with ADI-PEG 20 and subcutaneous cytarabine significantly lowered marrow blasts in all the xenografts, some of which failed to respond to either agent alone. When AML blasts were treated with the combination in vitro, synergy in cell killing was demonstrated in 3 of 3 samples.

This report finds that the expression of ASS1 is heterogeneous in AML disease populations. Comparisons of the efficacy of immunologic and reverse transcriptase-polymerase chain reaction assays of ASS1 levels indicated that the latter was the more sensitive candidate for developing a biomarker to identify AML samples that may be sensitive to arginine deprivation. Questions regarding the mechanisms of cell killing by arginine deprivation alone and how it augments active agents such as cytarabine await more definitive answers that likely will vary with disease type and accompanying agents; so too does the understanding of mechanisms by which tumors will become resistant to this approach. More than a dozen clinical trials testing arginine deprivation using ADI-PEG 20 have been initiated, mainly in solid tumors and as a single agent.8 The information in this paper provides a compelling rationale to initiate clinical trials that will determine whether a cohort of AML is truly addicted to arginine.

Conflict-of-interest disclosure: The author declares no competing financial interests.

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Comment on Lorenz et al, page 4069

CLEC-2: the inside story

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In this issue of Blood, Lorenz et al elucidate the mechanisms by which antibody-mediated targeting of platelet C-type lectinlike receptor 2 (CLEC-2) induces receptor downregulation and thrombocytopenia. This information is important because antibody-mediated targeting of CLEC-2 may have therapeutic utility as antithrombotic therapy, especially if thrombocytopenia can be avoided.1

Excessive platelet aggregation at sites of atherosclerotic plaque rupture can result in formation of pathological thrombi that reduce or obstruct blood flow to downstream tissues and cause tissue ischemia or infarction.2 Consequently, antiplatelet drugs have been developed for treatment of acute thrombotic events. Platelet activation and aggregation at sites of vessel damage is, however, also normally required for cessation of bleeding. It is therefore not surprising that antithrombotic therapies that target platelets are often associated with a disconcertingly high risk for bleeding. The search therefore continues for antithrombotic therapies that do not cause bleeding.

The multiple cell surface receptors that are capable of activating platelets fall into 2 main categories depending on whether they signal through activation of heterotrimeric G proteins, such as Goq or Goi, or nonreceptor tyrosine kinases (NRTKs) such as Src-family kinases (SFKs) and spleen tyrosine kinase (Syk).3 The major platelet G-protein–coupled receptors (GPCRs) include the P2Y1 and P2Y12 receptors for adenosine S′-diphosphate (ADP), the protease-activated receptors (PARs) for thrombin (PAR1 and PAR3 or 4), and the thromboxane/prostaglandin endoperoxide receptor for thromboxane A2. Antiplatelet agents that target the major GPCRs are currently in use, and an elevated risk for bleeding is a well-known side effect associated with each of them.4 The major NRTK-coupled platelet-activating receptors include the glycoprotein VI (GPVI)/Fc receptor γ-chain (GPVI/FcRy) collagen receptor complex, the C-type lectinlike receptor for podoplanin, CLEC-2, and (in humans) the low-affinity receptor for the Fc portion of the immunoglobulin γ heavy chain, FcγRIIA.5 The first 2 of these receptors represent especially interesting targets for antithrombotic therapy because knockout mice whose platelets fail to express either the GPVI/FcRy complex or CLEC-2 exhibit...
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