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Titans awake: HMAs for virus-driven ATL

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In this issue of *Blood*, Watanabe et al identify a set of 22 genes, including negative regulators of T-cell receptor (TCR) signaling, downregulated with promoter hypermethylation in human T-cell lymphotropic virus-1 (HTLV-1) infected CD4$^+$ T cells. The authors then show that the use of hypomethylating agents (HMAs) inhibits adult T-cell leukemia-lymphoma (ATL) growth in xenograft mice.[1]

ATL is a highly malignant neoplasm that originates from CD4$^+$ T cells infected by HTLV-1, 20 to 50 years postinfection. HTLV-1 is endemic in parts of Japan, the Caribbean, Central and South America, Central Africa, Romania, Iran, and Papua New Guinea. HTLV-1 confers a 3% to 5% lifetime risk for ATL in the estimated 10 to 20 million people carrying the virus.[2] HTLV-1 randomly inserts as a retroviral provirus into the genome of infected cells. In addition to canonical *gag, pol*, and *env* genes, HTLV-1 encodes several accessory genes; 2 of these, *tax* and *HBZ* (see figure panel A), are oncogenic in transgenic mice. The complex Tax protein interactome, which includes kinases and E3 ligases, activates NF-κB, cyclin-dependant kinases, autophagy, and anti-apoptosis factors to enhance host cell proliferation. The HBZ protein modulates Tax activities and inhibits senescence and apoptosis.[3] Early in infection, Tax orchestrates widespread PRC2-mediated histone odification (ie, H3K27me3), downregulating targeted promoters.[4] This probably precedes CpG methylation changes that are the focus of Watanabe et al. Chromatin remodeling eventually affects the HTLV-1 5′ LTR, inducing viral latency and silencing *tax*, but not transcription of *HBZ* from the 3′ LTR (see figure panel A). The self-reinforcing epigenome thus established produces a stably disturbed transcriptome diabolically similar to that induced by Tax itself and seems to ensure long-term host cell persistence in Tax's absence. This phenomenon might have been selected by Tax's high immunogenicity.[4] The discovery of an aging-related somatic mutation signature in ATL suggested that neoplasia develops over decades, long after HTLV-1 latency, selecting for mutations (both Tax and HBZ inhibit p53[3]) plus an epigenome that de-represses TCR signaling to promote ATL expansion[5] (see figure panel A).

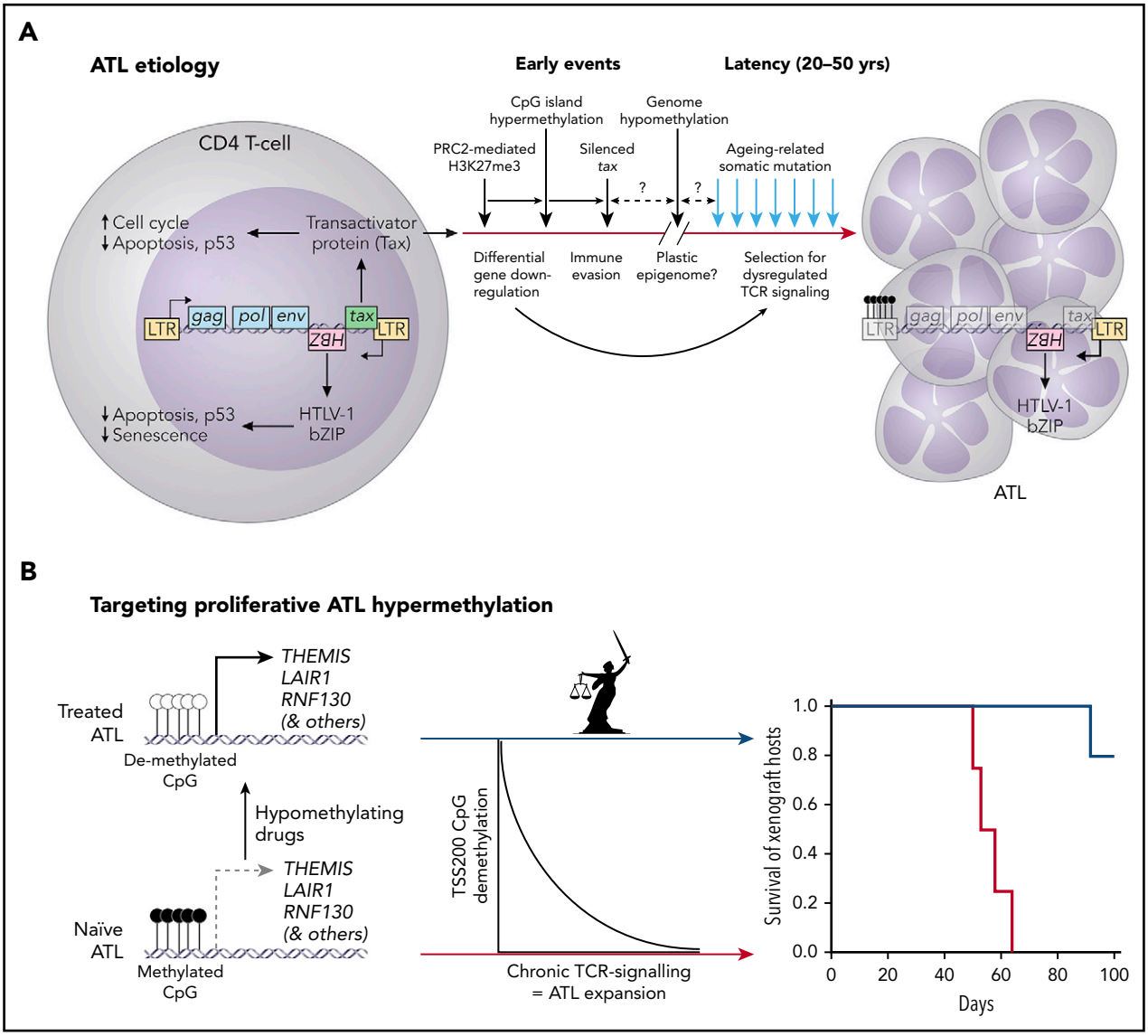
Diversity in clinical features and prognosis led to subclassification of ATL into smoldering, chronic and acute leukemic forms, and ATL lymphoma.[6] Although smoldering and chronic ATL are relatively indolent (4-year overall survival rates, 52% and 36%), about one-half progress to aggressive ATL (acute and lymphoma; 4-year overall survival rates, 11% and 16%).[7] Therapies include zidovudine plus interferon-alfa, monoclonal anti-CCR4 therapy, multiagent chemotherapy, or allogeneic hematopoietic stem cell transplantation, but cure is rarely achieved.[8]

Watanabe et al used Infinium BeadArrays to interrogate CpG methylation in HTLV-1 infected and uninfected CD4 T cells sorted by CADM1 and CD7 expression from healthy controls (n = 9), asymptomatic carriers (n = 3), and smoldering (n = 3), chronic (n = 4), and acute (n = 3) ATL patients. HTLV-1-infected cells progress from CADM1$^-$/CD7$^+$ (healthy T) through a CADM1$^+$/CD7^{dim} population to a CADM1$^+$/CD7$^-$ population that increases from indolent to acute ATL. HTLV-1-infected T cells were distinguished by CpG hypomethylation profiles using 20 000 CpG probes randomly sampled from the total of 470 870. Although global hypomethylation characterized infected cells, there was no significant difference in global hypomethylation levels between clinical groups, so its biological significance as well as its etiology remain unclear.

Delving deeper, 12 025 hypermethylated and 33 581 hypomethylated regions were identified in infected vs healthy T cells that were shared in all ATL patients; aggressive and indolent ATL clustered using the hypermethylated but not hypomethylated regions. These included 1207 hypermethylated CpG islands lying within 200 bp 5′ of transcription start sites ("TSS200"), with increasing hypermethylation as ATL progressed. A set of 22 downregulated genes with corresponding TSS200 hypermethylation included negative regulators of TCR signaling: *LAIR*, *RNF130*, and *THEMIS*. Dysregulation of TCR signaling is an established ATL hallmark.[5] Downstream effectors of TCR signaling were shown to be either persistent or constitutively active in ATL cell lines and amenable to downregulation by forced expression of *THEMIS*.

These data motivated experiments to reverse hypermethylation using clinically available hypomethylating agents: azacitidine (AZA) and decitabine (DAC), plus 2 new decitabine prodrugs, OR12 and OR21, which have the potential for oral bioavailability.[9] Encouraged by their in vitro data, Watanabe et al inoculated MT-2 ATL cells into mice subcutaneously and administered AZA, DAC, or OR21 intraperitoneally. They observed tumor reduction by all three HMAs, albeit with variable hematopoietic toxicity (least to most; AZA, OR21, DAC) and hypomethylation of *THEMIS* TSS200 (least to most; AZA, OR21, DAC) at the doses used. With a view to potential long-term oral therapy, OR-21 was used (intraperitoneally, twice weekly for 100 days) to treat mice bearing peritoneal hCD45$^+$/CADM1$^+$ xenografts established by inoculating peripheral blood mononuclear cells from a patient with chronic ATL. OR21 produced a striking improvement in morbidity and mortality (see figure panel B).

These discoveries were possible because the authors focused their analysis on shared differentially methylated regions in infected/uninfected cells in patients across the disease spectrum; the devil lies in finding relevant detail. Disregarding the small cohort size, it is encouraging that epigenetically downregulated genes converged on pathways already implicated by orthogonal means and demonstrably amenable to therapeutic manipulation. The degree to which hypomethylation or addiction to TCR signaling dictate drug



(A) The etiology of HTLV-1 infection (splicing and scale are ignored in the simplified virus genome) and conversion to ATL. (B) ATL etiology involves promoter CpG hypermethylation that silences downregulators of TCR signaling, including Themis, the Titaness personification of natural law. Expansion of chronic ATL xenografts in mice was significantly inhibited by HMA therapy with OR-21.

efficacy remains debatable; AZA was equally efficacious in reducing tumor volume despite significantly lower effect on CpG methylation in the *THEMIS* promoter than DAC or OR-21. Parenteral formulations of AZA and DAC are in regular clinical use for higher risk myelodysplastic syndrome, chronic myelomonocytic leukemia and low-blast acute myeloid leukemia (AML) and are an alternative for newly diagnosed AML in the elderly who have comorbidities that preclude use of intensive induction chemotherapy. Because HMA therapy is continued for as long as patients derive benefit, oral formulations are preferable, and oral AZA (CC-486) and decitabine and cytidine deaminase inhibitor combinations (eg, ASTX727) have advanced through to phase 3 trials, with the former showing promise as maintenance therapy following AML remission.[10] OR-21 was shown to be bioavailable following intraduodenal administration to macaques and is potentially another oral HMA.[1,9] Despite the limitations of their xenograft model, Watanabe et al demonstrate that a slumbering Themis can be roused to restore her natural law. Their data are a compelling precursor for clinical evaluation of HMAs in ATL.

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MYELOID NEOPLASIA

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Why do Tregs suddenly disappear in aplastic anemia?

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In this issue of *Blood*, Lim et al show that regulatory T cells (Tregs) from aplastic anemia (AA) patients are susceptible to Fas ligand (FasL)-mediated apoptosis, which may explain, in part, their diminution at disease onset.[1] The cell death can be overcome in vitro and in vivo by interleukin-2 (IL-2) supplementation. This work builds on the previous observation of the same group, which showed 2 subpopulations of Tregs with distinct phenotypes in AA: Treg A, with a naive phenotype (low proliferative index), and Treg B, with a memory phenotype (moderate/high proliferative index) that correlated with response to immunosuppressive therapy (IST).[2]

It is well established that acquired AA is characterized by a proinflammatory state whereby activated $CD4^+$ (T helper 1 [Th1], Th17), $CD8^+$ T cells and associated proteins/cytokines result in stem and progenitor cell damage and their elimination.[3] Various degrees of bone marrow failure ensue, with pancytopenia a hallmark of disease. If severe, consequences of pancytopenia can be dire when untreated. Along with proinflammatory signals, lack of regulation constitutes an important disturbance in autoimmune diseases, in general, which is also seen in AA.[2,4] The mechanisms that result in this reduction are not well established.

In the current study, the investigators explore the mechanisms that underlie the imbalance and functional impairment between the Treg subsets in a small cohort of AA patients (n = 19) and healthy donors (n = 25). Apoptosis-related genes and pathways were preferentially upregulated in Treg B (compared with Treg A) and associated with a greater induction of cell death following FasL exposure. IL-2 rescued Treg B undergoing FasL-mediated apoptosis. These expanded cells maintained suppressive properties in proliferation assays in vitro and in a graft-versus-host disease (GVHD) animal model in vivo. Although Treg A was more resistant to FasL, it did not expand in low IL-2 concentrations in vitro; however, at higher doses, Treg A and Treg B expanded in culture and acquired a more memory phenotype. During this expansion, increased expression of phosphorylated BCL-2 was observed, providing a prosurvival signal abating FasL-mediated apoptosis for both Treg populations. These observations provide a mechanistic insight into Treg depletion in AA. But how can they be translated into the clinic?

For many decades, the primary focus in clinical protocol development in severe AA (SAA) has been to address the proinflammatory milieu and suppress it with lymphocytotoxic and immunomodulatory drugs. Nearly 30 years ago, the combination of horse anti-thymocyte globulin (h-ATG) and cyclosporine (CsA) was defined as standard IST.[5] Since then, several alternate immunosuppressive regimens have been developed; however, results have been disappointing because of inferior outcomes in response to h-ATG/CsA or prohibitive toxicity.[5] In the past decade, a nonimmunosuppressive approach, using stem cell stimulation with thrombopoietin receptor agonists, produced promising outcomes. Improvements in marrow function and blood counts were shown with eltrombopag as a single agent in IST-refractory SAA, as well as in the front line when combined with standard h-ATG/CsA.[5] Thus, the combination of IST and stem cell stimulation addresses the different facets of AA pathophysiology, producing a more complete hematologic recovery.

Could an intervention that improves immune regulation help to overcome the inhibitory signals to the marrow in AA? One possibility would be increasing Tregs in vivo. Toward this goal, different approaches have been sought in transplantation and in autoimmune disorders. One of the earlier promising strategies was to infuse autologous ex vivo–expanded Tregs; however, its development has been hindered by technical, scalability, and logistical problems, with no robust clinical data reported.[6] Exogenous administration of IL-2, as proposed by Lim et al, could be an alternate strategy. At lower doses, IL-2 activates and expands Tregs and has been investigated in several proof-of-concept, dose-finding, and phase 1/2 trials in vasculitis, refractory GVHD, type 1 diabetes, alopecia, and systemic lupus erythematosus.[7] In general, IL-2 was well tolerated, and an increase in Tregs was noted in most studies. Clinical benefit was observed; however, given the lack of a control group, IL-2's activity could not be determined. Its short half-life has been limiting in these earlier studies, and different formulations and associations with other Treg-promoting agents are being explored.

A more practical approach would be to use drugs with Treg-inducing properties. Unfortunately, this approach has not been encouraging in SAA. Sirolimus and rabbit anti-thymocyte globulin (ATG) are immunosuppressants that have shown to expand Tregs in vitro and in vivo[8,9];