

here by Kumar et al, only 12 of 24 patients had a pathogenic or likely pathogenic genetic variant. Hence, they propose a 4-point scoring system of biomarkers based on cTfh frequency, CD4 effector memory cell activation, frequency of naïve CD4⁺ T cells, and B-cell maturation defects seen as decreased CSMB cells noted in all 24 patients with pES, irrespective of their genetic etiology. However, this degree of obedient clustering of distinct immune profiles in patients with ES need to be reproducible in the hands of other investigators in larger cohorts. Most patients in their cohort of 24 had some degree of lymphoproliferation and pulmonary or gastrointestinal manifestations. These associations underscore the importance of looking for similar clinical clues in all patients presenting with suspected ES. These observations might help us better understand the pathobiology of pES and provide some rational and empirical basis for therapies guided by cellular phenotyping through immune profile-based scoring system, especially in the remainder of the patients with pES where no known genetic cause can yet be discerned. Today the eponymous syndrome named after Evans remains an omnibus placeholder clinical diagnosis just like another immune disorder known as common variable immune deficiency, where the main function is to help insurance providers code the patients for reimbursements. Complete and further diagnostic work-up of both adult and pediatric patients with ES can provide clues for treatments. This goal can be accomplished by multicenter collaborative clinical trials performing upfront genetic testing and cellular immunophenotyping of every patient presenting with multilineage autoimmune cytopenias as has been proposed by Kumar et al.

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LYMPHOID NEOPLASIA

Comment on Fishman et al, page 399

T/myeloid MPAL: origin and pathogenesis

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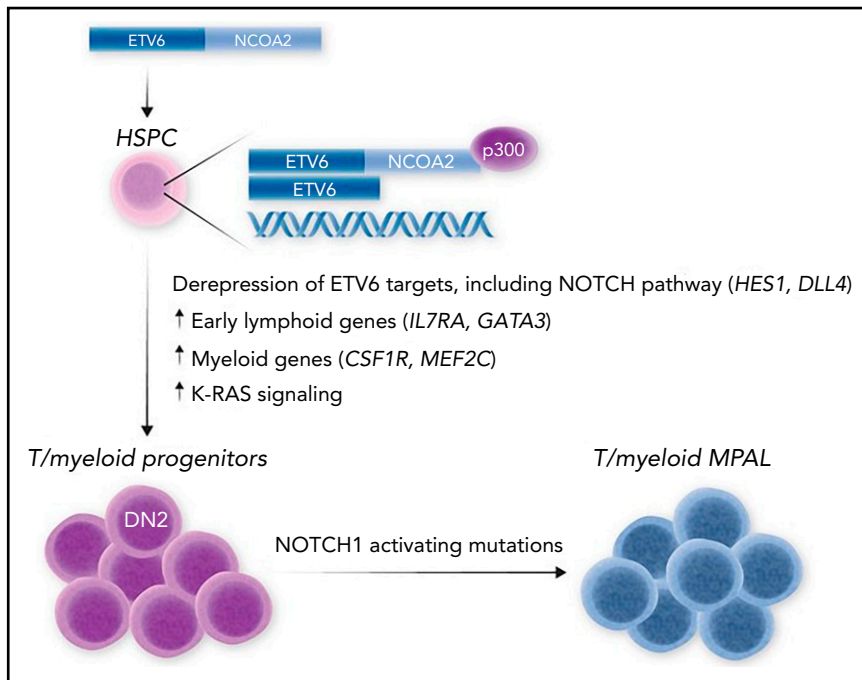
In this issue of *Blood*, Fishman et al have for the first time developed a pre-clinical model of T/myeloid mixed phenotype acute leukemia (MPAL) that faithfully recapitulates the human disease by using a fusion oncoprotein that they had previously identified in this leukemia subtype.¹ This work provides important insights into the cellular origin and molecular pathogenesis of this disease and provides, for the first time, a preclinical model that can be used to test new therapeutics.

MPAL is a rare leukemia subtype with poor prognosis that is characterized by immunophenotypic features of both myeloid and lymphoid lineages. The majority of patients with MPAL have features of either B-cell and myeloid lineages or T-cell and myeloid lineages (T/myeloid MPAL).² MPAL is particularly difficult to treat, in part because it is unclear whether MPAL should be treated as acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), or a combination of both.³ The cell of origin and molecular basis of this disease, in particular the causes of its biphenotypic nature, are unclear. This in turn makes optimal clinical care equally unclear.

Recent sequencing studies have defined the molecular landscape of T/myeloid MPAL and identified alterations in

transcriptional regulators as a defining feature of this disease, including recurrent mutually exclusive alterations or deletions in *WT1*, *ETV6*, *RUNX1*, and *CEBPA*, which are complemented by mutations in epigenetic regulators and activating mutations in signaling pathways.^{4,5} This has also revealed that this disease is distinct from T-cell ALL (T-ALL) and AML but shares significant molecular and genomic similarity to early T-cell precursor-like ALL (ETP-ALL), another subtype of immature leukemia with poor prognosis.^{4,6}

The ETS variant 6 (*ETV6*) gene is a member of the ETS family of transcription factors that encodes a transcriptional repressor. In addition to *ETV6* loss-of-function mutations in T/myeloid MPAL, several *ETV6* fusion oncogenes have



Model for T/myeloid MPAL induced by ETV6-NCOA2. Expression of the ETV6-NCOA2 fusion oncoprotein in HSPCs leads to formation of an aberrant transcriptional complex via binding to wild-type ETV6 and the transcriptional coactivator p300. This causes de-repression of ETV6 target genes, including NOTCH targets (*HES1* and *DLL4*) and early lymphoid genes (*IL7RA* and *GATA3*) that promote lymphoid commitment while maintaining the expression of myeloid regulatory genes (*CSF1* and *MEF2C*) that block further lymphoid differentiation. This causes differentiation arrest of multipotent T/myeloid progenitors, which accumulate further mutations, such as activating *NOTCH1* mutations, that result in overt T/myeloid MPAL.

been identified.^{4,7} One of these, *ETV6-NCOA2*, which fuses *ETV6* with the coactivator *NCOA2*, was first identified in 6 patients with T/myeloid MPAL and is associated with activating mutations in the key T-ALL oncogene *NOTCH1*.⁷

To determine the function of *ETV6-NCOA2*, Fishman et al ectopically expressed this fusion oncoprotein in murine hematopoietic stem and progenitor cells (HSPCs). Strikingly, this induced immature lymphoid genes in these cells, even when they were cultured in myeloid conditions, which implies an instructive role of this oncogene in driving lymphoid differentiation. Notably, this lymphoid program was not induced in cells transduced with *KAT6A-NCOA2* (also known as *MOZ-TIF2*), an *NCOA2* fusion oncogene associated with AML. This implies that the differential targeting of *NCOA2* by *ETV6* vs *KAT6A* DNA-binding domains is important in driving the lymphoid vs myeloid phenotype of leukemias containing these fusion oncoproteins. Indeed, when transferred in vivo, *ETV6-NCOA2*-transduced HSPCs induced T/myeloid MPAL that was highly similar to the human disease and was

accompanied by secondary mutations in *Notch1* (see figure). Experiments performed in human HSPCs showed similar results, with *ETV6-NCOA2* driving a lymphoid gene expression program. In this setting, *ETV6-NCOA2* could cooperate with nontransforming *NOTCH1* mutations to drive T/myeloid MPAL in human cells, which showed remarkable similarity to human T/myeloid MPAL xenografts that carried *ETV6-NCOA2* mutations. This confirms that *ETV6-NCOA2* cooperates with *NOTCH1* mutations in human T/myeloid MPAL and that *NOTCH1* is important in driving the leukemia phenotype.

Although *ETV6* encodes a transcriptional repressor, *ETV6-NCOA2* fuses the *ETV6* N-terminal pointed domain that mediates *ETV6* dimerization with the activation domains of *NCOA2*, implying that this fusion functions to de-repress *ETV6* target genes (see figure). Indeed, Fishman et al show that this oncoprotein binds to both wild-type *ETV6* and the coactivator p300, forming an aberrant transcriptional complex that binds and de-represses key *ETV6* targets, including the *NOTCH1*

pathway activators *HES1* and *DLL4*. This also leads to upregulation of early lymphoid genes such as *IL7RA* and *GATA3* that may be important in driving lymphoid specification of HSPCs. However, expression of key myeloid regulators is maintained, which results in a block in T-cell development at the DN2 stage, when these genes are normally downregulated to facilitate T-cell commitment and further T-cell development⁸ (see figure). These genes included *CSF1R* and *MEF2C*, which is known to be an important driver of ETP-ALL.⁹

Together, these results invoke a model whereby mutations that affect transcriptional regulators initiate T/myeloid MPAL in early hematopoietic progenitors. This is consistent with recent genomic analyses of MPAL, which showed that initiating mutations are acquired in early hematopoietic progenitors that retain myeloid and lymphoid potential.⁴ In the case of *ETV6-NCOA2* fusion, the result is to invoke a transcriptional program that causes lymphoid specification, whilst maintaining the expression of key myeloid genes that sustain myeloid potential and block further T-cell differentiation (see figure). This developmental arrest sets the stage for further mutations, such as in *NOTCH1*, that result in overt leukemogenesis. It remains to be shown whether this model applies to other *ETV6* fusion oncogenes that have been found in T/myeloid MPAL,⁴ to *ETV6*-inactivating mutations that have been found in both T/myeloid MPAL and ETP-ALL,^{4,10} and to other transcription factors such as *WT1*, *RUNX1*, and *CEBPA* that are recurrently mutated or deleted in T/myeloid MPAL. Nevertheless, these findings provide valuable insight into the pathogenesis of this unique leukemia subtype, as well as preclinical models that will help to identify new therapeutic vulnerabilities in this poor-prognosis disease.

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Comment on Orellana-Noia et al, page 413

CNS prophylaxis in DLBCL: time to say goodbye?

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In this issue of *Blood*, Orellana-Noia et al report that central nervous system (CNS) relapse rates following either intrathecal (IT) or systemic (IV) administration of methotrexate (MTX) are not significantly different, shedding doubt on the overall efficacy of both approaches.¹

For patients with diffuse large B-cell lymphoma (DLBCL), the overall incidence of relapse or progression in the CNS has been estimated at 5% with percentages varying from 1% to >15% in different risk groups. Current guidelines recommend CNS prophylaxis with IT or IV MTX for patients with high CNS-international prognostic index (IPI).² In a large retrospective study, Orellana-Noia et al now compare CNS relapses occurring after single-route IT or IV MTX. Do their findings suggest that both routes of administration are comparably effective, or ineffective? What are the clinical implications?

Because the overall incidence of CNS disease in DLBCL is ~5%, not all patients are deemed high-risk candidates requiring prophylaxis. The CNS-IPI identifies a high-risk group carrying a 10% rate of CNS disease.³ Because this rate was not considered high enough to justify CNS

prophylaxis in every high-risk patient, the search for alternative predictors continued. Indeed, more recently, combinations of CNS-IPI and molecular characteristics (activated B-cell subtype, double-hit lymphoma, distinct genetic signatures) were reported to increase the risk of CNS relapse.^{4,5} Disappointingly, however, a very high-risk group unequivocally warranting aggressive CNS prophylaxis in any patient carrying these characteristics has not been identified so far.

As early as 2009, we and others started to report that IT MTX was not effective in preventing CNS relapse in the rituximab era.⁶ Similarly, studies investigating the systemic administration of MTX also gave equivocal results. Thus, although studies on prophylaxis with IT or IV MTX became increasingly controversial, guidelines and most clinicians continue to recommend MTX for prophylaxis to prevent secondary CNS involvement (see table).

The study by Orellana-Noia et al adds to our doubts on the current practice of prophylactically administering IV or IT MTX to patients with DLBCL. The authors report that CNS relapse rates do not significantly differ between patients receiving IT or IV MTX (5.4% vs 6.8%, $P = .4$) and, importantly, differences in CNS relapse rates by route of administration failed to show significant differences also when patient groups were stratified by CNS-IPI, National Comprehensive Cancer Network-IPI, and double-hit status. Interpretation of the data and arriving at appropriate recommendations are not easy. One extreme would be to completely abandon CNS prophylaxis because increasing numbers of studies failed to demonstrate a benefit of MTX administration regardless of the route (see table). A more cautious approach would possibly return to IT MTX because, if not more effective, it is undoubtedly less toxic than IV MTX. Both conclusions seem premature because all studies have limitations (eg, patients in the current study receiving IT MTX were mostly treated with dose-adjusted etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, and rituximab (DA-EPOCH-R) while patients given IV MTX mostly received rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP). Although the key message remained unchanged after this imbalance had been taken care of by statistical modeling, the retrospective nature of this and other studies does not definitely exclude that the results were influenced by known and unknown confounding factors.

The comparison of CNS relapses occurring after CHOP vs cyclophosphamide, doxorubicin, vincristine, etoposide, and prednisone (CHOEP) or R-CHOP vs CHOP was an early demonstration that not only the prophylactic regimen but also first-line therapy may influence the incidence of CNS relapses.^{7,8} We recently reported that rituximab, doxorubicin, cyclophosphamide, vindesine, bleomycin, prednisone (R-ACVBP) including 4 IT injections of MTX followed by consolidative CNS prophylaxis with IV MTX, rituximab, ifosfamide, etoposide, and cytosine arabinoside resulted in very low CNS relapses. Patients with age-adjusted International Prognostic Index (aIPI) 2 or 3 experienced a 3-year