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Individualizing therapy for the hematologic malignancies: the stuff of genes and dreams

Treatment for hematologic malignancies has traditionally included non-specific cytotoxic agents, such as anthracyclines vinca alkyloids, alkylating agents, and corticosteroids, most often in empiric combinations. In recent years, major advances in treatment have resulted from the availability of novel agents that either target surface antigens or cellular pathways.

Using such agents, strategies can be developed to achieve a major goal of the treatment of hematologic malignancies: to individualize therapy, increasing the likelihood of an efficacious result while minimizing the exposure of patients to unnecessary toxicities of treatments that are unlikely to achieve clinical benefit. This "dream" was first proposed by Paul Erlich as a "magic bullet" that would have a specific affinity for the "parasites" (e.g., microorganisms, cancer cells), but would spare normal tissues.[1] Nevertheless, it took a hundred years until monoclonal anibodies were available for the effective treatment of cancer. The first step was developing hybridoma technology to produce adequate quantities of monoclonal antibodies for clinical use.[2] Next was to identify tumor-specific cell antigens as targets for immunotherapy.[3] Nevertheless, further progress towards individualizing therapy only became possible with an increased understanding of the immunology, biology and genetics of the various malignancies. As a simple example, monoclonal antibodies are selected on the basis of tumors that express a specific antigen. Rituximab, the chimeric anti-CD20 monoclonal antibody, has revolutionized our management of patients with B-cell malignancies. However, its use is primarily restricted to diseases that express the appropriate surface antigen. The antibody exerts its effects through a variety of mechanisms including antibody dependent cellular cytotoxicity (ADCC), complement mediated cytotoxicity (CDC), and induction of apoptosis. ADCC requires binding of the Fc portion of the antibody molecule to the effector cells by the Fcgamma receptor. Patients who are homozygous for 158 valine/valine alleles of FcgammaRIIIa exhibit a higher affinity to human IgG1 which translates into a higher response rate to rituximab treatment than those with a phenylalanine allele.[4] However, the clinical impact of these polymorphisms may be dependent not only on the antibody being used, but the disease status and whether the antibody is being used alone or in combination with chemotherapy 5-7. Thus, we need to gain a better understanding of the complex-

ities of lymphoma biology: why do only some patients who express CD20 respond to rituximab and why do patients become resistant while still expressing the antigen? Several types of CD20 mutations have been identified; however, they were identified in only 22% of cases, and their clinical relevance remains unclear.[8]

One of the most individualized treatment approaches for lymphoma was anti-idiotype vaccines, the idiotype being the only truly specific B-cell marker. Data from Levy and coworkers 9 suggested that patients who developed a cellular or humoral immune response to such a vaccine experienced a longer time to disease progression than those who were unable to mount such a response. These encouraging data led to three randomized clinical trials to test the concept. Unfortunately, the first two to be completed failed to demonstrate any benefit from the vaccine 10 and the future of this technology is uncertain.

A number of lymphoma-associated genes have not only been implicated in the pathogenesis of the diseases, but may provide a therapeutic target. For example, overproduction of the Bcl-2 protein has been shown to be a poor prognostic factor in diffuse large B-cell NHL (DLBCL).[11,12] Nevertheless, Mounier *et al.*[13] demonstrated that the addition of rituximab to the cyclophosphamide, adriamycin vincristine, prednisone (CHOP) regimen (R-CHOP) was able to overcome this resistance to therapy with an improvement in survival for Bcl-2-positive cases; however, there was no benefit adding the antibody to CHOP in Bcl-2-negative patients. Winter et al from the Eastern Cooperative Oncology Group prospectively studied Bcl-6 protein expression determined by immunohistochemistry in samples from 199 patients with DLBCL. Bcl-6 is a transcription repressor required for the generation of germinal centers by B-lymphocytes resulting in immunoglobulin maturation. It also contributes to the germinal center B-cell phenotype of DLBCL. These investigators demonstrated that the addition of rituximab to CHOP improved the failure-free survival of DLBCL patients with tumors that were Bcl-6-negative, but with no effect on the Bcl-6 positive cases.[14] In contrast to other results, these investigators were unable to demonstrate that Bcl-2 expression exerted a negative effect on patient outcome. Winter et al updated and expanded these data at the 10th International Conference on Malignant Lymphoma (ICML) and included an evaluation of p21, a cyclin-dependent kinase inhibitor that is a downstream effector of p53. They also found that rituximab appeared to benefit patients with DLBCL whose tumors were positive for p21 but not those with p21 negative tumors. When combining the various markers, the best outcome was in patients with Bcl-2-negative/p21-positive tumors, while those with Bcl-2-positive/p21-negative patients had the worst outcome. Results with other combinations were intermediate between the two.[15] An international group of investigators included in the Lunenberg Consortium have been evaluating various biologic factors for their ability to improve on the predictability of the International Prognostic Index (IPI).[16] They were able to identify 7 factors with clinical relevance in DLBCL: Ki-67, MUM1, Bcl-2, Bcl-6, CD5, CD10, and HLA-DR expression which, when validated, will be incorporated into a new prognostic system to better predict outcome and, eventually, direct treatment.[17] Biological parameters have also been evaluated in other lymphoma histologic subtypes. For example, DNA microarray signatures may predict patients most likely to respond to rituximab in follicular lymphomas.[18] Recently, Hartmann *et al*.[19] evaluated the expression of 33 genes with potential prognostic importance in patients with mantle cell lymphoma using

quantitative RT-PCR. They identified and validated a 5-gene model to predict survival: the genes included *RAN, MYC, TNFRSF10B, 2*, and *SLC29A2*. They hypothesized that this test could eventually be adopted into a risk-directed strategy for patients with mantle cell lymphoma.

Transcription factors are critical in malignant transformation of cells through activation or repression of downstream target genes. Thus, these factors are potential therapeutic targets since the result of blocking their activity could result in a loss of malignant cells of their advantage in growth and survival. Thus, interfering with Bcl-6 directed pathways could potentially reprogram tumors to exhibit a normal phenotype. A number of small molecules has been generated by Melnick and coworkers that specifically kill BCL-6 positive DLBCL cells through an effect on the BCL6 target genes, but with no effect on control genes. Such agents would be of a high level of interest for clinical trials.[20]

The pattern of gene expression may not only be important as a diagnostic and prognostic technology, but may also direct therapy. Using DNA microarrays, DLBCL can be distinguished into several clinically important subgroups with varying responsiveness to therapy and outcomes.[21,22] For example, Rosenwald and coworkers 21 demonstrated that DLBCL can be distinguished into two main categories based on gene expression profiling, a germinal center B cell (GCB) type and a less favorable activated B cell (ABC) type. The majority of genes that predicted poor survival belonged to the proliferation gene expression signature, more highly expressed in dividing cells. These observations supported the rationale for an infusional chemotherapy regimen such as DA-R-EPOCH which is now being compared with R-CHOP in CALGB 50303. A crucial part of this study will be the DNA microarray results which will hopefully determine whether a gene signature can predict which therapy is more likely to be effective in individual patients.

Monti *et al*.[22] have identified a subset of B-cell receptor (BCR) DLBCLs that demonstrate increased expression of BCL6 transcription factors. BCR DLBCL exhibit spleen tyrosine kinase (SYK) dependent tonic (non-induced) and ligand-induced BCR signaling which may provide a survival pathway for the malignant lymphocytes.[23] This pathway could potentially be exploited to therapeutic advantage. Friedberg and coworkers recently presented their preliminary data from a clinical trial of Fostamatinib disodium, an oral SYK inhibitor.[24] Their series included 68 patients with relapsed or refractory DLBCL (N=23), follicular lymphoma (n=21) and "other" (n=24; 11 cases of SLL/CLL, 9 MCL, 1 lymphoplasmacytic and 1 marginal zone lymphoma). Fostamatinib disodium was well-tolerated; neutropenia was the most prominent serious adverse event. There were no significant gastrointestinal effects and no patient experienced alopecia. Eight patients in the phase 2 portion of the study required dose modification to 150 mg twice daily. The overall response rates were 21% (DLBCL), 10% (follicular lymphoma) and 54% for the SLL/CLL cohort. Based on these encouraging results, further study of this agent are warranted.

Recent attention has also focused on novel agents that target the epigenetic alterations in lymphomas. Inappropriate silencing of genes can result from two potential mechanisms: transcriptional repression by mutated or aberrantly expressed transcription factors, and epigenetic silencing by hypermethylation of tumor suppressor or DNA repair genes. The consequence is the genetic reprogramming of cells to exhibit a malignant phenotype. The cells inherit alterations in gene expression that are not caused by changes in the primary DNA sequence, but which are increasingly being

recognized for their roles in carcinogenesis. These epigenetic changes may involve covalent modifications of amino acid residues in the histones that are wrapped around the DNA, with changes in the methylation status of cytosine bases in the context of CpG dinucleotides within the DNA itself. Methylation of CpG-islands in the promoter regions of genes has been associated with heritable silencing of normal genes. In contrast to genetic alterations, gene silencing by epigenetic modifications is potentially reversible. Agents that inhibit cytosine methylation and histone deacetylation can result in decondensation, demethylation and reestablishment of gene transcription. Thus, DNA methylation and histone modifications are attractive targets for the development and implementation of new clinical strategies. A number of drugs are already in clinical trials to target these mechanisms. Histone deacetylase inhibitors in clinical trials include depsipeptide, vorinostat, panobinostat, and MGCD0103, the first two in T-cell lymphomas, the latter two being most effective in Hodgkin lymphomas.[25-28]

Cancer cells, in general, and lymphoma cell in particular are characterized by defects in programmed cell death, or apoptosis. There are primarily two pathways that lead to apoptosis, an extrinsic pathway triggered by the death receptor domains, and a mitochondrial-based intrinsic pathway that involves a balance between the Bcl-2 family proteins and BAX and other proapoptotic proteins, with activation of caspases resulting in cell death. A number of agents are in clinic trials that are directed at various sites in the apoptotic machinery and which have demonstrated clinical activity. Of note is oblimersen sodium (bcl-2 antisense oligonucleotide) which, when added to fludarabine and cyclophosphamide in relapsed and refractory chronic lymphocytic leukemia, improves the major response rate (complete remission plus nodular partial emission) when compared with chemotherapy alone, with a longer duration of response, and, in fludarabine sensitive patients, has been demonstrated to also prolong survival 29. Other agents with demonstrated activity either *in vitro* or *in vivo* include ABT-263 and YM-155, obatoclax, and AT-101.

To achieve the goal of individualized therapy, it is essential to include laboratory correlative studies along with the therapeutic clinical trials. For example, in the Cancer and Leukemia Group B, tissue microarrays are generated from all patients on study as well as receptor polymorphism analysis for patients with follicular lymphoma on antibody-based trials. Pharmacogenetics are included in other studies as there is increasing evidence suggesting that germline polymorphisms related to the metabolism, transport, therapeutic targets and/or pathways of anticancer agents might help predict therapeutic outcome.

Conclusions

In the past, the diagnosis of a lymphoma was based solely on its morphologic appearance under the microscope. The distinction among various subtypes became possible with the availability of immunohistochemistry. Adding additional features such as genetics, age and even tumor location, the number of subtypes of lymphoma has increased to more than 60 30. Nevertheless, marked differences in response to treatment and outcome are apparent even within a very specific diagnosis. In the future, instead of assigning a diagnosis and, therefore prognosis, using the particular morphologic, immunologic, and clinical features of the lymphoma, the designation will be based on the results of genetic, molecular, and biological studies. Thus, a patient's lymphoma will be defined by the individualized, targeted treatment to which it is most likely to respond.

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