

granulomatous disease, etc) where larger quantities of hematopoietic stem cells (HSCs) available for gene transduction could improve persistence following transfer come to mind immediately, as do novel strategies combining HSPCs and other blood cells used to confer tolerance to solid organ transplants.<sup>8</sup> Perhaps this platform could be used to monitor response to immunosuppressive therapy in patients with aplastic anemia. Even in the allogeneic blood cell transplant realm, where donors currently undergo painful bone marrow harvest or an inconvenient and transiently painful 5- to 6-day treatment with granulocyte colony-stimulating factor (G-CSF), there is a need for less toxic and more rapid HSPC mobilization techniques.

The platform described here by Wang and colleagues sought to identify novel regulatory pathways involved in HSPC mobilization (see figure). In addition to ARHGAP25, a number of additional candidates were discovered and await their further validation. However, there are several additional experiments not yet performed that could be envisaged. The authors studied the most common method used to procure HSPCs from peripheral blood of patients: cyclophosphamide followed by G-CSF. Although certainly clinically relevant, it would be of interest to determine whether there are differences in HSPC phosphoproteomic profile following other stimuli including agents blocking the CXCR4/CXCL12 interaction or antagonists of VLA-4/VCAM1, among other agents, particularly because some data suggest that although HSPC mobilization may be the final common end point, mobilization methods may differ substantially in the manner in which they affect the bone marrow microenvironment.<sup>9</sup> The platform described could also be exploited to study HSC engraftment and HSC response to other environmental stimuli (eg, radiation) and, importantly, to better understand intracellular pathways in hematological malignancies such as acute myeloid leukemia, where persistence of drug-resistant leukemic stem cells following chemotherapy has been postulated to be a major source for treatment failure.<sup>10</sup> So in this regard, the importance of this article lies not only in the unleashing of another pathway regulating HSPC mobilization, but also in demonstrating the potential of combining high-speed

multicolor flow cytometry with high-throughput mass spectrometry for evaluating other rare cell populations under a variety of conditions.

The key to unlocking several mysteries of HSPC behavior may be within our grasp. We now have methods available to interrogate the molecular processes governing functions of rare cell populations during steady state and following extrinsic stimuli. The studies reported here by Wang and colleagues are hopefully just the beginning of the search for novel targets that will improve the lives of our patients.

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

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## ● ● ● LYMPHOID NEOPLASIA

Comment on Vallois et al, page 1490

# New mutations for nodal lymphomas of TFH origin

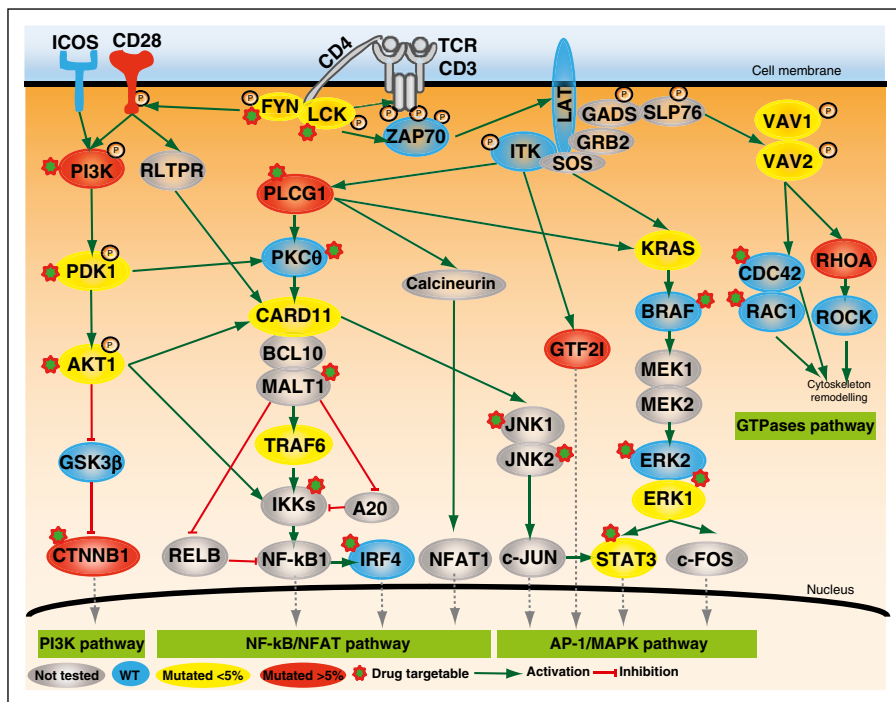
**José Fernández-Piquer** CENTRO DE BIOLOGÍA MOLECULAR SEVERO OCHOA; CENTRO DE INVESTIGACIÓN BIOMÉDICA EN RED DE ENFERMEDADES RARAS; INSTITUTO DE INVESTIGACIÓN SANITARIA FUNDACIÓN JIMÉNEZ DÍAZ

In this issue of *Blood*, Vallois et al show that genome and transcriptome analyses focused on T-cell receptor (TCR)-related genes can identify new druggable targets particularly valuable in peripheral T-cell lymphomas (PTCLs), in which the pathogenesis is poorly understood and treatment outcome is unfavorable.<sup>1</sup>

**N**odal lymphomas with a T-follicular helper (TFH) immunophenotype include angioimmunoblastic T-cell lymphomas (AITL) and a subset of PTCL not otherwise specified. Recent advances in this field have led the World Health Organization to revise the entity of both nodal and extranodal T-cell and natural killer cell neoplasms, proposing some revisions and new provisional entities.<sup>2</sup> Clinically, PTCLs treated with conventional therapies have a poor clinical outcome. Although stem cell transplantation

is effective in prolonging disease-free survival in young patients, early disease progression makes even this option difficult.<sup>3</sup>

Several lines of evidence had previously suggested that TCR activation might be involved in PTCL pathogenesis. For example, the ITK-SYK fusion found in PTCLs mimics the TCR-triggering antigen-independent activation of the TCR-signaling pathway. Therefore, based on multiple studies suggesting that this pathway can induce a T-cell lymphoproliferative disorder, the



Mutated genes involved in TCR costimulation or intracellular signaling. Positive and inhibitory interactions are depicted as solid green and red arrows, respectively. The protein symbols of mutated genes appear inside colored ovals depending on the frequency of mutation (yellow, <5%; red, >5%). Those of wild-type (WT) genes appear in blue ovals, and not-sequenced genes are in gray. PI3K, phosphatidylinositol 3-kinase. See Figure 2 in the article by Vallois et al that begins on page 1490.

authors focused on investigating new putative mutations in the costimulatory/TCR cascade working on the hypothesis that abnormal TCR-signaling costimulation of signaling may be relevant to PTCL pathogenesis. In this way, they first applied whole-exome/genome sequencing in a limited discovery cohort. Then, they designed an elegant targeted deep-sequencing approach, consisting of a panel enriched in TCR-signaling elements, which allowed them to capture a broader spectrum of mutations than those detectable using Sanger sequencing and which facilitated finding more information about the frequency of mutated alleles in an extended cohort. Besides *RHOA* mutations, which had been previously described in 70% of AITL and TFH-like PTCLs,<sup>4</sup> they discovered that nearly half of the patients (49%) in this extended cohort harbored mutually exclusive mutations in other TCR-related genes in particular *PLCG1* (14.1%), *CD28* (9.4%), mutated exclusively in AITL, *PI3K* elements (7%), *CTNNB1* (6%), and *GTF2I* (6%) (see figure).

To evaluate the importance of these mutations, they functionally assessed mutations in the *RHOA* gene by pull-down experiments and luciferase reporter assays. Additionally, *PLCG1* and *CARD11* variants

were functionally analyzed by their ability to induce *MALT1* protease activity and were tested in luciferase-based assays. Interestingly, the results of these functional analyses revealed that most mutations could be classified as gain-of-function activating mutations. Indeed, the integration of mutational analyses with gene expression profiles allowed them to verify that the TCR-mutated samples exhibited enrichment for molecular signatures reflecting T-cell activation and proliferation.

How do these mutations evolve over the course of disease and in response to chemotherapy? Direct comparisons between samples at diagnosis and at relapses could shed considerable light on the impact of these mutations in lymphoma progression and clinical outcome in the context of intratumor heterogeneity. Therefore, the authors performed preliminary analyses of paired samples in 4 relapsed patients and concluded that the mutational heterogeneity that characterizes AITL samples at diagnosis is essentially conserved after treatment, although 2 patients exhibited 1 additional mutation at relapse involving *CTNNB1* and *VAV1* genes. Because these analyses were done in a very limited number of sample pairs, additional efforts, by comparing data from targeted gene

sequencing and patterns of gene expression in a larger number of paired samples, would be instructive. In this context, it would be reasonable to think that tumors exhibiting intratumor heterogeneity at diagnosis might be genetically different in relapsed samples due to selection of cell clones enriched in driver genes responsible for resistance.<sup>5</sup>

Another interesting area needing exploration is epigenetics. Because highly recurrent mutations in PTCLs (including AITLs) involve epigenetic modifiers as *TET2*, *DNMT3A*, and *IDH2*,<sup>1,4</sup> mapping and analysis of the chromatin state could be a powerful means to detect additional changes of regulatory sequences and activity, which critically contribute to the progression of these lymphomas.<sup>6</sup>

The authors have integrated genome data into a clinically useful model to conclude that there are no significant differences in the main clinical characteristics or overall survival between TCR-mutated and non-TCR-mutated patients. However, TCR-mutated patients receiving conventional chemotherapy had an increased risk of early progression compared with nonmutated patients. Therefore, it seems clear that using drugs capable of reducing TCR activation, as suggested in this article, may be a very useful choice for treating these types of T-cell lymphomas.

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## **New mutations for nodal lymphomas of TFH origin**

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