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Introduction

In 2011, approximately 274,930 people in the United States were living with or in remission from leukemia, of which 44,600 were newly diagnosed. Generally, chemotherapy can achieve complete responses and even in some cases long-term remission, but a hematopoietic stem cell transplant (HSCT) is the most reliable curative modality. The elicitation of a graft-vs-leukemia immunologic response is thought to be the main reason for this success. However, many patients are not candidates for this therapeutic option, and relapse rates are high, resulting in limited options.

Over the years, one of the more exciting and yet enigmatic concepts recurrent in the development and improvement of cancer treatment is the implication that the immune system can be harnessed and directed into a precision attack against a neoplasm. Over 100 years ago, primitive experimentation suggested that elicitation of an inflammatory response against a bacterial challenge in patients with cancer could subsequently provoke a protective immunologic response against the cancer as well. Yet, with another century's worth of acquired knowledge unraveling many of the intricacies of our immune system and the underlying defects leading to malignancy, we still are unable to recruit the immune system reproducibly and reliably into a potent protective antitumor response. That said, we have indeed made significant inroads into the development of effective strategies to elicit potent antitumor immunity.

Developing Strategies in the Immunotherapy of Leukemias

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Background: In the current treatment paradigms for leukemias, hematopoietic stem cell transplant (HSCT) is considered the best option with a curative potential although more often than not it simply delays disease progression. Advances are needed, both in current therapies and in the development of new strategies. Partly from studying the nuances of the curative potential of stem cell transplant, we have come to appreciate the relevance of the immune response and the potential of immunotherapy.

Methods: This review article summarizes the recent advances in the field of immunology and immunotherapy for leukemia.

Results: In passive immunotherapy, recent progress in chimeric T-cell antigen receptor technology has been encouraging. In active immunotherapy, a cancer vaccine may potentially enhance HSCT. An overview of various clinical studies of peptide vaccination strategies focusing on molecular targets such as the Wilms' tumor gene 1 (WT1), proteinase 3 (PR3), and receptor for hyaluronan acid-mediated motility (RHAMM) is provided. Cell-based vaccination strategies are also briefly explored.

Conclusions: The immune system clearly has the capacity to recognize and react to leukemic cells, and recent evidence directs our attention to the importance of mounting inflammatory and CD4 T-cell responses to complement and support the cytotoxic activity elicited by peptide vaccines.
Studies in animal models first led to the discovery that tumors were not immunologically silent entities but rather harbored tumor-associated antigens (TAAs), which could prime the development of both humoral and cell-mediated immunity. Although expression of many of these antigens, such as the cancer-testes antigens (CTAs), is not restricted to neoplasms, some antigens, such as the BCR-ABL fusion protein, are clearly unique to malignant cells. In the context of B-cell lymphomas, the variable region of the antibody, uniquely expressed in the clonal population, is also a therapeutic target. Over the past three decades, the list of known TAAs, many of which are tumor-specific, has grown impressively. Although we have yet to exploit any of these TAAs to develop a successful tumor vaccine to the degree of standard of care, they have helped immeasurably to shape our understanding of tumor immunology and may still retain relevance in the future design of efficacious immunotherapies.

The development of a potent and protective antitumor response undoubtedly requires both humoral and cell-mediated components. Antibody-mediated therapies offer the advantage of ex vivo synthesis of a universal agent, which circumvents immune compatibility and major histocompatibility complex (MHC) limitations. Thus, it is no surprise that the first-generation immunotherapeutics utilized a passive immunity approach. Early attempts at protein- or peptide-based vaccine strategies intent on immunizing against TAAs failed to overcome the inherent tumor-induced suppression of the antitumor response.

### Passive Immunotherapy

Passive immunotherapy aims to harness the potency and precision of the immune system without necessarily engaging the endogenous mechanisms that would elicit a sustained memory response. In the late 1990s, the development and implementation of rituximab, a chimeric humanized antibody recognizing CD20 on the surface of B lymphocytes, established a clear beneficial role for immunotherapy in hematologic malignancies; the benefits of including rituximab in the front-line treatment of B-cell malignancies are well established, and they have paved the way for the development of additional biologics. Over the past decade, new monoclonal antibodies targeting different surface antigens, as well as modifications and enhancements of CD20-targeting monoclonal antibodies, have been introduced into therapeutic algorithms, yielding modest but significant improvements in progression-free and overall survival when incorporated into standard front-line regimens. Immuno-modulatory monoclonal antibody therapies directed against targets such as cytotoxic T-lymphocyte antigen 4 (CTLA4) and programmed cell death protein 1 (PD-1) on leukocytes as opposed to tumor cells, intended to modulate and enhance immune reactivity, have been explored in solid tumors. The recent approval by the US Food and Drug Administration (FDA) of the anti-CTLA4 antibody ipilimumab for the treatment of melanoma emphasizes the efficacy of this approach to immunotherapy. Thus far, preliminary studies in the setting of hematologic malignancies have yielded some intriguing, if not promising, results.

Although the capacity to engineer monoclonal antibodies has existed for decades with the advent of hybridoma technology, thus facilitating the exploitation of the humoral arm of the immune system in the development of immunotherapeutic agents, engaging the T-cell arm of the immune system in a passive immunotherapeutic approach presents a much more complex challenge; the counterpart to the antibody, the T-cell receptor (TCR), is not a soluble secreted factor but rather a cell-surface molecule. Furthermore, immunotherapeutic interventions intent on artificially amplifying specific clonal T-cell responses require a considerably more tailored approach, as the TCR-mediated recognition of its cognate antigen is dependent on the MHC molecule presenting the peptide epitope. Adoptive transfer models, requiring harvesting followed by ex vivo expansion and activation of antigen-specific T cells, are labor-intensive, overly taxing, and ultimately prohibitive to standardization of a treatment protocol. That stated, there has been renewed emphasis on engaging the T-cell response in tumor immunotherapy, leading to some important developments. Ideally, these cellular-based strategies will instill a certain degree of compartmental immunologic memory, incorporating perhaps the most essential aspect of active immunization.

Chimeric T-cell antigen receptors (CARs) have overcome many of the obstacles inherent in T-cell immunotherapy by circumnavigating the challenges posed by MHC restriction, in terms of antigen presentation as well as potential alloimmunization and graft-vs-host responses. With CARs, a single-chain Fv variable fragment (scFv) from a monoclonal antibody that provides antigen specificity is incorporated into a transmembrane molecule, which also includes the signaling component zeta chain of the TCR complex. Expression of these vectors in T cells leads to the induction of cytotoxic responses independent of MHC-restricted antigen presentation. Recent further investigation comparing several target cell types (including cytotoxic CD8 T cells, natural killer T cells, and γδ T cells to determine an optimal effector cell population to maximize antitumor activity) indicated roughly equivalent efficacy among the three lymphocytic subtypes. To apply this technology to hematologic malignancies, CARs have been designed to recognize several surface antigens including CD19 and CD20, and clinical testing has yielded intriguing results. Furthermore, preclinical data suggest the viability of targeting CD22 and receptor tyrosine kinase-like orphan receptor 1 (ROR1) as well as CD30 in Hodgkin lymphoma and CD33 in acute myeloid leukemia (AML).

Although cytotoxic T lymphocytes (CTLs) modified to express CARs could elicit tumor-specific lysis,
first-generation constructs provided only limited benefit, mainly because of suboptimal in vivo clonal expansion and persistence. Second- and third-generation constructs have sought to overcome this limitation by incorporating costimulatory signaling into the chimeric receptor complex including CD28 and CD137 (also known as 4-1BB).

Using a murine xenograft model, Hoyos et al. showed that antitumor effects of a CD19-CAR system could be amplified by inclusion of CD28 costimulatory signaling along with forced production of interleukin-15 (IL-15). In their system, T cells from patients with chronic lymphocytic leukemia (CLL) modified to express the CD19/CD28 CAR and produce IL-15 demonstrated superior survival and trafficking capacity to tumor sites and tumoricidal function when introduced into severe combined immunodeficient (SCID) mice inoculated with either Daudi or Raji tumor cells.

The first clinical evidence to support the efficacy and viability of the CAR technology as a therapeutic modality was published in 2010 by Kochenderfer et al. In their case report, a patient with relapsed refractory stage IVB follicular lymphoma achieved an impressive partial response, durable to 32 weeks, upon receiving retrovirally engineered CD19-specific CAR lymphocyte infusions after lymphodepletion with cyclophosphamide and fludarabine and postinfusional IL-2 immune amplification. Subsequently, in 2011, Porter et al. reported on a patient with refractory CLL (1 of 3 treated in this pilot clinical trial) who received a low dose of autologous T cells modified ex vivo with a lentiviral vector encoding a CD19-specific CAR coupled to CD3zeta signal transduction and CD137 costimulation after a preconditioning regimen of pentostatin and cyclophosphamide to deplete lymphocytes. Such treatment resulted in a significant clonal expansion of the transferred T cells along with a durable presence and a sustained complete response.

Subsequently, Brentjens et al. published their data on the first 10 patients with refractory CLL or relapsed/refractory B-cell acute lymphoblastic leukemia treated with their second-generation CAR coupling CD19 specificity with CD28 costimulation. In their study, CAR T lymphocytes were well tolerated and demonstrated effective trafficking to tumor sites with retention of cytotoxic activity. However, ultimately only 1 of the 10 patients experienced a marked response with significant reduction in lymphadenopathy, and 2 others demonstrated transient stable disease. Similar to the Porter data, patients in this study who received prior conditioning with cyclophosphamide exhibited enhanced persistence of the infused modified lymphocytes. The authors attributed these disappointing results in part to the fact that all of these patients were heavily pretreated and suggested that CAR adoptive transfer therapy may demonstrate more substantial clinical benefit in patients with minimal residual disease. Nonetheless, these two most recent clinical studies demonstrated the feasibility and potential of passive T-cell immunity in the treatment of hematologic malignancies. There is now recent evidence to suggest that CAR T-cell therapies may perhaps bridge the gap between passive and active immunization, with immunologic memory being established after infusion.

Although the CD19- and CD20-specific CARs serve as an encouraging first step toward a potentially curative intervention, there is room for optimization, either in terms of improving the CD19 and CD20 vectors or defining better target antigens. In accordance, our repertoire of targets has been expanding as we begin to develop CARs for the treatment of other hematologic malignancies.

Peinert et al. have developed a CAR recognizing the Lewis Y carbohydrate antigen, potentially relevant in myeloma and AML, which has demonstrated in vitro and in vivo cytotoxic activity in a murine xenograft system. Also, Marin et al. published their preclinical data on the development of a third-generation CAR complexing a CD33-specific CAR with CD28 and OX-40 costimulatory signaling. They demonstrated that cytokine-induced killer cells inherited enhanced proliferative, migratory, and lytic capacity as well as increased cytokine production directed at a variety of leukemic cell lines in an in vitro assay system.

Similarly, Hudecek et al. provided interesting preclinical data suggesting the potential relevance of the ROR1 antigen, expressed on the cell surface in both CLL and mantle cell lymphoma, as a targetable antigen. They demonstrated that lentiviral transfection of sort-purified CD8+CD45RO+CD62L+ central memory T cells with a CAR-encoding specificity for ROR1 produced enhanced CTL cytotoxicity and interferon gamma (IFN-γ) production in vitro. CD23-specific CARs have demonstrated increased cytotoxicity directed against both CLL tumor cell lines and primary isolated tumor cells in an in vitro as well as in vivo murine Rag2(−/−)γc(−/−) xenograft mouse model, in work published by Giordano Attianese et al. The developmental pipeline is obviously filling as we look to improve upon this exciting adoptive transfer therapy.

Although the main focus continues to be placed on the optimization of immunologic activation and persistence, adequate expansion, and localization of the modified lymphocytes, safety is also an important concern. As we seek to improve upon the durability of these infused lymphocytes, we also need to consider the fact that there may come a time when the cells need to be eradicated rapidly from circulation. Many of the molecular targets being explored are not unique to the tumor cells, and thus, the potential for graft-vs-host responses is real. Furthermore, these autologous T cells maintain their endogenous TCR, and, in bypassing the natural safeguards inherent in MHC restriction, we run the risk of inadvertently activating and expanding a T-cell population that carries an MHC-restricted specificity for an autoantigen, thereby potentiating autoimmune manifestations.
Several adaptations have been developed to prevent or limit the risk of such occurrences. CAR vectors that encode a suicide gene component have now been developed to allow for rapid elimination of the CAR-modified cells.\(^{31,39}\) Additionally, more precise selection of lymphocytes for transfection, such as selecting cytotoxic lymphocytes enriched for viral specificity by antigenic maturation, in effect selects the cross-reactivity of the modified T cells while potentially conferring additional benefits such as selecting for memory populations that possess inherent longevity.\(^{40}\)

The development of CAR technology into a viable cancer therapy began more than 20 years ago, and we are just now seeing the potential benefits realized in the clinical setting. There are still some enormous steps to take to translate this concept into a viable and practical tumor treatment protocol, but recent advances are encouraging and ideally will fuel a surge in continued and expedited development.

**Active Immunotherapy**

In the evolving concept of immunotherapy in cancer treatment, the optimal strategy to generate a potentially curative intervention is active immunization. Unlike passive immunity, active immunization would provoke a cognate immune response that would engage the adaptive response and lead to the establishment of immunologic memory. Ideally, the development of an anamnestic response would provide sustained protection and reactivity against any leukemic recurrence long before a relapse would be clinically apparent.

The best proof of principle we have to date lies in our improved understanding of the benefits and mechanisms of action underlying the often sustained remissions associated with allogeneic HSCT in leukemia and lymphoma. Although initially designed and implemented as a method of bone marrow rescue after lethally high-dose chemotherapy, allogeneic HSCT is now understood to promote a graft-vs-leukemia response, which can be further augmented with donor lymphocyte infusion in the face of leukemic relapse after transplant. HSCT is potentially our best chance to cure most hematologic malignancies, but it is a high-risk, high-reward approach, and stringent MHC requirements prevent many patients from qualifying for HSCT. Thus, the development of a protocol that could engage active immunity similar to HSCT, for instance a cancer vaccine, could potentially provide the efficacy of HSCT while reducing or eliminating the limitations to accessibility and inherent risk of the treatment itself.

**Peptide Vaccination Strategies**

Perhaps the most intuitively obvious peptide vaccine target is the BCR-ABL neoantigen formed in the t(9;22) translocation, which is associated with 95% of chronic myeloid leukemia (CML) cases. Tumor immunogenicity associated with CML is well established by the graft-vs-leukemia responses measured in patients with CML treated with HSCT and further emphasized by the efficacy of allogeneic donor lymphocyte infusion in cases of relapse after transplant.\(^{41,42}\) Logically, the immune recognition of the Philadelphia chromosome-positive leukemic cells should be directed, at least in part, against the novel antigenic epitopes introduced by the mutation. The 210 kDa BCR-ABL fusion protein can be associated with one of several identified breakpoint mutations, the most common of which are the b3a2 and b2a2 variant transcripts.

The immunologic relevance of the BCR-ABL fusion protein has been extensively studied, and specific reactive epitopes have been defined.\(^{43-46}\) Also, cognate T-cell responses in both the cytotoxic and helper compartments have been elicited and measured.\(^{47-52}\) Peptide-restricted cytotoxic lymphocyte responses have been demonstrated in in vitro culture conditions and have been measured in patients with CML as well as in patients after HSCT.\(^{53-55}\) The inversely proportional relationship that has been demonstrated between endogenous CML-specific T-lymphocyte responses and leukemic burden,\(^{53,54}\) as well as the reported inverse relationship between the incidence of CML and certain HLA haplotypes (HLA-A3, HLA-B8, and HLA-DR4),\(^{56,57}\) suggests that the immunologic reactivity associated with CML is not merely a laboratory phenomenon but a clinically relevant process.

Several clinical trials have explored the efficacy of immunizing against the neoepitopes introduced by the translocation in the chronic phase of disease. The first phase I study evaluated a combination of four class I A2.1-, A3-, A11-, and B8-restricted peptides introduced in combination with a class II-restricted epitope and QS-21 adjuvant in 12 patients treated concomitantly with IFN-α.\(^{55}\) The vaccine strategy proved to be safe and well tolerated, but additional assessments of efficacy demonstrated peptide-specific proliferative responses in 50% of the patients challenged with the higher doses, and specific cytotoxic responses were not evidenced in this study.

In a continuation of the important observations from this phase I study, the group published phase II trial results describing measurable delayed-type hypersensitivity (DTH) and peptide-specific CD4 T-cell proliferative and IFN-γ production responses in 11 of 14 patients with chronic-phase CML.\(^{58}\) CD8 T-cell responses were measurable in a smaller subgroup of the immune responders. Molecular responses were also assessed, with noted decreases, at least transiently, in 50% of the participants. None of these patients was treatment-naïve, with prior or concurrent treatments including IFN-α, hydroxyurea, HSCT, or imatinib. In this study, patients received five vaccinations consisting of the same six-peptide combination (five class I peptides along with one class II peptide in the QS-21 adjuvant).

In another phase II trial by Rojas et al.,\(^{59}\) 19 patients with chronic-phase CML were vaccinated with a combination of an A3- or B8-restricted class I peptide
combined with the pan-DR epitope (PADRE) to elicit helper responses. All patients enrolled were in complete hematologic response and maintained on a stable dose of imatinib. All demonstrated immunologic responses, with 14 of 19 patients developing measurable BCR-ABL peptide-directed T-cell responses detected in the context of IFN-γ production, and the immunologic responses correlated with concurrent molecular responses based on BCR-ABL transcript levels. In this study, vaccination was ineffective in the 5 patients who had not achieved a major cytogenetic response.

Employing a vaccine generated with a combination of native and heteroclitic peptides, Maslak et al. conducted a pilot trial exploring the efficacy of immunization in patients with CML achieving either major or complete cytogenetic remission on imatinib therapy. Their aim was to test whether synthetic analog peptides could enhance CTL responses to the native breakpoint epitopes. Participants with a b3a2 breakpoint mutation received a combination of five peptides, including two heteroclitic HLA-A2 class I peptides, two native class I peptides, and one native class II peptide, in the vaccine cocktail; patients with a b2a2 breakpoint mutation were vaccinated with a combination of a single heteroclitic HLA-A2 class I peptide along with a native class II peptide. Of the 13 patients included in the trial, 11 completed the planned 11 vaccinations over a 12-month period, and 4 of 7 patients positive for HLA-A0201 mounted measurable specific and cross-reactive CTL responses based on IFN-γ production and flow cytometric tetramer analysis. Unfortunately, none of the in vitro immunologic activity measured in this study correlated with clinical benefit, nor were they able to provide evidence of cytolytic activity against fresh CML cell isolates.

Building on the premises of the prior Maslak study, Jain et al. evaluated the impact of synthetic analog peptide vaccination in 10 patients with CML who had achieved a complete cytogenetic response on imatinib therapy in a small phase II trial. Patients were again challenged with a similar cocktail of native and heteroclitic class I and class II peptides, receiving 15 immunizations over a 12-month period. Three patients achieved a 1-log reduction in BCR-ABL transcript levels, and 3 additional patients achieved a major molecular response. Immunologic analysis revealed a corresponding transient decrease in regulatory T-cell numbers in patients demonstrating a molecular response.

Bocchia et al. reported on an interim analysis of a multicenter study of 57 patients with CML. All had achieved a complete cytogenetic response on imatinib and received a five-peptide vaccine coupled with granulocyte-macrophage colony-stimulating factor (GM-CSF). Their data suggest that in the 43 evaluable patients, 67% demonstrated a peptide-specific CD4+ T-cell response, and 48% of these patients also had a significant molecular response, with a > 50% reduction in BCR-ABL transcript levels.

To enhance peptide immunogenicity and perhaps engage HLA-0201–restricted CTL responses, which have proven to be difficult to elicit, vaccines comprising heteroclitic class I peptides have also been developed. Overall, we have only begun to assess the impact of peptide vaccination regimens on the management of CML in the tyrosine kinase inhibitor era, with many important questions remaining unanswered; some are as direct as identifying the optimal vaccine regimens and adjuvants, whereas others are more complex challenges, such as overcoming the limitations and inefficacy in vaccination beyond the state of chronic-phase CML with minimal residual disease. In light of the paradigmatic shift in the treatment of CML brought about by the introduction of imatinib, we have yet to elucidate the ideal role of immunotherapy in conjunction with tyrosine kinase inhibitor therapy in the goal of obtaining a final cure for this disease.

More recently, AML has been extensively studied in the context of vaccine immunotherapy. In fact, the immunologic recognition of AML and the clinical observation of immune responses to irradiated leukemic cells were documented over 40 years ago. With CML, identification of certain specific mutations in AML, such as the promyelocytic leukemia gene-retinoic acid receptor-α (PML-RARA) mutation in acute promyelocytic leukemia, has led to the development of highly effective therapeutic agents that have profoundly changed the management of the disease. However, unlike CML, AML represents a more heterogeneous disease. HSCT remains the best option, if any, for AML for cure or durable remission. Similar investigation into the antigenicity of the PML-RARA fusion product has failed to yield a proven immunogenic neoantigen epitope, although murine studies have demonstrated some early potential. With the current treatment options lacking effectiveness and durability, efforts to define and develop therapeutic vaccines have intensified. Several TAAs, which have received attention based on their overexpression in the context of malignancy, have been proposed as potential targets (Table). Many of them may be overexpressed in chronic myeloid and lymphoid leukemias as well, thus increasing their potential therapeutic value.

The Wilms’ tumor suppressor gene 1 (WT1) has become an increasingly relevant and attractive molecular target in the expanding field of immunothera-

Table. — Selected Tumor-associated Antigens Targeted for Immunotherapy in Leukemia

<table>
<thead>
<tr>
<th>Antigen Name</th>
<th>Description</th>
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<tbody>
<tr>
<td>Wilms’ tumor suppressor gene 1 (WT1)</td>
<td>Proteinase 3 (PR3)</td>
</tr>
<tr>
<td>Receptor for hyaluronan acid-mediated motility (RHAMM)</td>
<td>Preferential antigen of melanoma (PRAME)</td>
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<tr>
<td>Flt3L</td>
<td>Telomerase reverse transcriptase in humans (hTERT)</td>
</tr>
<tr>
<td>CML28</td>
<td>Survivin</td>
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peptides in leukemia. Immunologic activity, including WT1-specific antibodies and CTL responses, has been detected in association with a variety of hematologic and solid tumor malignancies.\textsuperscript{82,83} Furthermore, WT1 expression correlates with disease progression and has been implicated as a prognostic indicator in several hematologic malignancies.\textsuperscript{84,85}

In a phase I trial, Oka et al\textsuperscript{69} established the efficacy of WT1 as a potential immunotherapeutic target. They demonstrated that vaccination with a WT1 peptide along with Montanide ISA-51 adjuvant could precipitate tumor regression associated with a detectable expansion of WT1-specific CTL populations in 26 participants with breast cancer, lung cancer, myelodysplastic syndromes (MDS), or AML. Of the 20 evaluable patients in this study, 12 demonstrated a clinical response that could be correlated to an increase in immunologic activity directed against the WT1 antigen.

In 2009, Keilholz et al\textsuperscript{86} published the results of their phase II study of 19 patients with untreated or relapsed/refractory AML or high-risk MDS, further exploring the immunogenicity and cytolytic impact of WT1 peptide vaccination. As with the prior study, participation was limited to patients with an HLA-A2 haplotype to accommodate tetramer analysis to assess immunologic response. Peptide vaccinations were delivered with keyhole limpet hemocyanin hapten adjuvant along with GM-CSF. Of the 14 participants without detectable WT1-specific CTLs at baseline, 8 mounted a measurable response by week 10, while failure of clonotypic expansion of T cells appeared to be associated with higher marrow blast percentages. Clinically, 10 patients with AML in the study had objective responses interpreted as stable disease, and 4 additional patients demonstrated clinical benefit after initial disease progression, supporting a potential role for WT1 vaccination strategies in AML.

Expanding on the positive results of these trials, Maslak et al\textsuperscript{87} conducted a pilot study to evaluate a multivalent peptide vaccine combining two class I HLA-A0201–restricted peptides, including one heteroclitic peptide and four HLA-DR.B1–restricted class II peptides along with Montanide ISA-51 adjuvant in 10 patients with AML in complete remission but with measurable WT1 transcript levels. Only 3 of the 10 participants were HLA-A0201–positive, but all 3 demonstrated an increase in WT1-specific CTL function and proliferation; 7 patients demonstrated CD4+ proliferative responses after vaccination, suggestive of a WT1-specific response. All of these findings should encourage further exploration of this vaccine strategy.

In addition, a recent phase I/II trial piloted the use of WT1-peptide–loaded autologous dendritic cells (DCs) in combination with keyhole limpet hemocyanin and zoledronate as a V9V2 T-cell adjuvant to elicit cytokine support. A sustained WT1-specific CTL clonotypic expansion was noted in 2 of 3 participants, but limited conclusions could be drawn from this study overall because of its small size.\textsuperscript{88}

To circumvent the limitations imposed by MHC restriction, which has hindered the development of cancer vaccines, a phase I/II trial reported on 10 patients with AML in hematologic remission who were challenged with autologous monocyte-derived DCs engineered to express and present WT1 antigen using electroporation to introduce WT1 mRNA.\textsuperscript{89} Two patients in partial remission were converted to complete remission, and 5 of the 10 patients, including these 2, demonstrated a reversion of the AML-associated tumor marker to normal. Immunologic responses were detected in the form of antigen-driven IFN-γ production and increased levels of circulating IL-2 and HLA-DR+ CD4+ T cells, and clinical responses appeared to correlate with the presence of high levels of activated natural killer cells. In summation, significant efforts have produced some intriguing and suggestive results to indicate a potential role for WT1 as a target antigen for immunotherapeutic interventions, but currently this role appears to be limited to patients with minimal residual disease.

Another emerging antigenic target demonstrating interesting potential is proteinase 3 (PR3), a neutral serine protease stored primarily in azurophilic granules and overexpressed in leukemic progenitor cells as well as in AML and CML blast populations. PR3-specific CTLs have been shown to possess in vitro lytic activity against primary isolated tumor cells, with 79% specific lysis of fresh isolated CML blast cells and 54% specific lysis of fresh isolated AML blast cells.\textsuperscript{72} PR3-specific CTLs also have demonstrated the ability to selectively inhibit in vitro colony-forming activity of marrow-derived cells from patients with CML but not from healthy donor marrow-derived cells.\textsuperscript{90} Furthermore, the presence of PR3-specific CTLs strongly correlated with remission of CML after IFN-α and HSCT, with circulating PR3-specific CTLs detected in 11 of 12 responders and none of 8 nonresponders.\textsuperscript{91} Similar results of persistent PR3-specific CTL reactivity were reported in 7 patients with CML maintaining a cytogenetic complete remission status after withdrawal of IFN-α.\textsuperscript{92} In a phase I/II trial of a PR3 vaccine in 66 leukemic or preleukemic patients (42 with AML, 13 with CML, and 11 with MDS) using Montanide ISA-51 and GM-CSF as adjuvants, a PR3-directed immune response was detectable in 25 of 53 patients (47%) with measurable disease, and clinical responses were observed in 9 of 25 of these immune responders (36%) vs only 3 of the 28 nonresponders.\textsuperscript{93,94} PR3-specific immune responses were also associated with a prolonged event-free survival (8.7 months vs 2.4 months; P = .03) and a trend toward longer overall survival.

Considering the demonstrated polyvalent antigenic nature of leukemia, Rezvani et al\textsuperscript{95} conducted a small phase I study on 8 patients with AML, CML, or MDS to explore the impact of simultaneous vaccination against two TAAs (WT1 and PR3), with the intent to generate a better sustained immunologic response,
RHAMM has been identified as an immunologically relevant antigen, strongly expressed in several hematologic malignancies, including CML, AML, MDS, and MM, and associated with both cellular and humoral immunity. In a phase I clinical trial evaluating 10 HLA-A2–positive patients with AML, MDS, or MM with detectable tumor expression of RHAMM, 7 of the 10 patients challenged with a single 500 μg peptide vaccination along with Montanide ISA-51 and GM-CSF demonstrated an expansion of RHAMM-specific CD8+ T cells. Furthermore, 3 of the 6 patients with AML or MDS achieved a clinical response, with 2 showing a significant reduction in bone marrow blast percentages and 1 with MDS becoming erythrocyte transfusion-independent. Of the 4 patients with MM, 2 demonstrated a reduction in serum-free light chain levels.

In a small clinical trial by the same group, building on these initial results, another 9 patients with MDS, AML, or MM were enrolled and received four biweekly vaccinations of 1,000 μg RHAMM-R3 peptide combined with Montanide ISA-51 and GM-CSF, with subsequent immune monitoring. Of the 9 patients, 4 developed detectable immune responses characterized by an expansion of RHAMM-R3–specific CD8 T cells, 2 patients experienced a decrease in FoxP3+ regulatory T cells, and 3 patients evidenced a clinical response in terms of bone marrow blast count reduction (1), peripheral cell count improvement (1), or serum-free light chain reduction (1). Overall, the results showed a diminished response compared with results seen in the prior study with the lower peptide dose of 300 μg.

Greiner et al similarly evaluated the immune and clinical responses to a series of four biweekly vaccinations with 300 μg of R3 peptide with Montanide ISA-51 and GM-CSF in 6 patients with CLL. In this study, 5 of the 6 patients demonstrated an immunologic response based on expansion of tetramer-specific CD8+ T cells, and 4 of the 6 patients were assessed as clinical responders based on >20% decrease in leukocyte count, although only 3 of the 6 patients demonstrated both clinical and immunologic responses. Furthermore, this study confirmed the finding that a regimen with multiple vaccinations was associated with an expansion of a regulatory T-cell population. Thus, although RHAMM constitutes another promising vaccine target in several leukemias, the preliminary results also raise the concern that the transient benefits seen in these studies may be due to the induction of a regulatory component, which could hinder the potency of the cytotoxic cellular immune response.

Cell-based Vaccination Strategies

Peptide-based vaccines attempt to elicit a precision strike against a tumor, with the intent to minimize nonspecific autoimmune activation by capitalizing on the identification of a known antigen unique to the cancer cell in terms of sequence or expression level. Perhaps the most glaring flaw in the logic behind this approach is that our identification of epitopes based
on the optimal molecular fit of a peptide in the HLA-binding groove does not necessarily coincide with identification of the most immunogenic epitope of the protein. In addition, we cannot be certain that the particular protein represents a dominant antigenic target in the antileukemic response capable of driving an effective and protective response.

The identification of TAAs, or even the optimal TAA (should it exist), may ultimately not be sufficient to elicit a protective immune response if the protein or peptide is not presented to the immune system in an inflammatory context. To maintain the universal applicability of peptide-based vaccines, most investigators employ peptides combined with adjuvant compounds to generate tumor immunity. Perhaps a more effective approach has been to develop cellular-based vaccines, employing DCs to deliver a peptide in the context of a strong immunogenic signal. The development of autologous DC vaccines, typically generated ex vivo from harvested peripheral blood monocytes, has served an invaluable role in shaping our understanding of the dynamics and obstacles in creating an effective tumor vaccine. However, the labor-intensive methodology makes this step essentially infeasible for large-scale implementation.

Alternative approaches intended to maximize universal applicability while maintaining antigenic diversity have led to the development of the bystander cell-based vaccine. The GVAX bystander vaccine utilizes K562 cells, an erythroleukemic tumor cell line lacking any MHC expression and engineered to secrete GM-CSF.

In a pilot study to determine whether this vaccine could improve molecular responses in patients with chronic-phase CML who had not achieved major cytologic remission despite continued imatinib therapy in excess of 1 year, 19 patients received four vaccinations at 3-week intervals. This treatment resulted in an interval decline in polymerase chain reaction (PCR) measurements of BCR-ABL in all 19 individuals and progressive decline in disease burden in 13 of the 19 patients (including 7 whose BCR-ABL PCR became undetectable). Borrello et al. reported on a phase II trial of vaccination with GVAX bystander cells mixed with autologous leukemic cells prior to autologous HSCT as postremission therapy for AML. Of 46 patients who achieved complete remission, 28 were vaccinated with GVAX and irradiated autologous leukemia cells harvested prior to induction chemotherapy, followed by a second leukapheresis to harvest primed lymphocytes just prior to HSCT. The primed lymphocytes along with the autologous stem cells were infused back into the individuals on day 0 of the transplant. The 3-year relapse-free survival and overall survival rates in the 48 patients who achieved complete remission were 47.4% and 57.4%, respectively. In comparison, the relapse-free and overall survival rates in the 28 patients receiving the immunotherapy were 61.8% and 73.4%, respectively. These findings suggest that the GVAX vaccine and primed lymphocyte infusion may offer clinical benefit and warrants further attention to determine how outcomes with this approach might compare with established outcomes associated with allogeneic HSCT in AML.

Given that leukemic cells possess a certain degree of antigen-presentation capacity, investigators have explored the modification of harvested tumor cells to express molecules such as CD40 to enhance their immunostimulatory capacity. Several groups have demonstrated that DCs could be generated from myeloid progenitor cells in patients with CML, which maintain expression of the BCL-ABL fusion protein. A phase I/II trial using autologous DC vaccination in 10 patients with CML who had not achieved an adequate cytogenetic response after treatment with either imatinib or IFN-α showed improvement in cytogenetic response in 4 of the individuals after treatment, with 3 of the 4 also demonstrating T-cell responses to leukemia-associated antigens.

Hus et al. conducted a small pilot study evaluating a vaccination strategy with autologous DCs pulsed with tumor lysates in 12 patients with CLL. Patients received up to 8 intradermal injections, with a clinical response consisting of a decrease in leukocyte count > 25% seen in 5 of the 12 individuals. Four of these patients also demonstrated specific CTL activity against either RHAMM or fibromodulin leukemia-associated antigens, and all 5 experienced a decrease in the frequency of CD4+CD25+FoxP3+ T-regulatory cells.

In terms of AML and MDS, a phase I clinical trial by Ho et al. studied the impact of vaccination with irradiated autologous leukemic cells engineered to secrete GM-CSF early after allogeneic HSCT. They demonstrated durable complete remission in 9 of 10 participants with high-risk AML or MDS, which compared favorably with complete remission in historic controls (2-year overall survival of 56% vs 21% for historic controls). In reality, autologous cell-based and DC-based vaccine strategies are exceedingly cumbersome to find routine application in the standard clinical management of leukemias. Conversely, in the setting of HSCT, where cell harvest and manipulation are already inherent in the process, cell-based vaccines may play an expanding and exciting role in the future.

Conclusions
In the past few decades, we have witnessed an amazing growth in our collective understanding of the intricate interactions between hematologic malignancies and our immune system. From a vague appreciation that tumor cells have some capacity to be immunogenic, we now realize that tumors are far from immunologically inert but rather establish a dynamic equilibrium with the immune system from early on. The challenges we face in developing immunotherapeutic approaches to malignancies are not to elicit a transient de novo response but instead to intervene on and alter the course
of an established immune response, with formidable regulatory elements fully engaged.

Our arsenal of immunomodulators is expanding, as is our understanding of the mechanisms of tumor immunity and tolerance. Hybridoma technology allowed us to harness the humoral response in order to develop reagents such as rituximab and alemtuzumab, which have altered our approach to hematologic malignancies, and now in this past year, we are finally witnessing the clinical application of the cellular counterpart to the monoclonal antibody in the chimeric T-cell antigen receptor. The immune system plays an important role in tumorigenesis and tumor progression, and as we improve our conception of this relationship, we are also witnessing the development of exciting new tools to intervene and disrupt the process.

References


