dangerous gene alterations in immunocompetent patients with DLBCL (Pasqualucci et al).

In this issue, Gaidano and colleagues (page 1833) report similar somatic gene changes (mainly point mutations with infrequent nucleotide deletions or insertions) in regulatory and occasionally coding regions of several proto-oncogenes of NHL cells from patients with AIDS. They describe clonal abnormalities in one of these genes in approximately 50% of cases and in two genes in approximately 25% of patients. The point mutations identified had characteristics consistent with those occurring during SHM and similar to those previously defined in patients with DLBCL and intact immune systems. Thus, mistargeting of the adaptive SHM mechanism appears to represent a general phenomenon leading to clonal deregulation and lymphoma. Since these clonal abnormalities may have a broader spectrum than those initially reported in NHL patients without AIDS, this maladaptive process may be even more common in the setting of immune compromise.

The SHM process involves expression of the activation-induced cytidine deaminase gene, which is necessary and sufficient for this process (Muramatsu et al, Cell. 2000; 102:553-563). Although not analyzed in the studies of Gaidano et al, others recently reported the expression of activation-induced cytidine deaminase in NHL (Greeve et al, Blood. 2003;101:3574-3580). SHM and activation-induced cytidine deaminase may be linked to lymphomagenesis by generating double-strand DNA (dsDNA) breaks, which could initiate chromosomal translocations (Kuppers and Dalla-Favera, Oncogene. 2001;20:5580-5594) and the mistargeted and dangerous mutations described by Gaidano et al and Pasqualucci et al.

Thus, the seminal observations of the Dalla-Favera laboratory implicate aberrant targeting of the SHM process in the initiation of several aggressive B-cell lymphomas. Since the occurrence, over time, of new mutations in the same proto-oncogenes was occasionally identified, this mistargeting may also lead to the accumulation of additional genetic lesions and to the evolution of even more aggressive disease. If so, the SHM process and activation-induced cytidine deaminase may be therapeutic targets to limit lymphoma progression.

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Ara-G Fas L–itates T-cell death

Irreversible DNA damage is the hallmark of traditional cytotoxic chemotherapy. Antimetabolites exert their impact through interrupting the DNA replication and repair processes that are required for normal cellular function. Unfortunately, these processes are necessary for both normal and malignant cells and have resulted in fairly narrow therapeutic indexes for many agents. Efforts to find selectivity have focused on differences between normal and malignant cell populations in terms of rates of proliferation, regulation of apoptosis and cell cycle, and ability to repair DNA damage. However, the redundancy of these critical growth and survival pathways in all cell types continues to thwart our abilities to discriminate and target the malignant clone.

Rodriguez and colleagues (page 1842) offer an important model to better understand the mechanisms of arabinosylguanine (ara-G)–induced lymphocyte cell death, specifically the death of T cells, and they have shed important light on the mechanisms that contribute to the drug's selectivity. Provocatively, ara-G works by at least 2 complementary mechanisms. One is the classical inhibition of DNA synthesis for all nucleoside analogs. Ara-G also exploits a unique feature of T-cell biology, the autoregulation (in part through Fas/FasL-induced apoptosis) that is fundamental to the development of a normal, functioning immune system. However, the authors' insight creates important new questions needed in order to further exploit and broaden the applicability of these findings: (1) Does ara-G trigger expression and/or liberation of multiple proapoptotic factors by additional mechanisms? (2) Do other nucleoside analogs trigger similar bystander effects? How? Is this also restricted to T cells or other lineages as well? (3) How can the selectivity of T-cell death through ara-G be translated clinically?

T-cell malignancies and inherited disorders remain a small fraction of malignancies, even within their respective rare disease categories. Our bias is that broader application of this finding may be found as a treatment approach for other T-cell regulation disorders such as autoimmunity and graft-versus-host disease. We also suggest that ara-G's full potential will be realized through the development of rational combinations and sequences of drugs that exploit discrete pathways in selective and complementary fashions.

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AML: clustering genes to predict outcome

Acute myeloid leukemia (AML) is a heterogeneous group of hematopoietic malignancies with diverse genetic abnormalities and phenotypes. Currently, treatment decisions are based on the French-American-British (FAB) classification scheme, which uses largely morphologic characteristics, as well as immunophenotyping and cytogenetic analyses to identify different subtypes of the disease associated with better or worse prognosis. In this issue, Yagi and colleagues (page 1849) have used microarray-based assays to identify gene expression patterns that correlate with prognosis in a collection of pediatric AML patients. The authors assayed the expression of more than 12 000 genes in bone marrow and blood samples and used various data analysis methods to identify groups, or clusters, of patients with distinct phenotypes. Although the study was performed with only 54 patients divided amongst several FAB subgroups, the results have several important implications for the development of new prognostic tests and for the analysis of microarray data in patient samples. First, the simplistic approach of

hierarchical clustering was unable to distinguish groups of genes that could predict outcome. However, by using more powerful statistical approaches, the researchers were able to identify a set of 35 genes that were highly predictive for good or bad prognosis. The list includes regulators of cell cycle and apoptosis that could be targets for novel therapeutic agents.

Another interesting finding reported by Yagi and colleagues concerns the relationship between the standard FAB classifications and the gene expression data. Although the FAB subtypes are relatively good predictors of prognosis, when gene sets that correlated with the FAB subtypes were identified, the resulting gene lists were poor predictors of outcome, suggesting that the FAB subtypes and the gene expression profiles measure fundamentally distinct features of the leukemic cells that are difficult to compare. The findings raise interesting questions about the relationship of genetic and morphologic indicators and suggest that microarray-based approaches will open new avenues in the treatment of AML.

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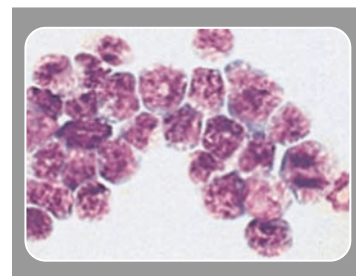
More is not always better

The t(15;17) is present in more than 95% of patients with acute promyelocytic leukemia (APL). This translocation generates 2 fusion proteins: promyelocytic leukemia–retinoic acid receptor alpha (PML-RARα) and RARα-PML. To understand the development of APL and to search for more effective treatments of this disease, several PML-RARα APL transgenic mouse models have been generated using myeloid specific regulatory elements, such as cathepsin G and myeloid-related protein 8 (MRP8), to direct PML-RARα expression into early myeloid cells. All of these PML-RARα mice develop a myeloproliferative syndrome early in life, and 15% to 20% of these mice develop an APL-like disease after 6 to 14 months (see Grisolano et al, Blood. 1997; 89:376-387; Brown et al, PNAS. 1997;94: 2551-2556; and He et al, PNAS. 1997;94: 5302-5307). Additional expression of the t(15;17) reciprocal fusion protein RARα-PML can increase the percentage of APL in transgenic mice by 4-fold. However, the long latency remains. These results indicate that PML-RARα is necessary but not sufficient for APL development. Additional mutations are probably required for leukemia to occur.

The current explanation of how PML-RARα is involved in leukemogenesis centers on the dominant-negative effect of PML-RARα. PML-RARα contains the DNA and retinoic acid (RA) ligand-binding domains of wild-type RARα. In the absence of a ligand, both RARα and PML-RARα repress transcription due to the interaction with nuclear receptor corepressor/silencing mediator for retinoid and thyroid receptor (NCoR/SMRT) and form complexes with histone deacetylase (HDAC). Removing acetyl groups by HDACs increases the positive charge on proteins and enhances protein interactions with negatively charged DNA to keep chromatin in a more compacted confirmation, which does not favor the initiation of transcription. Furthermore, removing acetyl groups may also enhance the binding of repressor proteins. In the presence of physiologic concentrations of RA, RA binds to RAR and changes the conformation of RARα, replacing the HDAC complex with transcription coactivators, including histone acetylase (HAT) complex. Acetylation of histones changes chromatin structure to favor gene expression. The PML portion of PML-RARα contains the oligomerization domain of PML. The oligomerized PML-RARα forms a more stable complex with NCoR/SMRT and HDAC. Dissociation of this complex requires a much higher concentration of RA. This theory explains why a high dosage of RA can be effectively used to treat APL. It also suggests that relatively higher levels of PML-RARα expression may be more effective at initiating APL development.

It has been difficult to detect PML-RARα expression in the above mentioned PML-RARα transgenic mice. Therefore, Westervelt and colleagues (page 1857) hypothesized that increasing the expression of PML-RARα may enhance the penetration of APL development in transgenic mice because the upstream fragment of the human cathepsin G used in their previously reported PML-RARα transgenic mice may lack critical regulatory elements required for high-level transgene expression. In order to fully capture cathepsin regulatory elements to express PML-RARα in early myeloid cells, Westervelt et al generated another PML-RARα mouse model by knocking the PML-RARα cDNA into the cathepsin G 5′ untranslated region. In contrast to the 15% to 20% penetration of APL in previously



reported PML-RARα transgenic mouse models, more than 90% of PML-RARα knock-in mice developed APL, although the latency was similar to other transgenic models. These results suggested that the new knock-in model provides a level of PML-RARα expression that is more optimal for APL development, and most of us would have predicted that this level would be higher. However, when real-time reverse transcriptase–polymerase chain reaction (RT-PCR) analysis was used to compare the expression of PML-RARα in bone marrow cells and APL cells from this knock-in model (vs their human cathepsin G–PML-RARα transgenic mouse model) it was surprising to discover that PML-RARα was expressed at an extremely low level in the knock-in mice—less than 3% of the expression in the transgenic mice. This result goes directly against the original hypothesis (based on the dominant-negative effect of the interaction of PML-RARα with the HDAC complex) that more PML-RARα expression will enhance the development of