these questions. Patients receiving abatacept with a C_{trough_1} < 39 µg/mL showed a rate of acute GVHD comparable to those receiving placebo and, concordantly, the immunological reconstitution followed the same slope.

Why do patients receiving abatacept for HSCT achieved higher plasma drug concentrations than patients with rheumatologic diseases? The current belief is that it is related to the expansion of the antigen-presenting cells in the latter group and their relative depletion during the early posttransplant phases in the former.[6] However, in the context of the transplant setting, the antigen-presenting cells and B-cell numbers early after abatacept administration were not correlated with PK parameters.

Abatacept also reduced steroid-refractory acute GVHD occurrence, thus limiting the use of secondary immunosuppressive agents. Detailed data on this issue are not available, and further analyses are needed to investigate the impact of abatacept on the exposure to other immunosuppressants. Likewise, why the 7/8 cohort showed higher exposure to abatacept in comparison with 8/8 group is currently not understood. However, it does explain the unexpectedly favorable outcome of the mismatched cohort in this trial.

The relationship between exposure to abatacept and acute GVHD was so cogent and persuasive that the readers cannot help but wonder whether it is already legitimate to escalate the dose in those patients with lower abatacept exposure, with the goal of providing a PK-driven approach for tailored GVHD prophylaxis. My answer is concordant with the authors' proposal, discouraging dose adjustments in the routine clinical practice but testing this hypothesis in a dedicated trial.

Finally, the article from Takahashi et al does not find any correlation between PK parameters and chronic GVHD, which was not reduced by abatacept in the ABA2 trial. This may be due to the schedule used in the transplantation setting, with intensive but brief administration course, mainly targeted to prevent acute GVHD. GVHD prevention is at the heart of a very delicate balance between graft versus leukemia and GVHD itself. Intensifying GVHD prevention can affect infections, immune reconstitution, and, more importantly, relapse. With the caution due to the limited power of the study, infections and relapse did not increase after abatacept administration, and, even more intriguingly, Takahashi et al found that they were not affected by increased exposure to abatacept. Thus, higher exposure to abatacept minimized the risk of acute GVHD without increasing the undesirable "collateral damage," such as relapse and viral infections (see figure). Using a drug at inappropriate dosage may increase the risk of missing its great potential. It has already occurred, at least in part, in the history of GVHD prevention (ie, ATLG/ATG) in which optimal dosing is still an open issue.[8,9]

An ancient aphorism from Paracelsus, a Swiss physician, alchemist and astrologer (1493-1541), says that "alchemy serves to separate the true from the false": PK analysis and therapeutic drug monitoring should be part of routine transplant care. It is time to do it.

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LYMPHOID NEOPLASIA

Comment on Gao et al, page 711

Insights into the genomics of iAMP21-ALL

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In this issue of *Blood*, Gao et al describe the genomic landscape of pediatric acute lymphoblastic leukemia with intrachromosomal amplification of chromosome 21 (iAMP21-ALL).[1]

iAMP21-ALL is a relatively recently identified ALL subtype, first reported as a distinct entity in 2003[2,3] after occasional earlier case reports. It comprises ≈2% of childhood ALL, and was initially recognized because use of fluorescence in situ hybridization (FISH) probes to identify the *ETV6*::*RUNX1* translocation detected cases with increased numbers of signals associated with the *RUNX1* probe.[4] Both the number of extra *RUNX1* signals and the amplified region of chromosome 21 observed on karyotype are variable, posing challenges for both defining the diagnostic criteria and understanding the critical molecular driver of leukemogenesis. Uncertainty about the role of iAMP21 as a driver lesion in ALL also arises in a small subset of cases in which

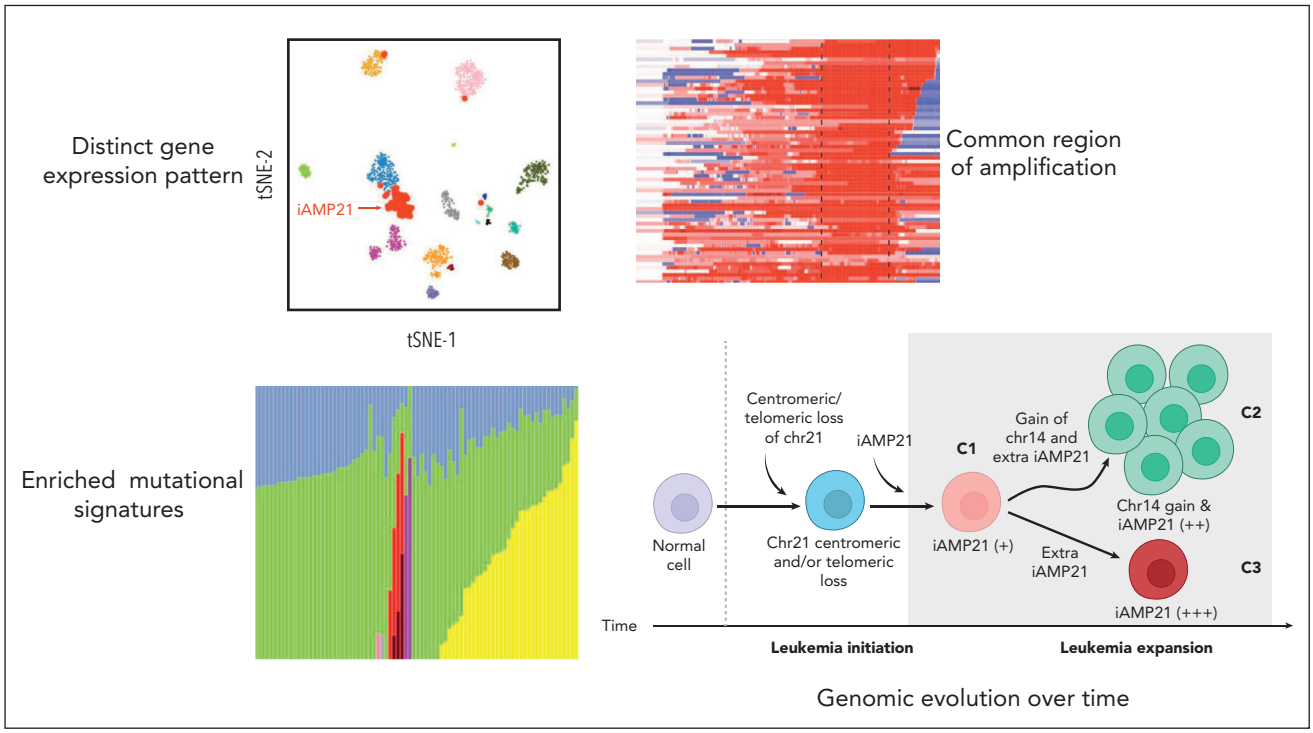
iAMP21 is identified as co-occurring with other ALL subtype-defining genetic lesions, including *ETV6*::*RUNX1*, high hyperdiploidy, *BCR*::*ABL1*, and *P2RY8*::*CRLF2*.[4-6] Clinically, iAMP21-ALL is associated with an older age and lower white blood cell count at diagnosis, and relatively unfavorable outcomes, although survival is improved when patients are treated on higher-intensity treatment regimens.[7,8] Thus, accurate identification of iAMP21-ALL cases is important because intensification of therapy partially compensates for the unfavorable prognosis associated with this subtype. In addition, an improved understanding of the key molecular drivers of iAMP21-ALL may inform development of targeted therapeutic strategies to improve outcomes.

In the present study, Gao et al assembled an impressive cohort of 124 pediatric cases from St. Jude Children's Research Hospital, the Children's Oncology Group, and UK clinical trials, for comprehensive genomic characterization by whole-genome sequencing (WGS) and/or whole-transcriptome sequencing (WTS). Although 72 of these cases were previously reported,[5] the expanded cohort reported here has enhanced power to provide a detailed characterization of the biology of iAMP21-ALL (see figure).

A key contribution of this study is to leverage the combined WGS and WTS analyses to define a common region of amplification in iAMP21-ALL, encompassing 71 genes, and to identify 43 genes in this region with differential expression compared with non–iAMP21-ALL cases. Many of these differentially expressed genes have been previously implicated in leukemia pathogenesis (eg, *CHAF1B*, *DYRK1A*, *ERG*, *HMGN1*, and *RUNX1*). More important, bioinformatic analyses did not identify any smaller subset of genes as key drivers, suggesting that coordinated deregulation of multiple genes is required for leukemogenesis in iAMP21-ALL.

In addition to defining the common region of amplification and key differentially expressed genes in iAMP21-ALL, the large size of this study permitted further characterization of the complexity of this ALL subtype. It has previously been recognized that 2 specific constitutional abnormalities of chromosome 21 (Robertsonian translocations between chromosomes 15 and 21 and ring chromosomes involving chromosome 21) are associated with an increased risk of iAMP21-ALL, likely because the abnormal chromosome 21 structure initiates formation of the iAMP21.[9] Here, Gao et al use WGS to provide detailed characterization of the pattern of chromosome 21 copy number alterations in cases with these constitutional abnormalities, demonstrating that it differs from the mechanism of successive break-fusion-break cycles that generates iAMP21 in typical cases. They go on to identify an additional "Robertsonian-like" subgroup, with a copy number profile that resembles the Robertsonian variant, but without evidence of a constitutional Robertsonian translocation. Using WTS, they demonstrate that all 3 of these variant subgroups share the iAMP21 gene expression signature, supporting the appropriateness of classifying them as iAMP21-ALL. Conversely, they demonstrate that cases with other subtype-defining alterations (*DUX4* rearrangement and *ETV6*::*RUNX1*) do not share the iAMP21 gene expression profile, and are therefore more appropriately classified as bearing iAMP21 as a secondary rather than primary alteration.

A key clinical implication of this study is that the original definition of iAMP21-ALL based solely on FISH (at least 5 *RUNX1* signals per interphase cell, with ≥3 occurring on a single abnormal chromosome 21) is insufficient. Although this definition correctly classifies most cases, it does not identify variant cases with rearrangements



Genomic characterization of iAMP21-ALL. Chr, chromosome.

of portions of chromosome 21 to other chromosomes, nor cases where *RUNX1* is not contained in the highest region of amplification. The authors advocate for use of copy number profiling by single-nucleotide polymorphism array or WGS, as either a stand-alone diagnostic test or a back-up for evaluation of complex or atypical cases. This aligns with another recent report that found that 9% of 207 iAMP21-ALL cases were missed by the FISH-based definition, but identified by chromosomal microarray testing.[6]

Gao et al also use single-cell and mutational signature analyses to provide some intriguing insights into the timing of events in the pathogenesis of iAMP21-ALL. Their data suggest that the formation of the iAMP21 chromosome is an early event that evolves progressively over time, and intriguingly, that the iAMP21-ALL subtype is enriched for a UV-mutational signature. They propose a putative model that would account for this signature, positing that UV-mediated mutagenesis may occur during peripheral circulation of pre-leukemic iAMP21-chromosome–positive cells. Further research is needed to explain the functional basis for iAMP21-ALL mutational signatures.

Overall, this study elegantly integrates WGS and WTS data to provide a detailed characterization of iAMP21-ALL, defining the key region of amplification and the set of differentially expressed genes; highlighting the importance of copy number profiling to identify variant cases; and suggesting the timing of acquisition of somatic alterations. Because 91 of 124 (73%) of the cases in this cohort were of European ancestry, additional studies of racially and ethnically diverse populations are needed to investigate possible ancestry-associated differences in the genomics and/or clinical features of iAMP21-ALL. Further studies are also needed to investigate potential targeted therapeutic strategies based on the upregulated pathways identified in this study and others.

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THROMBOSIS AND HEMOSTASIS

Comment on Han et al, page 724

Improving clots in trauma

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In this issue of *Blood*, Han et al report in vitro studies and an in vivo rat model of polytrauma that demonstrate that leukocyte inflammation contributes to trauma-induced coagulopathy by oxidation and digestion of fibrinogen and that suppression of inflammatory processes blocked the modifications of fibrinogen and trauma-induced coagulopathy.[1]

Your blood coagulates beautifully.
—Ernest Hemingway,
A Farewell to Arms

Considering that the responses to physical trauma have been fundamental to all vertebrate organisms for millions of years, it is surprising that we know so little about basic mechanisms. In fact, there is evidence that mammalian evolution included the development of a more efficient hemostatic system to deal with trauma.[2] Human survival from injury also requires an appropriate inflammatory and immune response.

This research study uses in vitro and in vivo models of polytrauma to gain insight into the effects of inflammation on clotting. Polytrauma is defined as injuries to multiple body parts and organ systems and is associated with hemorrhagic shock as well as increased mortality and morbidity. Major tissue injury and hemorrhagic shock increase inflammation and also contribute to trauma-induced coagulopathy, a major disturbance of coagulation leading to increased bleeding associated with trauma, which greatly elevates early bleeding mortality.

Trauma-induced coagulopathy is a complex process involving many pathways, but loss and consumption of fibrinogen are a cornerstone of the pathology.[3] Moreover, it has been previously demonstrated that fibrinogen is both depleted and selectively oxidized in human trauma patients with trauma-induced coagulopathy.[4] This article clearly demonstrates that modification of specific regions of fibrinogen through oxidative processes and subsequent degradation is fundamental to trauma-induced coagulopathy.