● ● ● CLINICAL TRIALS AND OBSERVATIONS

Targeting WT1 in hematologic malignancies?

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In this issue of *Blood*, Tawara et al report the first results of a novel treatment approach with T-cell receptor transduced autologous T cells targeting a restricted Wilms' tumor 1 (WT1)–derived epitope in patients with refractory acute myeloid leukemia (AML) and high-risk myelodysplastic syndrome (MDS).[1]

The WT1 protein is a zinc finger transcription factor that has been implicated in cell proliferation, differentiation, apoptosis, and organ development.[2] It was initially described as a tumor suppressor gene but later identified as a true oncogene,[3] and a modulator of tumor angiogenesis and progression.[4] WT1 is a tumor-associated antigen that has been used as a target for immunotherapy by adoptive transfer of antigen-specific T lymphocytes and/or vaccination.[5,6] These studies demonstrate the possibility of specific WT1-directed immune responses, particularly in hematologic malignancies.[7-9]

T-cell receptor (TCR) transduced T cells recognize malignant cells in the context of an HLA-restricted, epitope-specific manner. In this study, Tawara and coworkers administered 2 doses of autologous HLA*2402-restricted WT1$_{235-243}$–specific TCR-redirected T cells to patients with refractory AML or high-risk MDS in a dose-escalation phase 1 trial.[1] In addition, following the T-cell transfer, these patients received 2 injections of a mutated WT1 peptide vaccine with adjuvant targeting the same WT1 HLA*2402-restricted 235-243 epitope. Impressive increments of WT1-specific T-cell frequencies were observed in vivo as

evaluated using MHC-tetramer analyses. Increases of up to 16% of CD8$^+$ T cells were seen, with simultaneous transient decreases in the blast population.

This study confirms the safety of adoptively transferred WT1 TCR-transduced autologous T lymphocytes and describes a notable persistence of these cells in vivo. However, the clinical responses have been limited, and it will, therefore, be critical to build and expand on the results of this phase 1 study. First, generation and expansion of αβTCR-transduced T cells for adoptive transfer to the desired, more effective dose levels remain a logistical challenge. In this phase 1 dose escalation trial, none of the patients of a planned cohort 3, intended to receive 5×10^9 cells per dose, actually received treatment at this dose level. These patients received one-fifth of the intended dose. Furthermore, experience administering T cells transduced with the CD19-chimeric antigen receptor for patients with B-cell malignancies have convincingly shown that preceding lymphodepleting chemotherapy markedly improves T-cell persistence and clinical responses in vivo.[10] In the current study using WT1 TCR-transduced T cells, only a few patients received low-dose chemotherapy prior to T-cell infusion. Therefore, one approach to improve clinical

outcome in this setting would be to condition patients with lymphodepleting chemotherapy prior to T-cell infusion.

Second, despite the authors' conclusion that the use of the WT1 vaccine may not have significantly contributed to the overall response, a combination adoptive T-cell transfer and vaccine approach to enhance or maintain an initial immune response is desirable and logical. In this study, the authors administered only 2 T-cell infusions followed by 2 subcutaneous injections of mutated WT1 vaccine. It appears that repeated dosing of T cells and additional injections of the WT1 vaccine might also enhance T-cell persistence and clinical response in this patient population. Alternatively, the addition of a checkpoint inhibitor may provide a tool to enhance tumor-specific immune responses and improve clinical outcome. Overall, this study offers a new platform to enhance the specific targeting of WT1 in patients with myeloid malignancies using combination immune-based therapies.

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

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● ● ● **IMMUNOBIOLOGY AND IMMUNOTHERAPY**

Human and mouse leukocytes: different clockwork

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In this issue of Blood, *Zhao et al use a humanized mouse model to investigate the mechanisms driving daily oscillations in circulating human and murine leukocytes.[1] In the same mice, they find human and murine circulating leukocytes displaying inverted oscillations, reproducing the trafficking pattern previously observed in both species. A novel network regulating circadian leukocyte trafficking is proposed. It involves interspecies differences of stress-kinase regulation of reactive oxygen species (ROS), hypoxia–inducible factor 1α (HIF-1α) and clock gene–dependent regulation of the CXCL12 receptor CXCR4. This study underscores the crosstalk of the genetic clock with metabolism and ROS in the regulation of leukocyte migration and reveals new mechanistic players.*

Circadian rhythms allow for the organism's adjustment to basic day/night changes, such as activity/sleep or feeding cycles. These are governed at the organismal level by the pacemaker in the brain, the suprachiasmatic nucleus, which receives light input through the retinohypothalamic tract and synchronizes peripheral organs via the autonomic nervous system and the hypothalamic-pituitary-adrenal axis on a daily basis. At the cellular level, peripheral oscillators exist in many cell types and regulate metabolism, proliferation, and function. Different clocks interact with each other to ensure robust responses. Core clock genes, such as *BMAL1/ARNTL1*, regulate the transcription of multiple genes, including other clock genes that drive transcription-translation loops over 24 hours (reviewed in Curtis et al[2] and Takahashi[3]).

Oscillations previously found both in the number and the activity of hematopoietic progenitors and leukocytes might have important implications for regeneration and response to infections.[4-6] For instance, oscillations of *Bmal1* expression in inflammatory monocytes regulate chemokine genes and immune response.[7] Inverted oscillations in circulating leukocytes have been previously reported in (nocturnal) mice and (diurnal) humans.[4-6] In both species, leukocytes are preferentially released from the bone marrow into circulation during the resting period. However, the mechanisms explaining interspecies differences in leukocyte trafficking have remained elusive.

Zhao et al create hematopoietic chimeric mice to study the trafficking of human and murine leukocytes. The model consists of neonatal NOD-SCID IL-2Rγ$^{-/-}$ (NSG) mice sublethally irradiated and intrahepatically transplanted with CD34$^+$ human fetal liver cells. Then, 8- to 12-week-old mice carrying 30% to 50% human CD45$^+$ cells are selected for circadian studies.

Strikingly, the same chimeric mice show inverted trafficking patterns for human and murine leukocytes, reproducing the interspecies differences. Previous studies in mice and humans[4,5] have shown oscillations of the CXCL12-CXCR4 signaling pathway,

a key regulator of leukocyte migration. In C57BL/6 mice, previous studies have shown oscillations in bone marrow Cxcl12 expression.[4,8] Zhao et al do not find obvious *Cxcl12* messenger RNA oscillations in NSG mice. However, because the number of experimental mice studied was lower, the sampling less frequent, and the time points performed at other times, it remains unclear whether there are differences from the strain/immunodeficiency and/or the transplant setting or not. A similar consideration applies to *BMAL1* messenger RNA expression, which does not seem to oscillate in mouse or human leukocytes in the chimeric mice, but has been previously shown to oscillate in leukocyte subsets.[7]

Regardless, the sharp difference of mouse/human leukocyte trafficking in the same environment argues for key cell-autonomous mechanisms. Consistent with previous studies,[5,8] they find oscillations in CXCR4 expression in antiphase with circulating leukocytes. Blockade of Cxcl12 (which is murine derived in the humanized model) blunts oscillations of both murine and human leukocytes. Blockade of the human receptor has similar consequences on the human leukocytes, pointing toward a major role of CXCR4. To understand how the murine and human receptors are differentially regulated during circadian cycles, the authors profile clock gene expression in leukocytes. Intriguingly, in the humanized model, the peripheral oscillator appears to be present in mouse leukocytes, but not in human leukocytes. This interesting difference points toward species-specific regulation of CXCR4 (and possibly other adhesion receptors) in relation to the genetic clock, leaving a fertile area for future studies.

The authors find that CXCR4 oscillations are abolished in BMAL1-deficient leukocytes, consistent with previous findings in mice.[5] Therefore, they hypothesize that a clock network–independent BMAL1 function regulates CXCR4. Because HIF-1α expression follows circadian oscillations, regulates CXCR4, and binds BMAL1, the authors measure HIF-1α expression and find direct correlations with CXCR4 expression. Because HIF-1α is regulated by ROS, they measure ROS levels in human and murine leukocytes and find inverted expression patterns during circadian cycles. Treatment with the ROS inhibitor *N*-acetylcysteine abrogated oscillations in HIF-1α, CXCR4, and circulating leukocytes. They