expression studies in finding correlations between genetic lesions and prognostic biomarkers and, more importantly, in finding functionally critical drivers of leukemogenesis.5–7

In this issue of Blood, Harvey and co-workers now confirm that children with B-precursor ALL, who have been classified as high risk on the basis of the simple clinical National Cancer Institute criteria age and high white blood cells at the time of diagnosis, can be subdivided into subgroups by a combination of mRNA expression analyses and assessment of genomic DNA copy number aberrations.8 Two of these subgroups coincided with known cytogenetically defined risk groups. Two other subgroups were remarkable in that they identified patients with an excellent prognosis of 4-year event-free survival (EFS) of > 90% or a particularly bad prognosis of 4-year EFS of ~ 30%. The favorable subgroup was defined by the high expression of 4 outlier genes and ERG deletions. The unfavorable group was characterized by (1) high expression of 4 genes and (2) DNA deletions, rearrangements, and mutations of another 6 gene loci including CRLF1, RAG, JAK2, and IKZF1 (thus confirming the prognostic significance of these genes and a BCR-ABL–like expression signature in pediatric ALL5,6,9–12 and also confirming the power of combined mRNA expression and DNA analyses in determining individual risk profiles).

Although the definition of the subgroups represents a remarkable success, critical questions remain. The first relates to the influence of therapy on the risk profile. This is well illustrated by the case of pediatric T-ALL, where the favorable impact of activating NOTCH1 mutations depends on the subset(s) of the different treatment protocols.13–15 While the role of CRLF1 has been documented in both Childhood Oncology Group8,16 and Berlin-Frankfurt-Münster protocols,17 the general role of the other changes in different treatment protocols still requires testing. A second issue relates to the small size of the subgroups of a disease that is already rare. Tailoring and testing treatment concepts in the context of such a personalized medicinal approach will require studies that are designed on a wide international scale: this will challenge the different cultures that underlie medicine in general, and the organization of clinical studies including different regulatory agencies in particular.

Finally, while it is helpful to define very-high-risk groups in pediatric ALL, identifying druggable pathways of high-risk ALL and developing logical new treatments for this most difficult group of patients will be essential.

In this issue, Harvey and colleagues state that they will focus on identifying hitherto unidentified altered kinases that may contribute to the BCR-ABL–like expression profile of high-risk childhood ALL that may also represent new targets for therapeutic intervention. Alternatively, one may want to look at the potential of epigenetic strategies to reverse the block in B-cell differentiation in this group of patients. In any case, finding new options for the unfortunate 20% of children with ALL is what future patient generations will expect from us.

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

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Filling a void in Gray Platelets

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The Gray Platelet Syndrome (GPS) is the paradigm of clinical α-granule deficiency. Yet, its description had been limited to case reports. Gunay-Aygun et al now provide the first comprehensive study of GPS and assign the causative gene to a locus on chromosome 3.1

In 1971, Raccuglia described a patient with thrombocytopenia whose platelets included a high percentage of large cells that were nearly devoid of granules, rendering them gray upon Wright staining.2 He termed the disorder the Gray Platelet Syndrome. The sine quanon of GPS is the identification by electron microscopy of a large number of empty

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PLATELETS & THROMBOPOIESIS

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In 1971, Raccuglia described a patient with thrombocytopenia whose platelets included a high percentage of large cells that were nearly devoid of granules, rendering them gray upon Wright staining.2 He termed the disorder the Gray Platelet Syndrome. The sine quanon of GPS is the identification by electron microscopy of a large number of empty
However, elevated B12 may serve as a useful marker for determining whether electron microscopy is required in the setting of suspected GPS.

The study shows that the bleeding tendency in GPS is complex, ranging from insignificant to severe. Thrombocytopenia is uniformly observed in affected individuals. In several cases, however, the bleeding tendency is out of proportion with the degree of thrombocytopenia. Platelet aggregation is normal in the majority of patients tested. This observation supports the premise that α-granules do not serve a critical function in ex vivo aggregation under conditions in which adequate fibrinogen is provided in plasma. This observation does not rule out, however, that α-granule contents are important for hemostasis in vivo, as suggested by previous observations of increased bleeding times. The observation that 7 of the 8 GPS patients with a severe bleeding tendency were women with menometrorrhagia may be related to thrombocytopenia and/or factors within α-granules that are required for endometrial hemostasis and perhaps proliferation.

The substantial interest in GPS has been driven by the anticipation that determination of its molecular underpinnings will provide new information about α-granule biogenesis. As an experiment of nature, GPS has been challenging. Although mouse models have been useful in understanding the molecular basis of dense granule release, few rodent models of isolated α-granule deficiency exist.

Mice lacking the hematopoietic zinc finger transcription factor and RNA-binding protein, Hzf, produce platelets with empty α-granules and represent a good model of α-granule deficiency. However, sequencing of the orthologous Hzf gene in 5 patients with GPS demonstrated no defect. The dearth of existing knowledge regarding α-granule formation has limited candidate gene approaches.

The small number of affected individuals has hampered previous efforts to identify the putative GPS gene by molecular genetics. Having assembled the majority of known GPS families, Gunay-Aygun et al used genome-wide linkage analysis to map the GPS gene. Although their study falls short of identifying the causative gene, it demonstrates that GPS has an autosomal recessive mode of inheritance in at least 22 of 25 cases and that the causative gene maps to a 9.4-MB interval on chromosome 3p. Thus, it is likely that the majority of cases of GPS have a common genetic etiology. Unfortunately, the linked region on chromosome 3 has a very low rate of recombination and no recombination events were identified to reduce the list of 165 candidate genes. Sequencing of 69% of exons within candidate genes failed to demonstrate pathologic mutations.

Some clues to the underlying mechanism of GPS have been provided by careful analysis of the phenotype. The defect occurs at the level of the megakaryocyte, which contains empty α-granules that are transported to platelets. All other organelles appear normal. The empty α-granules express membrane proteins, contain some endocytosed plasma proteins, and appear to be capable of activation-dependent fusion with surface-connected membranes. Proteomic analyses of α-granule proteins from patients with GPS confirm that soluble endogenous proteins are markedly decreased or undetectable, while endocytosed soluble and membrane-bound proteins are present. These observations imply a defect in the production, packing, and/or sorting of endogenous α-granule proteins.

Studies of megakaryocytes from GPS patients indicate a defect in α-granule maturation. Endogenous α-granule proteins are present in the early stages of megakaryopoiesis (days 5 and 6), but are progressively lost (days 9–11). In day-12 megakaryocytes from GPS patients, von Willebrand factor is observed in small vesicles near the Golgi, but mature α-granules fail to form. Elevation in plasma
levels of endogenous α-granule proteins (PF4 and β-thromboglobulin) supports a packing or sorting defect rather than a defect in protein synthesis.\(^2,^3\) By analogy, mice lacking chondroitin sulfate have α-granules deficient in PF4, β-thromboglobulin, and platelet-derived growth factor because of a granule-packing defect\(^4\) and patients lacking the vesicle-sorting protein VPS33B have α-granule deficiency.\(^10\) Defects in protein packing, vesicle trafficking, granule acidification, membrane retrieval, or protein processing could prevent normal granule maturation and result in shunting to a default pathway of constitutive secretion. The large number of genes that could potentially contribute to α-granule maturation complications identification of likely culprits from the long list of candidate genes located in the linked interval on chromosome 3p. We can only hope that improvements in sequencing technologies will make identification of the causative gene in GPS more black and white.

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**Comment on Huang et al, page 5002**

**IVIg conducts DC-platelet nuptials**

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The anti-inflammatory effects of IVIg have been adoptively transferred using IVIg-primed cells in autoimmunity and inflammatory states (reviewed in Crow et al\(^1\) and Lazarus\(^2\)). Although dendritic cells (DCs) have entered the spotlight in this process, the downstream target of these cells has been unknown. In this issue of *Blood*, Huang and colleagues observe that IVIg-primed DCs can surprisingly induce downstream regulatory effects in murine ITP at the level of the platelet rather than the phagocyte.\(^3\)

IVIg-directed DC-platelet nuptials. IVIg effects in autoimmunity and inflammation can be mediated by DCs. IVIg first primes the initiator DCs\(^4\) in an FcγRII-dependent manner.\(^2,^3\) These cells then propagate a still- poorly described intermediary pathway which may involve the function of several interacting cells. The propagation and/or inhibition stages appear to be dependent on the presence of FcγRII and P-selectin. The specific cell that expresses these molecules in terms of IVIg action remains unclear. Platelets have only been thought of as passive victims in ITP; however, Huang et al now show that an important downstream target affected by IVIg-primed DCs could be the platelet rather than the macrophage. A result of this IVIg pathway is the “marking” of the ITP platelet which has reduced ability to engage the phagocytic M6. How the platelet is exactly affected remains unknown but could involve some modification of the platelet or a potential involvement of P-selectin or its ligand PSGL-1. (Professional illustration by Alice Y. Chen.)

**Intravenous immunoglobulin (IVIg)** has beneficial effects in a wide array of inflammatory states and autoimmune diseases. Although much remains to be unveiled about all of the details surrounding the mechanistic effects of IVIg, it has been proposed that activating Fcγ receptors on the surface of DCs are a primary target of IVIg.\(^4\) A specific role for FcγRII-dependent signaling has also been implicated in IVIg action in murine immune thrombocytopenia (ITP).\(^5\) What has been missing is an understanding of the downstream pathway whereby these IVIg-primed DCs mediate their inhibitory effects. Although the downstream mechanistic pathway induced by DCs is likely complex, the general assumption was that platelets were only the victims in ITP and that the target of this immune regulation would more logically affect the macrophage. In sharp contrast to this...
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