

## EDITORIALS



## Microenvironmental Protection in Diffuse Large-B-Cell Lymphoma

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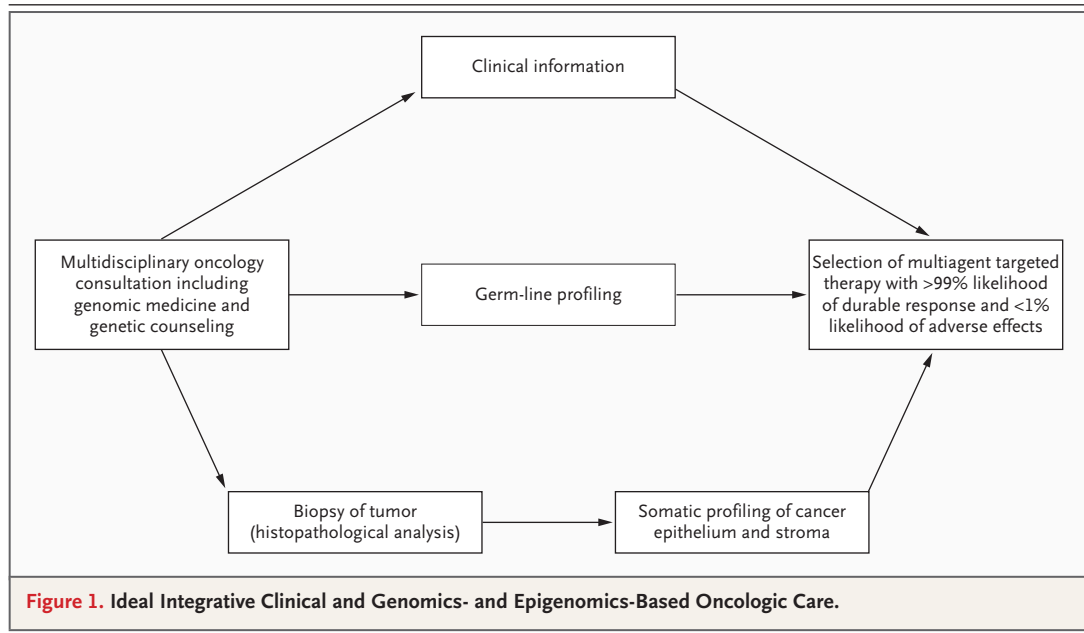
The recent coming of age of genomic techniques and bioinformatics tools has led to an unprecedented proliferation of publications on genome-wide association studies and expression profiling for use in genetic analysis, molecular classification, and prognostication of various diseases. Such reports, however, have often resulted in confusion and, at times, disappointment. The use of expensive methods to subclassify histologic variants of tumors as accurately as \$15 immunohistochemical assays, the inability to replicate more than 98% of published genomewide association studies, and multiple retrospective studies using single-group series with small sample sizes all contribute to these sentiments. For these reasons, the article by Lenz and his collaborators<sup>1</sup> in this issue of the *Journal* is welcome. They found three gene-expression signatures that were associated with survival among patients with diffuse large-B-cell lymphoma. Two different expression profiles, each reflecting different pathways that are characteristic of the stroma (i.e., the tumor microenvironment), differentiated the best prognosis from the worst prognosis.

The combination of cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) is a potential cure for diffuse large-B-cell lymphoma. The addition of rituximab, a monoclonal antibody against the B-cell transmembrane protein CD20, to CHOP (R-CHOP), improves overall survival by 10 to 15%. Although it is phenotypically uniform, diffuse large-B-cell lymphoma is heterogeneous at the molecular level, which led the authors to hypothesize that rituximab improves survival only in certain subgroups of diffuse large-B-cell lymphoma. Ample clues suggest that this postulate should be correct: Among patients with cyto-

genetically defined as well as various molecularly defined subgroups of diffuse large-B-cell lymphoma, the 5-year overall survival rates after CHOP are vastly different,<sup>2,3</sup> and different cell lines of diffuse large-B-cell lymphoma that are exposed to rituximab respond differently.<sup>2</sup>

Lenz and colleagues studied pretreatment biopsy specimens from 181 patients treated only with CHOP or a CHOP-like regimen (the training group) and specimens from 233 patients treated with R-CHOP (the validation group).<sup>1</sup> They looked for new survival-associated expression signatures at the  $P < 0.01$  level in the training group, and they found two, which they called germinal-center B-cell and stromal-1. To refine the signatures, the authors reorganized all the genes in them by hierarchical clustering according to their expression levels. This exercise resulted in five clusters of coordinately expressed genes, which optimized the likelihood that genes within each cluster would be functionally related and have some physiological relevance. When compared with a bivariate (germinal-center B-cell vs. stromal-1) survival model, these five clusters revealed that one of the five signatures was far superior to the others in adding predictive significance to the bivariate model for the R-CHOP cohort. The stromal-1 signature was associated with a good outcome. However, the new signature, which was refined into three gene clusters, one of which the authors call the stromal-2 signature, was found to be highly associated with a poor outcome.

In a trivariate model, Lenz and colleagues found strong associations with overall and 3-year progression-free survival in the R-CHOP cohort. They used the model to generate a survival-predictor score. Model scores were used to divide the



R-CHOP cohort into quartile groups, with 3-year survival rates ranging from 89% in the best survival quartile to 48% in the worst survival quartile and 3-year progression-free survival rates of 84% in the top quartile to 33% in the bottom quartile. Most importantly, the authors showed that the gene-expression model and the International Prognostic Index enhance each other and are in fact, adjunctive tools.

Lenz and colleagues were able to sort the malignant component from the nonmalignant tumor stroma using the CD19 status of the cells. In this manner, they proved that the stromal-1 and stromal-2 gene-expression signatures originated from the stromal component. This is an important differentiation, because the converse finding — that the malignant lymphoma cells were producing mesenchymal and extracellular-matrix markers — would give a (surprising) clue to the pathogenesis of diffuse large-B-cell lymphoma.

That the tumor stroma could affect a neoplasm is not a new concept; it perhaps dates to more than two decades ago.<sup>4</sup> Over the past 8 years, moreover, multiple independent investigations have uncovered genomic and epigenomic alterations in a broad range of solid-tumor stromal cells in breast, colon, and head and neck carcinomas, and even in the stroma of inflammatory bowel disease.<sup>5-10</sup>

It is also obvious that the cells that compose tumor stroma are themselves heterogeneous. For

example, when researchers examined CD10+ breast-tumor stromal cells, which consist of myofibroblasts and rare fibroblast-like cells, only epigenetic, but not genetic, alterations were detected.<sup>10</sup> However, when CD10- tumor stromal fibroblasts were selected, genomic alterations were evident.

Like diffuse large-B-cell lymphoma, stromal alterations in breast and head and neck cancers are associated with clinical outcome. In breast cancer, for example, somatic mutations in the tumor-suppressor gene *TP53* in the stroma, but not in the neoplastic epithelium, have been found to be associated with regional nodal metastases.<sup>8</sup> In the absence of *TP53* mutations in the stroma, loss of heterozygosity at five particular genomic markers, three of which harbor p53 downstream molecules or targets, in the stroma was similarly associated with nodal metastases. Indeed, although *TP53* mutations in tumor stromal fibroblasts accelerate tumorigenesis, they also appear to sensitize tumors against doxorubicin and cisplatin.<sup>11</sup>

In diffuse large-B-cell lymphoma, the stromal-1 signature, which was associated with a favorable outcome, contains genes encoding components of the extracellular matrix and those of the monocytic lineage found in T cells and natural killer cells.<sup>1</sup> In contrast, the stromal-2 signature, which was associated with a poor outcome, contains genes encoding molecules related to angiogenesis. The association of this signature with a poor prog-

nosis thus makes sense. One wonders whether an antiangiogenic agent should be added to the treatment of patients with a lymphoma containing the stromal-2 signature.

The goal of this kind of research is to integrate germ-line, or host genomic and epigenomic information, with corresponding information from the cancer to predict outcome and assess risks in selecting the best therapy with the highest durable response rate and the lowest toxicity (Fig. 1). With the recognition of the importance of the tumor stroma in tumor invasion, disease progression, and clinical outcome, somatic profiling of the neoplasia must now take into account the tumor microenvironment (Fig. 1). The challenge in all these integrative endeavors is that genetic data are only as good as the documentation and annotation of the clinical phenotype and outcome.<sup>12</sup>

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1. Lenz G, Wright G, Dave SS, et al. Stromal gene signatures in large-B-cell lymphomas. *N Engl J Med* 2008;359:2313-23.
2. Brusamolino E, Maffioli M, Bonfichi M, Vitolo U. Front-line therapy for nonlocalized diffuse large B-cell lymphoma: what

has been demonstrated and what is yet to be established. *Future Oncol* 2008;4:199-210.

3. Natkunam Y, Farinha P, Hsi ED, et al. LMO2 protein expression predicts survival in patients with diffuse large B-cell lymphoma treated with anthracycline-based chemotherapy with and without rituximab. *J Clin Oncol* 2008;26:447-54.
4. Bissell MJ, Barcellos-Hoff MH. The influence of extracellular matrix on gene expression: is structure the message? *J Cell Sci Suppl* 1987;8:327-43.
5. Kurose K, Gilley K, Matsumoto S, Watson PH, Zhou XP, Eng C. Frequent somatic mutations in *PTEN* and *TP53* are mutually exclusive in the stroma of breast carcinomas. *Nat Genet* 2002;32:355-7. [Erratum, *Nat Genet* 2002;32:681.]
6. Fukino K, Shen L, Patocs A, Mutter GL, Eng C. Genomic instability within tumor stroma and clinicopathological characteristics of sporadic primary invasive breast carcinoma. *JAMA* 2007;297:2103-11.
7. Weber F, Xu Y, Zhang L, et al. Microenvironmental genomic alterations and clinicopathological behavior in head and neck squamous cell carcinoma. *JAMA* 2007;297:187-95.
8. Patocs A, Zhang L, Xu Y, et al. Breast-cancer stromal cells with *TP53* mutations and nodal metastases. *N Engl J Med* 2007;357:2543-51.
9. Bian Y, Knobloch TJ, Sadim M, et al. Somatic acquisition of *TGFBR1\*6A* by epithelial and stromal cells during head and neck and colon cancer development. *Hum Mol Genet* 2007;16:3128-35.
10. Allinen M, Beroukhim R, Cai L, et al. Molecular characterization of the tumor microenvironment in breast cancer. *Cancer Cell* 2004;6:17-32.
11. Lafkas D, Trimis G, Papavassiliou AG, Kiaris H. p53 Mutations in stromal fibroblasts sensitize tumors against chemotherapy. *Int J Cancer* 2008;123:967-71.
12. Zbuk KM, Eng C. Cancer phenomics: *RET* and *PTEN* as illustrative models. *Nat Rev Cancer* 2007;7:35-45.

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## The Genetics of Speech and Language Impairments

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Without instruction, most children master the complexities of spoken language by the age of 6 or 7 years. About 5% of apparently healthy children, however, struggle to acquire basic competence in one or more aspects of spoken language and are classified as having specific language impairment.

Genetic factors have an important role in many such cases.<sup>1,2</sup> Children with specific language impairment are four times as likely to have a family history of the disorder as are children who do not have such an impairment,<sup>3</sup> and the concordance rate for the disorder is almost twice as great for monozygotic twins as for dizygotic twins.<sup>4</sup> More than 10 susceptibility loci have been identified. More often than not, loci that are robustly linked to specific language impairment in one study show no linkage in other studies, and all these loci have been linked to other neurodevelopmental disorders.<sup>5</sup> Are these

reported associations real? If so, which genes underlie these linkages, and what is their mechanistic effect?

Perhaps one reason that linkage studies have implicated different loci for specific language impairment is that each group of investigators has used different case definitions. Since the subjects are selected in different ways and different measures are used to define language impairment, the discovery of different loci would not be unexpected. Studies of relatively homogeneous groups of children with specific language impairment, whose disorders would appear to have a common cause, would seem to be more likely to yield a robust genetic result. Causes that have been proposed for receptive specific language impairment include deficits in short-term auditory memory, auditory sequencing, and rapid auditory processing.