were no CAR-T cells at 1 month after infusion in the first CLL cohort, compared with retention of CAR-T cells up to 6 weeks after infusion in the blood and marrow of patients treated in the second CLL and ALL cohorts. When these CAR-T cells were collected 8 days after infusion and cultured with antigen-expressing fibroblasts, they exhibited marked expansion and cytotoxic effects. Furthermore, these T cells were found to have infiltrated tumor beds in the 1 patient who died shortly after T-cell infusion.

This study is one of a number of studies investigating the use of second-generation anti-CD19 CAR-T cells in B-cell neoplasms. The results of a series of 3 patients with refractory CLL treated with CD19-CD137 (41BB) CAR-T cells have been reported. While all 3 patients treated in this study had a large tumor burden, 1 achieved a partial response and 2 achieved complete responses that were ongoing 7 to 11 months after treatment. All patients experienced tumor lysis syndrome 7 to 21 days after T-cell infusion, and B-cell aplasia and hypogammaglobulinemia were observed. CAR-T cells were evident in the blood and bone marrow for at least 4 months after treatment, and these cells maintained their effector functions.

These protocols differ in their use of alternate costimulatory molecules, the conditioning regimen, and the number of CAR-T cells infused, but together they demonstrate the safety and potential efficacy of a novel anticancer treatment strategy. The different approaches used in these studies may impact significantly on the efficacy of this new technology and more patients need to be treated to identify the optimal treatment protocol. Given the small numbers treated to date, we do not yet know the possible unanticipated toxicities, nor can we predict how reproducible these findings will be in more patients.

CAR targets must be present on the surface of tumor cells, but also should contribute to their malignant phenotype and have limited expression on healthy host tissues to avoid tumor immunomodulation. The ability of these cells to persist is attractive from an antitumor perspective, but we do not yet know how long they will persist and how long their affects on healthy tissue may last; CAR-T cells with a central memory phenotype have been described. These cautions aside, if these results can be replicated in further studies in larger numbers of patients, this technology could be modified to be used in a variety of hematologic and solid malignancies.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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LYMPHOID NEOPLASIA

Comment on Chao et al, page 4890

Lymphoma spread? Target CD47-SIRPα!

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The transmembrane protein CD47 is a potential therapeutic target for treatment of a variety of hematologic malignancies. In this issue of Blood, Chao and colleagues use a human non-Hodgkin lymphoma (NHL) xenotransplant mouse model to demonstrate that CD47 is involved in dissemination of NHL and that blocking its interaction with the signal regulatory protein α (SIRPα) by anti-CD47 antibody therapy can prevent spread of this lymphoma.1

CD47, formerly known as integrin-associated protein (IAP), is a ubiquitously expressed penta-transmembrane domain Ig-like protein. Apart from its ability to associate with integrins, it serves as a receptor for the extracellular matrix protein Thrombospondin. Furthermore, CD47 is a ligand for SIRPα, an inhibitory ITIM-motif receptor prominently expressed by phagocytic cells. This interaction inhibits phagocytosis by macrophages. Not surprisingly, given this diversity of interacting partners, CD47 plays an important role in a wide variety of biologic processes, including leukocyte motility, adhesion and migration, phagocytosis and recognition of “self,” and as such (xeno) transplant rejection and hematopoietic stem cell engraftment.2

In previous studies, the groups of Weissman and Majeti reported that CD47 is upregulated on circulating hematopoietic stem cells during inflammation-mediated mobilization and on myeloid leukemic cells, enabling them to evade phagocytosis by macrophages.3

Furthermore, they established that increased expression of CD47 on the self-renewing leukemia stem cells in acute myeloid leukemia (AML) and on acute lymphoblastic leukemia (ALL) cells is an independent poor prognostic factor, and targeting of CD47 with a blocking antibody in a human xenograft mouse model depleted AML and ALL by enabling phagocytosis of the malignant cells.4 Recently, Chao and colleagues demonstrated that CD47 is also overexpressed on multiple B-cell NHL subtypes, including diffuse large B-cell lymphoma (DLBCL), B-cell chronic lymphocytic leukemia (CLL), mantle cell lymphoma (MCL), and follicular lymphoma (FL), with adverse prognostic in DLBCL, CLL, and MCL.5 Using human NHL xenograft mouse models with the human Burkitt lymphoma cell line Raji and primary DLBCL and FL cells, either injected intravenously (“disseminated model”) or subcutaneously (“localized model”); Raji only), they showed that anti-CD47 treatment reduced lymphoma growth and prolonged...
Various types of NHL overexpress CD47, which correlates with poor prognosis. In this issue of Blood, Chao et al show that CD47 is more prominently expressed by disseminated lymphoma cells and that targeting CD47 with a blocking antibody or by RNAi impairs chemokine-controlled migration, promotes macrophage-mediated phagocytosis and prevents dissemination of the malignant B cells (right).

Next, the authors explored the possible mechanism(s) underlying this impaired dissemination. Given the critical role of chemokines and integrins in lymphoma dissemination, combined with the interaction of CD47 with integrins, they investigated if the observed effect may involve chemokine-controlled migration and/or integrin-mediated adhesion. Interestingly, their results convincingly show that silencing or blocking of CD47 severely impaired migration of the Raji cells toward the chemokines CXCL12 and CXCL13. Basal integrin-mediated adhesion was not affected but, unfortunately, chemokine-induced integrin activation was not studied. Future experiments will have to elucidate the underlying molecular mechanism of this novel feature of CD47 (eg, CXCR4/5 signaling, cytoskeletal reorganization, or integrin activation). Furthermore, by phagocyte depletion with the bisphosphonate clodronate and by means of anti–CD47 antibodies capable or not of blocking the interaction of CD47 with the phagocytosis inhibitory receptor SIRPα, compelling evidence was provided that the impaired dissemination involves enhanced macrophage-mediated phagocytosis.

These findings are particularly interesting given the potential application of anti–CD47 therapy as treatment for lymphoma or leukemia. Obviously, there may be many pitfalls on the road to a possible clinical application. Regarding specificity and clinical safety, however, it is important to note that despite the ubiquitous expression of CD47, CD47-deficient mice have a rather mild phenotype, a blocking anti–mouse CD47 antibody does not exert toxicity in mice, and a blocking anti-human CD47 does not affect phagocytosis of normal human PB cells and CD34+ BM cells in vitro. This apparent selectivity of anti-CD47 for malignant cells most likely involves the differential co-expression of a pro-phagocytic signal, for example, of the membrane protein Calreticulin (also overexpressed in NHL). However, given the critical role of the CD47–SIRPα interaction in discriminating “non-self” from “self” or “eat me” from “do not eat me,” let’s not forget that in the xenografted mice, the mouse phagocytes will be more eager to attack and eat the “non-self” malignant human B cells. Therefore, in the end, the specificity, safety, efficacy and the underlying mechanism of action of the blocking anti-CD47 antibody therapy can only be reliably determined in a completely human setting, that is, in clinical trials.

If clinical trials turn out to be safe and promising, there will also be many interesting options for rational combination therapy. Apart from the rather obvious combination of anti–CD47 with rituximab, there may be more enticing candidates. An example? Some novel efficacious small molecule drugs that target the BCR signaling pathway, that is, pharmacologic inhibitors of Syk (R788/R406), Btk (PCI-32765), and PI3K (CAL-101), show an unexpected mode of action in clinical trials with NHL. In, for example, CLL, rather than directly killing the cells the rapidly reduced lymphadenopathy is accompanied by transient lymphocytosis. Given the role of Btk, Syk, and PI3K in both BCR- and chemokine-controlled integrin-mediated adhesion and migration of B cells, the observed transient lymphocytosis and tumor regression may be the direct consequence of overcoming BCR- and/or chemokine–controlled retention of the malignant B cells in their tumor microenvironment (lymph nodes and bone marrow), thereby depriving the cells of critical

**CD47** is an integrin-associated protein and a ligand for the phagocytosis-inhibitory receptor SIRPα (left). Various types of NHL overexpress CD47, which correlates with poor prognosis. In this issue of Blood, Chao et al show that CD47 is more prominently expressed by disseminated lymphoma cells and that targeting CD47 with a blocking antibody or by RNAi impairs chemokine-controlled migration, promotes macrophage-mediated phagocytosis and prevents dissemination of the malignant B cells (right).
growth- and survival signals. Indeed, recent studies with CLL and MCL have provided support for this explanation (Buchner et al11, M. F. M. de Rooij, J. J. Buggy, and M.S., manuscript in preparation). In this perspective, it is tempting to speculate that combining these agents with anti-CD47 may turn out to be a highly efficacious treatment for NHL patients: once the malignant B cells have been forced out of their protective growth- and survival-supporting microenvironment into the circulation, they are more accessible and vulnerable for the action of anti-CD47, preventing their dissemination and priming the fully exposed malignant B cells for being attacked and eaten by the macrophages. This would make a promising rational combination therapy for complete eradication of lymphoma, spread or not!

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

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In the present studies, patients with truncating mutations of CXCR4 who presented with the characteristic features of WHIM syndrome were treated with daily intramuscular injections of plerixafor over an escalating dose range. The baseline data in both study populations confirmed the profound neutropenia and lymphopenia that is characteristic of the disorder. A clinical response was observed in both cohorts, even at the lowest plerixafor dose, as assessed by serial complete blood counts, and dose responsiveness was observed up to the maximal dose used. Interestingly, lymphocyte populations showed a more robust response to treatment with neutrophils, with normalized or supranormal counts obtained even at submaximal doses. Neutrophil counts were responsive to treatment but never normalized, even at maximal plerixafor dosing. This pattern was unexpected as neutrophil mobilization in healthy subjects was greater than that of lymphocytes. Nonetheless, the neutrophil counts attained were in excess of 300 neutrophils/μL of blood in both studies, suggesting that therapeutic dosing was attainable within the range reported in these studies. In the study by McDermott and colleagues,1 drug pharmacokinetic and pharmacodynamic properties were confirmed to be similar in the WHIM patients as in previously reported healthy controls. The safety profile after 1 week of use in both patient cohorts was acceptable, supporting further investigation of plerixafor as a therapeutic agent in WHIM syndrome.

In addition to the safety data presented, the results of the current clinical studies confirmed an interesting discrepancy between the relative responsiveness of lymphocyte, monocyte, and neutrophil populations in control versus WHIM syndrome subjects. In particular, B lymphocytes were highly mobilized from nearly undetectable levels to supranormal levels. It is not clear yet from which compartment lymphocytes were released by plerixafor treatment, but McDermott and colleagues speculate that the source is also the bone marrow.1 The effect of chronic plerixafor lymphocyte mobilization on immune function in WHIM patients was not addressed in these studies, but the stage is now set for efficacy studies in which immune function can be characterized during a therapeutic trial. Clinical efficacy is likely with regard to prevention of neutropenia-related bacterial infections in light of the results obtained in both studies.

COMMENT ON McDERMOTT ET AL, PAGE 4957, AND ON DALE ET AL, PAGE 4963

Released on a WHIM

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In this issue of Blood, 2 groups (McDermott et al1 and Dale et al2) independently report the results of phase 1 clinical trials using the CXCR4-specific chemokine receptor antagonist plerixafor (Mozobil) to target the hyperfunctional CXCR4 signaling axis in patients with the rare immunodeficiency disease WHIM syndrome.

Warts, hypogammaglobulinemia, immunodeficiency, and myelokathexis (WHIM) syndrome is an unusual disorder whose cardinal features are severe neutropenia despite an abundance of mature neutrophils in the bone marrow (myelokathexis), lymphopenia, and susceptibility to human papillomavirus infection. Initial speculation regarding the mechanism of neutropenia centered on inappropriate retention of mature neutrophils versus premature apoptosis. Genetic studies have revealed that the disease is caused by terminal truncations of the chemokine receptor CXCR4 cytoplasmic domain, a domain important for receptor down-regulation. This discovery suggested that hyperactivation of the mutant receptor was the underlying pathogenic mechanism and that inhibition of signaling might be therapeutically effective (see figure). The highly specific CXCR4 antagonist plerixafor is currently approved as a stem cell mobilizing agent. The use of this agent as treatment for WHIM syndrome required establishing the safety of CXCR4 antagonism for chronic use and testing whether partial blockade would be effective in mobilizing hematopoietic populations that carried mutant CXCR4 receptors.

On McDermott et al, page 4957, and on Dale et al, page 4963

PHAGOCYTES & GRANULOCYTES

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