

regulates splicing? What is its interaction with the core spliceosome? How is RBM15's association with specific genes regulated? What is the dominant-negative mechanism by which Mpl-TR acts to decrease HSC maintenance? Answers to these questions not only will elucidate critical aspects of the splicing machinery, but will be relevant for our understanding of the role of the RBM15-MKL1 fusion protein in AMKL.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

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● ● ● LYMPHOID NEOPLASIA

Comment on Mir et al, page 992

Serum cytokines in follicular lymphoma

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In this issue of *Blood*, Mir et al present data on the prognostic role of cytokines, chemokines, and their ligands measured in serum in patients with follicular lymphoma (FL).¹ This lymphoma has a variable course, and reliable markers for predicting outcome are needed.

FL is the second most common type of non-Hodgkin lymphoma with a highly variable course and with several treatment options. Both clinical features and biological factors, such as properties of the tumor and its microenvironment, have been shown to influence outcome.²⁻⁴ The tumor microenvironment consists of tumor-reactive T cells, follicular dendritic cells, and macrophages, and their activity might be mirrored in the serum. High numbers of CD8⁺ T cells have been associated with good prognosis, whereas CD4⁺ T cells (especially inside the follicles) are mostly associated with poor prognosis.²⁻⁴ The entire composition of the microenvironment and the architectural pattern (and not individual subsets) might be best associated with outcome.^{5,6} However, data on the prognostic importance of the tumor microenvironment are conflicting, mainly due to heterogeneity in study design and end points, patient selection, and technical aspects of immune cell quantification (flow cytometry or immunohistochemistry with manual or computer-assisted scoring),⁷ and because

patients are heterogeneously treated.⁸ As an example, the addition of the monoclonal anti-CD20 antibody, rituximab, to chemotherapy, has been shown to abrogate the negative impact of macrophages.⁹

Serum markers have previously been suggested to contribute to or mirror the tumor microenvironment in FL.¹⁰ Serum levels of IL-1R1, IL-6, IL-7, IL-10, IL-13, TNF- α , vascular endothelial growth factor (VEGF), and platelet-derived growth factor were increased in 60 FL patients compared with controls. Multivariate analysis identified early stage and high TGF- β levels as independent predictors of improved overall survival, while high lactate dehydrogenase and VEGF levels were independently associated with poorer progression-free survival.¹⁰

Mir et al now report data on serum levels of multiple cytokines and chemokines, and their receptors, in 2 large patient cohorts.¹ They have used a multiplex enzyme-linked immunosorbent assay and found that elevated levels of IL-2R, IL-1R1, and CXCL9 are associated with shorter event-free survival

(EFS) in FL patients treated with chemotherapy or chemoimmunotherapy, whereas IL-1R1 and also IL-12 were associated with a shorter EFS in patients in a wait-and-watch cohort, as well as in patients treated with rituximab monotherapy. These serum factors seem independent of the FL international prognostic index, a validated prognosticator, and might be of great clinical impact, especially as they are easily accessible in blood.

The same research group has previously shown that soluble IL-2R α facilitates IL-2-mediated immune responses and that elevated soluble IL-2R levels before treatment were associated with reduced survival in FL patients.¹¹ The prognostic relevance of these findings are now extended to more serum markers in 209 patients, prospectively enrolled on the University of Iowa/Mayo Clinic Specialized Program of Research Excellence Molecular Epidemiology Resource and confirmed in a meta-analysis, also including 183 patients from 3 South West Oncology Group trials.¹

A problem with the interpretation of the data is that the endpoint used is EFS, which might not be solid enough, and no survival data are presented. The predictive effect of the level of serum markers on response to anti-CD20 antibodies, in this report rituximab, cannot be evaluated because the results are not presented in relation to rituximab administration. Patients with wait-and-watch are grouped together with those receiving rituximab monotherapy and no delineation is made between those with rituximab+chemotherapy vs those few with chemotherapy alone.

The conclusion that IL-12 and IL-1R1 are predictive for poor outcome in FL patients who are initially observed (or getting rituximab monotherapy) is of interest, but prospective trials are needed to evaluate if early immunochemotherapy will affect the prognosis for patients with such high levels.

In newly diagnosed patients treated with more intensive regimens, Mir et al found that the elevated IL-2R is associated with short EFS.¹ The role of cytokines in lymphoma biology seems complex. A subset of lymphoma B cells express the IL-2R, and IL-2 is a critical homeostatic cytokine required for development, expansion, and activity of regulatory T cells. IL-2 is also necessary for the development of cytotoxic

T-cell function and has been used for the systemic immune therapy to amplify antitumor immunity. However, in lymphoma, the role of IL-2 is not well defined.

The total “cytokine milieu” might be of importance for T cell-mediated antitumor immunity. The role of IL-1, an inflammatory cytokine, and its receptor seem complex in lymphoma, and in the paper by Mir et al, an elevated IL-1R1 was a negative prognostic factor in both patient cohorts, while IL-1 was below the limits of detection.¹ Additionally CXCL9, which can act as a macrophage-derived CXCL12 synergy-inducing chemokine to which CXCR4 positive malignant B cells can respond, was found to be associated with shorter EFS in patients with more aggressive disease.

In conclusion, the evaluation of the cytokine profile in blood is of great interest as it might mirror the release from tumor cells and from cells in the microenvironment, reflecting lymphoma activity and host response. The biological relevance remains unclear and prospective trials are needed to show its role for prognostication and future risk-adapted therapy.

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

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PLATELETS & THROMBOPOIESIS

Comment on Lecchi et al, page 1006

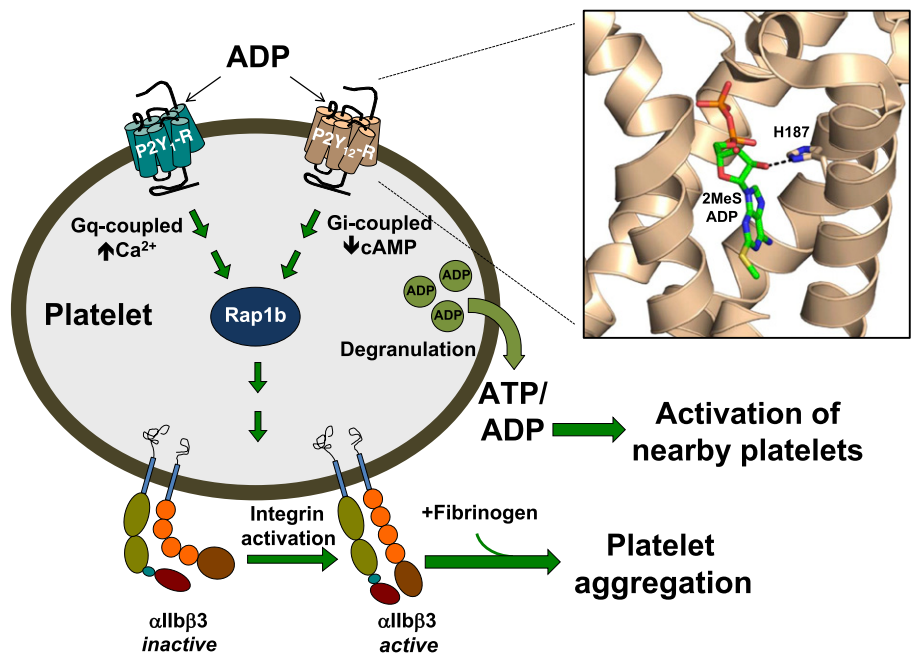
Insights into platelet P2Y₁₂ receptor activation

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In this issue of *Blood*, Lecchi et al report that lifelong abnormal bleeding episodes in 2 brothers are the result of a homozygous mutation in the gene encoding the P2Y₁₂ receptor that disrupts adenosine diphosphate (ADP)-promoted platelet aggregation.¹

The platelet activity of ADP arises from concomitant stimulation of 2 different P2Y receptor subtypes: the Gq-coupled P2Y₁

receptor and the Gi-coupled P2Y₁₂ receptor (see figure). The P2Y₁ receptor induces platelet shape change and initiates platelet



ADP simultaneously binds to 2 P2Y receptors, P2Y₁ and P2Y₁₂, leading to sustained activation of Rap1b and a conformational change of αIIbβ3 integrins from an inactive to an active form. Activated αIIbβ3 binds fibrinogen to form a platelet aggregate. Activation of the platelet also promotes degranulation, thereby releasing ATP and ADP to activate nearby platelets and amplify aggregation. (Inset) His187 in the P2Y₁₂ receptor is hydrogen bonded to the 2'-OH of 2MeSADP, which ultimately leads to activation of P2Y₁₂ receptor-mediated signaling pathways. The 2 brothers with a severe bleeding history detailed by Lecchi et al have a homologous His187Gln mutation that disrupts receptor activation. cAMP, cyclic adenosine 5'-monophosphate.