hematopoietic stem cell transplantation (AlloSCT), which is only applicable in a minority of patients, the clinical benefit of these systemic treatments should be carefully balanced against their toxicity and side effects. However, the decision of whether systemic chemotherapy or one of these nonchemotherapeutic systemic agents should be used in these patients is difficult to make, because comparative studies have not been done. The study by Hughes et al fills this gap by comparing retrospectively the efficacy of many chemotherapeutic and nonchemotherapeutic systemic agents in a group of 198 patients with MF and SS.

A particular strength of the study is the selection of time to next treatment (TTNT) as the primary end point. In current trials, the extent of skin disease is evaluated using detailed scoring systems such as the modified severity-weighted assessment tool (mSWAT). However, this measurement tool was only recently adopted and its use is limited in a retrospective study. The TTNT used by the authors provides genuine insight into the durability of responses as assessed in daily practice, and it provides a clinically relevant measure of effectiveness.

Previous studies showed that aggressive systemic chemotherapy in the early stages of MF is associated with considerable morbidity but does not result in increased survival. In the present study, it was found that in patients with only patches and plaques (stages IA–IB), and also in patients with only skin tumors but no extracutaneous disease (stage IIB), α-interferon gave significantly longer TTNT than HDAG and chemotherapy. In addition, in patients with advanced stages of disease with lymph node and blood involvement (stages IVA–IVB), treatment with α-interferon and HDAG resulted in longer TTNT than did systemic chemotherapy. Also when stratified by skin (T) score, α-interferon provided significantly better disease control than chemotherapy for T1 (patches and plaques <10% body surface area), T2 (patches and plaques >10% body surface area), and T4 (erythrodermic disease). Finally, extracorporeal photopheresis was especially effective in erythrodermic (T4) patients.

The relatively favorable clinical results of immunomodulatory agents (PUVA, α-interferon, HDAG, extracorporeal photopheresis) illustrate that immunomodulation can be an effective strategy in controlling disease and are in line with the presence of an active immune response against tumor cells in MF and SS. Indeed, previous studies have shown an active cytotoxic immune response in MF lesions, and rapid progression of disease has been observed in patients who were mistakenly treated with immunosuppressive agents.

In a small number of patients, AlloSCT was performed, resulting in excellent disease control. Recent retrospective studies evaluated AlloSCT results in MF/SS patients and reported an overall survival at 2 years ranging from 45% to 76%, and of those patients, 50% to 80% remained disease free. With further development of safe and effective AlloSCT protocols, it is likely that more MF/SS patients will be selected for this treatment, which makes it even more pertinent to avoid cumulative toxicity from relatively ineffective chemotherapy early in the disease course that may later hinder AlloSCT.

The results from this study confirm that current chemotherapy regimens have modest efficacy in MF/SS, and they argue against the routine use of systemic chemotherapy in patients before immunomodulatory therapies have been used. Based on these results, future prospective studies using more sophisticated clinical staging systems such as mSWAT will be instrumental in further quantifying the therapeutic effectiveness of different treatment regimens. Ultimately, recommendations from these studies should be incorporated into guidelines that can help treating physicians select optimal treatment for patients whose disease can no longer be controlled with SDT.

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Comment on Kolstad et al, page 82

Dendritic cells and lymphoma cells: come together right now

Nina Bhardwaj and Joshua D. Brody

In this issue of Blood, Kolstad et al report an elegantly designed and well-implemented study that showed intratumoral injection of ex vivo-produced immature dendritic cells (iDCs), granulocyte macrophage colony-stimulating...
factor (GM-CSF), and low-dose rituximab into an irradiated tumor elicits objective responses at untreated sites and that these clinical remissions correlate with the induction of tumor-reactive CD8 T-cell responses.\(^1\)

In 1973, Ralph Steinman and Zanvil Cohn at Rockefeller University described a novel and rare leukocyte subset in lymphoid organs that “constantly puts out and retracts small cytoplasmic dendrites.”\(^2\) The unique morphology of these DCs was gradually shown to underlie a unique function: a quantitatively and qualitatively enhanced capacity for presenting antigens to T cells. Studies of these professional antigen-presenting cells (APCs) progressed from in vitro to in vivo, from mice to humans, and from preclinical experiments to clinical trials showing that antigen-pulsed DC vaccination induces antigen-specific T cells in a setting in which antigen alone does not.\(^3\)

Effective preclinical antipathogen DC vaccines motivated the development of anticancer DC vaccines in numerous histologies, including melanoma, prostate, and kidney cancer, some of which yield immune responses and even survival benefit, although DC-based vaccines for lymphoma have particularly demonstrated objective clinical responses, even when used as monotherapy.\(^1,4,7\) Although these small studies similarly reproduce the anticancer effect of DC-based lymphoma vaccines, the differences—and the hopes for greater future success—are in the details. Each approach aims to ultimately present tumor-associated antigens on a sufficient number of activated DCs, although with differing methods for (1) DC production, (2) tumor antigen loading, and (3) DC activation (see figure). Each of these steps can be accomplished ex vivo or in vivo: the latter with pragmatic advantages, and the former with greater opportunities to assess and control each step.

In 2002, investigators from Stanford published final results of a study using ex vivo idiotype (Id)-pulsed, Percoll, and metrizamide gradient-purified DCs in 35 lymphoma patients, demonstrating induction of T-cell proliferative responses to Id protein, as well as complete and molecular remissions.\(^4\) Although most patients mounted anti-Id T-cell responses, there was no clear correlation between T-cell response and clinical benefit. In 2009, investigators from Milan attempted to both obviate the need for antigen identification/purification and broaden the spectrum of target tumor-associated antigens by using ex vivo monocyte-derived (GM-CSF- and interleukin [IL]-4-treated) DCs pulsed with autologous, apoptotic (heat-shock, \(\gamma\)-irradiated, and ultraviolet C-treated) tumor cells. There were 6 partial and complete responses, and these correlated with reductions in peripheral blood regulatory T cells (Tregs) and increases in natural killer cells, although there was no clear relationship between the induction of tumor-specific T-cell responses and clinical benefit.\(^5\) Two subsequent studies from Stanford sought to circumvent the need for ex vivo DC production/antigen exposure by using low-dose radiotherapy to load antigens from dying tumor cells onto the scarce peritumoral DCs followed by intratumoral injections of a Toll-like receptor 9 (TLR9) agonist to activate both these antigen-loaded DCs and possibly residual malignant B cells themselves to function as amateur APCs. These studies demonstrated partial and complete clinical responses, the correlation of in vitro tumor induction of Tregs with worse clinical response, and the potential to achieve more rapid and greater magnitude responses with repeated booster vaccination.\(^6,7\)

Kolstad et al from Oslo University Hospital show the utility of using ex vivo-produced immature monocyte-derived DCs (similar to the Milan approach) but then facilitating tumor antigen uptake and activation of these DCs in vivo at an irradiated tumor site (similar to the recent Stanford approach), along with GM-CSF and low-dose rituximab, to enhance Fc receptor-mediated phagocytosis. The
present study assessed clinical response by standard (International Working Group) criteria excluding the irradiated site and assessed tumor-specific CD4 and CD8 T-cell responses using a straightforward flow cytometry-based proliferation assay after 5-day coculture with the autologous tumor. The authors demonstrate a significant proportion of partial and complete responses (36% of all patients) and—perhaps most significantly—a statistically significant correlation of tumor-specific CD8 T-cell responses with clinical response, in some distinction to the aforementioned studies. The successful correlation of immunologic and clinical responses—if reproducible—represents a major step in accelerating the future of the field, creating opportunity for the use of immune response as a rapidly assessable surrogate end point (eg, comparing multiple iterations of the vaccine maneuver); assessment of vaccine efficacy when clinical end points would be confounded (eg, when used in combination or adjuvant settings); and analysis of mechanism (eg, probing the flow cytometrically defined tumor-reactive T cells for clonality [by high-throughput TRBV sequencing], reactivity to patient-specific genomically determined neoantigens, and expression of costimulatory or checkpoint molecules to suggest rational combination therapies).

The pragmatic consideration of in vivo DC loading demonstrated in this study is also a meaningful advance in the field as it reduces resource intense ex vivo processing to 2 individualized products (in the Stanford DC-Id and Milan DC-apoptotic tumor studies) to 1 individualized product (immature DCs). To further simplify the bringing together of large numbers of DCs with tumor antigens, our group developed a novel iteration of the approach that produces, loads, and activates DCs entirely in vivo. This study (#NCT01976585) uses intratumorally administered Flt3L to increase intratumoral DCs, followed by low-dose radiotherapy and an intratumorally administered TLR3 agonist, to generate tumor antigen-loaded DCs with no ex vivo processing. Preliminary results demonstrated partial and complete responses of patients with bulky and leukemic-phase low-grade lymphoma. A similar approach using Flt3L and a DC-targeting antibody-tumor antigen conjugate is being assessed by the Cancer Immunotherapy Trials Network for patients with advanced stage melanoma (#NCT02129075).

Bringing DCs and tumor antigens together is achievable and—as confirmed now in technically distinct but conceptually similar studies—can induce objective remissions in patients with low-grade lymphoma with a remarkable safety profile. The commendable study by Kolstad et al both fortifies and advances progress in this field. Their approach is both effective in its current form and extremely promising due to its potential for further optimization. Together, these DC vaccine studies improve our understanding of assessing meaningful antitumor immune responses and improve our outlook for safe and effective antitumor immunotherapy for our patients.

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CDK6 is a regulator of stem cells “Egr” to wake up

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In this issue of Blood, Scheicher et al show that cyclin-dependent kinase 6 (CDK6) has a novel role in the regulation of both normal and leukemic stem cells. It acts as a transcriptional suppressor of Egr1, enabling it to control both normal and leukemic stem cell activation.1

Mammalian CDKs are widely recognized for their well-established role in orchestrating the steps of cell-cycle progression.2 CDK4 and CDK6 form a complex with cyclin D to promote the G1 to S phase progression through the phosphorylation of retinoblastoma (Rb) protein and transcription factors with roles in proliferation and differentiation. CDK4 and CDK6 have been found to be aberrantly regulated in many tumor types, including mixed lineage leukemia (MLL)-rearranged leukemia,3,4 positioning potential CDK4/6 as therapeutic targets.4 Loss of CDK6 produces defects in hematopoietic cell proliferation and minor anemia.5 Quiescent hematopoietic stem cells (HSCs) can be activated to enter the cell cycle by stress conditions.6 These observations suggest that CDK6 is required for expansion of certain differentiated compartments rather than for proliferation of early hematopoietic precursors. Like HSCs, leukemia stem cells (LSCs) are quiescent and enter the cell cycle as required to repopulate the leukemia, a property thought to confer resistance to chemotherapy.7 Because of the critical role of HSCs and LSCs in providing a reservoir for hematopoiesis and leukemogenesis, respectively, Scheicher et al investigated the role of CDK6 in HSCs and LSCs.

The authors found that CDK6+//+ HSCs are unable to compete effectively with CDK6−/−.
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