One of the questions that is discussed by the authors is whether treatment intensity is maintained long enough. Less therapy reduction is proposed for familial/genetic HLH (FHL) patients beyond week 6. However, data supporting this proposal are limited: 6 out of 8 patients (4 with confirmed FHL) who died on days 43 to 59 had refractory or reactivated HLH. It is not clear how many of them achieved an initial full remission and if they had deviations from the etoposide dosing scheme, which were allowed in the protocol. Of note, HLH-2004, like HLH-94, was a pediatric study. Also, patients with underlying diseases were excluded. This is relevant because etoposide-based therapy is widely used for the treatment of HLH in older patients and patients with underlying diseases, who would not have been eligible for the HLH-94 study. Although rapid treatment with etoposide can be lifesaving in these patient groups as well, not all patients fulfilling the HLH-2004 diagnostic criteria require etoposide. Moreover, in some cases, modification of dosing and treatment duration may be appropriate. In support of this, a subgroup analysis in HLH-2004 indicated that some of the deaths within the first 2 months of treatment, occurring predominantly in patients without familial disease, may have been treatment related. This group of patients with secondary HLH can be much better defined due to the more extensive genetic analysis in the HLH-2004 study. Future analysis of the study data should use the opportunity to provide more information on this patient subgroup.

Importantly, exciting new therapeutic options that are more precisely targeted to the immunological disease pathophysiology are currently being tested in pilot studies, including alemtuzumab, tocilizumab, the JAK1/2 inhibitor ruxolitinib, and an anti–interferon-γ monoclonal antibody. The initial results from these studies are promising, but given the many different etiologies of HLH, the role of these new options in therapy will require careful study. In particular, from a world-wide perspective, it is likely that etoposide-based protocols will remain in use for a number of years to come. Therefore, the HLH steering committee of the Histiocyte Society is currently issuing recommendations on the use of etoposide-based therapy in different HLH contexts based on >20 years of experience with etoposide. Whether the new treatments result in outcome differences of a size that allows for a sufficiently powered randomized trial in such a rare disease remains to be seen. In any case, international collaboration, as exemplified in HLH-94 and HLH-2004, will be a crucial basis for further progress in the treatment of patients with this severe condition.

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● ● ● MYELOID NEOPLASIA

Comment on Zhao et al, page 2762

Single-cell dissection of monosomy 7 syndromes

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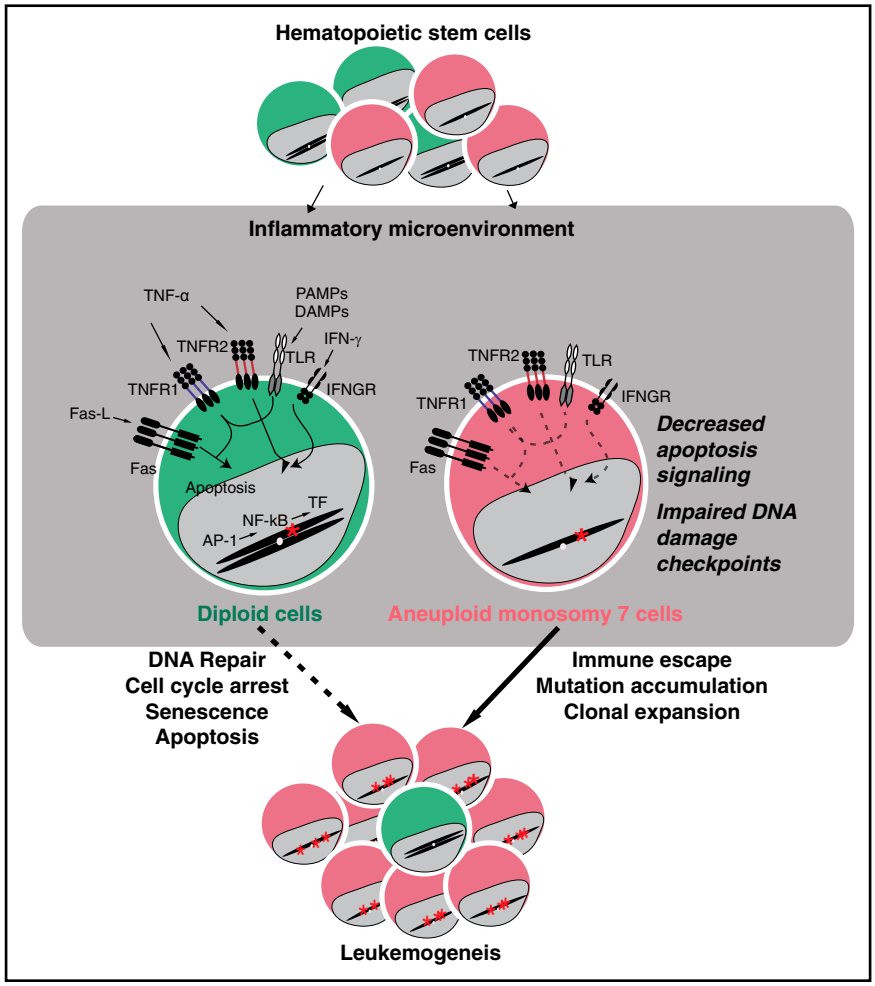
In this issue of *Blood*, Zhao et al perform single-cell RNA sequencing (scRNA-seq) of bone marrow–derived CD34$^+$ cells from patients with monosomy 7. Identifying cells with chromosome 7 aberrations, they uncover reduced transcription of genes upholding genomic integrity and concomitant accumulation of somatic mutations.[1]

In 1960, Nowell and Hungerford[2] discovered a small chromosome in the white blood cells of patients with chronic myelogenous leukemia (CML), signifying the first chromosomal abnormality associated with cancer. Cytogenetic analyses have since revealed that chromosomal abnormalities are a common feature of many cancers, with distinct aberrations being associated with specific forms of cancer. Myeloid malignancies encompass a heterogeneous group of clonal hematopoietic stem- and progenitor-cell (HSPC) disorders.[3] In addition to CML, they include myeloproliferative neoplasms (MPNs), myelodysplastic syndrome (MDS), and acute myeloid leukemia (AML). Acquired HSPC chromosomal abnormalities are 1 of the main risk factors for developing myeloid malignancies, characterized by impaired hematopoiesis and cytopenias. The incidence of myeloid malignancies increases with age. In the elderly, del(5q) represents the most common chromosomal aberration, followed by the complete or partial loss of chromosome 7, termed monosomy 7 (−7). In children, approximately 20% of leukemias are of myeloid origin, with −7 representing a common chromosomal aberration. Importantly, −7 is associated with refractory cytopenias and a high risk of progression to AML.[4] Dissecting how abnormalities affecting large chromosomal regions mechanistically give rise to distinct cancers is challenging. Because chromosomal composition varies among species, animal models are not useful.

Human genetics have provided insights into −7. Inherited bone marrow failure syndromes caused by mutations in genes required for DNA repair, chromosomal stability, and telomere elongation are associated with an increased risk of acquiring somatic mutations that often manifest as



Schematic representation of mechanisms underlying aberrant expansion of aneuploid hematopoietic stem cells with −7. During bone marrow failure, hematopietic stem cells are embedded in an inflammatory environment. Although diploid stem cells (green) that accumulate mutations usually are subject to cell-cycle arrest, apoptosis, and effective immune surveillance and eradication, aneuploid stem cells with −7 (red) display downregulation of genes implicated in the regulation of DNA damage checkpoints, cell cycle, and apoptosis, which thereby can facilitate their accumulation of additional mutations and aberrant expansion, ultimately leading to leukemogenesis. AP-1, activator protein 1; DAMP, damage-associated molecular pattern; Fas-L, Fas ligand; IFN-γ, interferon γ; IFNGR, IFN-γ receptor; PAMP, pathogen-associated molecular pattern; TF, transcription factor; TLR, Toll-like receptor; TNF, tumor necrosis factor; TNFR, TNF receptor. The figure has been adapted from Figure 6 in the article by Zhao et al beginning on page 2762.

myeloid malignancies. Furthermore, several rare autosomal-dominant or de novo monogenic causes of familial MDS/AML have recently been elucidated. Interestingly, heterozygous *GATA2* germline mutations are associated bone marrow failure as well as MPNs, MDS, and AML, predominately with −7. Overall, *GATA2* haploinsufficiency explains 37% of pediatric cases of −7.[5] Providing the first human examples of adaptation by aneuploidy, de novo or inherited gain-of-function mutations in *SAMD9* or *SAMD9L* encoded on chromosome 7q frequently acquire 7q segmental uniparental disomy in HSPCs and their progeny, whereas others develop −7 and subsequent MDS or AML.[6,7] In addition, recurrent microdeletions or mutations in chromosome 7 loci encoding *SAMD9/SAMD9L*, *CUX1*, *LUC7L2*, *CUL1*, and *EZH2* have been found in myeloid neoplasms.[8] Despite these insights, understanding how −7 in stem cells contributes to the development of myeloid malignancies remains enigmatic. Stem cells are scarce and hence difficult to study. Moreover, in vitro models of human cellular differentiation cannot account for the microenvironment or immune surveillance.

Harnessing new molecular techniques for scRNA-seq, Zhao et al sorted and sequenced a total of 391 HSPCs from 4 healthy donors and 588 HSPCs from 5 patients, 3 with −7, 1 with additional trisomy 1, and 2 with trisomy 8 as determined by clinical cytogenetics. Through statistical analyses of single-cell transcriptome data, they were able to resolve HSPCs into specific subtypes based upon gene expression profiles. Moreover, using 3 independent bioinformatical methods, they detected aneuploid cells with reduced expression of genes encoded by chromosome 7 genes or higher expression of those encoded by chromosome 1 or 8. The patterns of decreased or increased gene expression were consistent with the defined chromosomal abnormalities in each patient and the relative frequencies of such aberrant cells. Analyses uncovered abnormal differentiation profiles of −7 HSPCs. Comparing gene expression in HSPCs bioinformatically assigned as −7 with diploid cells, Zhao et al uncovered prominently downregulated genes and gene pathways in addition to a limited number of upregulated genes. Marked −7 cell heterogeneity was noted among patients. Nonetheless, comparing $CD38^+$ HSPCs in all 3 donors with −7 revealed some common transcriptional features generally categorized into functional groups implicated in DNA damage checkpoint, cell cycle, apoptosis, immune response, and hematopoietic differentiation, respectively (see figure). On the basis of these findings, they hypothesized that the downregulation of genes required for maintenance of DNA stability might result in genomic instability. Indeed, analyzing a single patient in whom a sufficient number of −7 cells were sequenced, they detected a higher rate of single-nucleotide polymorphisms in more differentiated $CD34^+$ $CD38^+$ HSPCs relative to diploid counterparts and more quiescent $CD34^+CD38^-$ HSPCs with −7.

Stemming from aberrant HSPCs, myeloid malignancies are increasingly viewed as a spectrum of diseases, ranging from clonal hematopoiesis to MDS and AML. The findings by Zhao et al elegantly offer insight into HSPC biology in the context of −7. Their work, as well as recent work by Giustacchini et al[9] investigating stem cells in *BCR-ABL* CML, provides the first example of how scRNA-seq enables in vivo studies of rare aberrant hematopoietic stem cells, illuminating dysregulated cellular pathways that may facilitate transformation to myeloid malignancies. Their data sets represent resources for investigating the early steps of human HSPC differentiation and gaining insights into early leukemogenesis induction at

the single-cell level. Notably, data obtained so far are correlative. Future efforts need to focus on validating findings in greater numbers of patients, in addition to identifying more definitive causal relationships between genes and function. Combined with insights from systematic efforts to delineate how distinct chromosomal abnormalities as well as gene mutations interact to determine transformation to leukemia,[10] evaluation of HSPC genetics at the single-cell level may in the future prove useful for monitoring treatment and prognosis of patients with myeloid neoplasms. Furthermore, knowing the functional consequences of chromosome gain or loss will help in exploring gene targets for new directed therapies.

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● ● ● PLATELETS AND THROMBOPOIESIS

Comment on Münzer et al, page 2774

CK2: a key regulator of thrombopoiesis

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In this issue of *Blood*, Münzer et al used a specific platelet/megakaryocyte (MK) deletion of casein kinase (*CK*) 2β mouse model to elegantly demonstrate that CK2 regulates both platelet formation and activation by altering microtubule structure, which results in impaired intracellular Ca^{2+} release.[1]

CK2 is a ubiquitous, constitutively active serine threonine kinase. It is a tetrameric protein composed of 2 regulatory β chains that dimerize to bind to 2 homologous catalytic chains (α and α′). In contrast with other kinases, CK2 has hundreds of potential substrates and is involved in many cellular processes, including survival, proliferation, transcription, and signaling.[2] The importance of these functions is demonstrated by the embryonic lethality of *ck2β* knockout mice.

CK2 is expressed in blood cells, including platelets, yet few studies have investigated the role of CK2 in the function of hematopoietic cells. CK2 is involved in the growth and proliferation of numerous cancers, including acute lymphocytic leukemia, acute myeloid leukemia, and their chronic forms (chronic lymphocytic leukemia and chronic myeloid leukemia), mainly by inducing an overactivation of certain signaling pathways such as the phosphatidylinositol 3-kinase (PI3K)/AKT, JAK/STAT, and NF-κB pathways.[3] This has led to the development of several CK2 inhibitors. Keeping in mind that most of these CK2 inhibitors may exert off-target effects, they have been useful in showing that CK2 is involved in platelet activation (aggregation and secretion) through the regulation of PI3K signaling.[4]

To precisely study the effects of CK2 in both thrombopoiesis and platelet functions, Münzer et al performed a megakaryocyte/platelet-specific genetic deletion of *ck2β* ($ck2\beta^{-/-}$) in mice.[1] This led to 2 important discoveries (see figure): (1) CK2 is involved in platelet production and survival. The *ck2β* knockout induced a macrothrombocytopenia resulting from an MK fragmentation inside the bone marrow, which led to ineffective platelet production and a slight decrease in platelet half-life. The decrease in platelet count stimulates megakaryopoiesis in bone marrow as well as in the spleen but without noticeable development of fibrosis. (2) CK2 regulates Ca^{2+}-triggered platelet activation and exerts an important role in thrombus formation under high shear.

Thrombocytopenic phenotypes related to premature MK fragmentation in the bone marrow have rarely been described. The first demonstration was in *Wasp*-deficient mice in which it was reported that MK fragmentation might be linked to an MK trafficking defect.[5] Subsequently, it was shown in patients that a germ line mutation in cytochrome C led to thrombocytopenia by platelet-like release in the marrow as a result of an increased MK apoptosis.[6] Finally, it was recently shown that the double *cdc42/rac1* knockout leads to macrothrombocytopenia with MK fragmentation in the marrow. This fragmentation was related to a defect in microtubule organization, but surprisingly, this double knockout barely affected the actin cytoskeleton.[7]

In the case of this conditional *ck2β* knockout, it was expected that thrombocytopenia would be related to an increase in apoptosis. This is because in hematopoietic cells, CK2 regulates the PI3K/AKT pathway and thus BAD, a key molecule in platelet survival and production. However, in the study by Münzer et al, the authors demonstrated that in their *ck2β* knockout mice, thrombocytopenia is the consequence of an alteration in microtubule dynamics. Indeed, it has recently been emphasized that CK2 plays an important role in the regulation of microtubule cytoskeleton and in morphogenesis.[8] In particular, CK2 is associated with