macrophages produced more CXCL13 than old macrophages and that CXCR5, the receptor for CXCL13, was more highly expressed in B-ALL cells transplanted into young recipients. Inhibition or blockade of CXCL13 reduced growth of B-ALL cocultured with young, but not old, macrophages, whereas CXCL13 treatment was sufficient to promote faster B-ALL growth in vitro and in vivo. In agreement, mouse recipients of *Cxcr5*$^{-/-}$ B-ALL cell transplants had reduced leukemic burden and increased survival when compared with those receiving wild-type leukemia cells. Furthermore, systemic depletion of macrophages and other phagocytes with clodronate-loaded liposomes reduced the leukemic burden and prolonged survival exclusively in young mice. These rigorous experiments demonstrate a key role for the CXCL13-CXCR5 axis in B-ALL and that this pathway is only functional in the young bone marrow microenvironment, most likely via CXCL13-producing macrophages (see figure). Therefore, the age of the microenvironment controls B-ALL progression.

The aging of the hematopoietic system results in extensive remodeling of the microenvironment, with changes in the frequencies and function of its cellular components and redistribution of hematopoietic stem cells within.[7,8] Zanetti et al demonstrate that this remodeling directly leads to changes in the biology of the leukemia. We anticipate that aging-induced remodeling of the microenvironment also affects leukemia initiation, progression, and chemotherapy resistance in other hematological malignancies and provides a rationale for some of the differences observed between leukemias in pediatric and geriatric patients.

Is this CXCL13-CXCR5 proleukemic pathway also functional and significant in humans? Zanetti et al found increased frequency of CD68^{+} macrophages and CXCL13 protein in bone sections from pediatric patients when compared with adult patients with B-ALL. In retrospective analyses of published B-ALL patient cohorts, they found that low CXCR5 levels correlated with better outcomes for some B-ALL subsets. The major limitation of these analyses is that the number of patients examined is too small to draw firm conclusions. Nevertheless, these findings provide a strong rationale for investigating whether CXCR5/CXCL13 expression can be used as predictive markers and whether this axis can be therapeutically targeted in B-ALL.

In addition to its direct clinical implications, the findings by Zanetti et al also raise exciting new questions. What is the role of CXCL13-producing macrophages in the young bone marrow? How is CXCL13 production, or CXCL13-producing macrophages, lost during aging? Answering these is a necessity, to ascertain whether this macrophage pathway can be therapeutically blocked without deleterious effects on hematopoiesis.

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MYELOID NEOPLASIA

Comment on Awada et al, page 1885

Machine learning finds new AML subtypes

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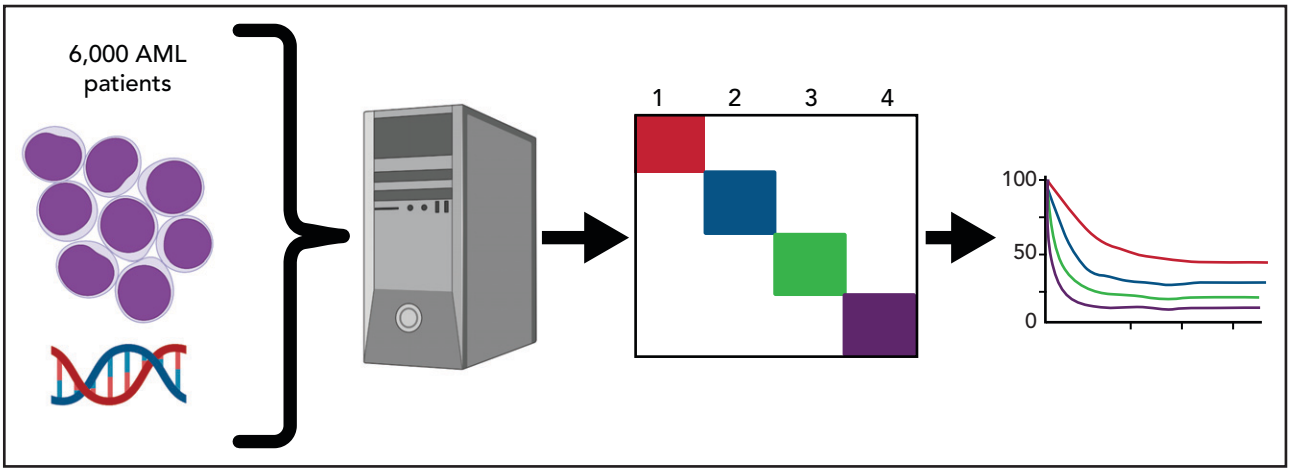
As applications for machine learning in medicine continue to expand, Awada et al demonstrate the utility of artificial intelligence algorithms for predicting prognosis in acute myeloid leukemia (AML) by defining novel molecular groupings.[1]

This is a fine example of a clinically actionable classification system based on machine learning that would not have been obvious to an experienced hematologist or in routine statistical methods. Machine learning is rapidly transforming medicine in radiology by automating the detection of clinically important patterns found within pixelated images,[2] but its exact role in cancer management is still in its infancy.

AML is an excellent test bed for combinatorial biomarker testing in cancer: at least 50% of patients have a relatively stable karyotype, a low somatic mutation burden, and a high consistency of mutation recurrence.[3-5] This means that the next new adult patient with de novo AML is likely to harbor at least 1 of the 22 most frequent somatic mutations, such as *FLT3*, *NPM1*, or *TET2*. Clinicians have known for more than a decade that mutations in combination matter. Patients with *NPM1* mutation but without *FLT3-ITD* have a better prognosis than those with *NPM1* and *FLT3-ITD*. Biallelic *CEBPA*-mutated AML has a better prognosis than one *CEBPA* mutation.[6] But going beyond these examples and including



Genomic lesions including somatic mutations and translocations from >6000 AML patients were aggregated. Machine learning identified 4 underlying genomic clusters based on molecular features that were then shown to have differing survival rates, implying underlying differences in biology.

more complex combinatorial mutation groupings is difficult and requires large sample sizes and computational algorithms. We also know from preleukemic stem cell studies that the acquisition order of mutation probably matters a great deal in AML (ie, whether you get a *RUNX1* mutation first or a *TET2* mutation first).[7] Therefore, if we are to improve precision prognosis prediction, then we cannot consider genomic lesions in isolation.

The need for complementary prognosis classifiers is urgent: many normal karyotype patients, for instance, can simply not be categorized as high or low risk. These AMLs may lack biallelic *CEBPA* or *FLT3-ITD* but have a selection of somatic mutations (such as *EZH2*, *IDH2*, or *GATA2*) that, when considered in isolation, have not been shown to be particularly high risk or low risk. In this issue, Awada et al from the Maciejewski group at Cleveland Clinic elegantly demonstrate the power of machine-learning–type algorithms to reveal previously unrecognized subgroups of AML that are not obviously apparent. Their predictive model is available as a web-based open source resource (https://drmz.shinyapps.io/local_app/), although they emphasize that their proposed schema is complementary to rather than a replacement of current classifications.[8]

Awada et al chose to use a simple machine-learning algorithm, Bayesian latent class analysis, applied to 6788 patients for whom genomic data were available (see figure). Latent class analysis tries to fit mutations into 1 underlying "latent" disease type vs another until a classification is found in which no 2 diseases share the same associated features. The method is commonly used in analyzing data from questionnaires with yes/no responses or attributing symptoms to an underlying disease process. For instance, lack of smell plus fever plus dry cough is more likely to be COVID-19 than common rhinovirus based on Bayesian probability. Because it does assume any prior associations and does not take into account morphology or antecedent conditions (such as myelodysplasia), machine learning allowed the team to build a model of AML disease subtypes based on the probability of common molecular features belonging, or not, to a separate underlying "disease."

Machine crunching was able to divide all patients into 4 major subgroups, which were then tested for prognostic value, that should show differences if indeed the new groupings represent distinct biology. The first (genomic cluster 1) had a median survival time of 34 months and comprised normal karyotype AML enriched for *NPM1*, *DNMT3A*, *FLT3-ITD*, and *IDH2* R140 mutations but absent *ASXL1*, *EZH2*, *TP53*, and *RUNX1*. The second group (genomic cluster 2) had a median survival of 26 months and comprised biallelic *CEBPA*, *IDH2* R172K with the absence of *NPM1*, *ASXL1*, *RUNX1*, and *TP53*. Cluster 3 (including *SF3B1*, *SRSF2*, and *EZH2* mutations) and category 4 (abnormal karyotype) had median survival times of 15.8 months and 9 months, respectively. Importantly, cross-validation studies showed an accuracy of 0.97.

Interestingly, de novo and secondary AML did not separate out cleanly between the 4 clusters, alluding to the possibility that morphological criteria or prior history of a blood disorder may not be the best possible criteria for classification of this heterogenous disease. It is also noteworthy that *IDH2* R140 mutations, but not *IDH2* R172K or *IDH1* mutations, were important classifiers for cluster 1, implying subtle differences in biology and metabolism. *DNMT3A*, *IDH2* R140, and *TET2* mutations were important determinants of cluster 2 but only if *NPM1* mutation was not present, suggesting that the presence of the enigmatic *NPM1* mutation dominates the phenotype. *RUNX1* mutations were especially important in attributing membership to cluster 3 and held the second-highest global importance as a classifying marker, underscoring its inclusion as a provisional disease entity in the recent World Health Organization classification.[9] Also of note, complex karyotype AML could be found in cluster 3 if the critical delineating lesions, −5/del(5q), −17/del(17p), and *TP53* mutation, were not present, suggesting that some complex karyotype AML may not always be associated with worst outcome.

Future work will be needed to validate machine-learning groupings prospectively in other cohorts and to study the underlying biology of these potentially distinct molecular entities, including in the leukemia stem cell and at clonal hematopoiesis.[10] Another limitation is the semi-

arbitrary restraining of their model to just 4 groupings: other parameters or algorithms could likely build alternative groupings. No doubt we will see machine-learning classifications applied to many other cancer types in the near future, as well as a different set of classifiers for targeted therapies beyond "7 + 3" and allogeneic transplantation. The most obvious benefit will be for patients who do not have an obviously good or bad "bellwether" biomarker for clinical decision-making.

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IMMUNOBIOLOGY AND IMMUNOTHERAPY

Comment on Martinez et al, page 1896

Grasping the sword of Damocles

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In this issue of *Blood*, Martinez et al report the efficacy of hematopoietic stem cell transplantation (HSCT) for children with a rare immune deficiency and autoinflammation disorder termed POMP-related autoinflammation and immune dysregulation (PRAID).[1]

A large majority of HSCTs worldwide are performed in adults with bone marrow malignancy.[2] The decision to perform such transplantations is typically informed by a significant body of experience indicating appropriate use of transplantation and, in the best of circumstances, data from well-designed and -conducted clinical trials. The smaller numbers of children undergoing HSCT and the diversity of indications mean that data are often much more limited in this setting, and determining appropriateness of HSCT can be difficult. Children with immune deficiency and dysregulation disorders present a particular challenge. In many disorders, there is significant phenotypic diversity, and the severity of the phenotype may be poorly predicted by laboratory testing. Also, reasonably effective supportive care is available for many disorders. Lastly, and perhaps most troublingly, in very infrequent disorders, it may be unclear which, if any, parts of the phenotype may be ameliorated by HSCT. Taken together, these variables can make the decision to proceed to transplantation in a small child with an incompletely understood immune disorder agonizingly difficult for both parents and physician, and the consequences of a regretted decision can be severe.

Immune dysregulatory disorders are particularly complex in determining the likelihood of improvement with HSCT, because restoration of completely normal immunity after HSCT may require interaction with host tissues that will not be replaced by allogeneic hematopoietic stem cells.[3] PRAID is an example of a group of disorders known as proteosome associated autoinflammatory syndromes, and POMP is a chaperone for proteasome assembly.[4] Proteasomes perform a range of key cellular functions, including the regulation of protein homeostasis, major histocompatibility class I antigen processing, cell-cycle proliferation, and signaling. Proteasomal defects generally lead to a markedly increased type 1 interferon signature, clinically manifested as autoinflammation, which can be ameliorated by immune suppression, and infections resulting from ineffective immunity, which are worsened by immune suppression, presenting a significant therapeutic struggle.

Martinez et al performed transplantation in 2 children with PRAID, both of whom faced a difficult-to-resolve conflict between the need to provide immune suppression for autoinflammation and the need to avoid immune suppression because of life-threatening infections (see figure). They report normalization of $CD8^+$, $CD4^+$, and memory T-cell proportions and restoration of T-cell cytokine secretion, with improved diversity of the T-cell repertoire after HSCT. Most importantly, the children experienced significant clinical benefit, with resolution of both autoinflammation and severe infections, and both are surviving, almost 3 and 5 years after HSCT, respectively.

This report is an example of 1 case or a small number of cases importantly informing both clinical practice and our understanding of the biology of a disease. A physician might have reasonably been concerned that T-cell recovery would have been limited by persistent proteasome deficiency in the cortical thymic epithelium, but this was not observed, which might reflect repopulation of the thymus with donor dendritic cells after HSCT. Overexpression of type 1 interferon–regulated