To the editor:

**Immune responses against the mutated region of cytoplasmatic NPM1 might contribute to the favorable clinical outcome of AML patients with NPM1 mutations (NPM1\textsuperscript{mut})**

Immune responses directed against epitopes derived from the mutated region of nucleophosmin 1 (NPM1) by NPM1\textsuperscript{mut}-specific CD8\textsuperscript{+} cytotoxic T cells (CTLs) might be involved in the rejection of NPM1\textsuperscript{mut} myeloid leukemic blasts. NPM1 mutations are one of the most frequent molecular alterations in acute myeloid leukemia (AML) and are an important prognostic marker.\textsuperscript{1} The mutations cause an abnormal shift of the NPM1 protein from the nucleus to the cytoplasm, described by Falini et al.\textsuperscript{2} AML patients with NPM1\textsuperscript{mut}, but without FLT3 internal tandem duplication (ITD) mutation, show improved overall survival.\textsuperscript{3} NPM1\textsuperscript{mut}/FLT3-ITD–negative patients do not seem to benefit from allogeneic stem cell transplantation in first-line treatment; however, this issue is still under evaluation, further clinical trials are ongoing, and also minimal residual disease (MRD) has to be considered in treatment decision.\textsuperscript{3,4} The functional role of NPM1\textsuperscript{mut} for the improved clinical outcome is still under evaluation. Immune responses to NPM1\textsuperscript{mut} may contribute to the favorable prognosis of this AML subtype. Recently, we described specific T-cell responses of CD4\textsuperscript{+} and CD8\textsuperscript{+} T cells against epitopes derived from mutated regions of NPM1.\textsuperscript{5} Two NPM1\textsuperscript{mut}-derived peptides, called #1 and #3, induced specific T-cell responses in patients with NPM1\textsuperscript{mut} (33% and 44%, respectively). NPM1\textsuperscript{mut} AML patients showed a significantly higher frequency of CTL responses against peptide #3 compared with healthy volunteers (P = .046).\textsuperscript{5} Several leukemia-associated antigens (LAAs) have been defined, most importantly RHAMM, Proteinase 3, and Wilms’ tumor antigen 1 (WT-1). These antigens were tested in clinical peptide vaccination trials.\textsuperscript{6} Immunologic and clinical responses were detected in patients with different hematologic malignancies.\textsuperscript{7,8} Berneman et al\textsuperscript{9} discussed NPM1\textsuperscript{mut} as a further important LAA to attack AML and leukemic stem cells by autologous T cells.

In this work, we performed survival analysis of 25 NPM1\textsuperscript{mut} patients (Figure 1A), analyzed by enzyme-linked immunospot comparing cases with or without specific T-cell responses. Our data suggest a better overall survival of patients with specific CTL responses against peptide #1 or #3 (P = .004; Figure 1B).

**Figure 1. Survival analysis of NPM1\textsuperscript{mut} patients.** (A) Kaplan Meier plot with the survival analysis of 25 NPM1\textsuperscript{mut} patients. (B) Overall survival of patients with specific CTL responses against peptides #1 or #3. Blue, patients with an immune response; green, patients without any specific CTL response. (C) Overall survival in dependence on the specific epitope. Blue, peptide #1; green, peptide #3; yellow, peptides #1 and #3; purple, no peptide.

Immune responses seem to differ in dependence on the epitopes (P = .026; Figure 1C), although this finding has to be interpreted with caution due to the low number of patients. The survival rate of all NPM1\textsuperscript{mut} patients is lower due to other molecular alterations (like FLT3-ITD in 11 of 25 NPM1\textsuperscript{mut} patients) and the inclusion of elderly patients (7 of 25 were older than 60 years of age). Due to its exquisite specificity in leukemia, NPM1\textsuperscript{mut} might constitute an ideal target structure for individualized immunotherapeutic approaches. Analysis with material from larger controlled clinical trials has to be performed. Nevertheless, these data suggest that immune responses might contribute to the clinical outcome. Therefore, immunotherapeutic approaches present a promising strategy for NPM1\textsuperscript{mut} patients for maintenance treatment or with persistent MRD. In an AML patient with NPM1\textsuperscript{mut} and molecular relapse, we demonstrated polyspecific CTL responses against several known LAAs, also NPM1 #3, after preemptive donor lymphocyte infusion.\textsuperscript{10} Importantly, the immune responses against LAAs were associated with MRD negativity.\textsuperscript{10} Such persistent responses against NPM1\textsuperscript{mut} epitopes provide a rationale for the development of preemptive maintenance strategies in AML patients with NPM1\textsuperscript{mut}.

Taken together, NPM1\textsuperscript{mut} might constitute an interesting target structure for individualized immunotherapeutic approaches in NPM1\textsuperscript{mut} AML patients. We hypothesize that immune responses against mutated NPM1 may contribute to the favorable prognosis.

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Minimal residual disease testing in multiple myeloma by flow cytometry: major heterogeneity

As more effective therapies become routinely used to treat multiple myeloma, standardized methods (such as flow cytometry) to determine the presence/absence of minimal residual disease (MRD) will likely play an increasingly important role in the clinical, research, and regulatory settings. Indeed, prior studies show that among patients who obtain a complete response, those who are MRD negative in their bone marrow by flow cytometry have better survival than those who are MRD negative. At this time, there are no established consensus criteria for MRD by flow cytometry of the bone marrow in multiple myeloma. To improve our understanding of MRD assessment practices, we conducted a survey of 30 major medical institutions in the United States. Directors of flow cytometry at each institution were sent an e-mail with 14 questions regarding their institution were sent an e-mail with 14 questions regarding their

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References

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