monoclonal free LC levels of 1000 mg/L in the serum and development of Bence Jones proteinuria similar to that in human patients (see figure). In addition, the LC deposition disease that developed in the mice mimics all of the human renal pathology, including nodular glomerulosclerosis, which could not be reproduced in the HC deposition disease model.[9] Furthermore, treatment with cyclophosphamide and bortezomib resulted in reduction of the plasma cells and serum-free LC concentration, ultimately decreasing the LC deposits in the kidney, albuminuria, kidney injury, and death from kidney failure.

This transgenic mouse model represents a major breakthrough and will no doubt contribute to a better understanding of MGRS-related diseases. This model was able to reproduce the human pathology in mice, and it showed prevention of renal damage with treatment using cyclophosphamide and bortezomib, which are often used to treat patients with LC deposition disease. Most importantly, it provided unambiguous evidence that cancer, multiple myeloma in this case, is not required for the development of MGRS-related diseases. When the concept of MGRS was introduced, the goal was to gain access to cytotoxic therapies to eradicate the clone that produces the monoclonal protein to preserve kidney function.[2] However, since the clones responsible for MGRS are usually indolent, it begs the question whether eradication of these small indolent clones is necessary if the monoclonal proteins can be removed or inhibited from interacting with the kidney.[10] The ability to test this question was made possible with the model developed by Bender et al. Models like these are essential for developing new alternative treatments that do not rely on cytotoxic drugs. Ironically, that would take us back to the doctrine that cytotoxic therapy is reserved for cancer. Scientifically, that is actually a good thing, since ideas and concepts are never meant to be stagnant. They need to constantly be challenged so they can be improved.

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

REFERENCES

1. Bender S, Ayala MV, Bonaud A, et al. Immunoglobulin light-chain toxicity in a mouse model of monoclonal immunoglobulin light-chain deposition disease. *Blood.* 2020; 136(14):1645-1656.

2. Leung N, Bridoux F, Hutchison CA, et al; International Kidney and Monoclonal Gammopathy Research Group. Monoclonal gammopathy of renal significance: when MGUS is no longer undetermined or insignificant. *Blood.* 2012; 120(22):4292-4295.

3. Rajkumar SV, Dimopoulos MA, Palumbo A, et al. International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. *Lancet Oncol.* 2014;15(12): e538-e548.

4. Hallek M, Cheson BD, Catovsky D, et al; International Workshop on Chronic Lymphocytic Leukemia. Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 guidelines. *Blood.* 2008;111(12): 5446-5456.

5. Kyle RA, Gertz MA. Primary systemic amyloidosis: clinical and laboratory features in 474 cases. *Semin Hematol.* 1995;32(1): 45-59.

6. Gertz MA. How to manage primary amyloidosis. *Leukemia.* 2012;26(2):191-198.

7. Solomon A, Weiss DT, Kattine AA. Nephrotoxic potential of Bence Jones proteins. *N Engl J Med.* 1991;324(26): 1845-1851.

8. Stokes MB, Valeri AM, Herlitz L, et al. Light chain proximal tubulopathy: Clinical and pathologic characteristics in the modern treatment era. *J Am Soc Nephrol.* 2016;27(5): 1555-1565.

9. Sirac C, Herrera GA, Sanders PW, et al. Animal models of monoclonal immunoglobulin-related renal diseases. *Nat Rev Nephrol.* 2018; 14(4):246-264.

10. Leung N, Bridoux F, Batuman V, et al. The evaluation of monoclonal gammopathy of renal significance: a consensus report of the International Kidney and Monoclonal Gammopathy Research Group. *Nat Rev Nephrol.* 2019;15(1):45-59.

LYMPHOID NEOPLASIA

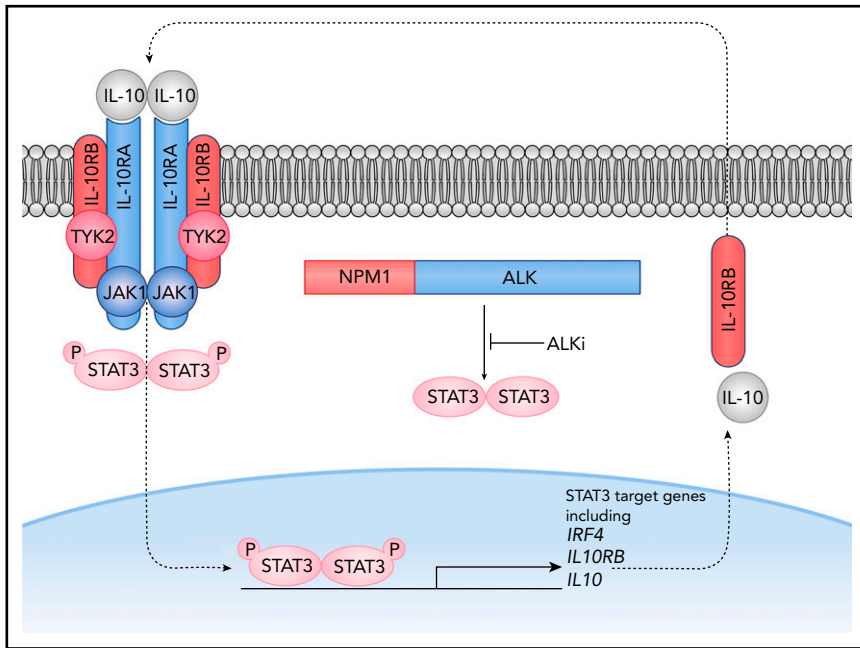# Drivers of crizotinib resistance in ALK+ ALCL

Guangzhen Hu and Andrew L. Feldman | Mayo Clinic

In this issue of *Blood*, Prokoph et al[1] seek to identify mechanisms by which ALK+ anaplastic large cell lymphoma (ALCL) cells develop resistance to the ALK inhibitor crizotinib. Their findings implicate a novel and potentially targetable interleukin-10 (IL-10) receptor-dependent autocrine loop that bypasses ALK signaling.

ALK+ ALCL is the most common T-cell non-Hodgkin lymphoma in children, the biology of which is driven predominantly by chromosomal rearrangements fusing the *ALK* tyrosine kinase gene to various partners, predominantly *NPM1*.[2] The resultant fusion protein, NPM-ALK, activates numerous cellular processes, including activation of the STAT3 transcription factor. Pediatric clinical trials have honed frontline combination chemotherapy regimens for ALK+ ALCL, but toxicity and a persistent subset of patients with poor outcomes remain significant challenges. NPM-ALK also can be targeted directly. Crizotinib is an ALK/MET/ROS1 inhibitor with demonstrated clinical efficacy in pediatric ALK+ ALCL; however, crizotinib resistance develops in some patients.[3] In a subset of these cases, resistance develops through acquisition of *ALK* mutations, which can be detected by sequencing and potentially targeted by newer-generation ALK inhibitors, such as alectinib, ceritinib, brigatinib, and lorlatinib.[4] However, non-ALK signaling pathways, such as the IGF-1R pathway, also may contribute to crizotinib resistance.[5] The role of these alternative signaling pathways in crizotinib resistance remains poorly understood.

To characterize mechanisms underlying crizotinib resistance in ALK+ ALCL, Prokoph et al performed a genome-wide CRISPR activation screen in 3 ALK+ ALCL cell lines and identified several candidate genes, including *STAT3, RORC, MYC, IRF4,* and *IL10RA.* The authors then evaluated these candidates by overexpressing them individually and by using a CRISPR knockout screen. The top gene candidate was *IL10RA,* which was overexpressed in

Overexpression of IL10RA leads to crizotinib resistance in ALK+ ALCL cells through an autocrine loop that bypasses the NPM-ALK fusion protein. IL-10 signaling leads to activation of STAT3, which in turn transcriptionally regulates *IL10* and *IL10RB* and promotes cell survival despite inhibition of ALK tyrosine kinase activity. P, phosphorylation. See Figure 5H in the article by Prokoph et al that begins on page 1657.

30% of crizotinib-resistant cell lines and was more highly expressed in a crizotinib-resistant patient sample without ALK mutations than in an *ALK*-mutated crizotinib-resistant patient sample or in samples from patients who relapsed on standard chemotherapy. The IL-10 receptor complex consists of a tetramer of IL10RA and IL10RB that interacts with JAK1 and TYK2 to activate STAT3 (see figure). Prokoph et al mechanistically demonstrated that the IL-10 signaling pathway modulated sensitivity of ALK+ ALCL cells to ALK inhibition via NPM-ALK–independent activation of STAT3. They further showed that STAT3 binds to the promoters of the *IL10*, *IL10RA*, and *IL10RB* genes, supporting the existence of a positive feedback loop that bypasses NPM-ALK. Although IL-10 signaling is common in ALK+ ALCL,[6,7] only a subset of patients develops crizotinib resistance, suggesting that only particularly strong IL-10 signaling is able to maintain STAT3 activity sufficient for cell survival when NPM-ALK is inhibited by crizotinib. Notably, the existence of ALK-independent mechanisms of STAT3 activation also has been demonstrated in ALK-negative ALCL,[8] although STAT3-negative ALCLs also exist.[9]

The findings of Prokoph et al characterize a novel and potentially targetable mechanism of crizotinib resistance in ALK+ ALCL cells. For example, JAK inhibitors, TYK2 inhibitors, and/or direct STAT3 inhibitors might contribute to regimens that treat or prevent some cases of ALK inhibitor resistance. Combining ALK inhibitors with chemotherapy also might lessen the incidence of resistance. It remains unclear, however, what proportion of clinical cases of crizotinib resistance involve the IL-10 autocrine loop demonstrated here, or what level of IL10RA expression and/or other conditions are needed in vivo for induction of this mechanism. In these studies, IL10RA was artificially overexpressed in vitro with or without IL-10 supplementation; further clinical studies will be required to assess the degree to which *IL10RA* overexpression by tumors represents a predominant and recurrent mechanism underlying resistance to crizotinib and perhaps other ALK inhibitors. The full diversity of ALK-independent escape mechanisms also remains unknown; survival pathways other than IL-10 signaling may exist and could relate to other gene candidates identified by the authors' screening approach.

In summary, Prokoph et al report an elegant approach to characterize crizotinib resistance mechanisms using in vitro screening in a relatively uncommon pediatric tumor with significant barriers to obtaining large numbers of clinical samples. Ongoing characterization of the spectrum of such mechanisms could lead to new salvage regimens as well as biomarker assays that help individualize management according to the mechanism of resistance. As the authors discuss, the precise mechanism driving *IL10RA* overexpression as a resistance mechanism requires further study. More broadly, the factors underlying the diversity of resistance mechanisms that develop among patients with the same disease receiving the same drug remain unclear. Understanding why different tumors acquire different mechanisms of resistance, while still others retain chemosensitivity, will facilitate development of more effective tyrosine kinase inhibitors, frontline combination therapies, and personalized strategies to predict and possibly prevent resistance.

Conflict-of-interest statement: A.L.F. receives research funding from Seattle Genetics and is an inventor of technology related to ALCL for which Mayo Clinic holds unlicensed patents. G.H. declares no competing financial interests. ■

REFERENCES

1. Prokoph N, Probst NA, Lee LC, et al. IL10RA modulates crizotinib sensitivity in NPM1-ALK+ anaplastic large cell lymphoma. *Blood.* 2020; 136(14):1657-1669.

2. Larose H, Burke GAA, Lowe EJ, Turner SD. From bench to bedside: the past, present and future of therapy for systemic paediatric ALCL, ALK. *Br J Haematol.* 2019;185(6): 1043-1054.

3. Sharma GG, Mota I, Mologni L, Patrucco E, Gambacorti-Passerini C, Chiarle R. Tumor resistance against ALK targeted therapy–where it comes from and where it goes. *Cancers (Basel).* 2018;10(3):E62.

4. Camidge DR. Next-generation ALK inhibitors: is the median the message? *Lancet Respir Med.* 2020;8(1):5-7.

5. Li Y, Wang K, Song N, et al. Activation of IGF-1R pathway and NPM-ALK G1269A mutation confer resistance to crizotinib treatment in NPM-ALK positive lymphoma. *Invest New Drugs.* 2020;38(3): 599-609.

6. Kasprzycka M, Marzec M, Liu X, Zhang Q, Wasik MA. Nucleophosmin/anaplastic lymphoma kinase (NPM/ALK) oncoprotein induces the T regulatory cell phenotype by activating STAT3. *Proc Natl Acad Sci USA.* 2006;103(26): 9964-9969.

7. Knörr F, Damm-Welk C, Ruf S, et al. Blood cytokine concentrations in pediatric patients with anaplastic lymphoma

kinase-positive anaplastic large cell lymphoma. *Haematologica.* 2018;103(3): 477-485.

8. Crescenzo R, Abate F, Lasorsa E, et al; European T-Cell Lymphoma Study Group, T-Cell Project: Prospective Collection of Data in Patients with Peripheral T-Cell Lymphoma and the AIRC 5xMille Consortium "Genetics-Driven Targeted Management of Lymphoid Malignancies". Convergent mutations and kinase fusions lead to oncogenic STAT3 activation in anaplastic large cell lymphoma. *Cancer Cell.* 2015;27(4):516-532.

9. Luchtel RA, Dasari S, Oishi N, et al. Molecular profiling reveals immunogenic cues in anaplastic large cell lymphomas with *DUSP22* rearrangements. *Blood.* 2018;132(13): 1386-1398.
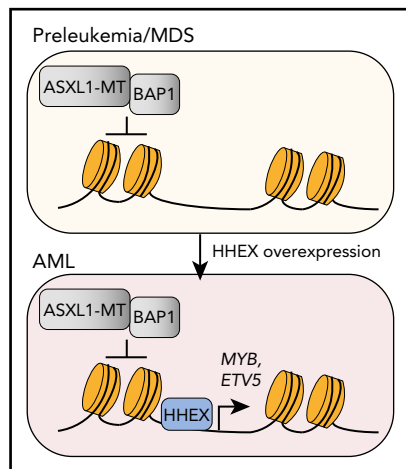

Comment on Takeda et al, page 1670

HHEX expression drives AML development

Daniel T. Starczynowski | Cincinnati Children's Hospital Medical Center

In this issue of *Blood*, Takeda and colleagues[1] reveal the importance of hematopoietically expressed homeobox (HHEX) in promoting acute myeloid leukemia (AML) transformation in cooperation with mutant additional sex combs-like 1 (ASXL1) and identify MYB and ETV5 as critical transcriptional targets shared by HHEX and mutant ASXL1.

AML results in the accumulation of immature clonal myeloid cells, driven by acquired genetic mutations in hematopoietic stem and progenitor cells (HSPCs). The largest class of recurrent AML gene mutations affect epigenetic modifiers, including DNMT3A, IDH1/2, TET2, EZH2, MLL/KMT2A, and ASXL1, which are often found as early initiating events.[2] Although mutations in any one of these epigenetic-modifying genes result in significant changes in the epigenetic landscape and gene expression in HSPCs, they are often insufficient to result in overt transformation to leukemia, suggesting that additional genetic and/or molecular changes are required for AML. For example, the cooccurrence of mutant DMNT3A and FLT3-ITD is associated with an unfavorable prognosis in patients and results in rapid and penetrant AML in mouse models.[3-5] In other cases, an initiating genetic mutation in the context of an altered cellular state coinciding with changes in the expression of critical genes is sufficient for leukemic cell transformation. Although frequently cooccurring gene mutations have been extensively studied in AML, much less is known about the relevant altered gene expression changes that cooperate with leukemia-initiating gene mutations.

ASXL1 is one of the most frequently mutated genes in myeloid malignancies, occurring in 5% to 11% of patients with AML. ASXL1 belongs to a family of chromatin-binding Polycomb proteins and is involved in controlling gene expression by interacting with epigenetic regulators, such as Polycomb repressive complex 2 (PRC2) and BAP1. Interaction of ASXL1 and BAP1 at promoters results in monoubiquitination of histone H2A at lysine 119 (H2AK119ub), catalyzed by PRC1 complexes, and subsequent repression of target gene expression. The majority of ASXL1 mutations are frameshift or nonsense mutations that disrupt the C-terminal plant homeodomain finger region resulting in C-terminally truncated ASXL1 mutant proteins (referred herein as ASXL1-MT). ASXL1-MT retains its ability to interact with several of its epigenetic binding partners and induces aberrant histone modifications in a dominant-negative or gain-of-function manner. Thus, these epigenetic changes induced by ASXL1-MT are thought to contribute to the development of myeloid neoplasms.

The contribution of ASXL1 mutations has been illuminated by analysis of ASXL1-MT knockin (ASXL1-MT-KI) mice, which develop mild anemia, impaired erythroid differentiation, myeloid skewing, and dysplasia.[6] Although these hematologic changes are indicative of early-stage myelodysplastic syndromes (MDS), the ASXL1-MT-KI mice never develop overt leukemia, suggesting that additional elements are required for myeloid transformation of ASXL1-mutated cells. To identify cooperating molecular events with ASXL1-MT, the team performed a retrovirus-mediated insertional mutagenesis screen and identified *HHEX* as a candidate that may cooperate with ASXL1-MT to induce myeloid leukemia.

HHEX is a homeobox-containing transcriptional repressor that is overexpressed in various subtypes of AML, including those with ASXL1 mutations.[7] In the current study, the team investigated the potential cooperation between ASXL1-MT and HHEX overexpression in myeloid leukemogenesis. Utilizing retroviral models, overexpression of HHEX expanded ASXL1-MT–expressing HSPCs by preventing apoptosis and blocking differentiation. However, overexpression of HHEX had nominal effects on normal HSPCs. Importantly, overexpression of HHEX in ASXL1-MT HSPCs accelerated the development of AML, whereas HHEX depletion attenuated the leukemic cell

ASXL1 mutant protein (ASXL1-MT) is associated with preleukemic states, including MDS. However, cooperating genetic and/or molecular changes are required for progression of ASXL1-MT–expressing preleukemic cells to AML. HHEX, a homeobox-containing DNA-binding protein, is overexpressed in AML and cooperates with ASXL1-MT to induce AML. HHEX mediates leukemia cell transformation by cooperating with ASXL1-MT to induce expression of MYB and ETV5, which are critical target genes for AML cell viability and prevention of differentiation.