● ● ● **CLINICAL TRIALS**

Comment on Sutton et al, page 6109

Autografting CLL: the game is over!

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In this issue of *Blood*, Sutton et al report the results of a randomized clinical trial exploring the role of autologous stem cell transplantation (ASCT) in patients with chronic lymphocytic leukemia (CLL), showing that transplantation may increase the response rate and prolong the time to progression but that this does not result in a longer survival in comparison with chemotherapy treatment.[1]

FCR vs. ASCT for Chronic Lymphocytic Leukemia

FCR					
Study	N. patients	Age, y	CR (%)	EFS (%)	OS (%)
Tam (8)	300	57 (17-86)	72	51 at 6 y.	77 at 6 y.
Hallek (9)	408	61 (30-80)	44	PFS: 65 at 3 y. Median: 51.8 m.	87 at 3 y.
ASCT					
Michallet (3)	112	54 (31-65)	59*	42 at 5 y.	86 at 5 y.
Sutton (1)	52 in CR* 46 non CR *	56 (31-66)	44.6*	CR* pts: 79.8 at 3 y. Non CR* pts. 48.9 at 3 y.	96 at 3 y. 82 at 3 y.

(*) CR (complete response) status at randomization

FCR versus ASCT for CML.

Chronic lymphocytic leukemia is a frequent form of leukemia with heterogeneous biology and clinical course. Despite considerable progress in its treatment, CLL remains incurable. Because of this, and in common with many other hematologic malignancies with no effective therapy, ASCT has been used in an attempt to improve patients outcome. In the early 1990s some studies reported encouraging results and raised hope that this procedure could be useful in CLL.[2] This promoted additional phase 2 studies and retrospective analyses trying to shed light on the usefulness of ASCT in CLL. In addition, many patients with CLL were offered ASCT outside clinical studies in the belief that the procedure could be useful. Initial enthusiasm, however, was soon tempered because the observed results demonstrated no plateau in either event-free or overall survival.

Nevertheless, the acid test for any treatment procedure is randomized clinical trials, which have been long awaited for ASCT in CLL. In addition to the paper by Sutton and colleagues in this edition, *Blood* has recently published another similar trial.[3] Thus, results of ASCT in CLL from 2 randomized trials are finally available, and the authors of these studies have to be commended for having undertaken a necessary but difficult task.

The main conclusions that can be drawn from these studies confirm current notions about ASCT in CLL: (1) Whereas the event-free and progression-free survival are longer in patients who receive a transplant, unfortunately, this does not translate into improved overall survival. (2) The negative impact of biomarkers that confer resistance to conventional therapy (eg, *TP53* aberrations) are not overcome by autografts, which is not surprising if one considers that ASCT is simply chemotherapy by another name. This is in contrast with allogeneic stem transplantation, which actually overcomes poor prognostic markers.[4] (3) Patients who gain the highest benefit from autologous transplantation (ie, young subjects with low tumor mass, responding very well to therapy and no unfavorable prognostic factors) are also those most likely to respond to conventional therapy and to have prolonged control of their disease and long survival. And (4) ASCT is not effective as salvage therapy. In fact, allogeneic stem transplantation should be considered as a treatment possibility in any patient failing chemoimmunotherapy.[5-7]

Finally, the upfront therapy in these studies, although reasonable when planned, is suboptimal by current standards. In the past few years, studies led by Keating and coworkers at the M. D. Anderson Cancer Center have placed chemoimmunotherapy, namely the combination of FCR (ie, fludarabine, cyclophosphamide, rituximab) at center stage for CLL therapy.[8] Importantly, the superiority of FCR over FC as initial therapy in CLL was confirmed in a randomized trial conducted by the German CLL Study Group.[9] As a result, FCR is the new benchmark for any study trying to demonstrate an improvement in CLL therapy.

The question that has to be asked today is whether the results obtained by Sutton, Michallet, and their respective colleagues would have been different if FCR had been used.[1,3] In phase 2 studies from the Dana-Farber Cancer Institute, patients who received

a transplant after FCR in the final phase of the study had inferior outcome to those patients who were selected on the basis of their response to less aggressive chemotherapy.[10] Moreover, further ASCT randomized trials in CLL are difficult to envision in an era in which, in contrast with 15 years ago, effective therapies for this form of leukemia exist, with newer and hopefully yet more effective therapies being investigated or in the horizon.

Whereas great caution must be taken comparing the outcome of separate studies, it would appear that the outcomes in terms of event-free-survival and overall survival are similar for patients treated with FCR and those receiving ASCT (see table).

It seems, therefore, that the game for ASCT in CLL is indeed over. It remains to be seen whether other forms of cellular therapy (eg, manipulated autologous T cells) can eventually be effective or offer some additional, positive effect to current or forthcoming chemoimmunotherapy regimens.[11]

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● ● ● **THROMBOSIS & HEMOSTASIS**

A knockout for knockin

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Have you ever wondered what is next for genetically modified mouse research after knockout, floxed-out, and *Cre*-nased mice? If the study by Flick and colleagues in this issue of *Blood* is representative of what is yet to come, then we are in for a thrill.[1]

By introducing an engineered anticoagulant-selective prothrombin mutant within the endogenous prothrombin gene (F2), Flick and colleagues generated a multifaceted phenotype that opens doors for new dimensions in genetically modified mouse research. Endogenous expression of engineered WE-thrombin (W215A/E217A), which conveys only the anticoagulant activity profile of thrombin but not its procoagulant activity profile, permits investigators to "peel the layers of the onion" of thrombin's multi-substrate specificity while learning important lessons about thrombin's diverse activities in normal physiology and pathophysiology. This innovative approach presents numerous unique opportunities, but also identifies new challenges and highlights the need to refurbish our current view on structure-function of coagulation proteases.

Thrombin is the archetype multifunctional coagulation protease. Its eminent role in hemostasis, thrombosis, vascular biology, inflammation, angiogenesis, tumor biology, etc, undoubtedly relates to its multiple substrate specificity involving more than 20 different physiologic substrates. However, thrombin does not just proteolyse whatever it encounters; instead, it relies on a series of intricate mechanisms to regulate its substrate specificity depending on location and (micro)environmental factors. Learning when, how, and why thrombin employs these mechanisms in complex biologic systems, such as in vivo, provides a wealth of biologic insights that ultimately will facilitate discoveries of novel therapeutic approaches.

However, this is not just about thrombin. This is also about other coagulation proteases with well-documented additional activities beside their function in coagulation, such as activities on vascular cells.[2,3] This is about a growing awareness that coagulation proteases are not just part of a linear network of coagulation reactions but are in fact networking hubs collecting, integrating, and executing signals from multiple sources and through multiple activities. This is about refurbishing our views on coagulation proteases according to this network model, about novel conceptual advances that reinforce our views with in vivo proof of principal studies, and about better insights into the complex biology of protease specificity that may one day lead to the identification of new, improved, or safer therapeutic entities.

To understand how the WE mutations (W215A/E217A) affect thrombin's activities, it is important to realize which features comprise the basis of thrombin's substrate specificity.[4,5] These include: (1) active site interactions; (2) exosite interactions; (3) the use of cofactors, such as thrombomodulin, which shields thrombin's procoagulant exosite I and mediates a substrate specificity switch; and (4) the phenomenon of thrombin's Na^+-induced "allostery," characterized by a Na^+-containing "procoagulant conformation" and an "anticoagulant conformation" devoid of Na^+. The WE mutations located near the entrance to the active site cleft result in a collapse of the 215-217 polypeptide strand thereby blocking access of substrates to the active site.[4] The 215-217 blockade of the active site is stabilized in human WE-thrombin by adoption of the Na^+-devoid "anticoagulant conformation," causing an additional 20-fold drop in activity. Lack of 215-217 blockade stabilization in mouse WE-thrombin is the basis for the ~ 14-fold difference in relative anticoagulant potency between human and