turnover of circulating platelets, their proteome appears to be surprisingly stable. Another remarkable finding is that the protein profile does not correlate at all with earlier published transcriptome analyses, implicating that—at least in anucleate platelets—the protein composition is not a mere reflection of the mRNA content. Clearly, the quantitative protein list has many applications. It allows calculating stoichiometries between subunits of protein complexes and protein isoforms, and thus, will help to understand the biochemistry of these proteins. For instance, the finding that 3 phospholipase Cβ isoforms are present at similar copy numbers implies that all 3 will have relevant roles in agonist-induced Ca2+ signaling. The protein list contains many other surprises; for example, a relatively high expression of ubiquitination proteins in platelets. Not disputing the major achievements made by Burkhart et al, there is still room for improvement. Proteomics analysis starts with trypsin digestion of a cell lysate, which gives specific problems. For instance, this procedure may result in underestimation of glycoproteins and proteins with high hydrophobicity or with multiple transmembrane domains. In addition, other proteins (eg, proteases) may form trypsin digestion fragments that due to their size or sequence are difficult to analyze, while protein isoforms may produce identical or indefinable sets of peptide fragments. This explains why in the new list G-protein–coupled receptors and other important membrane proteins are underrepresented. In addition, it is still problematic to quantify proteins with copy numbers below 500 per platelet. Specific enrichment procedures need to be employed in this case. The procedure of protein quantification applied in the article by Burkhart et al uses the so-called normalized spectral peaks to protein copy numbers. The authors use the so-called normalized spectral peaks to protein copy numbers. The application in the article by Burkhart et al uses the so-called normalized spectral peaks to protein copy numbers. The underlying concept is that protein expression levels and posttranslational modifications in platelets are controlled by multiple physiologic processes in health and disease, and are modulated by medication (see figure). Conversely, the composition and modification of these proteins could affect these physiologic processes. What the precise consequences are of variation in the platelet proteome pattern will become clear in the near future.

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

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Comment on Jerez et al, page 3048

LGL: a disease rediscovered

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In this issue of Blood, Jerez et al describe mutations in STAT3 in 2 subtypes of large granular lymphocyte leukemia (LGL).1 Large granular lymphocyte leukemia (LGL) is a rare lymphoproliferative disorder of T cells (mostly CD8+ T cells) or natural killer (NK) cells with an unclear etiology.2 Recently, mutations in STAT3, a key signaling protein often mutated in tumors, were reported in 31 of 77 (40%) of T-LGL patients,3 implicating this molecule in the expansion and persistence of these cells. Jerez et al now extend these findings and show that 33 of 120 (28%) of T-LGL and 15 of 30 (50%) of NK-LGL exhibited a mutated STAT3 Src-homology 2 (SH2) domain.1 This study is remarkable for the large numbers of patients investigated with this rare disorder. Mutations in the same SH2 domain, critical for activation of this protein and hence downstream pathways, were identified in both T- and NK-LGL, suggesting a common pathogenic mechanism driving expansion and persistence of lymphocytes in both disease subtypes. One of the mutations now identified in LGL, which alters the tyrosine residue at position 640 into a phenylanaline (Y640F), was previously shown to represent a gain-of-function in hepatocellular carcinoma.4 Jerez et al show here, in a reporter assay, that another mutation, D661V, also increases the basal activity of STAT3, independently of upstream signals.5 The SH2 domain of other STAT family members plus mutational hotspots in activators of this protein are differentially regulated in acute coronary syndrome: a proteomic study. PLoS One. 2010;5(10):e13409. The precise consequences are of variation in the platelet proteome pattern will become clear in the near future.

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in T- and NK-LGL, ample material for future investigations.

Jerez et al focused on the SH2 domain in their search for mutations. However, some of the gain-of-function STAT3 mutations found in hepatocellular carcinoma are outside this domain, suggesting that all domains of the STAT3 gene should also be closely inspected before we call them truly wild-type. Detailed sequence analysis could also solve the current paradox that neither laboratory findings nor the overall survival seem to distinguish between patients with wild-type and mutant STAT3 SH2 domains.

Now that mutations in the STAT3 gene in LGL are identified, the next step is to explore the functional consequence of these alterations in CD8+ T cells and NK cells. The transduction of STAT3 mutants identified in LGL, which are presumably gain-of-function mutants, and, as a control, from Job syndrome patients, which represent loss-of-function or hypomorphic mutants, into CD8+ T cells/NK cells from healthy donors should educate us on the effect these variants have on gene expression, proliferation, and survival on cytokine withdrawal, and leukemic behavior in the form of extended survival in a xenogenic mouse model. Furthermore, such studies should uncover differences in the way T cells and NK cells handle STAT3 mutants.

Lastly, although T-LGL is characterized by the expansion of CD57+CD8+ T cells, we observed that the LGL clone is represented in both CD57+ and in CD57- fractions. Indeed, CD57- cells lacked proliferative capacity, but were readily generated from sorted CD57+ LGL cells. With the recent discovery of a stem cell memory T cell (TSCM), a burning question is whether the T-LGL clone originates in early memory cells and carries the mutant STAT3 gene. Such a finding would reflush the need to target STAT3 inhibitors on the true LGL stem cell.

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MYELOID NEOPLASIA

Comment on Lu et al, page 3098

Live and let (MPN cells) die!

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In this issue of Blood, Lu et al show that the combination of interferon-α (IFNα) and nutlin-3 is efficient to selectively impair proliferation and differentiation of malignant hematopoietic progenitor cells (HPCs) in myeloproliferative neoplasms (MPNs) in vitro. However, may hamper the clinical use of IFNα: adverse reactions, leading to treatment discontinuation in 20% to 30% of patients in the long term; and possible resistance of subclones carrying mutations in genes other than JAK2. These limitations could be overcome using combination therapy allowing reduction of the dose of IFNα (to improve tolerance) and simultaneous targeting of multiple oncogenic pathways.

p53 is one of the most frequently mutated oncogenes in cancer. Such mutations are very rare in chronic-phase MPN but may be acquired during their evolution to acute leukemia. In addition to genotoxic mutations, wild-type p53 function can be inactivated by increased degradation after binding to murine double minute 2 (MDM2), a negative regulator of the p53 pathway. Inhibition of MDM2 results in p53 stabilization, activation of cell-cycle arrest, and apoptosis pathways, that is, restoring the ability of cancer cells that had developed mechanisms to escape from programmed cell death to die. Recently, Nakatake et al reported that JAK2V617F-mutated cells exhibit an increased p53 degradation due to an increased MDM2 protein level. In addition, they found that inhibition of MDM2/p53 binding with nutlin-3, a small molecule inhibitor of this interaction, resulted in specific inhibition of the EPO-independent growth of JAK2-mutated cells, suggesting that MDM2 inhibition could reduce the malignant clone in MPNs.
LGL: a disease rediscovered

J. Joseph Melenhorst