For many years the gold standard for patients with MM not eligible for ASCT has been the combination of melphalan and prednisone (MP) or dexamethasone-based regimens. The overall response rate was < 50% with a CR rate of < 5%, a median duration of response of 1.5 years, and a median overall survival (OS) of ~ 3 years. Interestingly, for this population of patients, new combination regimens incorporating novel drugs such as MP-thalidomide (MPT), MP-bortezomib (MPV), MP-lenalidomide (MPR), or lenalidomide plus dexamethasone have resulted in an unprecedented CR rate of up to 15%, 30%, 24%, and 24%, respectively.[7] However, the impact of these CRs on event-free survival (EFS) and OS in the nontransplantation setting has not yet established.

In this issue of *Blood*, Gay et al report on the impact of response to therapy on progression-free survival (PFS) and OS in 1175 newly diagnosed patients with MM, not eligible for ASCT and enrolled in 3 multicenter trials, treated with either MP alone (332), MPT (332), MPV (235), or MPV followed by VT maintenance (254).[1] Concerning response, CR was achieved in 17%, VGPR in 19%, and PR in 35%. According to the treatment group, CR was attained in 49%, 31%, 15%, and 5% of patients treated with MPV-VT, MPV, MPT, and MP, respectively. After a median follow-up of 29 months, PFS and OS were significantly longer in patients who achieved CR versus those who attained VGPR or PR. Of interest, the PFS and OS were virtually identical in patients who achieved VGPR and PR. Finally, the achievement of CR was an independent predictor of longer PFS and OS irrespective of age, International Staging System stage, and treatment arm.

There is no doubt that, in the transplantation setting, the achievement of IFE-negative CR is a crucial step forward for long-lasting response and survival in MM.[8] Gay et al clearly demonstrate that the achievement of IFE-negative CR in elderly patients treated with MP plus novel antimyeloma agents has also a significant impact on PFS and OS.[1] Interestingly enough, in a recent transplantation series, the achievement of VGPR did not result in a better outcome than the achievement of PR.[9] It has been shown that approximately one-third of CRs achieved after ASCT in younger myeloma patients last for > 10 years, representing the so-called "cure fraction" or "operational cure."[8] Although the achievement of a PFS of 67% at 3 years in elderly patients with MM in the study of Gay et al is encouraging,[1] it must be considered that the follow-up is still too short with few patients at risk beyond 4 years from initiation of therapy, to know whether or not operational cures can be expected with primary therapy incorporating novel agents in elderly patients. Furthermore, with the availability of novel technologies, the achievement of IFE-negative CR should no longer be the ultimate goal in the treatment of MM. In this regard, the impact of sCR should be investigated. It has been recently reported that the achievement of CR with primary therapy including novel agents results in the emergence of oligoclonal bands in up to 60% of the patients.[10] Whether this phenomenon is because of a higher tumor reduction or a more robust immune reconstitution as well as its potential prognostic influence are unknown. Finally, sequential MRD measurements with MFC or molecular studies could be helpful in determining from what level of MRD further treatment is or not needed. Ideally, the treatment approach in elderly patients with MM should include a triple-agent induction regimen such as MPT or MPV followed by maintenance incorporating novel agents along with sequential MRD studies to establish for how long treatment is still of benefit.

Conflict-of-interest disclosure: J.B. has received honoraria for consultation, educational lectures, and research grants from Celgene and Janssen-Cilag. C.F.d.L has received honoraria for educational lectures from Janssen-Cilag. ■

REFERENCES

1. Gay F, Larocca A, Wijermans P, et al. Complete response correlates with long-term progression-free and overall survivals in elderly myeloma treated with novel agents: analysis of 1175 patients. *Blood*. 2011;117(11):3025-3031.

2. Bladé J, Samson D, Reece D, et al. Criteria for evaluating disease response and progression in patients with multiple myeloma treated by high-dose therapy and haemopoietic stem cell transplantation. Myeloma Subcommittee of the EBMT. European Group for Blood and Marrow Transplant. *Br J Haematol*. 1998;102(5):1115-1123.

3. Durie BG, Harousseau JL, Miguel JS, et al. International uniform response criteria for multiple myeloma. *Leukemia*. 2006;20(9):1467-1473.

4. Paiva B, Vidriales MB, Cerveró J, et al. Multiparameter flow cytometric remission is the most relevant prognostic factor for multiple myeloma patients who undergo autologous stem cell transplantation. *Blood*. 2008;112(10):4017-4023.

5. Ladetto M, Pagliano G, Ferrero S, et al. Major tumor shrinking and persistent molecular remissions after consolidation with bortezomib, thalidomide, and dexamethasone in patients with autografted myeloma. *J Clin Oncol*. 2010;28(12):2077-2084.

6. Fernández de Larrea C, Cibeira MT, Elena M, et al. Abnormal serum free light chain ratio in patients with multiple myeloma in complete remission has strong association with the presence of oligoclonal bands: implications for stringent complete remission definition. *Blood*. 2009;114(24):4954-4956.

7. Bladé J, Cibeira MT, Rosiñol L. Novel drugs for the treatment of multiple myeloma. *Haematologica*. 2010;95(5):702-704.

8. Bladé J, Rosiñol L, Cibeira MT, Rovira M, Carreras E. Hematopoietic stem cell transplantation for multiple myeloma beyond 2010. *Blood*. 2010;115(18):3655-3663.

9. Lahuerta JJ, Mateos MV, Martínez-López J, et al. Influence of pre- and post-transplantation responses on outcome of patients with multiple myeloma: sequential improvement of response and achievement of complete response are associated with longer survival. *J Clin Oncol*. 2008;26(35):5775-5782.

10. Fernández de Larrea C, Tovar N, Cibeira MT, et al. Emergence of oligoclonal bands in patients with multiple myeloma in complete remission after induction chemotherapy: association with the use of novel agents. *Haematologica*. 2011;96(1):171-173.

● ● ● LYMPHOID NEOPLASIA

PCNSL: biomarker better than biopsy?

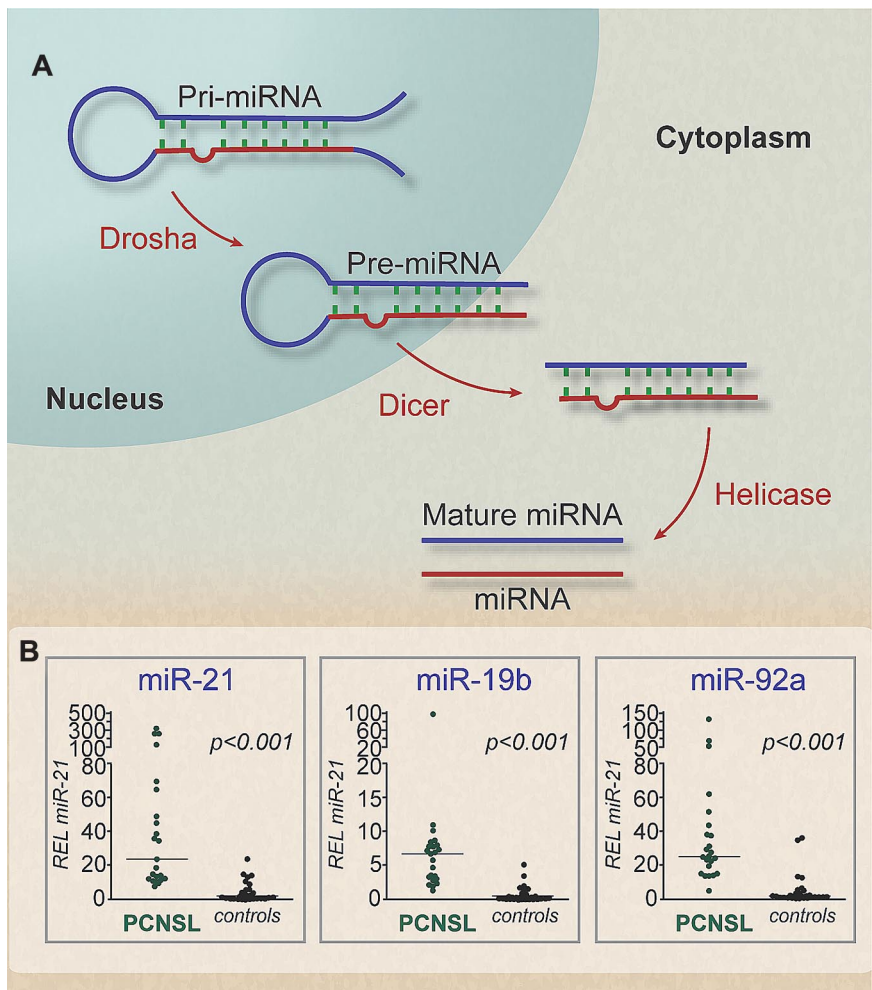**Gerald Illerhaus and Tracy Batchelor**   UNIVERSITY HOSPITAL FREIBURG; MASSACHUSETTS GENERAL HOSPITAL

In this issue of *Blood*, Baraniskin and colleagues report on microRNAs (miRNAs) as a possible biomarker for the diagnosis of primary central nervous system lymphoma (PCNSL).[1] Levels of *miR-21, miR-19, and miR-92a* were significantly increased in cerebrospinal fluid (CSF) samples from PCNSL patients compared with controls with inflammatory CNS disease or other neurologic disorders.

The diagnosis of PCNSL is most commonly achieved via stereotactic brain biopsy. Contemporary imaging methods (CT, MRI, PET) fail to reliably differentiate inflammatory processes, solid-tumor metastases, and primary or secondary CNSL. A misinterpretation of findings can lead to a delay in initiating therapy on the one hand, or to unnecessary

A

Pri-miRNA

Cytoplasm

Drosha

Pre-miRNA

Nucleus

Dicer

Helicase

Mature miRNA

miRNA

B

miR-21

miR-19b

miR-92a

REL miR-21

p<0.001

PCNSL *controls*

p<0.001

PCNSL *controls*

p<0.001

PCNSL *controls*

(A) Model for miRNA biogenesis. The initiation step is mediated by the Drosha complex in the nucleus. The product of this nuclear processing step is an ∼ 70-nucleotide pre-miRNA. After export, the cytoplasmic RNase III dicer participates in the second processing step (dicing) to produce miRNA duplexes. The duplex is separated by helicase and usually one strand is selected as the mature miRNA, whereas the other strand is degraded. (B) Scatter plots of expression levels of miR-21, miR-19b, and miR-92a in CSF samples from 23 PCNSL patients compared with 30 control patients with various neurologic disorders. Increased mean and median relative expression levels of miR-21, miR-19b, and miR-92a were demonstrated in PCNSL patients' CSF compared with controls. Professional illustration by Debra T. Dartez.

matory CNS diseases and other neurologic disorders. Despite their small patient sample (n = 23), using combined miRNA analyses they demonstrated that these candidate miRNAs have high sensitivity (95.7%) and specificity (96.7%) for PCNSL diagnosis (see figure panel B). The authors also demonstrated that miRNAs in the CSF exhibited remarkable stability with resistance to exogenous RNase, repeated freeze-thaw cycles, and long-term storage of CSF specimens. Possible explanations for this phenomenon include miRNA protection in exosomes or association of miRNA with other molecules (eg, CSF proteins). These features may enhance the practical utility of CSF miRNA for diagnostic purposes.

The article by Baraniskin et al advances the field of diagnostic markers in CNSL. Perhaps the analysis of miRNAs in the CSF of patients with suspected PCNSL will expand the diagnostic tools at our disposal, especially in patients in whom biopsy appears too risky or when histologic findings are equivocal. However, as these data were generated from a small number of patients, it will be up to future studies to validate the diagnostic utility of miRNAs in the PCNSL patient population. Finally, there is the intriguing possibility that miRNAs derived from primary brain tumors like PCNSL may also circulate in blood,[8] which could offer a readily accessible source of tumor-derived RNA for future study.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

resection of PCNSL on the other, in conjunction with related morbidity. Both of these situations can affect the patient's outcome dramatically. The success of stereotactic biopsy, while the histologic gold standard, depends on accessible lesions, and it is sometimes unfeasible when lesions lie close to or within critical brain structures. Although most brain biopsy procedures are safely performed there is up to a 7% risk of hemorrhage and up to a 35% risk of failure to achieve a definitive histologic diagnosis.[2] CSF examination can only provide definitive evidence of PCNSL in the presence of leptomeningeal dissemination and is < 50% sensitive for the diagnosis of PCNSL in this setting.[3] Antithrombin III,[4] soluble CD-27,[5] and free immunoglobu-

lin light chains[6] are CSF biomarkers that have not yet achieved general acceptance in clinical practice. miRNAs are short RNA molecules that bind the 3′-untranslated regions of mRNA transcripts (see figure panel A). They inhibit gene expression at a posttranscriptional level by interfering with the translational initiation or degradation of mRNA.[7]

Baraniskin et al were the first to define the role of miRNAs in the CSF of lymphoma patients. Taking a "candidate approach" and quantifying miRNA via qRT-PCR, the authors identified significant levels of miRNAs in the CSF of PCNSL patients. In particular, *miR-21, miR-19,* and *miR-92a* had diagnostic value in distinguishing PCNSL from inflam-

REFERENCES

1. Baraniskin A, Kuhnhenn J, Schlegel U, et al. Identification of microRNAs in the cerebrospinal fluid as marker for primary diffuse large B-cell lymphoma of the central nervous system. *Blood.* 2011;117(11):3140-3146.

2. Josephson SA, Papanastassiou AM, Berger MS, et al. The diagnostic utility of brain biopsy procedures in patients with rapidly deteriorating neurological conditions or dementia. *J Neurosurg.* 2007;106(1):72-75.

3. Chamberlain MC. Neoplastic meningitis: a guide to diagnosis and treatment. *Curr Opin Neurol.* 2000;13(6):641-648.

4. Roy S, Josephson SA, Fridlyand J, et al. Protein biomarker identification in the CSF of patients with CNS lymphoma. *J Clin Oncol.* 2008;26(1):96-105.

5. Kersten MJ, Evers LM, Dellemijn PL, et al. Elevation of cerebrospinal fluid soluble CD27 levels in patients with meningeal localization of lymphoid malignancies. *Blood.* 1996;87(5):1985-1989.

6. Schroers R, Baraniskin A, Heute C, et al. Detection of free immunoglobulin light chains in cerebrospinal fluids of

patients with central nervous system lymphomas. *Eur J Haematol*. 2010;85(3):236-242.

7. Bartel DP. MicroRNAs: target recognition and regulatory functions. *Cell*. 2009;136(2):215-233.

8. Skog J, Wurdinger T, van Rijn S, et al. Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. *Nat Cell Biol*. 2008;10(12):1470-1476.

● ● ● **THROMBOSIS & HEMOSTASIS**

Comment on Berckmans et al, page 3172

Salivary microvesicles clot blood

Peter L. Gross THROMBOSIS AND ATHEROSCLEROSIS RESEARCH INSTITUTE

The capacity of saliva to clot blood has been documented in the scriptures (Luke 16:21), folklore, and in the medical literature of the 1920s when Hunter described the ability of saliva to clot blood and proposed it as a means to attenuate bleeding from gastric ulcers.[1] In 1938, Glazko and Greenberg reported that saliva contains a cell–derived, protein-based thromboplastin,[2] which was later identified as tissue factor.
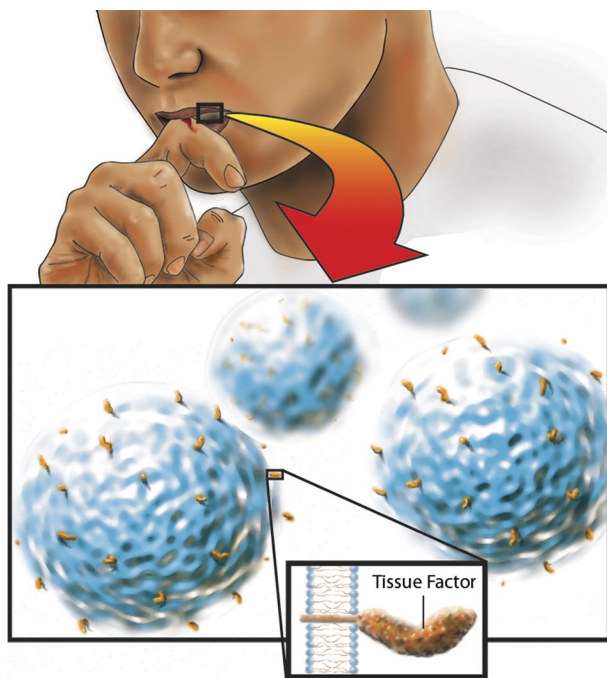
Tissue factor on microvesicles in saliva can cause blood to clot. This may provide the basis for the wound-licking reflex. Illustration by Nima Vaezzadeh.

Where is the source of tissue factor in saliva? In 1979, Zacharski and Rosenstein reported that almost one-quarter of the tissue factor procoagulant activity in saliva remains in the supernatant after centrifugation of the cells.[3] Using contemporary techniques, Berckmans and colleagues have now identified the source of the tissue factor procoagulant activity in the supernatant.[4] Salivary tissue factor is associated with microvesicles and exosomes. These are vesicles that are shed from cells.[5] Shedding occurs in resting cells, but increases with cell stimulation or apoptosis. The tissue factor–bearing vesicles in saliva are likely derived from cells in the salivary glands and the mouth because they express epithelial-cell and leukocyte markers on their surface (see figure). The concentration of tissue factor in these vesicles is at least 5-fold higher than that in blood, so there is more than enough to clot blood![6]

In this issue of *Blood*, Berckmans et al suggest that salivary tissue factor serves as an extra barrier between the blood and the outside milieu by promoting hemostasis and preventing infection. Blood pools in the saliva when the oral mucosa is injured. In the absence of flow, only low concentrations of tissue factor are required to trigger thrombin generation,[7] provided that there are an adequate number of normal platelets.[8] Could this explain, at least in part, why oral mucosal bleeding is more common with platelet disorders than with deficiencies of clotting factors?

As with all science, answers beget more questions. In mice, removal of the salivary glands decreases wound healing.[9] Is the poor wound healing the result of an absence of salivary tissue factor? Do the levels of salivary tissue factor change with aging or disease? Would strategies aimed at increasing salivary tissue factor reduce mucosal bleeding in patients with thrombocytopenia or gingivitis, or even those with gastric ulcers? These are questions for the future. Meanwhile, the work by Berckmans and colleagues provides some basis for why the wound-licking reflex may be beneficial. The clot-promoting activity of saliva may be offset by harm, including the introduction of oral bacteria into the wound.[10]

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

REFERENCES

1. Hunter JB. The action of saliva and gastric juice on the clotting of blood. *Br J Surg*. 1928;16:203-207.

2. Glazko AJ, Greenberg DM. The mechanism of the action of saliva in blood coagulation. *Am J Physiol*. 1938; 125:108-112.

3. Zacharski LR, Rosenstein R. Reduction of salivary tissue factor (thromboplastin) activity by warfarin therapy. *Blood*. 1979;53(3):366-374.

4. Berckmans RJ, Sturk A, Schaap MCL, Nieuwland R. Cell-derived vesicles exposing coagulant tissue factor in saliva. *Blood*. 2011;117(11):3172-3180.

5. Freyssinet JM. Cellular microparticles: what are they bad or good for? *J Thromb Haemost*. 2003;1(7):1655-1662.

6. Butenas S, Orfeo T, Mann KG. Tissue factor in coagulation: Which? Where? When? *Arterioscler Thromb Vasc Biol*. 2009;29(12):1989-1996.

7. Chatterjee MS, Denney WS, Jing H, Diamond SL. Systems biology of coagulation initiation: kinetics of thrombin generation in resting and activated human blood. *PLoS Comput Biol*. 2010;6(9). pii: e1000950.

8. Butenas S, Branda RF, van't Veer C, Cawthern KM, Mann KG. Platelets and phospholipids in tissue factor-initiated thrombin generation. *Thromb Haemost*. 2001; 86(2):660-667.

9. Bodner L, Knyszynski A, Adler-Kunin S, Danon D. The effect of selective desalivation on wound healing in mice. *Exp Gerontol*. 1991;26(4):357-363.

10. Weil HP, Fischer-Brugge U, Koch P. Potential hazard of wound licking. *N Engl J Med*. 2002;346(17):1336.