these mutations coexisted only rarely in the same leukemia.[6] Thus, although it is likely that the presence of NT5C2 in a relapse subclone is a surrogate for a relapse-driving process, the nature of this process remains to be identified.

Another intriguing hypothesis to explain the association of subclonal NT5C2 mutations with poor prognosis relapse involves a non-cell-autonomous mechanism. Could NT5C2-mutated cells enhance the fitness and chemotherapy resistance of adjacent leukemic cells? This possibility has recently been experimentally demonstrated. FLT3-mutated subclones enhanced the fitness of experimental KMT2A-MLLT3 fusion leukemias by secreting the macrophage migration inhibitory growth factor.[9] Is it possible that NT5C2-mutated cells in the bone marrow niche promote the survival and evolution of other leukemic subclones during remission of the primary ALL? Borrowing again from social sciences, this "collective impact" may be a general mechanism for coexistence or codependence of subclones that propagate leukemic cell resilience.

Beyond raising fascinating questions, the Barz et al study has 2 practical implications. First, molecular identification of NT5C2 may independently predict poor prognosis and may be used in risk stratification as an indicator for high-risk treatment. However, given the subclonal nature of NT5C2 mutations and their disappearance in subsequent relapses, specific therapy targeting the mutated NT5C2 cells[8] at the time of relapse is unlikely to be beneficial. Whether targeting NT5C2-mutated cells during high-risk ALL first-line maintenance therapy will reduce the risk of relapse remains to be determined.

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

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DOI 10.1182/blood.2020004990

LYMPHOID NEOPLASIA

Comment on Wong et al, page 934

Tearing ATL apart to find HTLV's sinister plans

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In this issue of *Blood*, Wong et al report that interferon regulatory factor 4 (IRF4) and nuclear factor κ light chain enhancer of activated B cells (NF-κB) drive maintenance of adult T-cell leukemia/lymphoma (ATL) by coordinately stimulating a transcriptional regulatory network normally intended for promoting T-cell immune functions.[1]

Despite carrying few genes, viruses can reshape the genetic landscape of normal cells and can start the process of transforming those cells into cancer cells through diverse mechanisms. A classic example is human T-cell lymphotropic virus type 1 (HTLV-1), which integrates into the genome of mature T cells and pushes them into becoming ATL cells.[2] The few proteins encoded by the limited HTLV-1 genome must cleverly hijack the normal cellular machinery of T cells so that HTLV-1 can thrive and reproduce. In T cells, the NF-κB pathway is primed to activate a transcriptional regulatory network of genes to drive cell proliferation in response to pathogens and other dangers. Uninfected T cells switch the NF-κB pathway on and off as needed. In contrast, HTLV-1–infected T cells express an oncoprotein called Tax that flips the switch to the on position.[2] If the infected T cells acquire mutations in the NF-κB pathway, the switch is flipped permanently to the on position, transforming precancerous cells into fully malignant ATL cells.[3] Fortunately, this happens in only about 5% of patients after more than 50 years of viral latency. But when ATL happens, the consequences can be devastating. Median survival is less than 1 year.

To improve outcomes, investigators have sought to better understand the ATL cancer drivers in the hopes of finding new vulnerabilities that might someday be targeted by drugs. Nakagawa et al[4] showed that a virally encoded oncoprotein called HTLV-1 basic leucine zipper factor (HBZ) induces the expression of the basic leucine zipper ATF-like transcription factor 3 (BATF3). BATF3 has a partner called IRF4 that is highly expressed and can be somatically mutated in ATL cells, driving T-cell proliferation.[3,5] Together, BATF and IRF factors form a transcription factor complex that induces genes important for various T-cell immune functions,[6] including *MYC*, one of the most essential ATL oncogenes.[4] Moreover, somatic mutations of the chemokine receptor *CCR4* enhance ATL cell migration and cell growth, which can be countered by therapeutic anti-CCR4 antibodies.[3,7] Although these earlier studies showed us how individual ATL players function, there was no unified understanding of how these players coordinate their actions to

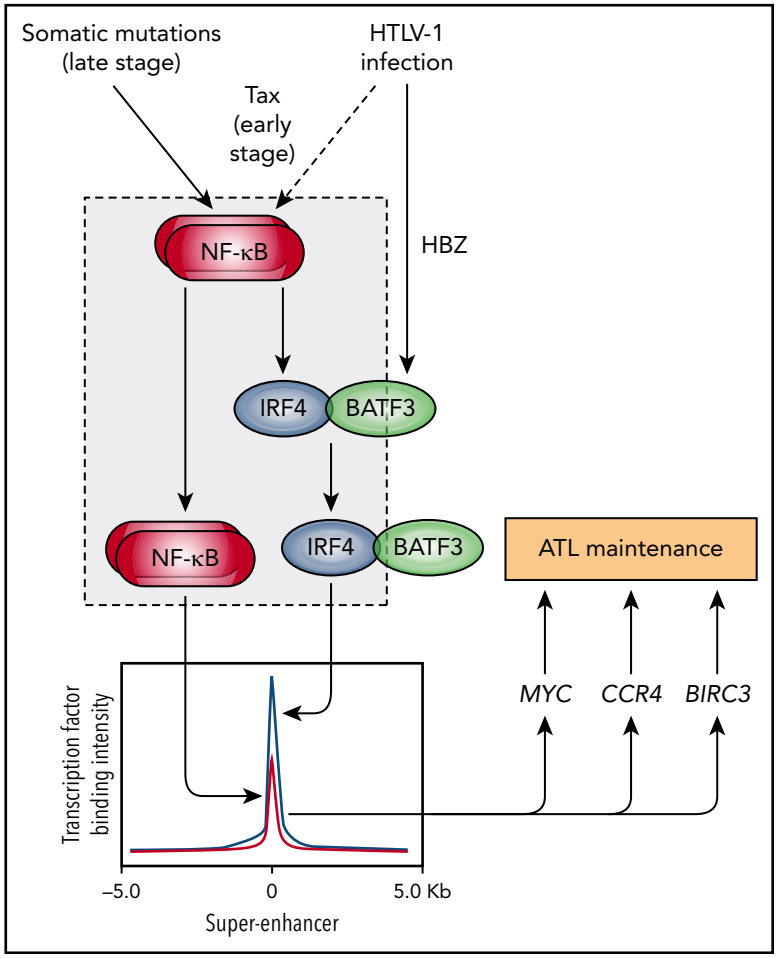

Diagram depicting a network of transcriptional interactions driving ATL maintenance that is initiated by HTLV-1 infection and then propagated by somatic mutations. The gray box indicates the network motif identified by Wong et al called a "coherent feed-forward loop" that is used by normal T cells to rapidly expand to fight foreign invaders but is hijacked and constitutively activated in ATL cells. This loop consists of the top-tier transcription factor NF-kB inducing the second-tier transcription factor IRF4 followed by cooperative binding and activation by the 2 factors of super-enhancers associated with major oncogenes (*MYC*, *CCR4*, and *BIRC3*).

promote ATL. The study by Wong et al formally establishes the existence and functional importance of an IRF4-NF-κB network motif by which several players join forces to drive ATL cell proliferation and survival.

The article by Wong et al refers to an impressive array of chromatin profiling, gene expression, and functional studies in ATL cell lines and patient samples that confirms previous observations of high IRF4 expression in ATL and essential roles of IRF4, MYC, and NF-κB for ATL oncogene expression and cell viability. When Wong et al examined the patterns of where NF-κB and IRF4 bind the tumor cell DNA and regulate gene expression, they saw remarkable convergence far higher than what would be expected by chance. For example, they identified a highly expressed ATL gene that was cooperatively induced by NF-κB and IRF4 called baculoviral IAP repeat containing 3 (*BIRC3*). BIRC3 is an E3 ubiquitin ligase and member of the IAP family of proteins that inhibits apoptosis.[8] Wong et al showed that BIRC3 was essential for ATL cell viability, establishing this protein as a novel ATL vulnerability. Besides *BIRC3*, additional nodes of convergence included *CCR4*, *MYC*, and several players in the T-cell receptor signaling pathway that activate NF-κB. Next, Wong et al delineated a key network connection in which NF-kB induced *IRF4* expression, which is consistent with previous studies in normal T cells.[9] This final link of the network established a pattern of regulation known in the jargon of mathematical modeling as a "type 1 coherent feed-forward loop".[10] In this pattern, transcription factor X induces transcription factor Y and then X and Y jointly induce gene Z (see figure). In all, Wong et al integrate new and old observations to reveal a unified perception of ATL oncogenesis consisting of viral inputs (Tax and HBZ), a propagating network motif (NF-kB and IRF4-BATF3), and oncogene outputs (*MYC*, *CCR4*, and *BIRC3*).

The Wong et al study was performed to stringent standards, resulting in high-quality signals, particularly for the chromatin profiling and gene expression data. Exceptional rigor was demonstrated by their use of multiple biological replicates, genetic tools, and cell lines that filled in gaps and confirmed and extended the literature. Even so, questions remain. Although the different ATL cell lines generally share the coherent feed-forward loop, it is unclear why there is heterogeneity. For example, the precise response elements that control *MYC* expression vary between cell lines, raising the possibility of additional players. In addition, mathematical simulations predict that the kinetics of the ATL feed-forward loop will be delayed when turning on and will be fast when shutting off. Although this feature seems attractive from a therapeutic standpoint, it is unclear how one can toggle the loop to the off state in the first place. Possibilities include small molecules that degrade key effectors or disrupt protein-protein interactions, the latter finding recent successes in breaking the menin-MLL interaction in myeloid leukemia. However, it remains unknown whether disrupting interactions between ATL players like IRF4 and BATF3 will show anti-leukemic effects or is even pharmacologically feasible.

In a remarkable and considerable undertaking, Wong et al stitched together several observations to define the satisfying architecture of a central ATL transcriptional network. We have learned that HTLV-1 is not only stealing the host cell's machinery to make copies of itself and express viral oncoproteins, it is also rewiring the host cell's transcriptional network to enhance its reproduction through cell division and subsequently ATL transformation. HTLV-1 evolved over thousands of years to hone its sinister plans to perfection. It is now up to us to find ways to expeditiously subvert the ATL network to treat this devastating disease.

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

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DOI 10.1182/blood.2020004998

MYELOID NEOPLASIA

Comment on Levy et al, page 948

Novel ET mutations: stuck in the MPL with you

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In this issue of *Blood*, Levy et al report on how novel mutations in the transmembrane (TM) domain of the thrombopoietin (TPO) receptor cause familial essential thrombocythemia and intriguingly link their potential mechanisms of action with that of receptor agonists.[1]

The hematopoietic cytokine TPO is the primary regulator of megakaryopoiesis, driving both megakaryocyte differentiation and progenitor expansion, as well as having essential roles in hematopoietic stem cell survival and proliferation.[2] The TPO receptor, TpoR (also known widely as MPL), is a member of the type I cytokine receptor family, sharing sequence and functional homologies with the receptors for erythropoietin, prolactin, and growth hormone.[3] In addition to its key roles in physiological hematopoiesis, TpoR has emerged as the common mechanistic link between the prevalent driver mutations in the Philadelphia chromosome negative myeloproliferative neoplasms (MPNs). Mutated *JAK2* and *CALR* interact with TpoR and are both reliant on the expression of physiological levels of the receptor to cause disease,[4] whereas a small number of MPN patients harbor mutations in TpoR itself, primarily in the TM and intracellular juxtamembrane (JM) regions (S505N and W515K, respectively), directly leading to receptor hyperactivity.[5,6] Gaining a complete understanding of how TpoR mutations alter receptor activity not only is important clinically but also provides a fascinating insight into cytokine receptor function, especially the role of the plasma membrane and membrane-associated regions of the receptor.

In this issue of *Blood*, Levy et al identify 2 familial essential thrombocythemia (ET) patients with novel double mutations of TpoR, L498W-H499C and H499Y-S505N, which highlight how subtle interactions between amino acids in the TM can result in potent changes in receptor activity (see figure). To understand the functional activity of these mutations, the authors generated cell lines expressing the TpoR mutations alone and in combination. The L498W mutation increases cytokine-independent STAT5 activity and cellular proliferation, both of which are potentiated by expressing L498W-H499C in combination, suggesting that H499C acts additively with the L498W mutation. As TpoR needs to form dimers to allow the cross-phosphorylation and activation of associated JAK2 molecules, the authors have applied a luciferase complementation assay that enables them to estimate the proportion of receptors that are in a dimeric state in the presence or absence of the newly identified mutations. Interestingly, both L498W and H499C increase the proportion of dimeric TpoR both alone and in combination; however, given the lack of factor-independent signaling and growth in cells expressing H499C, it would appear that in this case the dimeric complex fails to initiate signal transduction. Furthermore, both L498W and H499C are able to initiate signaling in the absence of the extracellular domain (truncated at position 489), suggesting that these mutations work by altering the configuration of the TM and cytosolic domains to allow interactions between the associated JAK2 molecules.

TpoR H499Y, which was found in combination with S505N in the second patient in this study, was also able to activate STAT5 and greatly increased S505N activity when expressed together. However, H499Y was unable to further enhance L498W activity while mutating either L498 or H499 enhanced signaling by the more common TpoR W515K mutation, highlighting the diversity in the mechanisms of action between mutations in the TpoR TM and intracellular JM regions.

Throughout the study, the authors draw potential parallels between the TM mutations and the mechanism of eltrombopag (Elt), a small molecule TpoR agonist used for the treatment of idiopathic thrombocytopenia purpura.[7] Although TpoR-H499 is essential for Elt activity,[8] it has been suggested previously that other amino acids upstream of H499 are equally important. A lack of Elt activity on the truncated TpoR supports this theory and leads the authors to investigate potentially important amino acids in the extracellular JM region of the receptor. Taking a structural modeling approach, W491 was identified as a key upstream residue. Being located on the same helical face as L498, S505, and W515 in the active dimer, the authors hypothesize that altering the position of