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The effect of azacitidine therapy on the M protein of MDS patients with concomitant MGUS

To the Editor:

Monoclonal gammopathy of undetermined significance (MGUS) is one of the most common pre-malignant disorders and affects approximately 3.5% of the population who are over 50 years of age. The pathophysiological concept considers that multiple myeloma (MM) evolves from MGUS in a large proportion of patients. Despite the proposed risk factors, there are still no reliable biomarkers to predict which MGUS patients will develop MM. The prevalence of MGUS in patients with myelodysplastic syndrome (MDS) ranges between 2% and 10%[1] and several agents are effective in the treatment of MDS and MGUS; however, the effect of azacitidine for MGUS has not yet been established in detail. The frequencies of TP53 mutations in MM/MGUS were 5%-10%, and the presence of TP53 mutant clones has been associated with adverse outcomes.[2] Another important distinction pertaining to the origin of plasma cell disorders is the involvement of interleukin (IL)-6. We herein examined the effects of azacitidine on the M protein of MDS patients with concomitant MGUS, and the association of IL-6 and TP53 mutations with MGUS.

Our retrospective study involved eight MDS patients with concomitant MGUS at the Japanese Red Cross Society Wakayama Medical Center between January 2010 and January 2018. Azacitidine was administered at 75 mg/m^2/day for 7 days every 28 days. Responses were evaluated every 2 cycles using blood counts and marrow aspirates. Serum M-protein was measured prior to the initiation and every 2 cycles of azacitidine treatment. The concentration of IL-6 was determined prior to the initiation and every 2 cycles of azacitidine treatment using enzyme-linked immunosorbent assay (ELISA) kits from Quantikine (R&D systems) based on the manufacturer's instructions. Bone marrow samples were obtained from all patients before the initiation and every 2 cycles of azacitidine treatment. Bone marrow sections (thickness of 2 μm) on SuperFrost microscope slides were de-paraffinized and pre-treated at 95°C for 7 min in citrate buffer (pH 6). In order to quantify and assess the distribution of hematopoietic cells and plasma cells, samples were stained for CD34 (Cell Marque Rocklin, CA) and CD138 (Dako Corporation, Carpinteria, CA). The DO-7 antibody (Dako Cytomation, Denmark), which labels wild-type and mutant-type p53 proteins, was used to detect p53 protein expression. The entire trephine section was assessed for p53 protein nuclear staining in hematopoietic progenitor cells, as previously described.[3] In order to minimize the possibility of false positive results, p53 protein expression was only considered to be positive if strong nuclear staining (score + 3) was present in at least 5% of hematopoietic cells in the entire BM section. G-band karyotyping, immunophenotyping, serum protein electrophoresis, and immunoelectrophoresis were performed using standard procedures.

Seven patients had IgG MGUS except for 1 who had IgA MGUS. The serum M-protein of all patients were ≥1.5 g/dL (median 2.6 g/dL, 1.8-2.8 g/dL), and the FLC ratios of all patients were 7.47 (0.03-17.2). Seven patients had high-intermediate risk MGUS, and 1 patient had high risk MGUS using the Mayo Clinic risk stratification model to predict progression. The median IL-6 level was 15.8 pg/mL (8.3-37 pg/mL). A bone marrow examination showed hypercellular marrow with myelodysplasia-related changes, numerous blasts, and plasma cells (Figure 1A). Strong nuclear p53 was detected in 5%-30% (Figure 1B) and CD138 immunostaining was noted in 5%-10% of plasma cells (Figure 1C) before the initiation of azacitidine treatment. A cytogenetic analysis revealed abnormal karyotypes in three patients, however, the specific chromosomal abnormalities, such as del(17p) and t(4;14), were not shown.

After two courses of azacitidine treatment, the median serum M protein levels (1.2 g/dL [0.7-1.7 g/dL]) and IL-6 levels (3.1 pg/mL [2.5-5.4 pg/mL]) decreased, and the FLC ratios of all patients were normalized. A repeat marrow examination showed decreases in the number of myeloblasts and plasma cells (Figure 1D). Strong nuclear p53 (Figure 1E) was decreased in hematopoietic cells and CD138 immunostaining was detected in a few plasma cells (Figure 1F). The azacitidine treatment was continued (median: 25 cycles [8-43 cycles]), and none of these patients showed progression of MGUS.

Azacitidine belongs to a class of cytosine analogues that was developed as an inhibitor of DNA methylation and has shown clinical efficacy toward MDS. In MM, DNA hypomethylation was reported as the predominant early change during myelomagenesis where it is gradually transformed to DNA hypomethylation in relapsed cases and during progression of the disease. Several reports showed that azacitidine was cytotoxic against MM cells and overcomes the growth and survival advantages provided by the BM microenvironment.[4]

The presence of TP53 mutations in MM/MGUS indicates a dismal prognosis: patients that exhibit a more aggressive disease course are more likely to have extramedullary disease and hypercalcemia and have shorter overall and progression-free survival. Mikulasova et al. reported that TP53 mutations were not detected in samples from patients with MGUS and may indicate that the presence of TP53 mutations are drivers of MM progression.[5]

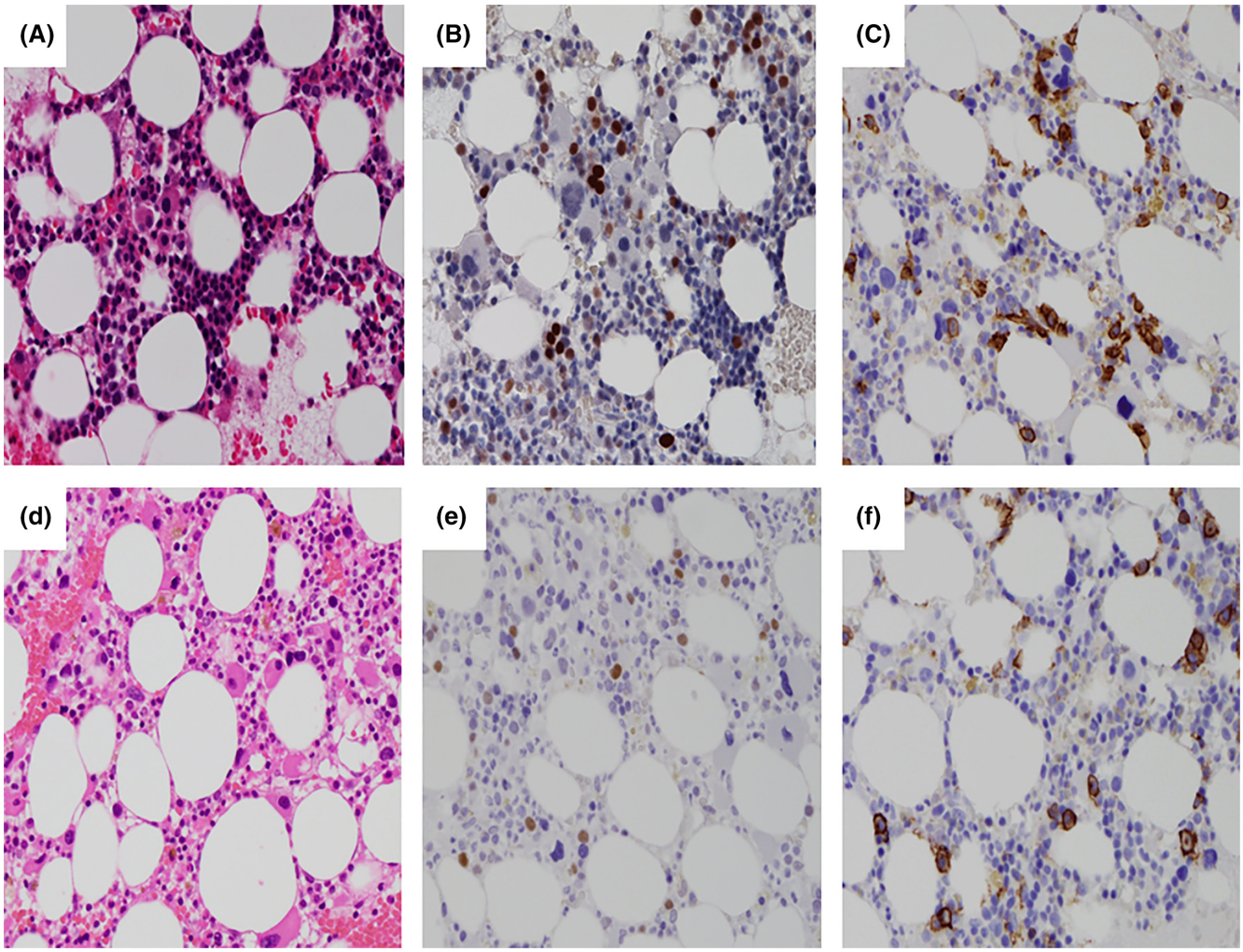

FIGURE 1 Bone marrow biopsies of the MGUS concomitant with MDS before (A, B, C) and after (D, E, F) azacitidine treatment (×40). A, Hypercellular marrow with myelodysplasia-related changes, numerous blasts and plasma cells. B, Strong nuclear p53 immunostaining in numerous hematopoietic cells. C, CD138 immunostaining in 10% of plasma cells. D, Hypercellular marrow with myelodysplasia-related changes and decreased blasts and plasma cells. E, Strong nuclear p53 immunostaining in a few hematopoietic cells. F, CD138 immunostaining in a few plasma cells

IL-6 is a potent human myeloma-cell growth factor, and its overproduction is known to play a critical role as an anti-apoptosis-inducing agent in MM. Khong et al. recently demonstrated that azacitidine exerts pleiotropic effects including the down-regulation of anti-apoptotic factors and JAK-STAT signaling as well as the inhibition of NFκB in MM cell lines.[6] In our study, the percentage of strongly p53-positive bone marrow cells was greater than 5% at the time of the concomitant occurrence of MGUS and MDS with increase of serum IL-6 levels; however, the levels of serum-M protein, IL-6 levels, the percentage of p53-positive and CD138-positive cells decreased after the treatment with azacitidine.

In this study, azacitidine was effective to delay the progression of MGUS. Although the mechanism underlying the progression of MGUS to MM is unknown, IL-6 and TP53 mutations appear to contribute to the pathophysiology of MGUS. In conclusion, azacitidine have clear activity against MGUS and should be considered in the treatment strategy.

CONFLICT OF INTEREST

Nothing to report.

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Incomplete implementation of guideline-based stroke prevention therapy in sickle cell disease

To the Editor:

Stroke is a devastating, but often preventable complication associated with sickle cell disease (SCD). The Stroke Prevention Trial in Sickle Cell Anemia (STOP) and Optimizing Primary Stroke Prevention in Sickle Cell Anemia (STOP 2) established that routine transcranial doppler ultrasound (TCD) screening with indefinite chronic red cell transfusions (CRCT) for children with abnormal TCD can significantly reduce stroke risk in SCD.[1,2] These data led to the "STOP protocol," which is strongly recommended in the 2014 National Institute of Heart, Lung, and Blood (NHLBI) guidelines and endorsed by the American Stroke Association. This protocol includes: (a) annual TCD screening and (b) indefinite CRCT for patients with abnormal TCD or previous cerebral infarct.[3] Subsequently, the TCD With Transfusions Changing to Hydroxyurea (TWiTCH) study demonstrated that children with SCD with abnormal TCD treated with CRCT could be safely transitioned to Hydroxyurea if there were no significant cerebrovascular abnormalities on neuroimaging.[4] However, individuals with prior stroke or cerebral vasculopathy must remain on indefinite CRCT for optimal stroke prevention.[5]

Despite these guidelines, CRCT is likely underutilized.[3] Barriers to CRCT implementation are likely multi-factorial, involving multiple patient and health care factors (eg, transition to adult care, socioeconomic/geographic variables).[6] No prior studies have examined barriers to lifelong CRCT implementation in individuals with SCD at high stroke risk. This study's goals were to: (i) describe the CRCT status of patients with SCD at high stroke risk in a large, Southeastern urban clinic and (ii) examine differences in patient-level characteristics related to CRCT implementation. We hypothesized that age, provider type, and proximity to comprehensive care would be associated with CRCT utilization. Treatment indication for CRCT and insurance status were exploratory variables.

This retrospective chart review study was approved by the Medical University of South Carolina's (MUSC) Institutional Review Board. Individuals with SCD at high stroke risk were identified using clinic rosters and previous stroke prevention trial registries from January 1, 2000 to December 31, 2014. *CRCT Status* was treatment status as of December 31, 2014: (i) patients currently receiving CRCT (transfusions >8×/year); (ii) patients not currently receiving CRCT; and (iii) patients lost to follow-up (LTFU) (absence of care at MUSC for 12 months) and no identification of "deceased" in the medical record. *Age* was determined as of December 31, 2014. *Provider Type* was separated into SCD specialist versus non-SCD provider (ie, primary care, no provider) according to last encounter. *Region* was based on last known zip code of residence. The Tri-County (counties closest to MUSC) was compared with all other regions of South Carolina. *Treatment Indication* (neurological indication) for CRCT was classified as prior stroke, abnormal TCD, and/or other indication. *Insurance Status* was classified as Medicare, Medicaid, Other Insurance, or None based on last encounter.

Patient characteristics were compared based on CRCT status using one-way ANOVA and Chi-square tests or Fisher's Exact tests (Table 1). Age was examined as a confounding variable using Chi-square tests or Fisher's Exact tests, and logistic regression was used to examine specific characteristics and CRCT status adjusting for variables confounded by age. Analyses were conducted in SAS version 9.4; significance level α was maintained at 0.05.

We identified 143 patients with SCD at high stroke risk, 9% (13 patients) of whom were confirmed deceased. Of these patients, 52% (68/130) were being treated with CRCT, 24% (31/130) were off CRCT therapy, and 24% (31/130) were LTFU. Initial treatment indication included: prior stroke (n = 81), abnormal TCD (n = 39), subarachnoid hemorrhage (n = 2), abnormal brain imaging (n = 3), seizure (n = 1), and multiple neurological indications (n = 1). The majority of patients not on CRCT (74%; 23/31) had CRCT stopped by provider choice, of whom 57% (13/23) were started on Hydroxyurea. These individuals had been receiving CRCT for greater than 5 years (exact duration is unknown) prior to termination. Historical encounters indicate some concern for iron overload in many patients, but not as the reason for stopping transfusion. No concerns for alloimmunization were noted. Further, many of these individuals had not undergone updated neuroimaging to assess for cerebrovascular changes. Patients currently on CRCT were significantly younger than patients off therapy or those LTFU. Over 91% of patients on CRCT had a SCD specialist versus 45% not on CRCT (P < .0001). Region did not differentiate those on CRCT versus off therapy; however, the majority of patients LTFU lived outside the Tri-County (P = .005). Results for CRCT indication showed 62% of patients on CRCT had prior stroke compared with 48% not on CRCT and 77% LTFU (P = .020). There were no significant differences for insurance status (P = .072).