Interestingly, like ibrutinib, vecabrutinib is also a potent ITK inhibitor (50% inhibitory concentration,14 nM). ITK is downstream of the T-cell receptor. The T cells present in the tumor microenvironment function as supporters of CLL, and it has also become clear that CLL cells actively recruit supportive regulatory T cells.[6] It has been reported that ibrutinib can reverse defects in T cells.[10] So the authors investigated whether vecabrutinib, like ibrutinib, has immunomodulatory effects. Treatment with either ibrutinib or vecabrutinib reduced the number of immunosuppressive $CD4^+$ regulatory T cells in Eμ-TCL1 mice, which suggests that vecabrutinib can reduce the supportive functions found in the microenvironment (see figure).

Because ibrutinib and vecabrutinib showed only a limited cytotoxic effect in CLL cells, combination with a drug that induces rapid apoptosis would be favorable. Venetoclax directly targets BCL-2 (a key regulator of programmed cell death) and is highly expressed in CLL cells. Jabaraj et al demonstrated that vecabrutinib, similar to ibrutinib, primes CLL cells to BCL-2 dependency (see figure). Subsequently, treatment with the combination of vecabrutinib and venetoclax resulted in prolonged survival of Eμ-TCL1 mice.

Approved BTKi-based regimens combined with BCL-2 inhibitor–based regimens are now well advanced in clinical trials,[9] and evaluation is needed to determine whether such combination therapies reduce the development of BTK and PLCγ2-mutated clones. Combining vecabrutinib with venetoclax could overcome the hurdle of development of BTK mutations and hopefully achieve long-term remissions, an essential step toward improving outcomes for patients with CLL.

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LYMPHOID NEOPLASIA

Comment on Johnston et al, page 889

GEP: time for prospective study in HL?

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In this issue of *Blood*, Johnston et al[1] use gene expression profiling (GEP) to investigate the tumor microenvironment in biopsies from children diagnosed with classical Hodgkin lymphoma (cHL). The study includes a comparison with an adult cohort and the development of a pediatric prognostic model.

From the late 1980s, GEP has been used by researchers to identify the cell of origin to better understand lymphoma pathology. Such studies were initially limited to the expression of a single gene but were soon expanded to a large panel of genes with improvements in the technologies used. The ability to use formalin-fixed, paraffin-embedded tissues enabled the use of GEP in the clinical setting. Two specific clinical domains were investigated: improved classification and subclassification of lymphomas and identification of new prognostic markers (see figure). The first approach was very successful for non-Hodgkin lymphomas (NHLs). Our understanding of rare lymphomas such as gray zone lymphoma, primary B-cell mediastinal lymphoma, or peripheral T-cell lymphomas, for instance, was greatly improved. Moreover, subclassification of more frequent lymphomas, like diffuse large B-cell lymphoma or follicular lymphoma, is now recognized as a clinically meaningful method to predict the outcome and development of targeted treatment.[2,3]

In cHL, one difficulty was the paucity of tumor cells. The first studies were done on cell lines or dissected cells in order to confirm the B-cell origin of cHL and how the pathology and GEP impact outcome.[4,5] However, the cells in the microenvironment are now recognized to play a major role in HL pathology and outcome.[6] Thus, GEP study of the clinical biopsy, not just the tumor cells, is needed.

Johnston et al first demonstrate significant differences in gene expression between pediatric and adult cHL with an enrichment of eosinophils, B cells, and mast-cells signatures in children, while macrophage and stromal cells signatures were more prominent in adults. This is a major point, even if it is still difficult to confirm that HL is intrinsically different

in children than adults. Given the different frequencies of histologic subtypes between adults and children, it would be of interest to correlate GEP with subtypes. Is this difference still present if we consider only children vs adults with nodular sclerosis subtype, for instance?

Further, they demonstrate, in a population of children affected with intermediate-risk cHL, that a previously published model predicting overall survival for adults could not be validated. They developed a GEP-based model reflective of the tumor microenvironment biology to predict event-free survival (EFS). This model was confirmed on an independent validation cohort (enriched for events) and is statistically independent of fever, albumin level, stage, and, most importantly, interim response. Of note, both cohorts were composed of children treated within the same Children's Oncology Group (COG) clinical trial.

Looking for new prognostic factors in pediatric HL is an important goal, especially in children classified as intermediate risk. This group is heterogeneous, and identifying those children who would benefit from first-line intensification has been an unmet need. To date, most clinical trials in cHL use classical risk factors for stratification, for example, anatomical stages (Ann Arbor classification) and inflammatory markers (B-signs, ESR). The major progress in this field has been the use of early response analysis, mostly after 2 cycles, by positron emission tomography (PET). Early response is recognized as a major prognostic factor for EFS and is currently used in treatment allocation in adult and pediatric trials.[7] Other approaches, including the use of circulating cell-free tumor DNA or GEP data, have provided interesting results in retrospective studies but are not currently used in prospective settings for treatment adjustment.

There are several problems with GEP-based clinical stratification. First, there is no consensus on the components which must be included in the models. Second, the prognostic value of a new factor should be compared with established prognostic factors, which is not the case in many studies. Another concern is the reproducibility of the model, especially using different treatments. For instance, a GEP-based model which was predictive for a population of adults with advanced-stage cHL treated with ABVD was not predictive if patients were treated with BEACOPP.[8] This is a concern as this potentially implies that any GEP-based model should be used only by the group designing it and implies that the treatment cannot be significantly changed. Lastly, such approaches must be feasible in a real-world setting and approved by health agencies for clinical use.

The GEP model designed by Johnston et al was shown to be predictive independent of other prognostic factors, which is a major point in its favor. But to be used clinically, this model should be validated in an independent cohort (eg, a population of children) classified in the same risk group but treated by different modalities. The value of this model in other subgroups of children (eg, patients classified in low- and high-risk groups) should also be studied. Finally, this study opens the possibility of prospectively using a GEP-based model within a clinical trial, assuming it is validated as outlined above.

It is useful to compare clinical prognostic markers in cHL with acute lymphoblastic leukemia (ALL) in children. In ALL, there are more studies on classical prognostic factors (clinical features and leukocyte counts), more accurate studies on leukemic cells biology (phenotype, karyotype, molecular studies), and more early response assessment (eg, minimal residual disease [MRD] studies). MRD status is now recognized as a key predictor of EFS.[9] This has led to the use of MRD as the main tool for clinical stratification. However, recent studies demonstrate that MRD status significance does vary with the biological subtype.[10] In pediatric cHL, there are many fewer patients and studies. Is the best approach to use a combination of classical prognostic factors, early response assessment by PET, and GEP? To know the answer to this question, collaborative, prospective studies are needed, much as the collaborative studies were performed in ALL.

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