et al.[21] in the current issue represents a significant step forward in this chain as it links biology to prognosis by showing that lower risk non-del(5q) MDS patients with *RPS14* haplo-insufficiency tend to have a prolonged survival. Be that as it may, the fact remains that defective ribosomal biogenesis, a previously unsuspected mechanism, is emerging as a surprise contender for the lead role in disrupting erythropoiesis in a variety of anemias. An entirely original subject of research has exploded on the MDS scene, one which links congenital and acquired anemias, and which is likely to produce exciting biological insights in the future, especially when combined with the emerging improved technology.

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NG and AR contributed equally to this work which was supported by grants from The Partnership for Cure and the M. Night Shyamalan foundation.

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Mutations of *NOTCH1*, *FBXW7*, and prognosis in T-lineage acute lymphoblastic leukemia

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Acute lymphoblastic leukemia (ALL) is the commonest childhood malignancy,[1] and a major cause of morbidity and mortality from hematopoietic malignancies in adults. While the outcome of treatment for ALL in children is generally favorable, with cure rates exceeding 80%, this is not the case in adults, given that relapse occurs in the majority of patients and carries a poor prognosis. Moreover, improvements in the out-

come of therapy of T-lineage ALL, which accounts for approximately 20% of cases of ALL, have lagged behind those of B-progenitor ALL. Consequently, there is great interest in identifying clinical, laboratory and genetic markers that may distinguish patients likely to be cured, who require less intensive treatment, from patients at high risk of relapse, who require aggressive or novel therapies.

From the genetic standpoint, T-lineage ALL is a heterogeneous disease and comprises a number of different subtypes harboring a range of sentinel chromosomal rearrangements, DNA copy number alterations, and point mutations. ALL has long been well-characterized at the cytogenetic level, and recurring chromosomal rearrangements observed in T-ALL include translocations juxtaposing regulatory elements at the T-cell antigen receptor gene loci (*TRB@* and *TRG@* at chromosome 7, and *TRA@* and *TRD@* at chromosome 14) to T-lineage transcription factors including *TAL1/SCL*, *LYL* and *HOX11*. Additional rearrangements include the *CALM-AF10* fusion, an interstitial deletion at chromosome 1p resulting in the *SIL-TAL1* fusion, and rearrangements of *MLL*.[2] It has been known for many years that submicroscopic DNA copy number alterations are also common in T-ALL, most notably deletion of the *CDKN2A/B* tumor suppressor locus, which is present in up to 75% of T-ALL cases.[3] The advent of high resolution single nucleotide polymorphism array and array-comparative genomic hybridization profiling of DNA copy number alterations has led to the identification of a number of additional lesions in T-ALL, including deletions of *PTEN*, *RB1*, deletions upstream of (and resulting in overexpression of) *LMO2*,[3,4] amplification of the oncogene *MYB*,[3] and amplifications of chromosome 9 accompanying fusion of *ABL1* (*NUP214-ABL1*).[5]

Although genome-wide analysis of DNA sequence variation has not yet been performed in T-ALL, several mutations have been identified by candidate gene analysis, most notably mutations involving *NOTCH1*, which result in ligand independent activation of this key regulator of T-cell fate,[6] and *FBXW7*, which encodes an E3 ubiquitin ligase whose functions include negative regulation of NOTCH1 signaling.[7] More recently, additional mutations have been identified in *PTEN*,[8] *JAK1*[9] and *WT1*.[10] *NOTCH1* mutations are especially common, occurring in over 50% of both pediatric and adult T-ALL cases, while *FBXW7* mutations are found in up to 20% of T-ALL cases. Thus, genetic lesions targeting multiple cellular pathways including T-lymphoid development, tumor suppression and cell cycle regulation, as well as PI3K/AKT signaling appear to be central events in the pathogenesis of T-ALL. As therapy fails in a substantial proportion of patients with this disease, a logical question is which, if any, of these lesions influence prognosis, and may be incorporated into clinical testing.

In a study published in this issue of the Journal, Baldus *et al.*[11] examined *NOTCH1* and *FBXW7* mutational status in adult T-ALL and investigated the relationship of these genetic lesions with outcome. This study is informative on several levels. Baldus *et al.* examined a relatively large cohort (N=126) of uniformly treated adult T-ALL patients. They identified *NOTCH1* and *FBXW7* mutations in 57% and 12% of patients, respectively – similar frequencies to those observed in the more widely studied setting of pediatric T-ALL. While multiple novel mutations were identified, the types of mutations are similar to those previously observed in children (for example the heterodimerization, PEST, and transactivation domains of *NOTCH1*) suggesting that the genetic pathogenesis of T-ALL is likely to be similar in children and adults. The authors then carefully examined associations between these mutations and clinicopathological variables and outcome. The findings here were largely negative. Overall, no significant association was observed between *NOTCH1/FBXW7* status and outcome (as assessed by remission rates, relapse rates, or event-free survival).

The authors then examined the relationship between these mutations and the maturational stage of the leukemias, and gene expression data. Notably, they observed that *NOTCH1/FBXW7* mutated cases had a more mature (thymic) immunophenotype, and *NOTCH1/FBXW7* wild-type cases more commonly had an immature CD4/CD8 double negative immunophenotype. They also examined the relationship of *NOTCH1/FBXW7* status and expression of two genes associated with prognosis in acute myeloid and lymphoblastic leukemia – *ERG* and *BAALC*. Elevated expression of *ERG* and *BAALC* has been associated with poor outcome in both ALL and acute myeloid leukemia.[12-14] Here the authors found a trend to an association between lack of *NOTCH1/FBXW7* mutations and poor outcome in the *ERG/BAALC* low expression/low risk group, although the numbers of patients in this stratified analysis were small.

What conclusions may be drawn from this analysis, and what role, if any, does mutational testing have in the clinical management of T-ALL? Several other recent studies have examined associations between *NOTCH1/FBXW7* status and T-ALL outcome and the results are mixed, with some studies observing associations with outcome, but others showing no or weak associations.[15-20] Many of these studies were relatively small. There would thus seem little justification in testing for these mutations in routine clinical care outside of the setting of trials designed to rigorously analyze associations with outcome. Moreover, these results should not be viewed in isolation. While many studies have now examined either cytogenetic alterations, DNA copy number alterations and/or mutational status of individual genes, studies comprehensively examining all known types of genomic alterations in adequately powered cohorts of T-ALL cases have not been performed. These are now urgently required to define the full complement of genetic alterations in T-ALL, and to examine associations between outcome and mutations targeting not just individual genes, but key pathways in leukemogenesis.

One of the most interesting findings of the study by Baldus *et al.* is that *NOTCH1/FBXW7* status is associated with the maturational stage of the leukemic cells and the expression of genes previously associated with leukemia outcome: *ERG* and *BAALC*.[12-14] The authors suggest that the degree of leukemic cell maturation may be intimately associated with susceptibility to chemotherapy, and this is supported by recent data demonstrating that the

presence of a very immature (early T-cell precursor) immunophenotype defines a subtype of T-ALL with a characteristic gene expression signature and extremely poor outcome.[21] These cases are characterized by genomic instability, although a unifying causative genetic alteration has not yet been identified. Nonetheless, elevated expression of genes including *ERG* is a feature of the gene expression profile of these *immature* T-ALL cases. Together, these findings suggest that specific genetic alterations arresting differentiation in T-ALL render leukemic cells less susceptible to chemotherapy, and that these lesions are accompanied by a characteristic gene expression signature (rather than just isolated elevated expression of *ERG* and *BAALC*). Similar observations have been made in B-progenitor ALL, in which mutation of the early lymphoid transcription factor IKAROS (*IKZF1*) is also associated with an *immature* gene expression profile, and poor outcome.[22] Moreover, these findings emphasize the importance of ongoing, intensive efforts to completely characterize all genetic mutations in this type of leukemia. Such a detailed understanding of the genetic landscape of ALL will be essential not only to identify prognostic markers, but also to identify rational targets for novel therapeutic intervention in this disease.

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