7. Thomas A, Mailankody S, Korde N, Kristinsson SY, Turesson I, Landgren O. Second malignancies after multiple myeloma: from 1960s to 2010s. *Blood*. 2012;119(12):2731-2737.

8. Ormerod A, Fausel CA, Abonour R, Kiel PJ. Observations of second primary malignancy in patients with multiple myeloma. *Clin Lymphoma Myeloma Leuk*. 2012;12(2):113-117.

9. Landgren O, Thomas A, Mailankody S. Myeloma and second primary cancers. *N Engl J Med*. 2011;365(23):2241-2242.

10. Mailankody S, Pfeiffer RM, Kristinsson SY, et al. Risk of acute myeloid leukemia and myelodysplastic syndromes after multiple myeloma and its precursor disease (MGUS). *Blood*. 2011;118(15):4086-4092.

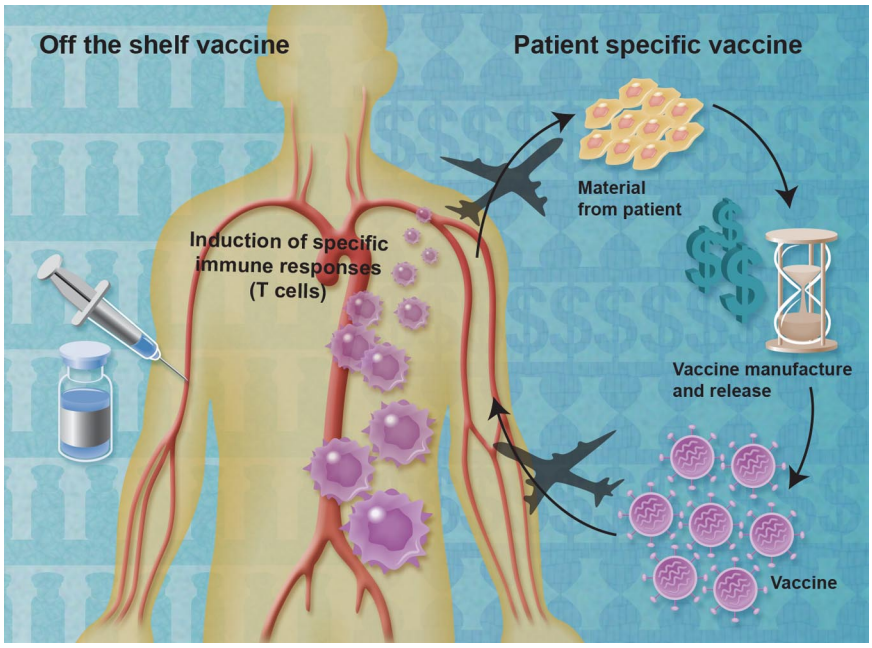
IMMUNOBIOLOGY

Comment on Weng et al, page 1613

Toward an off-the-shelf vaccine for B-cell malignancies

James L. Gulley NATIONAL CANCER INSTITUTE

While idiotype vaccines have shown promise for B-cell malignancies, production is cumbersome; thus, targeting a common antigen on malignant B cells using an off-the-shelf approach would provide significant logistical advantages.



Patient-specific therapeutic vaccines are generated from immune cells or tumor material obtained from cancer patients. This process is resource-intensive, but has yielded therapeutic vaccines with clinical benefit. In contrast, large numbers of doses of off-the-shelf vaccines can be manufactured at one time, then stored until needed. In theory, off-the-shelf vaccines should be cheaper to manufacture while simplifying time and supply lines. Professional illustration by Alice Y. Chen.

Bendandi et al demonstrated early on that complete molecular remission in lymphoma patients who had received a patient-specific idiotype vaccine is possible.[1] In a follow-up randomized phase 3 study, Schuster et al showed that vaccination with a patient-specific anti-idiotype vaccine led to improved disease-free survival in patients with follicular lymphoma.[2] Patients enrolled in study who achieved a complete response to chemotherapy were randomly assigned to vaccine with idiotype conjugated to keyhole limpet hemocyanin (KLH) with local GM-CSF versus KLH control. The median disease-free survival for vaccine-treated patients was 44.2 months versus 30.6 months for patients in the control arm (hazard ratio 0.62; $P = .047$). However, this patient-specific vaccine required a significant amount of time to produce (6-12 months was allowed). Only 69% of patients randomized remained in complete response to chemotherapy by the time vaccine was available, making the remaining 31% ultimately ineligible to receive vaccine or placebo. Nevertheless, the promising disease-free survival seen among vaccinated patients provides hope that therapeutic vaccines will prove to be a well-tolerated treatment associated with clinical benefit in the setting of minimal disease.

The complexity of making a patient-specific vaccine can lead to significant financial and temporal costs (see figure). The only currently approved therapeutic vaccine for cancer is sipuleucel-T, which showed a statistically significant and clinically meaningful 22% reduction in risk of death in patients with asymptomatic or minimally symptomatic metastatic castration-resistant prostate cancer.[3] The median 4.1-month improvement in survival was seen without significant side effects (only 1.5% of patients had to stop treatment because of toxicity). However, patients treated with sipuleucel-T must undergo apheresis at 3 time points and the vaccine is subsequently manufactured from the apheresis products at a central processing facility capable of rapid turnaround. This complexity adds to the price of the vaccine: $93 000 US for a complete course of treatment.[4] Several off-the-shelf therapeutic vaccines have shown preliminary evidence of efficacy,[5-7] providing hope that improvements in patient outcomes with this modality may lead to therapeutic options that are less resource-intense.

Identification of a common antigen on B-cell malignancies that is not present on normal B cells thus offers the potential for an off-the-shelf vaccine for lymphomas that avoids the resource-intense manufacture and release of patient-specific vaccines. In a convincing set of experiments described in this issue of *Blood*, Weng et al demonstrate that T-cell leukemia/lymphoma 1 (TCL1) oncoprotein is overexpressed on a wide range of human B-cell lymphomas, but only selectively expressed on normal B cells.[8] They demonstrate not only that TCL1 peptide-specific T cells could be generated from normal donors, but that TCL1-specific T cells were present in the blood of patients with lymphoma, and that these T cells could be expanded and, in an HLA-A2–restricted manner, could lyse autologous lymphoma cells but not normal B cells. This approach should help catalyze intensive translational efforts to rationally

design off-the-shelf therapeutic vaccines that, alone or in combination with other therapies, can efficiently propel clinically significant antilymphoma immunity in minimal disease settings without significant side effects.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

REFERENCES

1. Bendandi M, Gocke CD, Kobrin CB, et al. Complete molecular remissions induced by patient-specific vaccination plus granulocyte-monocyte colony-stimulating factor against lymphoma. *Nat Med*. 1999;5(10):1171-1177.
2. Schuster SJ, Neelapu SS, Gause BL, et al. Vaccination with patient-specific tumor-derived antigen in first remission improves disease-free survival in follicular lymphoma. *J Clin Oncol*. 2011;29(20):2787-2794.
3. Kantoff PW, Higano CS, Shore ND, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. *N Engl J Med*. 2010;363(5):411-422.
4. Gulley JL, Drake CG. Immunotherapy for prostate cancer: recent advances, lessons learned, and areas for further research. *Clin Cancer Res*. 2011;17(12):3884-3891.
5. Vansteenkiste J, Zielinski M, Linder A, et al. Final results of a multi-center, double-blind, randomized, placebo-controlled phase II study to assess the efficacy of MAGE-A3 immunotherapeutic as adjuvant therapy in stage IB/II non-small cell lung cancer (NSCLC) [abstract]. *J Clin Oncol*. 2007;25 (18S):Abstract 7554.
6. Kantoff PW, Schuetz TJ, Blumenstein BA, et al. Overall survival analysis of a phase II randomized controlled trial of a Poxviral-based PSA-targeted immunotherapy in metastatic castration-resistant prostate cancer. *J Clin Oncol*. 2010;28(7):1099-1105.
7. Butts C, Maksymiuk A, Goss G, et al. Updated survival analysis in patients with stage IIIB or IV non-small-cell lung cancer receiving BLP25 liposome vaccine (L-BLP25): phase IIB randomized, multicenter, open-label trial. *J Cancer Res Clin Oncol*. 2011;137(9):1337-1342.
8. Weng J, Rawal S, Chu F, et al. TCL1: a shared tumor-associated antigen for immunotherapy against B-cell lymphomas. *Blood*. 2012;120(8):1613-1623.

● ● ● IMMUNOBIOLOGY

Comment on Doubrovina et al, page 1633

What T cells see in WT-1

Jeffrey J. Molldrem MD ANDERSON CANCER CENTER

The Wilms tumor protein, WT-1, is a widely recognized tumor antigen that is aberrantly expressed in myeloid and lymphoid leukemia and in this issue of *Blood*, Doubrovina et al report the most extensive catalog heretofore of HLA-restricted immunogenic peptides derived from WT-1, which are recognized by CD8 and CD4 T cells.[1]

The investigators used a pool of overlapping 15-mer peptides from WT-1 to screen for cytokine production from autologous T cells from the blood of 56 healthy donors, and to subsequently identify the peptide sequences and HLA restrictions of the immunogenic epitopes. The authors identified 42 peptide epitopes from WT-1 including 41 new epitopes restricted to class I and class II HLA molecules. Importantly, peptide-specific T-cell responses were evident in nearly 80% of the donors, and T-cell lines specific for 29 of the 42 epitopes induced specific lysis of WT-1–expressing leukemic blasts. These results show that a large number of potentially immunogenic peptides are naturally processed and presented from WT-1 on the surface of leukemic blasts. The results suggest that these epitopes could be useful in immunotherapy strategies that target WT-1–expressing malignancies, including leukemia.

Wilms tumor is the most common renal neoplasm of children and 50% of individuals carrying a germ line mutation predisposing to Wilms tumor develop the disease. The WT-1 protein is a zinc finger transcription factor that is important in embryonic kidney and early genitourinary development. It acts as a tumor suppressor or oncogene depending on the cell type and promoter context. The *WT1* gene is composed of 10 exons that code for multiple isoforms of the WT-1 protein, with molecular weights of 45 to 49 kDa. Four major isoforms of WT-1 are the result of alternative splicing, while 8 minor isoforms result from different initiation sites. Some isoforms have a role in RNA processing rather than transcription regulation. Thus, WT-1 may be a critical antigen in the initiation or maintenance of a malignant phenotype.

WT-1 is a promising target antigen in many malignancies including leukemia because it is differentially expressed in a broad number of malignant cells. WT-1 also is a useful marker of residual myeloid leukemia, and it may be expressed in a subset of leukemia-initiating cells.[2,3] Until the current study, a modest number of WT-1 epitopes confined mostly to the HLA-A2, HLA-A24, and HLA-DR4 alleles had been identified as immunogenic.[4-6] Nevertheless, the results of clinical immunotherapy trials in leukemia patients have shown that T-cell responses can be induced against these epitopes and objective clinical responses have been observed in some patients. Therefore, it is reasonable to expect that if further epitopes that are restricted by additional HLA alleles could be identified, targeted immunotherapy could be extended to more patients, which may be clinically beneficial. Moreover, targeted immunotherapy approaches, such as those that target WT-1, are potentially less toxic than conventional treatments due to the specificity of the immune response and the absence of significant toxicity in the antigen-targeted therapy trials to date.

Nevertheless, there are caveats to the exciting potential for directly translating these findings to the clinic. While adaptive immunotherapy strategies necessarily target specific epitopes, identifying the relevant antigens is not the only, nor the most significant, obstacle to the development of new immunotherapy treatments for leukemia and other cancers. For example, even when we understand which epitopes are targeted on leukemic blasts, to eliminate leukemia the antigens must be expressed on the leukemia stem cell and the therapy must preferentially eliminate leukemia stem cells over normal hematopoietic stem cells. In addition, we must increase our understanding of how tolerance to these antigens is regulated. In particular, both central (thymic) and peripheral (lymph node) regulation of immunity to tumor-associated antigens such as WT-1, a self-antigen, must be understood. The role of immune checkpoint regulation, governed by interactions of surface molecules on antigen-presenting cells and T cells such as CD80/CTLA-4 and PD-L1/PD-1, are clinically highly relevant molecules that alter the threshold of T-cell activation, thereby favoring a milieu in which immunity to tumor antigens can develop. The role of regulatory T cells and B cells, and of myeloid-derived suppressor cells (MDSCs), is also critical for maintaining tolerance to tumor antigens. In addition, optimal strategies for therapeutically delivering the antigens (eg, antigen as peptide, protein, DNA, or cell-based system) and in what immunologic context (eg, modification of tolerance) and clinical context (eg, minimal residual disease state) still must be elucidated.