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 (NPM1mut)**

Immune responses directed against epitopes derived from the mutated region of nucleophosmin 1 (NPM1) by NPM1mut-specific CD8+ cytotoxic T cells (CTLs) might be involved in the rejection of NPM1mut myeloid leukemic blasts. NPM1 mutations are one of the most frequent molecular alterations in acute myeloid leukemia (AML) and are an important prognostic marker. The mutations cause an abnormal shift of the NPM1 protein from the nucleus to the cytoplasm, described by Falini et al. AML patients with NPM1mut, but without FLT3 internal tandem duplication (ITD) mutation, show improved overall survival. NPM1mut/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. The functional role of NPM1mut for the improved clinical outcome is still under evaluation. Immune responses to NPM1mut may contribute to favorable prognosis of this AML subtype. Recently, we described specific T-cell responses of CD4+ and CD8+ T cells against epitopes derived from mutated regions of NPM1. Two NPM1mut-derived peptides, called #1 and #3, induced specific T-cell responses in patients with NPM1mut (33% and 44%, respectively). NPM1mut AML patients showed a significantly higher frequency of CTL responses against peptide #3 compared with healthy volunteers ($P = .046$). 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. Immunologic and clinical responses were detected in patients with different hematologic malignancies. Berneman et al discussed NPM1mut 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 NPM1mut 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).

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 NPM1mut patients is lower due to other molecular alterations (like FLT3-ITD in 11 of 25 NPM1mut patients) and the inclusion of elderly patients (7 of 25 were older than 60 years of age). Due to its exquisite specificity in leukemia, NPM1mut might constitute an ideal target structure for individualized immunotherapeutic approaches. 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 NPM1mut patients for maintenance treatment or with persistent MRD. In an AML patient with NPM1mut and molecular relapse, we demonstrated polyspecific CTL responses against several known LAAs, also NPM1 #3, after preemptive donor lymphocyte infusion. Importantly, the immune responses against LAAs were associated with MRD negativity. Such persistent responses against NPM1mut epitopes provide a rationale for the development of preemptive maintenance strategies in AML patients with NPM1mut.

Taken together, NPM1mut might constitute an interesting target structure for individualized immunotherapeutic approaches in NPM1mut AML patients. We hypothesize that immune responses against mutated NPM1 may contribute to the favorable prognosis.

![Figure 1. Survival analysis of NPM1mut patients.](image)

Figure 1. Survival analysis of NPM1mut patients. (A) Kaplan Meier plot with the survival analysis of 25 NPM1mut 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.
To the editor:

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 measurement of MRD by flow cytometry of the bone marrow in multiple myeloma patients.

Twenty-six institutions responded; 11 (42%) said they perform MRD testing (Table 1). The number of events acquired for MRD testing by flow cytometry varied from 100 000 to 4 000 000, with most institutions (6/11; 55%) obtaining 100 000 to 500 000 events. The number of abnormal plasma cells needed to define MRD positivity ranged from 20 to 50. In 2008, the European Myeloma Network organized 2 flow cytometry workshops to identify specific indications for flow cytometry in patients with monoclonal gammopathies and to facilitate the development of consensus technical approaches. In our survey, we found the definition of abnormal plasma cells to differ substantially between institutions (Table 1), with some relying on CD19 and CD45 negativity with or without CD56 positivity to determine the extent of MRD despite previous studies showing that normal plasma cell subpopulations can be negative for CD19 and CD45 or CD56 positive.

More specific antigens such as CD27, CD81, and CD117 were used by fewer than half of the institutions. Our survey illustrates the heterogeneity in MRD testing of multiple myeloma by flow cytometry. There is considerable variation in the number of bone marrow cells analyzed (events) and number of abnormal plasma cells needed to define the presence of MRD, which affects maximum possible sensitivity. Furthermore, the maximum detection sensitivity ranged from 0.0005% to 0.02%, a 100-fold difference in sensitivity (Table 1). The variation in antibodies studied and definition of an abnormal plasma cell by flow cytometry affects ability to differentiate normal from neoplastic plasma cells.

As antimyeloma therapies become more effective, standardization of MRD testing by flow cytometry of the bone marrow will become increasingly important to allow better assessment of response and clinical prognostication. In addition to technical aspects, future clinical studies need to define the optimal timing to assess for MRD testing in
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