not expressed in human B cells, only TLR1, TLR2, TLR6, and TLR10 remain potential candidates for a nonredundant function in marginal zone–like B cells. Of these, TLR2 forms heterodimers with either TLR1 or TLR6 for recognition of triacyl and diacyl lipopeptides, respectively. TLR10 is thought to recognize lipoteichoic acid, either as a homodimer or a heterodimer with TLR1 or TLR2.[6] The high expression levels of TLR10 make this a more interesting candidate receptor.[1,2] However, the function of TLR10 has not been well established, because it is: (1) not present in the mouse, and (2) fails to activate typical TLR target genes.[6]

Insights into human T cell–dependent and –independent B-cell responses cannot be straightforwardly translated from mouse models. Mice have a different anatomical make up of their splenic marginal zone and they carry few memory B cells with mutated Ig genes, probably due to their specific-pathogen-free housing. The discovery of various new genetic defects in patients with primary immunodeficiencies has greatly enhanced possibilities to perform in vivo functional analysis of the human immune system. By showing that CD27^{+}IgM^{+}IgD^{+}, but not CD27^{+}IgD- memory B cells depend on MyD88-IRAK4-TIRAP signaling, Weller et al identified the first pathway that is specifically required for these cells.[1] Moreover, their results support the concept that pattern recognition receptors can signal for maturation in B cells that are not dependent on cognate T-cell help. Because (for obvious reasons) the authors were unable to directly study patients' spleens, it remains unclear whether the defect in circulating CD27^{+}IgM^{+}IgD^{+} B cells is the result of impaired responses in the marginal zone. Interestingly, these cells are markedly reduced, but not completely absent, in both CD40- and in MyD88/TIRAP/IRAK4-signaling deficient patients.[1,3] This could imply that both pathways are required for their homeostatic maintenance, or that in healthy individuals CD27^{+}IgM^{+}IgD^{+} B cells are a mixture of 2 subsets produced by 2 distinct pathways. In case of the latter, it can be anticipated that T–cell independently– derived memory B cells will display fewer somatic hypermutations and in vivo proliferation.[3] Comparative phenotyping or transcription profiling of CD27^{+}IgM^{+}IgD^{+} B cells in CD40L and MyD88/TIRAP/IRAK4 deficiency could facilitate the identification of novel markers

to discriminate these pathways in healthy individuals.

In addition to marginal zone responses, the approach of Weller et al can be applied to study TLR signaling in other T cell–independent responses. Recently, it was shown that CD27-IgA[+] memory B cells are not dependent on CD40 signaling and display a low replication history, high IgA2 subclass and Igλ usage, reminiscent of T cell–independent responses in the human intestinal tract.[3] It would be interesting to study whether these cells depend on similar or distinct signaling pathways as CD27^{+}IgM^{+}IgD^{+} memory B cells.

Splenic marginal zone B cells are implicated in the response against encapsulated bacteria. TIRAP-dependent TLR1, TLR2, and TLR10 recognize structures that are typically present in the bacterial cell wall. MyD88/IRAK4/TIRAP-deficient patients suffer from recurrent bacterial infections early in life, but seem to overcome this when growing older.[7] It is currently unclear whether the defect in CD27^{+}IgM^{+}IgD^{+} B cells contributes to the high frequency of infections, but the reduction of infection with older ages suggest that these patients develop (CD27^{+}IgD-) B-cell memory. Furthermore, recurrent bacterial infections in patients with an antibody deficiency are not reported to result from a specific defect in CD27^{+}IgM^{+}IgD^{+} B cells. Rather, these patients display defects in both CD27^{+}IgM^{+}IgD^{+} and CD27^{+}IgD-, or only CD27^{+}IgD- B cells.[8] Thus, further studies are required to assess the nonredundant role of CD27^{+}IgM^{+}IgD^{+} B cells in human immunity.

Besides inherited defects that impair TLR signaling, somatic mutations in MyD88 have recently been identified that constitutively activate MyD88-dependent signaling in lymphomas. Interestingly, these mutations have been identified in marginal zone lymphomas,[9] as well as in germinal center B-like diffuse large B-cell lymphomas.[10] Constitutive

MyD88-dependent signaling in these lymphomas is critical for their survival.[10] However, Weller et al here show a differential requirement of CD27^{+}IgM^{+}IgD^{+} and CD27^{+}IgD- B cells on TLR signaling.[1] This concept is important for further studies on T cell–independent B-cell activation, circulating marginal zone–like B cells, and human TLR10 function. Insights into these processes have direct implications for diagnosis and treatment of patients with immunodeficiencies or B-cell malignancies.

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● ● ● **CLINICAL TRIALS**

MDS: unraveling the mystery

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In this issue of *Blood*, Sekeres et al report a phase 2 study of lenalidomide and azacitidine for higher risk myelodysplastic syndromes (MDS).[1] High overall response and duration of response supports the concept of combination, multitargeted approaches rather than traditional chemotherapeutics for this disease.

MDS affect approximately 75 per 100 000 persons in the US over the age of 65 and is widely recognized as clonal disorders of diverse pathogenicities leading to bone marrow failure, cytopenias, infection and bleeding risks, leukemic transformation, and early death.[2] Until recently, that was essentially where our understanding ended, prompting some to adapt Churchill's adage that it is "… a riddle, wrapped in a mystery, inside an enigma; but perhaps there is a key…." Recent advances in molecular techniques can be well applied to this problem, helping us to unravel this mystery. Although conventional karyotyping may detect abnormalities in only approximately 50% of subjects, single nucleotide polymorphism arrays and comparative genomic hybridization can identify abnormalities in approximately 80%. An integrated look into the overlap of the multiple abnormalities identified in MDS has recently been presented by Lindsley and Ebert.[3] In those with 5q deletion, the haplo-insufficiency for this ribosomal protein gene leads to an abnormally elevated p53 drive and is associated with the increased apoptosis noted in this disorder. In addition, altered miRNA expression from that region triggers signaling cascades affecting the toll receptor pathway and nuclear factor-KB activation, leading to competitive growth advantage of the dysplastic cells. Numerous mutations have recently been identified that regulate methylation networks as well, including DNMT3A, IDH, TET2, and others, noting the effect changes have promoting the disease. Genome sequencing has also revealed recurrent somatic mutations in the spliceosome, with evidence that this machinery is involved in the epigenetic regulation of gene expression as well. Understanding the combinations and permutations of abnormalities in RNA splicing, epigenetic regulation, DNA repair, and kinase signaling may lead to new and better therapies for these heterogeneous disorders.

This improved understanding of abnormal molecular pathway regulation in MDS has led to initial forays into targeted therapies, resulting in 2 classes being approved for this disease: the imids and hypo-methylating agents. Lenalidomide inhibits haplo-deficient phosphatases and releases progenitors from p53 arrest.[4] Additional mechanisms include anti-angiogenesis via inhibition of bFGF-, VEGF-, and TNF-α–induced endothelial cell migration. Further, lenalidomide has multiple immunomodulatory effects involving stimulation of T-cell proliferation including natural killer (NK) cell number and function, and the production of multiple cytokines.[5,6] As a single agent it results in significant improvements, particularly in patients with chromosome 5q31.1 abnormalities. List and colleagues reported an 83% response rate in those with chromosome 5q31.1 abnormalities and 57% in those with normal karyotype, with 12% response in those with other karyotype changes.[7] Of the 20 with abnormal karyotypes identified, 50% attained a complete cytogenetic remission, which appeared to be durable.[7] Similarly, hypo-methylating agent benefits include transfusion independence, encouraging complete remission rates, and even improved overall survival in phase 3 prospective studies. Kantarjian and colleagues showed in a phase 3 study an overall response rate of 16% and another 47% hematopoietic improvement in those treated with 5-azacytidine compared with 7% with supportive care.[8] There was a median of 22 months until leukemic transformation or death in the treatment group versus 7 months in the supportive care arm as well ($P < .0001$). Similar benefits have also been reported for azacitidine.[8] These effects are mediated in part through depletion of methyl-cytosine resulting in reversal of hypermethylation of CpG islands in the promoter regions of certain genes, leading to the reversal of epigenetic silencing.

Studies effectively combining multiple targeted therapies given concurrently, as provided here by Sekeres et al, have been lacking. The rationale for such a study is clear as those with higher risk MDS seem to retain the multiple abnormal mechanisms present from the low-risk state from which they often evolved. Thus, using therapies that attack different mechanistic abnormalities may allow for synergistic benefits. This study treated 36 patients with azacytidine (75 mg/m² per day × 5 days) and lenalidomide (10 mg per day × 21 days [28-day cycle]), resulting in a 72% overall response rate (44% complete response) with a median duration of response over 17 months. Infections, cytopenias, and constitutional symptoms in the 10% range were the primary toxicities encountered. Building on recent information elucidating the role of multiple molecular abnormalities in MDS, Sekeres et al correlated their clinical work with a comprehensive genetic analysis of their subjects. Their data suggest patients with abnormalities in the methylation pathways have a higher rate of complete remissions with this combination therapy than would otherwise be expected. This type of molecular characterization of responders/nonresponders is what is needed as we increase our targeted therapy options and seek to personalize care choices in future studies.

Success of this combination targeted therapy approach is exciting and encouraging, although obstacles remain. While we continue to focus on maximum tolerated dosages for combination strategies, there is growing evidence for the relevance of both scheduling and ratio-metric dosing to optimize tumor kill. Because blood-borne cancers are not common, support for clinical studies to allow for rapid accrual is important but often lacking, particularly if the targeted therapies are made by different companies. Interpretation and extension of the clinical results is dependent upon completion of the important hypothesis-driven correlative science as noted in this report. Yet, increasingly, this funding is difficult to secure in the current research environment. The spiraling costs of health care may soon impact the planning of such novel combinations as well. Wang et al have recently noted the significant improvement in survival provided by hypo-methylating agents in this disease is associated with a dramatic increase in costs of care, indicating comparative effectiveness research may have a growing role in our future assessments of targeted therapy efficacy as well.[9] For these reasons, while it is an exciting time for cancer research, translating the significant advances in our understanding of the molecular pathogenesis of diseases to improved combination targeted therapies in the clinical arena will remain a challenge for our current generation of clinical trialists.

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● ● ● **PLATELETS & THROMBOPOIESIS**

Comment on Urban et al, page 5032

α-granules: a story in the making

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α-granules are by far the most abundant platelet granules. Yet little is known about how they are formed. In this issue of *Blood*, Urban et al now characterize platelets from patients with an inheritable α-granule defect, demonstrating a role for VPS16B in α-granule biogenesis and taking us one step closer to understanding how these elusive organelles are formed.[1]
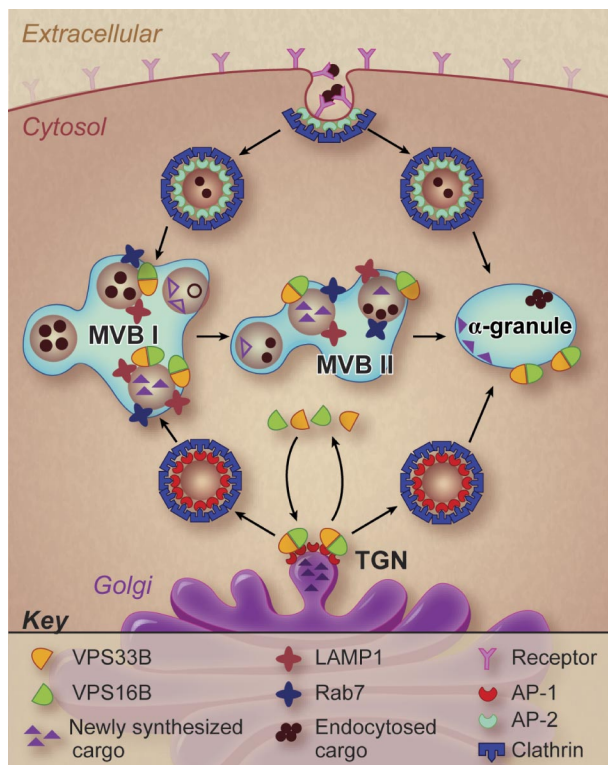
A working model of α-granule formation in megakaryocytes. Potential pathways of α-granule biogenesis are shown and possible sites of VPS33B and VPS16B action are indicated. Professional illustration by Debra T. Dartez.

Platelet granules were first observed in the late 19th century, when newly developed staining procedures were applied to the recently described platelet. With the application of electron microscopy to platelet biology in the 1960s, the diversity of platelet granules was first appreciated. Three classes of granules were described based on morphology: dense granules, lysosomes, and α-granules. Dense granules are endowed with membrane transporters and accumulate high concentrations of calcium, bioactive amines, adenine nucleo-

tides, and polyphosphate. Lysosomes contain enzymes involved in protein, carbohydrates, and lipid degradation. But the dominant platelet granule in number and total volume is the α-granule. They are approximately 10-fold more numerous than dense granules and comprise roughly 10% of the platelet volume. α-granules contain hundreds of different types of proteins, which may be sorted and stored into different subpopulation of α-granules. Diverse functions have been proposed for α-granules, including roles in hemostasis and thrombosis, inflammation, antimicrobial host defense, angiogenesis, and progression of malignancy.[2]

Despite the prominence of α-granules in platelet biology, little is known about their biogenesis. Many proteins involved in dense granule formation have been identified by studying dense granule defects in patients and mice with various degrees of occulocutaneous albinism and bleeding diatheses. In contrast, α-granule deficiency is extremely rare. Gray platelet syndrome (GPS) is the most well-recognized disorder of α-granules, but only 1 to 2 dozen families with GPS have been identified. Nonetheless, Kahr's group and others have recently demonstrated that mutations in *NBEAL2,* which encodes neurobeachin-like protein 2, cause GPS.[3-5] Even before this work, however, Kahr and colleagues had characterized another syndrome associated with α-granule deficiency termed arthrogryposis, renal dysfunction, and cholestasis (ARC) syndrome.[6] ARC syndrome is a rare autosomal recessive condition characterized by death in the first year of life. In addition to their other maladies, these patients have platelets that lack α-granules. The fact that ARC syndrome is caused by a defect in VPS33B implicated this protein in α-granule formation.[6]

Urban et al have now gone on to identify another protein involved in α-granule formation: VSP16B. The group first used a yeast 2-hybrid system to identify binding partners for VPS33B. They pulled out an uncharacterized gene product, C14orf133, which they subsequently identified as human VPS16B. Additional studies confirmed an association of platelet VPS33B with VPS16B. While conducting these experiments, a paper was published demonstrating that mutations in *C14orf133* were associated with ARC syndrome.[7] This observation provided proof-of-principle that not only did VPS16B bind