Many patients with HCL are asymptomatic and can be observed for months or years before requiring treatment, an exception being vHCL, which tends to present with high disease burden. Therapy of HCL is indicated when the patient develops one or more of the following conditions: significant cytopenias with related symptoms, symptomatic splenomegaly (or uncommonly adenopathy), constitutional symptoms. When treatment is warranted, the purine analogs cladribine and pentostatin have replaced splenectomy and interferon as the initial agents of choice.[4,5] Because of ease of administration and paucity of toxicities, a single cycle of cladribine has become the preferred standard treatment. The majority of patients achieve a durable response. However, a proportion of patients, irrespective of whether they are classical or vHCL, either fail to respond or rather rapidly relapse.

Thus, we come to the relevance of the 2 papers discussed. The question is whether it is possible to recognize at diagnosis which patient will fail cladribine and therefore who could be spared a useless therapy and considered for an alternative type of treatment. Following in the footsteps of chronic lymphocytic leukemia (CLL),[6] where patients have a biased use of immunoglobulin heavy chain variable (*IGHV*) genes and the prognosis is significantly different according to the presence or the absence of *IGHV* somatic mutations, the 2 studies have investigated IG genes in 2 large series of patients. Forconi and colleagues have prospectively studied 53 cases included in a multicenter Italian clinical trial of newly diagnosed HCL.[1] They prove that the presence of UM *IGHV* genes defines a minor subset of patients who are refractory to single-agent cladribine and have a more aggressive behavior (see top panel of the figure). In their series, 5 of 58 cases were cladribine failures and all 5 were UM (compared with only 1 of 53 beneficial response). Arons and colleagues have retrospectively studied 82 patients, most seeking trials for a relapsed/refractory disease and representing a poor prognosis population.[2] They have found that a high proportion of cases (14/82) were characterized by the use of the *VH4-34* gene, irrespective of whether they had a classic or vHCL (see bottom panel of the figure). All but one of the *VH4-34*$^+$ cases were UM. *VH4-34*$^+$ cases had strikingly significant lower response rate and progression-free survival after initial treatment with cladribine and experienced a shorter overall survival from diagnosis (see bottom panel of the figure). In the Italian series, 1 of 5 cladribine failure cases was *VH4-34*. Plausibly, the difference in the *VH4-34* frequency in the 2 studies reflects the differences in the patient populations (newly diagnosed vs poor prognosis) and in the study modality (prospective vs retrospective). The Italian study[1] reinforces the concept brought up by Arons et al.[2]

The take-home message is that the study of IG genes has to become an integral part of the diagnostic workup in HCL, irrespective of whether the patient has a classic or a vHCL. The reason is that if the IG genes are in the UM version and the IG genes used belong to the VH4-34 family, standard cladribine treatment may be ineffective. Accordingly, in these patients, new forms of treatment including monoclonal antibodies are warranted and deserve specific multicenter trials. Not surprisingly, the clinical behavior of UM-HCL parallels that of UM-CLL, but new interesting questions emerge. The road is now open for clinicians and biologists to understand why *VH4-34* is such a risky gene in HCL,[2] whether it has something to do with the autoimmune complications that may occur in HCL and also whether the rather high incidence of TP53 disfunction detected in the UM cases[1] may provide a clue to understanding the mechanism of cladribine resistance in the UM-HCL group and more generally to purine analogs.

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

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● ● ● PLATELETS & THROMBOPOIESIS

Comment on Falet et al, page 4729

Cracking the platelet WIP

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In this issue of *Blood*, Falet and colleagues report how deletion of the gene for the WASp interacting protein WIP in mice causes a major defect in GPVI signaling and activation of platelets, mediated by binding of IgAs to the platelet surface.[1]

Wiskott-Aldrich syndrome (WAS) is a rare X-linked disorder of the blood, which is largely a product of mutations in the gene encoding the WAS protein.[2] Patients present with a range of clinical phenotypes dependent on the nature of the mutation, but are often characterized by marked thrombocytopenia associated with reduced platelet volume and immunodeficiency with progressive lymphopenia. Several blood cell functions are altered, related to cytoskeletal function, including chemotactic migration, adhesion, and phagocytosis. However, the picture is complex because there is often an increase in granulocyte count, which is associated with autoimmune disease presentations, commonly including eczema, ulcerative colitis, and glomerular nephropathy with IgA deposits.

WASp is a 64-kDa protein with restricted expression in nonerythroid blood cells and is a major link between cellular signals and the cytoskeleton, regulating the Arp2/3 complex to nucleate new actin filaments.[3] WIP is a widely expressed, 63-kDa proline-rich protein that is centrally involved in actin nucleation through its interaction with WASp. Importantly, many of the missense mutations that are observed in WAS patients occur in the N-terminal WH1 binding domain of WASp, which is the region of the protein responsible

Phenotype	WAS (human)	WASp$^{-/-}$ (mouse)	WIP$^{-/-}$ (mouse)
Platelet count	↓↓[1]	↓	↓
IgA bound to platelet surface	✓	×	✓
GPVI loss induced by JAQ1	ND	ND	×[2]
Platelet expression of WASp	✓[3]	×	×
Platelet expression of WIP	ND	↓	×
Platelet responses to GPVI agonists[4]	✓	✓	×

Comparison of phenotypes between human WAS, mouse WASp$^{-/-}$, and mouse WIP$^{-/-}$ platelets. (1) WAS platelet volume is also reduced. (2) Anti-GPVI antibody JAQ1 induces shedding of surface GPVI in wild-type mouse platelets, and partially in those WIP$^{-/-}$ mice that do not have surface IgA bound. However, the majority (76%) of WIP$^{-/-}$ mice show IgA bound to the platelet surface, and this correlates with complete protection from GPVI shedding. (3) WAS patients show a variety of mutations in the WASp gene. Coding mutations most commonly arise in the N-terminal WH1 domain. (4) Responses measured included shape change, actin assembly, Arp2/3 activation, PLCγ2 phosphorylation, cytosolic calcium rise, α-granule secretion, and αIIbβ3 activation. ND indicates not determined.

for interacting with WIP. These mutations in particular correlate with X-linked thrombocytopenia, and therefore suggest that WIP is likely to be a biologically important binding partner of WASp.

The present study from Falet et al is the first to determine in detail the platelet phenotype seen in WIP$^{-/-}$ mice,[1] although some features of platelet viability have been previously demonstrated.[4] WIP has been shown previously to be required for T-cell proliferation and activation via the T-cell receptor/CD3 complex, and for formation of the immunologic synapse.[5] WIP$^{-/-}$ B cells, however, proliferate more extensively although their antibody response to T cell–dependent antigens is impaired.

In the present study by Falet and colleagues, the role of WIP in platelet function is addressed in the context of a hypothesis that the WIP knockout mouse should phenocopy the WASp knockout.[1] This is a reasonable basis for the study, considering the immunologic phenotype of WIP$^{-/-}$, and the close functional association between WIP and WASp, which is disrupted in many WAS patient mutations. The study elegantly dissects a role for WIP in regulating features of platelet viability and function. It also demonstrates that not all paths in biology are linear, and not all outcomes predictable, because although some aspects of WIP$^{-/-}$ platelet function equate to WASp$^{-/-}$ and WAS platelets, others do not.

For the purposes of comparison, the figure shown lays out the measured parameters and their outcomes for mouse WIP$^{-/-}$, mouse WASp$^{-/-}$, and human WAS platelets. The notable features of the phenotypes are that WIP$^{-/-}$ platelets also lack expression of WASp and are therefore double knockouts in these cells. On the other hand, WASp knockout platelets still show expression of WIP, although at a reduced level. Importantly, the majority of WIP$^{-/-}$, but not WASp$^{-/-}$, mice develop platelet-associated IgAs. The WIP$^{-/-}$ phenotype therefore has parallels with WAS patients, where IgA-mediated autoimmune disease is a feature and where platelets are commonly coated with antibodies.[6] The phenotypes, however, diverge at this point; WAS patients demonstrate a severe thrombocytopenia and WIP$^{-/-}$ mice have a more moderate reduction in platelet count. Whereas antibody coating may provide part of the explanation for increased platelet clearance in WAS patients, it is possible that in WIP$^{-/-}$ mice, IgA coating actually protects against splenic clearance. The picture is even more complicated in the WASp$^{-/-}$ mouse phenotype, where the platelet count is only moderately reduced with minimal antibody coating of these platelets.

Therefore antibody coating, per se, does not necessarily equate to a change in platelet clearance. It may be the difference in phenotypes between the knockout models, and WAS is a product of the exact surface antigens occupied by antibodies. This could explain the other major difference between WIP$^{-/-}$ platelets and those from WASp$^{-/-}$ mouse platelets or human WAS platelets, which is the ablation of glycoprotein VI (GPVI) responsiveness. Falet et al clearly demonstrate a very marked reduction in a multitude of responses to GPVI agonism in WIP$^{-/-}$ platelets, whereas those responses were normal in WASp$^{-/-}$ and in human WAS platelets. Therefore, this may be a product of antibody blockade of GPVI function. Because there was no shedding of GPVI in WIP$^{-/-}$, this would suggest that antibodies were not directed to GPVI itself. It is of course possible though that WIP plays a critical signaling role in mediating GPVI function in its own right, in a WASp-independent manner, and that IgA binding in the absence of WIP is a red herring from a functional point of view.

In summary, although the WIP$^{-/-}$ mouse phenocopies WAS immunologically quite well and may, therefore, explain some WAS patient syndromes in persons with no obvious WASp coding mutations, the platelet phenotype is only partially copied. This intriguing study from Falet et al leaves open further investigation into the mechanisms of the severe thrombocytopenia in WAS, the mechanisms of Ig coating of platelets in the patients and in the WIP$^{-/-}$ mouse, and finally the possibility of WASp-independent signaling functions of WIP.

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