CTCL.[6] Moreover, IL-12[7] and IFNγ, both products of innate immune cells (the former from myeloid DCs and the latter largely from natural killer cells), have significant activity for CTCL.[6] These cytokines can help drive and sustain a T helper type 1 (Th1) response that appears to be inhibited by the prevailing Th2 milieu that is typical of Sezary syndrome, the leukemic variant of CTCL. Therefore, agents that can activate the innate immune response with production of these cytokines are likely to ameliorate the immune abnormalities and to improve the disease manifestations.

In that regard, Wysocka and colleagues have demonstrated that several classes of TLR agonists have the capacity in vitro to potently activate innate immune cells of patients with advanced, refractory CTCL[8,9] (see figure). The cells of patients with high tumor burden Sezary syndrome produce high levels of INFα in response to Type A CpG-ODNs. Significant activation of natural killer cells and $CD8^+$ T-cells and a marked increase of natural killer cell cytolytic activity can also be observed. These effects can be synergistically enhanced if the patients' cells were initially primed with either IFNγ or IL-15. More remarkably, in vitro experimentation with members of the imidazoquinoline family, which are synthetic agonists for TLRs 7 and 8, and which appear to be significantly more potent than CpG-ODNs, broadly activated cellular immune responses of these patients. Similarly, marked synergism was observed with IFNγ priming of cells.

The in vitro potency of these TLR agonists has been translated into clinical benefit when either CpG-ODNs or imidazoquinolines have been administered to patients with CTCL. Kim et al, in a phase 1 study of a type B CpG administered subcutaneously, treated 28 highly refractory patients with advanced CTCL.[2] Although the maximal tolerated dose was never reached, and the lower doses, including the 6-mg dose that produced responses when combined with local lesional radiation in the latest study, were ineffective at producing disease responses, overall a 32% response rate was observed, including several complete responses. Imiquimod, a TLR7 agonist in the imidazoquinoline family, when applied as a cream directly to CTCL skin lesions can induce local immune activation that can be associated with lesion regression.[3] However, it has quite a low bioavailability leading to inconsistency of clinical response. Moreover, responses are critically dependent on numbers of resident pDCs within lesions that can be activated by imiquimod. A variety of factors can lead to diminished numbers and function of pDCs in the skin including the use of potent topical steroids and administration of ultraviolet light, each of which may induce apoptosis of dendritic cells. In addition, application of topical tacrilimus may inhibit the ability of resident pDCs to further activate surrounding normal T cells that are necessary for the induction of an adaptive immune response against the tumor cells.

Resiquimod, which is an agonist for TLR7 expressed on pDCs, as well as an agonist for TLR8 expressed on mDCs, is a promising member of the imidazoquinoline family that has yet to be put into clinical trial for CTCL.[9] Its bioavailability as a topical gel is 10-fold greater than that of imiquimod cream and its potency may be up to 100 times greater. It could potentially be used as a single agent with potency great enough to induce systemic immune activation after cutaneous application. It may also be ideal to use it in combination with IFNγ with which it can synergize to broadly activate Th1 type cellular immunity.

Multimodality approaches like that used by Kim et al, employing a proapoptotic stimulus along with an immune adjuvant, such as a TLR agonist, are well suited for the therapy of CTCL. Other means of inducing high levels of apoptosis of tumor cells that are currently considered standard of care for CTCL are PUVA and photopheresis. Each of these has been demonstrated to produce higher response rates when combined with immune-boosting agents such as interferon α. Now that much more powerful TLR agonists such as resiquimod are available for clinical testing, this is the opportune time to undertake a study of multimodality therapy employing these exciting immune stimulants.

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● ● ● GENE THERAPY

Comment on Nagai et al, page 368

Aurora: a new direction for a new dawn

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The use of gene transfer techniques to introduce TCR α/β genes that confer specificity for a target antigen offers the opportunity to produce large numbers of cancer-specific T cells for adoptive therapy.[1] In this issue of *Blood*, Nagai and colleagues examine the feasibility of adoptive therapy using lymphocytes genetically engineered to express the T-cell receptor (TCR) for the leukemia-associated antigen Aurora kinase A (AURKA).[2]

The success of adoptive immunotherapy in leukemia is hampered partly by lack of sufficient high-avidity antitumor T-cell precursors in most leukemia patients[3] and technical difficulties in timely preparation of sufficient numbers of leukemia antigen-

specific T cells for each patient. TCR gene transfer using retroviral vectors is an attractive strategy for redirecting the antigen specificity of polyclonal primary T cells to create tumor antigen-specific lymphocytes. This approach can potentially overcome the limitations of current adoptive T-cell therapies, which rely largely on the isolation and expansion of tumor-specific lymphocytes ex vivo from individual patients, and result in the generation of sufficient numbers of potent antitumor immune effectors for adoptive immunotherapy.

Pioneering work from Rosenberg and colleagues at the National Cancer Institute proved the feasibility of this approach in clinical trials of adoptive therapy with autologous TCR gene-transduced T cells directed against cancer-associated antigens for the treatment of metastatic melanoma and synovial cell sarcoma.[4,5] In the setting of hematologic malignancies, TCR gene therapy targeting a number of leukemia-associated antigens such as Wilms tumor gene product 1 (WT1),[6] and minor histocompatibility antigens such as HA-1 and HA-2[7] are currently being investigated in preclinical studies or in early-phase clinical trials.

Aurora kinase A is a candidate tumor-associated antigen that is widely expressed in various types of cancer, including hematologic malignancies; its expression in normal tissues is largely limited to the testis, making it an ideal target for immunotherapy.[8] Previously, Yasukawa and colleagues identified an immunogenic HLA-A2–restricted antigenic epitope of Aurora kinase A, $AURKA_{207-215}$, capable of inducing $CD8^+$ T cells with in vitro cytotoxicity against AURKA expressing leukemic cells.[9]

Here, Nagai and colleagues sought to examine the feasibility of generating engineered T cells bearing Aurora kinase-A specific TCR genes as a strategy for the treatment of leukemia. In elegant experiments, they clearly demonstrate that polycloncal $CD8^+$ T cells retrovirally transduced to express the HLA-A2–restricted $AURKA_{207-215}$ TCR α/β chains generated from the AURKA $CD8^+$ T-cell clone have specific recognition of AURKA-overexpressing human leukemic cells, both in vitro and in a xenogeneic mouse model of human leukemia. Importantly, $AURKA_{207-215}$–specific TCR-transduced $CD8^+$ T cells were selective in their recognition of leukemic cells and did not lyze HLA-A2 positive normal peripheral blood mononuclear cells or cord blood $CD34^+$ cells, suggesting that $CD8^+$ T cells targeting AURKA will not result in immune-mediated destruction of normal stem cells.

Intriguingly, Nagai et al show that $CD4^+$ T cells could be redirected, using the same TCR that recognized the HLA-A2–restricted $AURKA_{207-215}$ epitope, to recognize and secrete T helper 1 cytokines including IL-2, in response to $AURKA_{207-215}$–expressing targets. The cytotoxic antitumor effect of $CD8^+$ T cells is partly dependent on $CD4^+$ T cells, which provide $CD8^+$ T cells with growth factors such as IL-2 and can mediate the destruction of tumor cells either directly or indirectly.[10] It is therefore likely that the adoptive transfer of redirected $CD4^+$ T cells concurrently with $CD8^+$ T cells expressing the same tumor-specific TCR gene could enhance the in vivo expansion, persistence, and antitumor reactivity of adoptively transferred antigen-specific $CD8^+$ T cells in vivo. It remains to be determined whether such redirected helper $CD4^+$ T cells will also exert direct cytotoxicity against leukemic cells in vivo.

Taken together, these results suggest that adoptive therapy using redirected $CD4^+$ and $CD8^+$ T cells that recognize AURKA-derived epitopes, may be a promising strategy for the treatment of AURKA-expressing cancers. These encouraging preclinical data support the development of clinical trials to investigate the safety and utility of such an approach in patients with relapsed or refractory leukemia.

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● ● ● MYELOID NEOPLASIA

Comment on Frisch et al, page 540

Bad to the bone

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In this issue of *Blood*, Frisch and colleagues identify an unexpected effect of leukemia cells: alterations in bone homeostasis within the bone marrow hematopoietic microenvironment.[1]

Ever since descriptions of the various functional components of the hematopoietic stem cell niche, leukemia researchers have wondered whether leukemia stem cells (LSCs) require similar cell-extrinsic support for long-term maintenance of leukemia.[2] LSCs differ from normal hematopoietic stem cells (HSCs) in that they have activation of key pathways regulating self-renewal, proliferation, and survival. Activation of these pathways is sufficient in many models for full leukemogenesis, suggesting that the LSC niche may be an attractive, but functionally redundant contributor to LSC survival. However, recent key