patients, indicating that it is possible to classify disease before splenectomy, using a “liquid biopsy” approach.

Moving from genes to patterns of expression and to morphology, the authors show that the DMT cluster is characterized by an atypical morphology, whereas cases belonging to the other 3 categories display a more typical morphology. Subsequent analysis of expression of 1402 “microenvironmetal” genes by RNA sequencing added an additional layer to this novel molecular stratification, by dividing SMZL in immune suppressive and immune silent subsets. The first subset is characterized by expression of genes typical of T cells, type 2 macrophages, and coding for exhaustion markers. These findings are in line with the concept that infiltrating immune cells cannot react against the tumor. Conversely, the immune silent subset essentially lacks expression of microenvironment-related genes, with an overt dominance of pathways belonging to tumor B cells, underlining that these tumors are not invaded by T cells. Interestingly, these expression profiles are equally distributed among genetic profiles, raising the question of what controls microenvironment organization. Mouse models recapitulating the different genetic lesions will likely be instrumental in providing additional information on how tumor cells shape the microenvironment.6,7 In addition, a possible role of infectious agents, for SMZL typically hepatitis C virus, needs to be addressed to determine whether it can induce a T-cell-rich and exhausted, or a T-cell-depleted environment.

In summary, this comprehensive study reclassified SMZL, complementing classical diagnostic tests, such as immunohistochemistry and flow cytometry, with mutational analyses and gene expression profiles. Survival data show that this classification is effective in identifying disease subgroups with differing overall survival. Even if in the case of an indolent disease such as SMZL the clinical impact of genomic and transcriptomic (re)classification deriving from of this observation may be limited, it will certainly be a founding element for the design of novel therapeutic strategies, as is already happening in other more aggressive lymphomas.8

There are 2 main features of this work that make it applicable for clinical care. The first is the definition of a minimal set of 14 genes that created an accurate mutational classification of SMZL into the 4 subentities described previously. This panel is easily adaptable to routine diagnosis of pathology laboratories. The second feature is that, in principle, the panel could be run using peripheral blood samples. If confirmed by independent studies, these findings imply that mutational classification could be performed independent of splenectomy. Treatment of SMZL is currently shifting from splenectomy to more conservative approaches that target the B cell: future studies will tell whether different mutational profiles will respond to different targeted drugs.

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**LYMPHOID NEOPLASIA**

Comment on Sasaki et al, page 748

**JAK-ing up treatment for CRLF2-R ALL**

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In this issue of Blood, Sasaki and colleagues used a novel screening approach to identify the signaling pathways that B-cell precursor acute lymphoblastic leukemia (B-ALL) cells harboring cytokine receptor-like factor 2 rearrangements (CRLF2-r’s) depend on to survive, leading to new suggestions for therapy.1

In normal monocytes, dendritic cells, and basophils, CRLF2 partners with the interleukin 7 receptor α chain to form a receptor complex that interacts with the ligand thymic stromal lymphopoietin receptor to activate downstream pathways. CRLF2-r in B-ALL result in “ectopic” overexpression of the CRLF2 receptor complex in immature B cells, which do not normally express this protein. CRLF2-r B-ALL falls within the subgroup of “Philadelphia chromosome-like” ALL because of overactivation of multiple downstream signaling pathways that resemble those in Philadelphia chromosome–positive ALL.2

CRLF2-r arises mainly from two genetic alterations: IGH-CRLF2, which is a reciprocal translocation with the immunoglobulin heavy chain locus, and P2RY8-CRLF2, which is an interstitial deletion within the pseudoautosomal region 1 (known as PAR1) of chromosomes X and Y. Rarely, recurrent CRLF2 mutations occur. Downstream pathways activated by CRLF2-r reportedly include the JAK/STAT and PI3K/AKT/mTORC1 pathways. It is noteworthy that JAK mutations are
present in approximately half of CRLF2-\(r\) cases and that RAS-related genes (KRAS, NRAS, PTPN11, and NF1) are commonly mutated in CRLF2-\(r\) ALL.\(^3\)

In clinical practice, CRLF2-\(r\) and overexpression is common, reported in 10% to 15% of cases of B-ALL, particularly in teenagers and young adults.\(^3\) It is overrepresented in patients of Hispanic ethnicity and predicts a poor outcome.\(^5\) JAK inhibition with ruxolitinib is being evaluated in clinical trials, because of the perceived importance of the JAK-STAT pathway in this disease. However, in preclinical work, ruxolitinib has shown somewhat limited efficacy as a single agent,\(^6\) especially when compared with the confirmed utility of tyrosine kinase inhibitors in Ph-like ALL arising from ABL-class fusions.\(^7\)

In an elegant study examining the causes of ruxolitinib resistance in CRLF2-\(r\) ALL, Sasaki and colleagues used two cell lines expressing IGH-CRLF2, both of which also harbored the same JAK2 mutation, by using CRISPR screening, which allows for the simultaneous interrogation of many genes, with the goal of assessing the contribution of a given gene or pathway to a particular phenotype - in this case, ruxolitinib resistance. Essentially, the researchers wanted to determine which genes enable cells to survive despite the presence of ruxolitinib. They did this work in cells that either did or did not have RAS mutations, to also explore the relevance of the RAS pathway. The authors then performed signaling pathway studies to validate their screening findings. Finally, they tested novel drug combinations to block the identified pathways, both in vitro and in patient-derived xenograft mouse models, using CRLF2-\(r\) cells.

The work of Sasaki et al has several key conclusions. First, the findings agree with the work of others that the mTORC1 pathway plays a key role in the survival of cells harboring IGH-CRLF2.\(^8\) A second and perhaps unexpected finding is that survival of the CRLF2-\(r\) cells did not depend on genes of the STAT pathway, downstream of JAK. Third, mutations of the RAS pathway changed which other pathways the IGH-CRLF2 cells depended on to survive.

The findings are important because of the clinical implications and potential applications. First, inhibition of both RAS and mTORC1 pathways is likely to be critical in treating this disease entity. Of interest, signaling through both those pathways is activated by receptor tyrosine kinase-mediated signals, and, in Sasaki et al, a combination of ruxolitinib and gilteritinib, a Flt3 inhibitor with broad tyrosine kinase activity, clearly blocked those pathways. Second, the data suggest that ruxolitinib should not be the treatment of choice in RAS-mutated CRLF2-\(r\) ALL, but that treatment should instead be based on inhibiting RAS pathway-related genes. Finally, the data presented suggest that the MEK1/2 inhibitor trametinib can block downstream RAS signaling, but only when combined with gilteritinib, which inhibits receptor tyrosine kinase-mediated signals upstream of RAS. The combination, which effectively blocks both the RAS and mTORC1 pathways, had significant antileukemic activity in this study.

There are caveats to this work: in the mouse model, less robust anti-ALL activity was observed in the marrow compared with that in the blood and spleen. The suggestion that this difference stems from the particular relationship between the CRLF2-\(r\) ALL cells and the bone marrow microenvironment warrants further investigation. A very recent study using unbiased, high-throughput screening in CRLF2-\(r\) ALL, showed that ruxolitinib was synergistic with the standard-of-care chemotherapy drugs used in the treatment of ALL, particularly vincristine and dexamethasone.\(^9\) These agents can dissociate B-ALL cells from the protective influence of stromal cells in the microenvironment, as shown by work from my laboratory,\(^10\) further suggesting that cell-external factors are also relevant to drug resistance in CRLF2-\(r\) ALL. The second caveat is that the only model studied by Sasaki and colleagues was an IGH-CRLF2 model.

In summary, this interesting and important work suggests novel and rational directions toward improving therapy for CRLF2-\(r\) ALL by using existing approved agents and is of relevance to clinicians, trialists, and basic scientists.

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