using bone marrow cells. Careful selection of patients with IgM MGUS cells by flow cytometry or bead selection of cells should be applied to understand whether the genomic makeup of IgM MGUS with or without *MYD88* (L265P) mutations is the same entity. This hypothesis is suggested as less likely by the fact that clonal Ig rearrangements were seen in IgM MGUS cases without the *MYD88* (L265P) mutation, although this needs to be carefully excluded in future studies.

A more likely explanation is that the mutation is a progression event, leading to a question regarding the founding lesions that drive WM pathogenesis. So far, NGS data from other studies have failed to show other highly prevalent mutations or genetic abnormalities in WM.[3] Mutations of NF-κB–related genes have also been reported in WM, but they are not common,[6] and deletions of the long arm of chromosome 6 are seen in 50% of cases.[8] How can this be reconciled? One possibility is that there are various (perhaps many) pathways by which an aberrant IgM-producing clone can develop in the germinal center, but can remain subclinical (not detectable because of low concentration of IgM monoclonal proteins) or clinically silent (IgM MGUS). But then a *MYD88* (L265P) mutation occurs and accelerates (unmasks) such clones leading to the diagnosis of WM, smoldering WM, or even more advanced IgM MGUS. Even if the mutation was found to be subclonal in all IgM MGUS cases, this would still suggest it is a progression event.

Familial predisposition for WM is reported in a smaller subset of cases,[9] although it should be mentioned that in some of these families the predisposition includes IgM MGUS. Could the predisposition occur because of normal SNP variants or mutations of *MYD88* (L265P)? This is unknown, but it is a question in need of testing. An alternative hypothesis is that the familial predisposition occurs for these pre-WM clones, and the acquisition of *MYD88* (L265P) mutations occurs in a stochastic fashion but is common enough that phenotype results in familial clustering. It should also be remarked that WM is associated with a personal and family history of autoimmunity and allergies,[10] and, as such, normal variants of other immune regulatory molecules are possible.

In conclusion, these findings once more show the revolutionary power that next-generation sequencing has brought to cancer research. In this golden age of cancer genomic discovery, the *MYD88* (L265P) "touch" is happening!

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● ● ● CLINICAL TRIALS & OBSERVATIONS

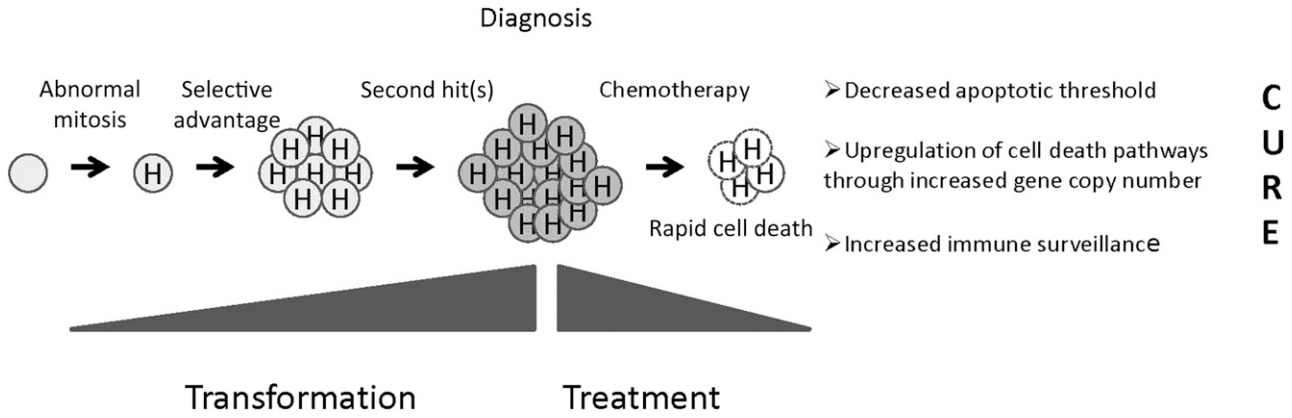
Comment on Dastugue et al, page 2415

Safety in numbers: hyperdiploidy and prognosis

William L. Carroll[1] [1]NEW YORK UNIVERSITY CANCER INSTITUTE

In this issue of *Blood*, Dastugue and colleagues report their findings examining the impact of hyperdiploidy on the outcome of children with B-cell acute lymphoblastic leukemia (ALL). Since the 1980s, it has been appreciated that 25% to 30% of B-cell ALL cases have a "high" hyperdiploid karyotype (generally defined as >50-67 chromosomes) and that this subset has an unusually good prognosis (reviewed by Paulsson and Johansson[1]). This feature has been used routinely to stratify patients into treatment groups. However, in spite of major progress understanding other biological subtypes of ALL, the underlying transforming pathways that define this major subgroup and those that account for the good response to therapy remain largely unknown. Moreover, controversy exists on whether the driver in both cases is the gain of specific chromosomes or whether it is due to the overall gain in chromosome number. Dastugue et al report that the best indicator of overall prognosis is ploidy assessed by karyotype and that prognosis is improved at higher modal chromosome numbers.[2]

Aneuploidy, an abnormal number of chromosomes, is a frequent observation in human cancer (reviewed by Gordon et al[3]). Evolution has led to protective mechanisms to ensure faithful replication and inheritance of genetic information so that cells with abnormal chromosome content are removed. Compensatory mechanisms must exist in cancer cells to compensate for the stress of aneuploidy. Cancer cells often display defects in pathways that regulate genome stability that result in aneuploidy. Thus, hyperdiploidy may be a consequence rather than a driver of



A precursor B cell undergoes an abnormal mitosis that results in hyperdiploidy. A selective advantage is conferred, resulting in a preleukemic clone, and second "hits" result in the fully malignant phenotype. Hyperdiploid cells display unusual sensitivity to chemotherapy through as-yet-undefined mechanisms.

malignancy. However, available data indicate the genomes of hyperdiploid ALL cells (and all childhood ALL cells) are relatively stable (note chromosome instability refers to the *rate* of karyotypic change). The great majority of cases of hyperdiploid ALL appear to be the result of the simultaneous gain of extra chromosomes in a single abnormal cell division.[4]

Evidence seems to favor a beneficial impact of hyperdiploidy in leukemogenesis (see Figure). Like most other forms of ALL, hyperdiploid clones appear to have a prenatal origin followed a long latency until the development of the truly transformed clone though acquisition of "second hits." What accounts for this selective advantage? The repertoire of altered expression of key growth and survival pathways could provide cells with a selective advantage. Clues to the identification of such pathways come from the pattern of chromosome gains in hyperdiploid ALL. It is important to note that chromosomes 21, X, 14, 6, 18, 4, 17, and 10 tend to be gained in blasts associated with lower modal karyotypes and retained as modal chromosome number increases.[5] Such selection could be due to the acquisition of novel beneficial properties associated with this subset of chromosomes or selection against other chromosomes because of negative attributes. Acquisition of chromosome 21 is seen in 95% of hyperdiploid cases, and children with constitutional trisomy 21 do display an increase in hematologic malignancies (but a decrease in solid tumors, curiously).

Although hyperdiploidy may be involved with leukemogenesis, it is clearly disadvantageous to the tumor after application of chemotherapy. This is in sharp contrast to the unfavorable impact of aneuploidy in many solid tumors, likely because of the presence of chromosomal instability. Numerous reports, including the paper by Dastugue et al in this issue, show 5-year event-free and survival rates of 84% to 90% and 93% to 95%, respectively, using contemporary treatments.[6,7] Hyperdiploidy is associated with other features associated with a good prognosis but it carries independent prognostic significance in multivariate analyses. The unique chemosensitivity of these cells has been demonstrated in ex vivo assays and by the high rate of end-induction remission. The study by Dastugue et al also showed that hyperdiploidy was associated with low minimal residual disease burden at end induction. Some disagreement exists about the prognostic relevance of individual chromosomes. Combined analysis of Pediatric Oncology Group and Children's Oncology Group studies showed the powerful effect of trisomies of chromosomes 4, 10, and 17 (triple trisomy) that led to routine incorporation into risk stratification (trisomies of 4 and 10 are in current use), while investigators from the Medical Research Council could not confirm this finding but showed that among high hyperdiploid cases those with trisomy 18 had a lower risk of relapse.[7,8] Much has been made of these discrepancies, but the incidence of trisomies 4, 10, 17, and 18 is proportional to increases in chromosome number. Thus it might be predicted that modal chromosome number should emerge as the most important variable and that is exactly the conclusion of the study by Dastugue et al.

The mechanism of enhanced chemosensitivity is unknown. Cells might be primed for cell death because of a decreased apoptotic threshold due to the stress associated with aneuploidy. Alternatively, increased gene and protein expression associated with increased copy number of individual genes involved in drug transport, metabolism, or common to many different agents (eg, executors of cell death) might enhance the effect of chemotherapy. In this case, "passenger lesions" associated with transformation could function as "drivers" of chemosensitivity. For example, elevated levels of the reduced folate carrier (hRFC, *SLC19A1*) located on chromosome 21 has been associated with a decreased risk of relapse. As expected, levels are higher in hyperdiploid cases, although this difference did not reach statistical significance compared with National Cancer Institute standard-risk patients without hyperdiploidy in one study.[9] Nonetheless, subtle differences in therapy among treatment groups may account for the relative importance of certain trisomies in published studies. Another provocative mechanism of enhanced cell death was illustrated by a recent study showing that immunosurveillance mechanisms exist to control ploidy.[10]

In summary, analysis of modal chromosome number remains a powerful predictor of outcome in childhood B-cell ALL and should be used routinely to stratify patients. The importance of individual trisomies may relate to subtle differences in treatment. DNA index may be used in place of karyotype in resource-poor areas,

and newer technology may be integrated in the future. Finally, we await a more detailed understanding of the biology of hyperdiploid ALL in the quest to develop targeted therapy.

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● ● ● HEMATOPOIESIS & STEM CELLS

Comment on Malinge et al, page 2440

Ikaros, Notch, and GATA1 cross paths during megakaryopoiesis

Ivan Maillard[1] [1]UNIVERSITY OF MICHIGAN

In this issue of *Blood*, Malinge et al[1] describe new reciprocal interactions between Ikaros, Notch, and GATA transcription factors during megakaryocyte development. Ikaros represses megakaryocytic genes and selected Notch targets, while being turned off by GATA1 upon terminal differentiation.

Both Ikaros and Notch proteins were first identified for their essential functions in lymphocyte development as well as for their dysregulation in lymphoid malignancies. The Krüppel-type zinc finger transcription factor Ikaros (encoded by *IKZF1*) regulates expression of multiple genes critical for the development of lymphoid lineages. In mice, Ikaros loss-of-function is associated with the emergence of aggressive T-cell lymphoblastic leukemias. In humans, high-risk B-cell acute lymphoblastic leukemias harbor recurrent genomic deletions at the *IKZF1* locus.[2] In addition, emerging work suggests that Ikaros also functions in the myeloid, erythroid, and megakaryocytic lineages. For example, an *Ikzf1-gfp* reporter allele revealed a strong correlation between high Ikaros expression in hematopoietic progenitors and myeloid cell fate, while low Ikaros expression identified progenitors committed to megakaryocytic and erythroid differentiation.[3] Moreover, Ikaros-deficient mice have thrombocytosis, suggesting that Ikaros can negatively regulate megakaryopoiesis.

Notch signaling was first studied for its essential role in T-cell development. In addition, activating *NOTCH1* mutations are present in a high proportion of T-cell acute lymphoblastic leukemias in mice and humans.[4] In this context, Notch and Ikaros appear to have antagonistic functions. Expression of dominant-negative Ikaros isoforms lacking DNA binding can cooperate with Notch activation in T-cell leukemia.[5] Ikaros may directly antagonize the effects of the Notch transcriptional activation complex at target gene loci. At least in mice, Ikaros can also repress transcription from internal *Notch1* promoter elements that drive expression of truncated constitutively active Notch receptors.[6,7] Interestingly, recent work indicates that Notch signaling also plays important functions in the myeloid and megakaryocytic lineages.[8,9] These observations have set the stage to investigate whether Notch and Ikaros interact and exert new functions outside of lymphoid progenitors.

In this issue, Malinge et al[1] focus their attention on the role of Ikaros in megakaryopoiesis. As a rationale to initiate this work, the authors built on their past observations that Notch signaling supports enhanced megakaryopoiesis in vitro and in vivo.[9] In addition, acute megakaryocytic leukemias (AMKL) carrying the recurrent OTT-MAL translocation were shown to activate an aberrant Notch signature.[10] Based on the hypothesis that Ikaros may antagonize Notch in these cells by analogy to lymphoid cells, Malinge et al[1] report that Ikaros overexpression inhibits both Notch-driven megakaryocyte specification and expansion of an AMKL cell line expressing the OTT-MAL fusion protein. When analyzing the mechanisms of this effect, however, the authors discovered that Ikaros had a broad impact on the megakaryocyte transcriptional network, including many Notch-independent effects. Together with other regulators of megakaryocytic differentiation, GATA1 expression was downregulated by Ikaros. Reciprocally, the abundance of Ikaros transcripts was reduced by GATA1 expression, while direct binding of GATA1 to the *Ikzf1* locus was identified by chromatin immunoprecipitation. These findings suggest that Ikaros expression is downregulated by the GATA2/GATA1 switch that occurs during megakaryopoiesis, perhaps to allow full expression of multiple genes repressed by Ikaros that are necessary for terminal differentiation. Interestingly, this phenomenon was specific to the megakaryocyte lineage and not observed in erythroid progenitors, in which Ikaros appeared to cooperate with GATA1 to promote erythroid differentiation. These observations suggest the existence of