inhibitor of microtubule polymerization used in autoinflammatory diseases, inhibited release of α-defensin-1 as well as reduced thrombus formation. This suggests that inhibiting neutrophil activation and presumably α-defensin-1 release may represent an effective antithrombotic strategy to prevent immunothrombosis without compromising hemostasis. Traditional anticoagulants or newer direct oral anticoagulants might not suffice in the absence of adjuvant therapy. Use of the activated FXII inhibitor corn trypsin inhibitor was likewise antithrombotic in this model, consistent with a role for contact activation in thrombosis; yet this result does not explicitly identify the relative contribution of neutrophil α-defensin-1 release in contact activation-mediated thrombosis. It remains to be clarified to what extent α-defensin-1 plays a role in other neutrophil processes and the relative amount of α-defensin-1 released by neutrophils during thrombus formation in humans.

The large, dense fibrin clots generated in part by α-defensin-1 raise basic questions whether this process is integral to (patho) physiologic host defense mechanisms. As fibrin can act as a protective film to prevent microbes from entering and proliferating in a clot,[10] α-defensin-1 enhancement of the dense fibrin network suggests that α-defensin-1 may act as an antimicrobial agent through direct and indirect physiological mechanisms. Alternatively, microbes like *Staphylococcus aureus* secrete coagulases, encapsulating the bacteria in fibrin to evade the immune response. Could further generation of fibrin by α-defensin-1 pathologically contribute to immune evasion or alternatively incite disseminated intravascular coagulopathy, a thrombohemorrhagic state seen in overt sepsis? The case may be that although too much is pathologic, a little is better than none at all (ie, the French red wine paradox).

In conclusion, Abu-Fanne et al present a novel mechanistic finding that contact activation stimulates neutrophil release of α-defensin-1, which exerts direct effects on fibrin polymerization and thrombus formation (see figure). It is important to note that although present in the paneth cells of the small intestine in mice, α-defensins are absent from mouse neutrophils. In contrast, α-defensins constitute 50% of the total content of azurophil granules in human neutrophils and are even still expressed in neutrophils of patients with immune-impaired diseases, including serine protease-deficient neutrophils in Papillon-Lefévre syndrome patients. Although in mice the α-defensin-1 pathway may be dispensable for the physiological function of neutrophils, it may be required for physiology in humans as an extension of innate immunity. Thus, future studies will need to mechanistically resolve whether the direct inhibition of α-defensin-1 before or after its release might be therapeutic. This exciting study provides rationale to target and inhibit contact activation, limiting immunothrombosis without compromising hemostasis and potentially preserving other immune host defense mechanisms.

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CLINICAL TRIALS AND OBSERVATIONS

Comment on Langerak et al, page 494

Highs and lows of minimal residual disease in CLL

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In this issue of *Blood*, Langerak et al report on the prognostic value of minimal residual disease (MRD) status in elderly patients with comorbid chronic lymphocytic leukemia (CLL) who were treated on the phase 3 CLL11 trial with chlorambucil plus rituximab (R-Chl) or chorambucil plus obinutuzumab (G-Chl).[1]

The authors found that high levels of MRD were independently associated with shorter progression-free survival (PFS) and overall survival (OS) and reported that the G-Chl regimen was superior to R-Chl in terms of inducing MRD-negative remissions.[2] On the basis of these findings and the growing pool of data about posttherapy MRD status in CLL patients, the authors suggest that MRD has the potential to become a surrogate marker for survival in clinical trials.

MRD in CLL is analyzed by quantifying the residual cancer cells after therapy is completed. The lowest level of MRD, MRD-negativity or MRD-undetectable (MRD-U), has been defined as the detection of <1 CLL cell per 10 000 leukocytes.[3] Some groups, such as Langerak et al, further

stratify patients into MRD-intermediate (MRD-I; 1-99 CLL cells per 10 000 leukocytes) and MRD-high or MRD-positive (MRD-P; ≥100 CLL cells per 10 000 leukocytes) groups.[1,4] Langerak et al found that more patients achieved MRD-U status (35.8%) when they were treated with G-Chl than when they were treated with R-Chl (3.3%).[1] In multivariable analyses, MRD-P status was a strong independent predictor of shorter PFS and OS.[1] The median PFS for MRD-U, MRD-I, and MRD-P groups was 56.4, 23.9, and 13.9 months, respectively. The median OS for MRD-U and MRD-I groups was not reached after 65.6 months, whereas the median OS for the MRD-P group was 60.0 months.[1]

In the CLL community, the clinical significance and importance of MRD status has experienced several highs and lows over the last several years as the options in the therapeutic landscape for CLL patients have evolved from standard chemoimmunotherapy regimens to more targeted agents. Initially, excitement about MRD status was high after publication of the significance of MRD status in young fit CLL patients treated on the CLL8 study with fludarabine and cyclosphosphamide with or without rituximab (FCR or FC).[4] Of evaluable patients, MRD-U status was achieved in 35% of the patients who received FC and 63% of the patients who received FCR.[4] Böttcher et al found that low MRD levels were significantly associated with prolonged PFS and OS.[4] The median PFS for MRD-U, MRD-I, and MRD-P groups was 68.7, 40.5, and 15.4 months, respectively. The median OS for the MRD-U group was not reached after 52.4 months, whereas the median OS for the MRD-I and MRD-P groups was 63.8 and 25.3 months, respectively.[4]

After the initial determination of the high importance of MRD, ibrutinib, an oral kinase inhibitor targeting Bruton tyrosine kinase, was introduced. Ibrutinib offered significant clinical benefit and prolongation of survival despite its ability to induce MRD-U status in <10% of patients.[5,6] This led to a low level of enthusiasm for and shift away from the importance of attaining MRD-U status.

Then, venetoclax, an oral inhibitor of BCL-2, was introduced. In a phase 2 study of high-risk CLL patients with del(17p) karyotype, 30% of patients were able to achieve MRD-U status after treatment with single-agent venetoclax.[7] When venetoclax was given in combination with rituximab as a part of the phase 3 MURANO study, an even higher number of relapsed and refractory CLL patients (62%) were able to achieve MRD-U status.[8] Again, MRD status was also independently associated with PFS, but the follow-up time was too limited to determine association with OS. Thus, enthusiasm for achievement of MRD-U status for patients with CLL returned to a high point.

Although there is general agreement that attainment of MRD-U status is a desirable outcome for patients with CLL depending on therapeutic option, the ideal future utility of MRD status has yet to be determined. However, there are several potential relevant uses for MRD status. The first is as a prognostic marker. As described, MRD is already used for this purpose in CLL clinical trials, but is not yet recommended in routine clinical practice.[3]

The second use is as a biomarker for direction of response-adapted therapy. Many novel trials use MRD status to direct subsequent therapy. For example, if MRD-U status is reached after a set number of FCR cycles or over time with ibrutinib, can therapy be stopped without clinical detriment? Could this limit treatment-related adverse events and financial toxicities? Alternatively, should detection of the re-emergence of disease after attaining MRD-U status be a cause to initiate therapy even in the absence of clinical symptoms?

The third use is as a replacement for clinical or radiologic response assessments. Interestingly, in recent clinical trials, some patients have not achieved complete remission by clinical or radiologic criteria but have achieved MRD-U status. Which is a more accurate assessment? Could the use of MRD status instead of radiologic assessments limit patient exposure to potentially toxic radiation?

And finally, MRD could be used as a surrogate end point for survival in clinical trials. Because CLL patients are living longer, trials to determine a difference in OS must be performed over many years. Ideally, investigators would like to have a shorter end point to demonstrate survival to limit the length and cost of clinical trials. Using MRD status as a surrogate end point has already been approved in Europe as a result of the European Research Initiative on CLL.[9] One of the first major studies to use MRD as a surrogate end point for CLL patients is the ongoing CLL13 study in which treatment-naive CLL patients are randomly assigned to FCR or bendamustine plus rituximab vs venetoclax plus rituximab vs obinutuzumab plus venetoclax vs ibrutinib plus venetoclax plus obinutuzumab (NCT02950051). MRD status at 15 months is the primary end point for the study. Using MRD status as a surrogate end point in CLL is not yet recommended in the United States, so this method needs further validation.

The article by Langerak et al has demonstrated that even in elderly and comorbid patients with CLL, MRD-U can be achieved and it correlates with improved survival. They demonstrate that obinutuzumab induces more MRD-U than rituximab. Therefore, combination therapies with the goal of attaining MRD-U have selected obinutuzumab as the preferred anti-CD20 monoclonal antibody. The CLL11 study on which these data are based served to recommend G-Chl as a standard comparator in trials for treatment-naïve elderly or comorbid CLL patients. The ongoing CLL14 study compares G-Chl with obinutuzumab plus venetoclax in this population, and preliminary reports suggest prolonged PFS for patients treated with the latter (NCT02242942). Because MRD status currently has high importance in the CLL community, publication of the final results and MRD data from the CLL14 study is highly anticipated. More data from prospective clinical trials similar to that reported by Langerak et al are needed to determine the final role for MRD in CLL.

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