only minor trauma or even spontaneous intracranial haemorrhages. Secondly, the diagnosis of congenital FXIII deficiency must be based on accurate laboratory tests. As shown in 1960, the usual screening tests for coagulopathies such as prothrombin time, activated partial thromboplastin time, and thrombin time, do show normal values in case of FXIII deficiency. Therefore, if clinical symptoms indicate a bleeding disorder, a complete evaluation of the clotting system is required including a specific test that detects FXIII deficiency. Nowadays clot solubility tests like those used by Duckert et al are no longer recommended because of the high number of undiagnosed or late-diagnosed FXIII deficiencies attributable to this test.[5] Correct diagnosis is as important as the correct treatment because every patient left undiagnosed will suffer from severe bleeding complications and probably death. Hence, diagnosis of congenital FXIII deficiency should not be delayed in any child with an unknown bleeding tendency especially when prolonged umbilical cord bleeding is observed after birth.

Treatment of patients with congenital FXIII deficiency normally consists of prophylactic administration of plasma-derived pasteurized FXIII concentrate every 4 to 6 weeks at a dosage ranging from 10 to 35 U/kg.[6] Prophylaxis is highly efficient because of the long half-life of FXIII. When prophylactic treatment is available the prognosis is very good, although there is a lifelong risk of bleeding.

Inbal et al have now taken the treatment of such patients a big step further because plasma-derived FXIII concentrates do carry a risk for infection with blood-borne pathogens but also allergic reactions. In their multinational, open-label, single-arm, phase 3 prophylaxis trial in patients with congenital FXIII A-subunit deficiency, they investigated the efficacy and safety of a new rFXIII manufactured in *Saccharomyces cerevisiae* (baker's yeast).[1] This rFXIII A-subunit associates in plasma with the endogenous FXIII B-subunit to form a stable FXIII heterotetramer (see figure) because FXIII circulates in plasma as a tetramer consisting of 2 catalytic A-subunits and 2 carrier B-subunits (A_2B_2).[7] In a phase 1 clinical trial, rFXIII had a half-life similar to that of native FXIII and was found to show a good safety profile.[8] Inbal et al also address the issue of the development of nonneutralizing antibodies, which is a major problem in patients with hemophilia. For their study, 41 patients were enrolled at 23 centers in 11 countries for a 52-week treatment period of monthly 35 IU/kg of rFXIII intravenously.[1] Throughout the treatment period, only 5 trauma-induced bleeding episodes in 4 patients required additional treatment with FXIII-containing products. Transient, nonneutralizing, low-titer anti-rFXIII antibodies developed in only 4 patients. However, these nonneutralizing antibodies declined below detection limits in all patients despite further exposure to rFXIII.[1]

These findings have important implications for the treatment of patients with congenital FXIII deficiency by providing a novel and safe treatment with rFXIII instead of plasma-derived products. This work by Inbal and colleagues establishes a new treatment standard for FXIII deficient patients.

What should we expect next from rFXIII? A favorable cost-effectiveness profile needs to be established so that rFXIII becomes widely used. In this context it must be noted that, especially in some developing countries, congenital FXIII deficiency is expected to be much higher because of traditional consanguineous marriages. Inherited bleeding disorders are a major problem in such countries and the safety of plasma-derived factor concentrates as well as multidisciplinary comprehensive care is not always guaranteed.

In the future, the availability of rFXIII also has possible implications in patients with acquired FXIII deficiency. Congenital FXIII deficiency is associated with a complete lack of circulating plasma FXIII A-subunit antigen, but clinically significant reductions in FXIII levels have also been reported in a number of conditions, such as major surgery, liver cirrhosis, graft-versus-host disease, sepsis, and chronic inflammatory bowel disease. In these acquired FXIII deficiency states, FXIII A-subunit levels can drop into a range of 20% to 50%.[9,10] The reduction is caused by decreased synthesis or consumption. Further studies are needed to see whether FXIII substitution therapy with rFXIII will be an option in patients with acquired FXIII deficiency.

In conclusion, Inbal et al describe a safe and novel treatment of patients with congenital FXIII. This is good news for all patients with this rare bleeding disorder. However, it is also important that all patients with this hemorrhagic diathesis are correctly investigated and diagnosed because congenital FXIII deficieny is probably the most underdiagnosed rare bleeding disorder in the world.

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● ● ● CLINICAL TRIALS

Comment on Sehn et al, page 5118, and on Salles et al, page 5126

CD20 antibodies: type II to tango?

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Although the chimeric anti-CD20 monoclonal antibody (mAb) rituximab has revolutionized the treatment of B-cell non-Hodgkin lymphoma (NHL), still many patients relapse and an increasing number become refractory to rituximab-containing therapy. This has initiated intense research to develop more potent anti-CD20 antibodies.

In this issue of *Blood*, Salles et al and Sehn et al report the results of the first two phase 1 trials with the next generation anti-CD20 mAb obinutuzumab (GA101).[1,2] Both studies show that obinutuzumab is well tolerated and has promising activity in a mixed group of heavily pretreated patients with relapsed or refractory NHL.

Obinutuzumab is a glycoengineered, humanized type II anti-CD20 antibody. As opposed to type I anti-CD20 mAbs (eg, rituximab) it induces no complement-dependent cytotoxicity. However, obinutuzumab is a much stronger inducer of direct cell death and antibody-dependent cellular cytotoxicity.[3] Compared with rituximab, obinutuzumab has superior activity in whole-blood B-cell depletion assays, in preclinical human lymphoma xenograft models, and in depleting B cells in nonhuman primates.[3] Unlike type I anti-CD20 antibodies it does not induce redistribution of CD20 into detergent-resistant lipid rafts but instead leads to strong homotypic adhesion and actin-dependent lysosome-mediated cell death.[4] Although it recognizes an overlapping CD20 epitope, obinutuzumab binds to CD20 in a different orientation and at a wider elbow angle than type I anti-CD20mAb,[5] which might be responsible for the different biologic characteristics of this type II anti-CD20 mAb.

Of course the key question is whether these differences translate into superior clinical activity. In their study of 21 patients, Salles et al achieved an overall response rate (ORR) of 33%. Interestingly, responses were only obtained in the follicular lymphoma patients, resulting in an ORR of 54% (31% complete response) in this subgroup.[1] Sehn et al treated 22 patients with obinutuzumab induction, followed by 2 years of maintenance for 8 patients. At the end of induction the ORR was 23%. In both studies response duration varied from 3 to 21 months.[2] These certainly are remarkable results in patients with a median of 4^2 to 5^1 prior treatments, including autologous stem cell transplantation. However, a major limitation of both studies is the small number of patients as well as the heterogeneity of the lymphoma subtypes included. This precludes robust conclusions about, for example, the correlation between response and tumor load or response and Fc-γ receptor polymorphism.[1] The same is true for the important question of whether obinutuzumab has activity in rituximab-refractory patients. This was found to be the case in 2 of 9^1 and 2 of 13^2 patients, respectively. A preliminary analysis of a randomized phase 2 study comparing 2 different doses of obinutuzumab in relapsed FL showed a 50% response rate in rituximab-refractory patients treated with the higher dose. However, again the numbers were very low (5 of 10 patients).[6] Although the range of obinutuzumab doses used in the Salles and Sehn studies is comparable, completely different induction schedules have been used, in line with the unfortunate tradition in the field of mAb treatment of lymphoma. Salles et al administered a total of 9 doses, starting with weekly infusions on days 1 and 8 and then once every 3 weeks, whereas Sehn et al gave 4 doses once a week. In both studies responses occurred in all dose groups, without clear evidence of a dose-response relationship. Sehn et al found consistently higher serum concentrations of obinutuzumab in responders, reactivating the chicken-or-the-egg discussion: is the response better because of higher serum levels or are these levels a reflection of a lower initial tumor load in the responders? The small patient cohort again does not allow solid answers to this question.

In both studies the safety profile was found to be comparable with what we know from treatment with rituximab. Most adverse events were infusion related, in general mild and predominantly restricted to the first infusion. Apparently the lack of complement activation by obinutuzumab does not prevent the occurrence of infusion-related reactions. Again, the small number of patients did not allow correlating measured cytokine levels and infusion-related reactions.[1]

Thus, although the data on obinutuzumab reported in this issue of *Blood* are novel and promising, they really can be regarded only as a starting point. Crucial for assessment of its future position is the direct head-to-head comparison with rituximab as well as analysis of the efficacy of obinutuzumab in rituximab-refractory patients. In the latter group the results with ofatumumab, a type I anti-CD20 antibody recognizing a different epitope and resulting in increased complement dependent cytotoxicity, have been rather disappointing.[7] Unfortunately, the molecular basis for rituximab-refractoriness is largely unknown, prohibiting rational approaches to reverse it. Several head-to-head comparisons are ongoing. The preliminary results of the GAUSS study, a randomized phase 2 study comparing obinutuzumab with rituximab in patients with relapsed indolent B-cell lymphoma, were recently presented.[8] Obinutuzumab resulted in higher overall response rates as assessed by both investigators (44% vs 38%; NS) and a blinded independent review facility (43% vs 28%; $P < .01$). Thus far there was no difference in progression-free survival. In this study induction treatment consisted of 4 weekly infusions of either obinutuzumab (1000 mg flat dose) or rituximab (375 mg/m^2). In patients without evidence of progression this was followed by maintenance treatment with obinutuzumab (1000 mg) or rituximab (375 mg/m^2), every 2 months for up to 2 years. Because in general 375 mg/m^2 will be roughly 25% less than 1000 mg, it is debatable whether this is the most appropriate way to compare these 2 mAbs. Two ongoing large, international, randomized phase 3 studies compare the combination of either obinutuzumab or rituximab with chemotherapy in previously untreated diffuse large B-cell lymphoma and treatment-naive indolent lymphoma patients, respectively. The German CLL Study Group runs a randomized phase 3 study in previously untreated CLL patients unfit for fludarabine-containing regimens, comparing chlorambucil, chlorambucil plus rituximab, and chlorambucil plus obinutuzumab. In the ongoing Gadolin study a total of 360 rituximab-refractory follicular lymphoma patients are randomized between bendamustine and bendamustine plus obinutuzumab followed by maintenance. The results of these pivotal trials are eagerly awaited.

In conclusion, it will take some years before we know whether obinutuzumab is indeed a useful extension of our therapeutic arsenal, that is, whether it improves the clinical outcome in patients with CD20-positive B-cell malignancies.

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Comment on Beekman et al, page 5071

From famine to feast: sending out the clones

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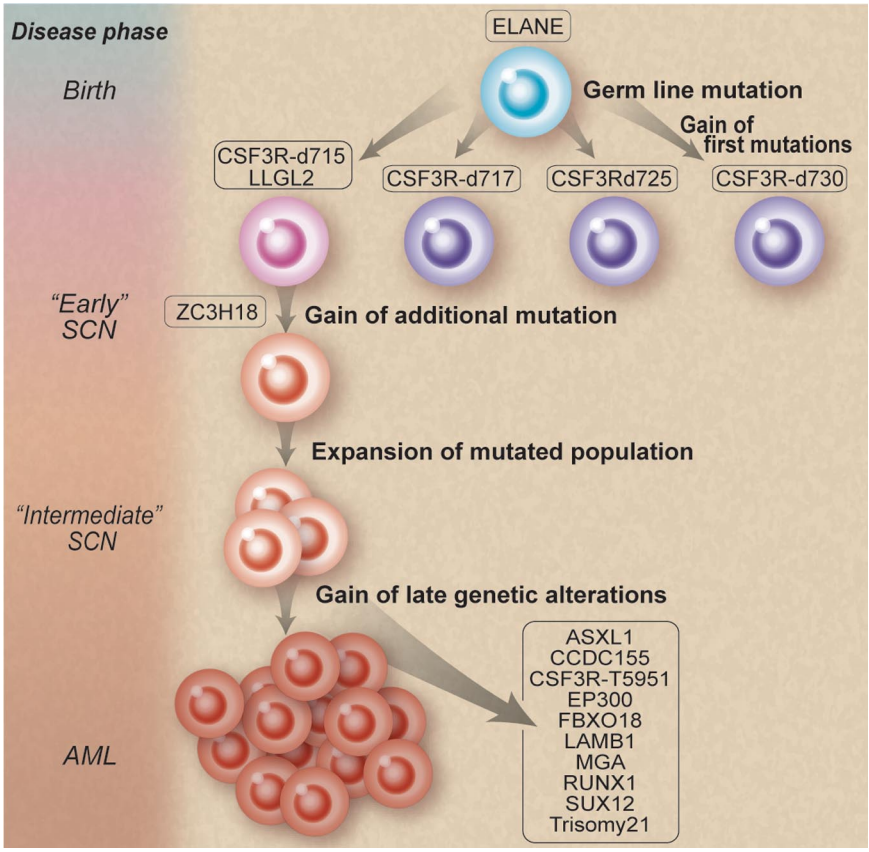
In this issue of *Blood*, Beekman et al provide compelling evidence for the multistep evolution of acute myeloid leukemia (AML) from severe congenital neutropenia (SCN) over a 17-year period. Moreover, they found that 5 different gain-of-function mutations in the granulocyte colony-stimulating factor receptor (GCSFR) arose during this transformation, suggesting that 2 mutations behaved as drivers for clonal outgrowth, while 3 others did not.[1]



Natural selection of clones. Mutations involving granulocyte colony-stimulating factor receptor (GCSFR) emerge during the course of severe congenital neutropenia (SCN) treated by recombinant human G-CSF. However, some mutations thrive and persist, while others die off. Professional illustration by Debra T. Dartez adapted from Figure 4 in Beekman et al, which begins on page 5071.

Sometimes referred to as Kostmann syndrome (after the Swedish pediatrician who described a family of individuals with neutropenia in 1956),[2] SCN is one of the rare inherited bone marrow failure syndromes. Typically presenting within the first few months of life, babies present with a life-threatening infection of the skin or gastrointestinal or respiratory organs. Classically, there is profound neutropenia ($< 200/\mu L$) in the periphery and, in the bone marrow, there is a developmental arrest at the promyelocyte stage. Despite broad spectrum antibiotics, many died from infection. Treatment with recombinant human granulocyte colony stimulating factor (filgrastim) in the early 1990s changed the natural history of the disease, enabling children to live into adulthood. Shortly after the widespread adoption of filgrastim as treatment of choice, Dong and colleagues described somatic nonsense mutations in the GCSFR among patients who developed myelodysplastic syndromes (MDS) or frank AML.[3] French and National Institutes of Health–based epidemiologic studies reported that an accumulated risk of developing MDS/AML was as high as 40%.[4,5] Like the other inherited bone marrow failure syndromes (such as Fanconi anemia, dyskeratosis congenita, and Shwachman-Diamond syndrome), SCN constitutes a cancer predisposition syndrome.

How does one go from famine (SCN) to feast (AML)? Working backward, Beekman et al isolated 12 somatic nonsynonymous mutations in the leukemic blasts of a patient. They then retrieved cryopreserved bone marrow specimens from 9 and 15 years before the development of AML. They found that 3 of these mutations, GCSFRd715, LLGL2, and ZC3H18, were present in low frequencies and coexisted in the same myeloid progenitors. They further demonstrated an increase in these clones over time from 15 to 9 years before the onset of AML, thereby supporting the notion of a selective outgrowth of clones harboring these 3 mutations. They also identified small amounts of other GCSFR mutations at 15 and 9 years before leukemia. Most fascinating of all is that only the GCSFRd715 persisted clonally. This demonstration provides supportive evidence for competitive outgrowth of the other mutants and that, true to evolution, some survive and others die off (see figure). Furthermore, they discovered a second "hit" to the GCSFRd715 involving a