[Lumiliximab With Fludarabine, Cyclophosphamide, and Rituximab Versus FCR Alone in Subjects With Relapsed Chronic Lymphocytic Leukemia] study).[8] If the results of this pivotal trial confirm the findings of Byrd et al,[7] it will have an important influence on the treatment strategy of patients with CLL.

In recent years, 4 drug combination therapies with FCR and alemtuzumab or FCR and mitoxantrone in refractory/relapsed CLL patients were also investigated[9,10] (see figure). However, the results were less impressive than those reported in the present study of Byrd et al[7] CFAR (FCR + alemtuzumab) immunochemotherapy was evaluated in heavily pretreated patients with up to 14 previous therapies.[9] Of the 74 patients, 18 (24%) achieved CR, 2 nodular partial response, and 28 partial response. The OR was 65%. In another randomized phase 2 study, Hillmen et al[10] compared an FCM (fludarabine + cyclophosphamide + mitoxantrone) regimen with FCM plus rituximab in previously treated CLL. In this study, a 4-drug regimen induced a higher CR and CR(i) rate (43%) than FCM alone (13%) (see figure). However, the study design did not allow for a statistical comparison of the 2 combinations.

In conclusion, despite the significant progress made in recent years, available therapies for refractory/relapsed CLL are only partially effective, and there is an obvious need to develop better strategies and new, more specific and active drugs.

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Comment on Lewandowski et al, page 443

Every cloud has a silver lining

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In this issue of *Blood*, Lewandowski and colleagues show that elevated extracellular ROS in the bone marrow are not just one of the signs of the damage induced by high doses of irradiation, but actually lead to up-regulation of VCAM-1 on endothelial cells, therefore mediating transplanted HSC homing and initial proliferation.[1]

Reactive oxygen species (ROS) are used to our advantage by immune cells, which release them in bursts as a lethal weapon against a number of pathogens, but otherwise they tend to play the role of the bad guys in most stem cell studies. Side products of each cell's respiratory chain, they inevitably accumulate as a result of active metabolism. As they generate several types of damage at the protein, lipid, and DNA levels, ROS take much of the blame for cell and tissue aging. The detrimental effect of intracellular ROS for hematopoietic stem cell function is widely accepted, and it has been shown that antioxidant agents prolong hematopoietic stem cell (HSC) life.[2] Although it is still debatable whether HSCs reside specifically in hypoxic niches in the bone marrow, it has been proposed that one of the advantages of a hypoxic niche would be to ensure that low ROS levels are maintained within the stem cell pool.[3] While studying HSC homing patterns in lethally irradiated recipient mice, Lewandowski et al unveil a positive role for extracellular ROS during the early stages of bone marrow transplantation. They use a newly developed fiber optic–based probe to detect HSCs along the femur bone marrow of live mice from hours to days after transplantation, and analyze their image sequences to obtain a comprehensive picture of femur bone marrow repopulation kinetics, so far studied only using tissue sections[4,5] or through localized bone thinning.[6] Live imaging of the whole bone cavity, similar to our previous analysis of bone marrow calvarium,[7] leads to efficient analysis of the behavior of small numbers of purified HSCs. In the femur, HSCs seed and engraft preferentially in the epiphysis and, in particular, the femoral head, rich in trabecular marrow and vasculature. It would be interesting to know whether the vessels in this area are differentially affected by or react differently to irradiation compared with the ones in the bone shaft.

Lewandowski et al detect high levels of ROS in the irradiated bone marrow cavities and, surprisingly, impaired marrow seeding and HSC proliferation when ROS levels are diminished by antioxidant treatment. So how can ROS have a positive effect? The answer is in the vascular up-regulation of VCAM-1, an adhesion molecule already known to be involved not only in leukocyte trafficking to sites of infection but also in HSC homing and function.[8] Antioxidant treatment reduces VCAM-1 expression and consequentially engraftment efficiency.

The presented data suggest that ROS, via modulation of VCAM-1 expression, contribute to the HSC sensing tissue damage and promptly starting the rescue process. Interestingly, the long-term outcome of HSC transplantation (ie, bone marrow complete engraftment and peripheral blood reconstitution) is the same whether the recipient mice are treated with antioxidants or not. The authors observe no difference in the outcome of secondary transplantations; however, previous work detected improved function of antioxidant-treated cells by the third serial transplantation. Bone marrow reconstitution after irradiation is highly demanding on HSCs, to the point that serially transplanted HSCs can be used to model stem cell aging. It is possible that ROS-mediated VCAM-1 induction evolved as a defense mechanism to ensure efficient response of all residual (or transplanted) HSCs, even though it triggers dangerously high levels of HSC metabolism. The consequential intracellular ROS accumulation would be but a little scar when the life of the irradiated subject is at stake.

Antioxidant treatment is widely regarded as a means to slow down HSC aging; however, we now know that it could hamper the initial steps of HSC engraftment after transplantation. The question now is whether it will be possible to identify a therapeutic window for antioxidant treatment in conjunction with bone marrow transplantation, allowing for HSC engraftment and rescue of the patient, but only minimally damaging the HSCs as a consequence of active cycling.

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Comment on Chen et al, page 706

Oxidative stress on VWF proteolysis

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In this issue of *Blood*, Chen and colleagues demonstrate that the rate of cleaving VWF by ADAMTS13 is significantly slowed when the residue Met^{1606} in the VWF A2 domain is oxidized.[1] The finding adds a new dimension to the complexity of regulating VWF cleavage and links the rate of VWF proteolysis to the state of oxidative stress.

Von Willebrand factor (VWF) multimers secreted from Weibel-Palade bodies of endothelial cells are enriched in ultra-large forms that are rapidly cleaved by the zinc metalloprotease ADAMTS13. Enzyme deficiency is associated with the development of thrombotic thrombocytopenic purpura as well as with conditions associated with systemic inflammation. There have been extensive studies on how VWF proteolysis is initiated and regulated, mostly focusing on identifying the interface between the substrate and metalloprotease, and specific mutations that alter the rate of cleavage. The current study has delineated a new regulatory mechanism. The study found that Met^{1606}, within the peptide bond (Tyr^{1605}-Met^{1606}) targeted by ADAMTS13, is oxidized to the sulfoxide by hypochlorous acid (HOCl) in vitro. The oxidative modification significantly slows the rate of cleaving an isolate A2 domain and purified VWF multimers by the metalloprotease. The study is significant because it provides the first experimental evidence that VWF proteolysis could be regulated by oxidative stress, which results from accumulation of reactive oxygen species including HOCl.[2] The study is also provocative because it raises several interesting and important questions. First, is Met^{1606} the only amino acid in VWF multimers that is subjected to oxidative modification? The answer is likely to be no, even though the current study is focused on the HOCl modification of a specific methionine residue. VWF contains a high percentage of cysteine residues (8.3%), some of which are in thiol forms that could be sensitive to regulations by redox in general and HOCl in particular. Consistent with the notion, an early report has shown that thiol(s) in the VWF A3 domain is targeted by a reductase activity associated with thrombospondin-1.[3] The question remains as to whether different reactive oxygen species differentially modify specific amino acids within a VWF multimer, leading to different functional outcomes. Second, does oxidation also affect VWF adhesion activity? The answer is likely to be yes. There is no direct evidence as to whether VWF with oxidized Met^{1606} is more or less adhesive, but converting thiols in VWF multimers to disulfide bonds is associated with enhanced VWF binding to platelets.[4] Third, is VWF oxidation by reactive oxygen species permanent or transient? A disulfide bond is traditionally considered to be a permanent basic posttranslational modification critical for maintaining the tertiary structure of a given protein. However, increasing evidence also suggests that the oxidative modification occurs extracellularly to nonstructural thiols in response to changes in a redox environment.[5] The blood redox system is composed of proteins and small-molecule thiols, and its balance can be changed in a variety of (patho)physiologic conditions. One well-known example is that the plasma redox potential is approximately 13 to 1 as measured by the ratio of reduced to oxidized glutathione.[6] However, this reducing state can be made transiently oxidizing in conditions such as oxidative stress. The question is whether the oxidative modification to VWF is reversible when the environment returns to a reducing state. Demonstrating such reversibility will answer a fundamental question as to