Gene-expression profiling has provided a powerful means for the identification of novel prognostic factors in malignancies. However, lack of widespread availability, challenging data analysis, and high cost have been barriers to the adoption of microarrays in routine clinical use. Application of reverse transcription–polymerase chain reaction (RT–PCR) presents a potential solution to these difficulties by focusing on a subset of genes with high predictive ability.

Our work1 and that of others2,3 has uncovered a strong role for immune signatures in the pathogenesis and progression of follicular lymphoma. Two immune signatures, one reflecting expression from T cells and the other reflecting expression from macrophages, have been shown to be associated with survival. The current study validates those findings and extends them by providing a valuable clinical tool that can be applied at the time of diagnosis.

The nature of immune signatures in follicular lymphoma clearly deserves further exploration. The current study suggests that simply the number of T cells has a strong influence on limiting disease progression. These T cells might represent regulatory T cells described previously.1 The T-cell signature could also signify the degree to which malignant B cells are able to interact normally with T cells. When that ability is lost, malignant B cells proliferate and fewer T cells are seen in the tumor field.

The number of macrophages in the biopsy has been shown to be associated with worse survival in patients with follicular lymphoma.4 The present study confirms those findings. Tumor-associated macrophages have been described in other malignancies as being associated with poorer survival.3 In addition to their immune function, macrophages mediate tissue repair and wound healing by playing a key role in epithelial migration, matrix modeling, and angiogenesis. The same functions are also important in the progression of tumors.

The association between high numbers of macrophages and poor prognosis suggests that the normal functions of macrophages may be subverted by malignancies in a way that promotes tumor progression and metastasis.

A fundamental assumption underlies all the studies that have explored molecular markers of prognosis in patients with follicular lymphoma: treatment does not affect survival. There is growing evidence that suggests a survival advantage for using rituximab in combination with chemotherapy. The impact of rituximab on expression of immune signatures and their prognostic value deserves further study.

This caveat aside, the study is valuable from 2 perspectives. First, it provides a useful method for the clinical translation of gene-expression-profiling results using RT–PCR, which is relatively low in cost and widely available. Second, it provides a practical means to prognosticate the course of disease, in addition to the follicular lymphoma international prognostic index and other clinical variables of outcome, for risk stratification in clinical trials.

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

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Comment on Xiang et al, page 4809; Loriaux et al, page 4788; and Tomasson et al, page 4797

Hitchhikers’ guide to the leukemia genome

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TKs have been implicated in the pathogenesis of diverse malignancies and often serve as excellent drug targets. Partial mutation scanning of the tyrosine kinase in AML has revealed a paucity of new causative abnormalities in a larger background of previously unknown single-nucleotide polymorphisms and somatically acquired passenger mutations that hitchhike a ride with the malignant clone.

Normally, tyrosine kinases (TKs) are tightly controlled signaling proteins that transmit, amplify, and elaborate extracellular signals in a manner that affects proliferation, survival, and differentiation of target cells. TKs that are deregulated as a consequence of somatically acquired mutations are widespread in hematological disorders, particularly chronic myelogenous leukemia, classic and atypical myeloproliferative disorders, acute lymphoblastic leukemia, acute myeloid leukemia (AML), and certain subsets of lymphoma. Activation occurs via 2 principal mechanisms: (1) fusion genes formed as a consequence of genomic rearrangements, and (2) point mutations or small insertion/deletions in regions that encode key regulatory domains. In AML, the latter type of mutation is more common, with KIT or FLT3 mutations being found in roughly 40% of cases. These abnormalities provide proliferative and antiapoptotic stimuli that co-operate with other mutations and gene fusions to produce the AML phenotype. Mutant RAS may provide similar stimuli in a further 10% of cases, but this leaves roughly half of AML cases for which the source of aberrant proliferative signals is completely unknown. Mutations in the other 90 tyrosine kinase genes encoded within the human genome are obvious candidates.

Three reports in this issue of Blood describe detailed analysis of TK genes in AML as part of a resequencing effort totaling more than 30 Mb from 282 patients. Rather than analyzing the entire tyrosine kinase, both studies targeted regions most likely to yield positive results. Tomasson and colleagues sequenced the kinase domains of 26 TKs selected largely on the basis that they were expressed in AML blasts as determined by array analysis (strikingly, the 2 most highly expressed TKs are the ones that are most commonly mutated: FLT3 and KIT). All remaining exons of JAK1 and TYK2 were also analyzed. Loriaux and colleagues looked at 85 TKs, but only sequenced exons encoding the activation loop (AL) and juxtamembrane (JM) domains because the majority of previously known activating TK mutations occur in these regions.

Tomasson and colleagues had constitutional DNA available from all cases and were able to determine that many of the non-synonymous changes were novel polymorphisms. One TYK2 polymorphism showed a different genotype distribution in patients compared...
with controls, suggesting that it may predispose carriers to AML. Only 4 somatically acquired nonsynonymous changes were identified: 2 in JAK1 and 1 each in NTRK1 and DDR1. The functional analysis of the 2 JAK1 mutations, described in the accompanying paper by Xiang and colleagues, indicates that they are not typical activating mutations because they do not transform Ba/F3 cells to growth factor independence; however, both mutants modified and increased signaling in response to upstream stimuli, suggesting (but not proving) that they may be causal. The 2 other acquired mutations were not functionally analyzed.

Loriaux et al found 30 nonsynonymous changes in 22 different genes, including an activating FLT3 mutation described in detail elsewhere1 and a previously described activating MET alteration. Constitutional DNA was not tested, so it unclear how many of the 30 changes were acquired; however, 20 variants were tested in Ba/F3 cells and only a single mutation (in FLT3) was found to be transforming.

Overall, these results are disappointing, at least for TK enthusiasts. Nevertheless, both approaches have limitations—a key TK may be expressed only in AML stem cells and not the great bulk of their progeny, or mutations may occur outside the AL, JM, or kinase domains. Furthermore, abnormalities in samples for which the percentage of blast cells was relatively low may have been missed. All TK exons will thus have to be sequenced in patients with a high blast percentage before the possibility of common recurrent TK mutations in AML can be dismissed. In addition, cytogenetically cryptic TK fusions may also be present, as well as other abnormalities that cannot be detected by exon sequencing, such as relatively large intragenic deletions or splicing abnormalities. Although a combination of approaches will be required to eliminate these possibilities, it is perhaps more likely that the proliferative stimulus, in many cases, will turn out to be provided by alterations in other signaling components.

The 3 studies used conventional Sanger sequencing, but they give a glimpse of the sort of data that is likely to emerge soon from the next generation of sequencers that have the capacity to generate billions of bases per week. These technologies will likely reveal hundreds of somatically acquired changes, only a small subset of which will be causal. Distinguishing the causal driver mutations from the irrelevant passengers may be possible in many cases by identifying recurrent changes in plausible candidate genes. However, high-throughput functional assays will also be required to assess the importance of individual mutations. As a complementary approach, it is interesting to see the application of shRNA screens, which, once adapted to primary cells, may become a powerful means of determining TK requirements for AML and other disorders.2

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