MM can be divided into nonoverlapping molecular subtypes, shown in circles, based on the primary genetic event, identified by black text. These appear to partially determine the profile of secondary genetic events, summarized in red text. Clustering of the copy number abnormalities identifies novel clusters with convergent evolution, such as cluster 7, identifying patients with a poor prognosis.

First, using several methods involving either a frequency-based approach or a functionally based approach, they have identified a total of 63 apparent driver genes, with 17 potentially actionable. This includes several novel putative oncogenes (PTPN11, PRKD2, SF3B1, IDH1, and IDH2) and several putative tumor suppressor genes (UBR5, HUWE1), all of which are mutated only infrequently in MM tumors. They also identify important pathways based on the 63 genes: MEX/ERK (50%), NF-κB signaling (14%), G1/S cell cycle transition (5%), epigenetic regulators (24%), and RNA processing (18%).

Second, they provide multiple examples of putative secondary genetic events that are nonrandomly associated with tumors that have different primary events (see figure). In one case, it appears that both synonymous and nonsynonymous mutations in CCND1 are predicated by the t(11;14) translocation, which dysregulates CCND1. Also, mostly nonsynonymous mutations in MAF are present uniquely in tumors with the t(14;16) translocation. Similarly, FGFR3 has activating mutations only in t(14;14) tumors, in which FGFR3 is upregulated by the translocation. This also may be true for unique mutations at aa594 in BRAF in t(14;16) tumors because of an APOBEC mutational signature that is associated with t(14;16) tumors. In other tumors, it is unclear why certain genetic events are more prevalent; for example, 11q+, FAM46C mutations, MYC rearrangements in hyperdiploid tumors; or PRDK2 and DIS3 mutations predominantly in t(4;14) tumors; or BRAF, DIS3, and ATM mutations in t(14;20) tumors. Their data also confirm one of their earlier observations that NRAS mutations are found predominately in t(11;14) and hyperdiploid tumors, whereas KRAS mutations are more randomly distributed among tumors with different primary events. Curiously, there is a predominance of mutations at codon 61 of NRAS vs more evenly distributed mutations at codons 12, 13, and 61 of KRAS. In addition to the nonrandom association of secondary events in different types of MM tumors, the figure also shows an example of the convergent evolution of secondary events (1q+, 11q−, 13q−, DIS3 mutation) in nonhyperdiploid MM tumors with different primary events.

Third, based on variant allele frequencies, they show that oncogenic mutations are more likely to be clonal than tumor suppressor gene mutations, with the notable exception of TP53. They suggest that the higher variant allele frequency suggests an earlier time of the mutation or a greater selection pressure for oncogenic mutations.

Fourth, they show that an increasing number of driver abnormalities are associated with a poor prognosis. Driver abnormalities include not only mutated driver genes, but also translocations, chromosomal and segmental chromosome gains and losses, loss of heterozygosity, and APOBEC mutational signature. Given the low prevalence of mutated driver genes in tumors, it seems likely that these other events are largely responsible for differing clinical outcomes. The recent study by Bolli et al clearly shows that it is mostly these other abnormalities and not mutated driver genes that effect clinical prognosis. In fact, both papers find that TP53 is the only mutated driver gene that is a very strong predictor of clinical outcomes.

In conclusion, this is an impressive collection of clinical and genomic data on 1273 newly diagnosed MM tumors that will be available for others to use for additional insights.

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

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TPO-logy accepted

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In this issue of Blood, Xu et al unequivocally show by using a series of elegant experiments that platelet GPlbα mediates hepatic thrombopoietin (TPO) production.1

The work by Xu et al challenges the prevailing model, which posits that TPO production by hepatocytes is constitutive and circulating TPO concentration is inversely proportional to the “Mpl mass” contributed by the total number of...
Recent data give credence to the assertion that TPO regulation is far more complex than previously known. Studies clearly show that the proinflammatory cytokine interleukin-6 (IL-6) stimulates hepatic TPO synthesis, providing a regulated pathway to increase platelet production during acute inflammatory responses in humans and in in vitro models.3 Recent investigations identified that asialylated senile platelets and the hepatocyte-specific Ashwell-Morell receptor (AMR) are the long elusive physiological ligand-receptor pair regulating hepatic TPO messenger RNA (mRNA) production by recruiting JAK2 and STAT3.4 This finding is further supported by data showing that platelets lacking the sialyltransferase ST3GalIV (St3ga14−/−) platelets are exclusively and completely cleared by the AMR.4

GPIbα has been identified as a major counterreceptor on St3ga14−/− platelets for the AMR.5 However, this conclusion was only indirectly supported by enzymatic removal of the N-terminal region of GPIbα from the platelet surface. Xu et al unequivocally show by using a series of elegant experiments that platelet GPIbα mediates TPO production. This conclusion is based on the following data: (1) circulating TPO is significantly decreased in GPIbα-deficient Bernard-Soulier syndrome patients and in Gp1b−/− mice, compared with controls (see figure); (2) lower TPO levels in GPIbα-deficient conditions were not attributable to increased TPO clearance by platelets lacking GPIbα, but rather via decreased hepatic TPO mRNA transcription and production. This conclusion was nicely supported by the fact that wild type, but not Gp1b−/− platelet transfusions rescued both hepatic TPO mRNA and circulating TPO levels in Gp1b−/− mice. Further, in vitro hepatocyte cocultures with platelets or GPIbα-coupled beads confirmed the disruption of platelet-mediated hepatic TPO generation in the absence of GPIbα.

Recent studies have highlighted the role of glycan modifications on platelets in mediating their clearance.6 In circulation, loss of terminal sialic acid (neuraminic acid) from the platelet surface has been linked with senescent platelet removal.4 The evidence provided by Xu et al that GPIbα glycans are recognized by the AMR to initiate hepatic TPO production is somewhat indirect. The authors show

megakaryocytes and platelets. Decades of research support the notion that TPO production is constitutive and TPO serum levels are maintained solely by its uptake and metabolism by platelets and megakaryocytes.2 Clear evidence supports this reciprocal relationship between platelet count and circulating TPO levels, for example in bone marrow transplant patients, in Mpl−/− mice, and in models of acute immune or chemotherapy-induced thrombocytopenia.

However, human and mouse data support another TPO regulatory model where platelets are sensed to regulate circulating TPO levels. For example, serum TPO levels are lower than expected in patients with immune thrombocytopenia and high in patients with essential thrombocythemia.5 Moreover, serum TPO levels are normal in Nfe2−/− mice despite severe thrombocytopenia, and mice deficient for Bak and Bax have normal to slightly increased serum TPO levels despite significantly increased platelet count.5 Hence, these data support the notion that platelet TPO metabolism is not the sole determinant of plasma TPO levels regulated in a complex manner.
that although treatment of Gp1b<sup>−/−</sup>
platelets with neuraminidase caused signif-
nicant desialylation, desialylated Gp1b<sup>−/−</sup>
platelets failed to rescue impaired he-
patic TPO production both in vivo and in vitro. The data support the notion that GPIbα, independent of other recep-
tors and platelet desialylation, is a pre-
requisite for hepatic TPO production. Additional evidence in support of this
notion was provided by recapitulating
impaired hepatic TPO production in
IL-4Rx/GPIbα transgenic mice, which lack the extracellular GPIbα domain, as well as with antibodies targeting extracellular portions of GPIbα. These results demonstrated that the N-terminus of GPIbα is required for platelet-mediated hepatic
TPO generation.

Platelet GPIbα is abundantly decorated
with sialic acid moieties, accounting for perhaps 70% to 80% of the total sialic acid on the platelet surface. However, unlike human GPIbα, the murine GPIbα amino acid sequence lacks any N-glycosylation consensus sequences. Grewal et al demonstrated that even in mice lacking GPIbα, neuraminidase treatment leads to efficient platelet clearance, albeit at a slower rate than in control mice.6 The data suggest that the glycans on GPIbα are necessary to set off a rapid rate of AMR-dependent
clearance, but the exposed galactose
moieties on other platelet glycoproteins
present counterreceptors for the AMR or other asialoglycoprotein receptors.

The AMR presents a complex receptor
that prefers complex N-linked glycans that are clearly absent from mouse
platelets on GPIbα.7 Hence, other binding
partners could be involved in AMR-
mediated platelet clearance. For exam-
ple, platelet bound von Willebrand factor, the ultimate GPIbα ligand, which expresses multiple N- and O-linked glycans in hu-
man and mice, could clearly contribute to
the AMR-platelet recognitions.8 It is un-
clear whether asialylated GPIbα O-linked
glycans contribute to AMR-mediated
platelet clearance. A recent study dem-
onstrated that hepatic AMR promotes preferential adherence to and phagocy-
tosis of asialylated and/or platelets that lack O-glycans, that is, platelets from
hematopoietic cell conditional C1galt<sup>−/−</sup>
mice, by Kupffer cells through the C-type
lectin receptor CLEC4F.9 These findings provide further insight into an essen-
tial role for core 1 O-glycosylation of
platelets in their clearance in the liver. It
remains to be determined if removal of sialic acid moieties (which come in differ-
ent flavors) from O-linked glycans play a role in AMR-platelet recognition.
These intriguing observations require further investigation to fully understand
the contribution of the published phe-
nomena to platelet clearance and TPO
production.

Despite the limitations of the here pub-
lished findings, the data reveal a novel
nonredundant regulatory role of platelets in hepatic TPO homeostasis, which fur-
thers our understanding of TPO regula-
tion and may have important implications in
diseases related to GPIbα such as
Bernard-Soulier syndrome and auto-
and alloimmune-mediated thrombocytopenias.

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**CLINICAL TRIALS AND OBSERVATIONS**

**Comment on Magoulas et al, page 658**

**Endosome trafficking: blood and more**

**Gary M. Kupfer | Yale University School of Medicine**

In the day-to-day practice of hematology, we are often asked to comment on
seemingly innocuous findings such as a low neutrophil count. With the evo-
lution of the modern genetic age, we have gained the ability to provide
explanations for pathophysiological findings, thus adding to the base of
knowledge regarding hematopoiesis. In this issue of *Blood*, Magoulas et al
add to our knowledge with their thorough analysis of a rare consanguineous
family, proving again the utility of studying rare disease for the benefit of
wider biologic knowledge.1 We have thus gained a deeper understanding
of myelofibrosis (MF), a disease found mostly in older people, by studying it
in the young.

MF is generally a variant of myeloprolif-
erative neoplasms that affects mainly
those older than 50 years and is asso-
ciated primarily with mutations in
genes related to myeloproliferative ne-
oplasm such as *JAK2, MPL*, and *CALR.*
Approximately 10% of MF cases have no
definable genetic cause. In pediatrics, MF
is an extremely rare disease, and cases
typically culminate in leukemia.4 The case
presented by Magoulas et al is marked
by the presence of MF in the context of
multiple congenital abnormalities, in-
cluding severe neurocognitive dysfunction.