children with acute lymphoblastic leukemia who have a superior clinical outcome. *Blood*. 2006;108(1):97-102.

3. Vinhas E, Lucena-Silva N, Pedrosa F. Implementation of a simplified flow cytometric assays for minimal residual disease monitoring in childhood acute lymphoblastic leukemia. *Cytometry B Clin Cytom*. 2018;94(1):94-99.

4. Arora RS, Eden T, Pizer B. The problem of treatment abandonment in children from developing countries with cancer. *Pediatr Blood Cancer*. 2007;49(7):941-946.

5. Cohen P, Friedrich P, Lam C, et al. Global access to essential medicines for childhood cancer: a cross-sectional survey. *J Glob Oncol*. 2018;4:1-11.

6. Hunger S, Reyes D, Negrin O, et al. Decreased early mortality and increased survival with less intensive therapy for acute lymphoblastic leukemia in the Dominican Republic [abstract]. *Pediatr Blood Cancer*. 2011;57(5):761.

7. Inaba H, Greaves M, Mullighan CG. Acute lymphoblastic leukaemia. *Lancet*. 2013;381(9881):1943-1955.

8. Borowitz MJ, Devidas M, Hunger SP, et al; Children's Oncology Group. Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia and its relationship to other prognostic factors: a Children's Oncology Group study. *Blood*. 2008;111(12):5477-5485.

DOI 10.1182/blood.2020005292

LYMPHOID NEOPLASIA

Comment on Yoshida et al, page 1467

CD28 fusions: an opportunity for young ATL?

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In this issue of *Blood*, Yoshida et al identify concurrent fusions of CD28 with CTLA4 and ICOS in younger Japanese patients with adult T-cell leukemia/lymphoma (ATL).[1]

With chemotherapy-based treatment approaches, overall survival in ATL has not significantly improved in the nearly 40 years since human T-cell lymphotropic virus and ATL were first described.[2] Therefore, the identification of a novel, measurable, and druggable target is exciting.

The median age at presentation with ATL is ~70 years in Japanese patients, whereas ATL arising in patients living in the United States, Europe, the Caribbean, and South America occurs at age 45 to 55 years.[3] It is not understood whether the differences in age at presentation reflect differences in disease biology, underlying host genetics, immune response to the tumor or virus, or environmental factors.

Yoshida et al hypothesize that the tumors of younger patients with ATL would contain distinct genetic alterations, similar to other cancers that present in younger individuals, such as ETV6-RUNX1 fusion seen in childhood acute lymphoblastic leukemia. They identify concurrent CTLA4-CD28 and ICOS-CD28 fusions in 37.5% of ATL cases (3 of 8 cases) in those age <50 years, the presence of which did not affect survival. This is in contrast to earlier reports of peripheral T-cell lymphoma (PTCL) and ATL, where the presence of both fusions was rare.[4,5]

CD28 and ICOS are costimulatory molecules that potentiate T-cell activation on binding their respective ligands CD80/CD86 and ICOSL. In contrast, ligation of the coinhibitory CD28 homolog CTLA4 inhibits T-cell activation through binding ligands of CD28 with higher affinity, sequestering CD80/CD86 and initiating an inhibitory signaling cascade. The CTLA4-CD28 fusion consists of the extracellular and transmembrane domains of CTLA4 and the intracellular signaling domain of CD28, whereas the ICOS-CD28 fusion combines only the signal peptide from ICOS with the extracellular and intracellular domains of CD28. In other PTCL tumors, this ICOS-CD28 fusion was associated with CD28 overexpression and ICOS haploinsufficiency.[6]

Yoshida et al demonstrate in vitro that the expression of CTLA4-CD28 and, to a lesser degree, CTLA4-ICOS fusions could induce cellular proliferation when cocultured with cells expressing CD80 and CD86. In ATL cases with both fusions present, immunohistochemistry demonstrated that ATL tumor cells express CD80 and macrophages in the tumor microenvironment express CD86, suggesting that both intra- and intercellular actions could drive cellular proliferation in vivo. The authors report that cases with fusions had gene expression signatures associated with AKT and RAF signaling and, strikingly, LAG3 downregulation, which is known to negatively downregulate T-cell proliferation. Finally, the in vitro CD80/CD86-driven proliferation of cells expressing CTLA4-CD28 fusion could be suppressed with a CTLA4-blocking antibody in a dose-dependent manner.

Chemotherapy alone rarely cures ATL, and there is a desperate need for new and better therapies and for an understanding of how to apply our current therapies more effectively. For example, mogamulizumab is associated with better responses in leukemic rather than nodal disease, even more so in the presence of CCR4-activating mutations.[7] Similarly, responses to combination therapy with zidovudine and interferon-α are significantly better in leukemic rather than nodal ATL.[8,9] This work suggests that there may be a rationale for targeting cases with CD28 fusions with an anti-CTLA4 antibody and/or targeting its downstream effectors, such as the phosphatidylinositol 3-kinase pathway.

Of course, these observations were made in a small number of cases, and it remains unclear why these dual fusions were not observed in a larger Japanese cohort,[4,10] which also included a small number of cases who presented at age <50 years. It is logical that these fusions should be investigated systematically in cohorts arising in the United States, South America, and Europe, where so-called young ATL is frequently seen. Presumably because of the small number of cases here, the age cutoff at 50 years is arbitrary, but in a larger cohort, perhaps a true biological entity may be defined. Understanding these biological differences and how to best select treatments and apply new therapies will be crucial to improving survival outcomes.

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

REFERENCES

1. Yoshida N, Shigemori K, Donaldson N, et al. Genomic landscape of young ATLL patients identifies frequent targetable

CD28 fusions. *Blood*. 2020;135(17):1467-1471.

2. Katsuya H, Ishitsuka K, Utsunomiya A, et al. Treatment and survival among 1594 patients with ATL. *Blood*. 2015;126(24):2570-2578.

3. Iwanaga M, Watanabe T, Yamaguchi K. Adult T-cell leukemia: a review of epidemiological evidence. *Front Microbiol*. 2012;3:322.

4. Kataoka K, Nagata Y, Kitanaka A, et al. Integrated molecular analysis of adult T cell leukemia/lymphoma. *Nat Genet*. 2015;47(11):1304-1315.

5. Vallois D, Dupuy A, Lemonnier F, et al. RNA fusions involving *CD28* are rare in peripheral T-cell lymphomas and concentrate mainly in those derived from follicular helper T cells. *Haematologica*. 2018;103(8):e360-e363.

6. Rohr J, Guo S, Huo J, et al. Recurrent activating mutations of CD28 in peripheral T-cell lymphomas. *Leukemia*. 2016;30(5):1062-1070.

7. Sakamoto Y, Ishida T, Masaki A, et al. CCR4 mutations associated with superior outcome of adult T-cell leukemia/lymphoma under mogamulizumab treatment. *Blood*. 2018;132(7):758-761.

8. Bazarbachi A, Plumelle Y, Carlos Ramos J, et al. Meta-analysis on the use of zidovudine and interferon-alfa in adult T-cell leukemia/lymphoma showing improved survival in the leukemic subtypes. *J Clin Oncol*. 2010;28(27):4177-4183.

9. Cook LB, Fuji S, Hermine O, et al. Revised adult T-cell leukemia-lymphoma international consensus meeting report. *J Clin Oncol*. 2019;37(8):677-687.

10. Kataoka K, Iwanaga M, Yasunaga JI, et al. Prognostic relevance of integrated genetic profiling in adult T-cell leukemia/lymphoma. *Blood*. 2018;131(2):215-225.

DOI 10.1182/blood.2020004958

MYELOID NEOPLASIA

Comment on Long et al, page 1472

The Fox(o) and the HDAC

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In this issue of *Blood*, Long et al[1] describe a FOXO1/3-HDAC8-p53–mediated pathway leading to resistance and persistence of FLT3-ITD$^+$ acute myeloid leukemia (AML) stem cells after FLT3-inhibition.

A fox, known for his cunning and wiliness, frequently features in many fables by the Greek slave Aesop and others. In the fable "The Fox and the Hare," the hare stares at the fox expressing his wonder about the fox's insidiousness. The fox replies by inviting the hare for dinner that evening, so they may discuss the subject. The hare accepts the invitation, but upon arrival at the fox's house realizes that only plates and bowls were laid on the dining table, but no food. The hare cries out what a fool he has been to accept the invitation, as he was meant to serve as dinner, and runs away.

FOXO(e)s, transcription factors, which normally act as tumor suppressors,[2] slyly may also promote leukemogenesis.[3] In fact, the stem cell in AML features several cunning fox(o)es, which lead to stem cell resistance to standard chemotherapeutic regimen, as well as their persistence. Modulations of signaling pathways or autophagy, (over) expression of certain proteins or enzymes such as drug efflux pumps, topoisomerase and others, gene alterations in genes, such as Fms-like tyrosine kinase 3 (*FLT3*), etc, microRNAs influencing cell damage, cell cycle, or apoptosis, and cell death,[4] immune evasion mechanisms or interactions with the tumor microenvironment,[5] may lead to resistance to chemotherapy. However, various mechanisms of resistance also occur in AML treated with targeted therapies, such as tyrosine kinase inhibitors (TKIs).

AML associated with internal tandem duplications in *FLT3* (FLT3-ITD$^+$ AML) affects approximately one-third of AML patients, whereby FLT3-ITD portends a poor prognosis, leading to increased risk of relapse and inferior overall survival.[6] The advent of well-tolerated FLT3-targeting TKI, however, has improved the outcome of patients with FLT3-ITD$^+$ AML, when used in combination therapies, although they do not eliminate leukemia stem cells. In 2017, midostaurin, a multikinase inhibitor, was approved for frontline treatment of AML patients with mutations of *FLT3* in combination with standard chemotherapy. Later, the FLT3 inhibitor gilteritinib was approved, and quizartinib is in clinical trial. However, resistance to FLT3 inhibitors, especially when used as monotherapy, seems to be the cause for the unsatisfactory clinical response. Predominant mechanisms of resistance are mutations in the activation loop or the "gatekeeping domain" in the tyrosine kinase domain of *FLT3*,[7] activation of prosurvival signaling pathways, but mutations in other genes, such *TET2* or *IDH1*, have also been observed, particularly in patients treated with the FLT3 inhibitor crenolanib.[8] In addition, the bone marrow microenvironment and its production of FLT3 ligand or fibroblast growth factor 2 has been implicated in the resistance of FLT3-ITD$^+$ AML cells to FLT3 inhibitors.[9]

In this article by Long et al, the authors unravel a novel pathway of resistance of FLT3-ITD$^+$ AML cells to FLT3 inhibitors via forkhead box protein (FOXO1/3)-mediated upregulation of histone deacetylase 8 (HDAC8). Upregulation of HDAC8 is shown to inactivate the tumor suppressor protein p53, thereby promoting the persistence of FLT3-ITD$^+$ AML cells in the presence of an FLT3 inhibitor (see figure).

Using 2 different cell lines with mutated *FLT3* and primary human AML cells, the authors demonstrate that treatment with quizartinib leads to upregulation of HDAC8, whose perturbed expression has previously been associated with poor prognosis in various cancers.[10] HDACs, in general, regulate genome stability and gene expression by the modification of histones and chromatin remodeling. Targeting of HDAC8 in combination with FLT3 inhibitor therapy increased the death of FLT3-ITD$^+$ AML cells and prolonged survival in immunosuppressed nonobese diabetic/severe combined immunodeficiency mice transplanted with *FLT3*-mutated cell lines. Gene expression analysis of *FLT3*-mutated cells treated with an HDAC inhibitor revealed significant upregulation of the p53 pathway. Conversely, depletion of p53 prevented the apoptosis of FLT3-ITD$^+$ AML cells treated with the HDAC and/or FLT3 inhibitor. Hypothesizing that increased expression of HDAC8 was due to enhanced transcription, the authors elegantly identified the role of the transcription factors FOXO1/3 for the upregulation of HDAC8 and the promotion of resistance to FLT3 inhibitors. Targeting HDAC8 also activated p53. In xenotransplantation studies with human FLT3-ITD$^+$ AML cells, the authors confirmed that pharmacological targeting of HDAC8 and FLT3 led to a superior outcome compared with treatment with the