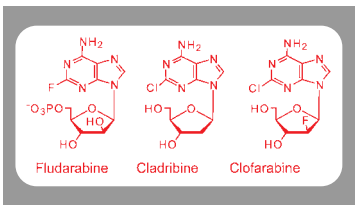
PNAs for AML

The introduction of the purine nucleoside analogs (PNAs) including fludarabine, pentostatin, and cladribine, either alone or in combination with other agents, has produced durable remissions and cures of patients with the chronic lymphoproliferative disorders chronic lymphocytic leukemia and hairy cell leukemia.

These successes as well as preclinical studies suggested that the PNAs may also have a role in the treatment of acute myelogenous leukemia (AML). Synergistic interaction between the PNAs and the cytosine nucleoside analog, cytarabine, an active agent in AML, has also been demonstrated.



Cladribine has been shown to produce a complete remission in 24% of newly diagnosed pediatric AML cases in a study by Krance et al.[1] However, this activity was not duplicated in adults with AML in a study by Gordon et al[2] where no complete remissions were achieved in 15 patients with relapsed or refractory AML and prolonged myelosuppression was noted. Fludarabine has been combined with cytarabine with or without the addition of granulocyte-colony stimulating factor (G-CSF) and/or an anthracycline, but retrospective studies have not clearly demonstrated an advantage of fludarabine-cytarabine regimens over traditional anthracycline-cytarabine combinations.[3]

Clofarabine (Cl-Fara-A, 2-chloro-2′-fluorodeoxy-9-β-D-arabinofuranosyladenine) was synthesized over 10 years ago as a second-generation PNA to overcome some of the limitations and incorporate the best qualities of both fludarabine and cladribine. Clofarabine is structurally identical to cladribine except for the substitution of fluorine for a hydrogen atom at the C-2′ position of the arabinofuranosyl moiety. A phase 1 study by Kantarjian et al[4] of clofarabine established a phase 2 dose of 40 mg/m^2 intravenously over 1 hour for 5 days for patients with acute leukemia. In this issue, Kantarjian and colleagues (page 2379) from MD Anderson Cancer Center report on a phase 2 trial of clofarabine in a group of 62 patients with relapsed and refractory AML (n = 31), myelodysplastic syndrome (MDS; n = 8), chronic myelogenous leukemia in blastic phase (CMLBP; n = 11), and acute lymphocytic leukemia (n = 12). Complete remissions were achieved in 20 patients (32%) and 9 patients had a complete remission without platelet recovery (CRp). In AML, higher responses were seen in patients with longer first complete remissions (7/8, 87%) and in patients in second or subsequent relapse (8/12, 67%). Responses were seen in 50% or more of patients with MDS and CMLBP but in only 2 of 12 patients with ALL. Severe but reversible liver dysfunction was noted in 15% to 25% of patients. The accumulation of clofarabine triphosphate in blast cells was greater in responding patients than nonresponders. This study suggested that clofarabine has significant activity in myeloid disorders. However, in contrast to these encouraging results, a recent multi-institutional trial of clofarabine by Foran et al[5] produced only 1 CRp out of 29 evaluable patients.

In summary, the jury is still out on the ultimate role of PNAs in the therapy of AML in adults. Continued study of clofarabine and other PNAs in combination with other active agents should clarify their role in the treatment of patients with AML. Ultimately, to use a time-worn phrase, randomized trials will be required to definitively establish the role of these agents in AML treatment.

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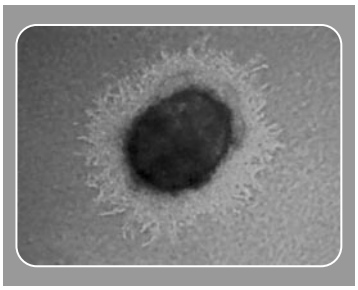
Putting a Hex on blood and endothelium

Homeobox genes are a large family of transcriptional regulators that confer positional identity along the anterior-posterior body axis. Several homeobox genes play roles in blood cell proliferation and in leukemogenesis. *Hex* is a homeobox gene that has previously been shown to be an early marker of anterior-posterior asymmetry in the vertebrate embryo.[1] It was also found to be transiently expressed in yolk sac blood islands.

The intimate temporal and spatial association of the vertebrate embryo's first blood cells with endothelial cells in blood islands of the yolk sac has long suggested that these 2 tissues can arise from a common hemangioblast precursor. This concept has been supported by the coexpression of many genes in blood cells and endothelium and by the functional role of genes such as *SCL*, *LMO-2*, and *flk-1* both in vascular and hematopoietic development. While direct evidence for a hemangioblast cell in the embryo is still lacking, a growing body of work with cultures of murine embryonic stem cells has defined a precursor, termed blast colony-forming cell (blast-CFC), that has both hematopoietic and endothelial cell potential.[2] The in vitro culture of embryonic stem cells as embryoid bodies has become a powerful model system for the investigation of early embryonic events, particularly the transition from epiblast cells to mesodermal fates. Several genes, including *flk-1*, *SCL*, and *Runx-1* are important for blast-CFC development in embryoid bodies but little is known about other genes that regulate their subsequent differentiation into hematopoietic and vascular lineages.[3]

In this issue, Guo and colleagues (page 2428) report that Hex plays a functional role in the initial development of the hematopoietic and vascular systems. Investigating embryoid bodies derived from murine embryonic stem cells lacking Hex expression, the authors show that there is a significant drop in definitive hematopoietic precursors as well as defects in endothelial cell outgrowth. Interestingly, there was no reduction in primitive erythroid progenitors, indicating that Hex joins the ranks of transcriptional regulators, including Runx-1 and c-myb, that differentially regulate primitive versus definitive erythropoiesis.[4] Unlike Runx-1,[5] deficiency of Hex does not reduce



blast-CFC numbers, indicating that Hex does not function at the level of the hemangioblast. These results point to a role for Hex in the differentiation of definitive hematopoietic cells and of endothelial cells and provide further indirect evidence that both of these lineages can arise from a common hemangioblast precursor.

Finally, the authors determine that Hex-deficient embryonic stem cells do not fully contribute to the lymphoid and myeloid cell compartments in adult chimeric mice. Are the roles of Hex in blood and endothelium related to a common embryonic precursor? Are the functions of Hex in embryonic and adult hematopoiesis similar? The answers to these and other vexing questions await future studies of Hex.

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T-cell differentiation: Notch another step

The idea that the Notch receptor family might play a role in hematopoiesis was first suggested when an activated form of the receptor was noted in a rare subset of childhood acute lymphoblastic leukemia (ALL). Since that time Notch and its associated family of membrane-bound ligands have been implicated as key modulators of differentiation virtually throughout the hematopoietic cascade. Decision points influenced by Notch have been defined at the stem cell level with self-renewal versus differentiation, in the myeloid lineage with macrophage versus dendritic cell differentiation, and at multiple steps in lymphoid differentiation including T- versus B-cell commitment. Understanding how Notch affects individual steps in the complex sequence of T-cell differentiation requires isolating these events, separating them from cumulative preceding effects of Notch activation.

García-Peydró and colleagues (page 2444) have provided new insight by doing just that, taking subsets of human thymocytes, transducing them with an activated form of the Notch1 receptor, and replacing them in an ex vivo thymic culture that permits further differentiation. They specifically studied the events occurring before antigen-mediated positive or negative selection. This interval in T-cell differentiation involves a complex series of steps with limited known external influences. Among these steps is whether the T-cell receptor ultimately expressed on the mature T cell will bear the dimeric alpha and beta chains or the gamma and delta chains, a choice that substantially impacts T-cell function. García-Peydró et al demonstrate that Notch1 activation induces significant favoring of the gamma-delta T-cell receptors, skewing differentiation fate prior to antigen specification. Thus, Notch signaling is capable of strongly influencing T-cell outcome prior to an impact of antigenic context. By favoring the gamma-delta cell population, Notch increases a set of lymphocytes associated with antitumor immunity and autoimmune phenomena.

While these studies pose an additional potential role for Notch in the composition of the adaptive immune system, they leave open the question of whether and how Notch is activated in physiologic settings to influence immune function. There is now no question that Notch has T-cell differentiation stage–specific effects; a concept furthered by the elegant experiments of García-Peydró et al. But how Notch ligands are expressed within the microenvironments in which T-cell differentiation is occurring, whether expression of these ligands is modulated, and whether thymocytes in vivo are responding to Notch ligand–mediated cues are yet to be fully elucidated. Armed with greater understanding of where in the differentiation process Notch may play a role, the spade work can now begin of defining just where interventions involving Notch-ligand interactions can make a difference and whether this is indeed a molecule to target for interventions in immunologic disease.

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ALK in NHL: To B (cell) or not to B (cell)? Characterization of the entity "ALK$^+$ DLBCL"

Expression of anaplastic lymphoma kinase (ALK) fusions in non-Hodgkin lymphoma (NHL) has been considered by many investigators to occur only in T or null cell CD30$^+$ anaplastic large cell lymphomas (CD30$^+$ ALCLs).[1-3] Although a significant