A deep molecular response of splenic marginal zone lymphoma to front-line checkpoint blockade

Splenic marginal zone lymphoma (SMZL) is an uncommon form of B-cell non-Hodgkin lymphoma (B-NHL) that frequently presents with prominent splenomegaly, often with circulating malignant lymphocytes but with modest lymphadenopathy. As SMZL is generally considered an incurable disease, treatment is often deferred until the manifestation of cytopenias, symptomatic splenomegaly, or constitutional symptoms warrant intervention. The current approach to frontline treatment typically involves rituximab given with or without chemotherapy, treatment of associated conditions such as hepatitis C infection, and less commonly splenectomy. Immune checkpoint blockade with agents such as anti-PD-1 antibodies have been effective in certain subtypes of B-NHL, but the use of these agents in patients with previously untreated SMZL has not yet been explored.

A 77 year-old man with a history of stage IIc melanoma treated with wide local excision with curative intent presented to the hematology clinic in June 2016 for evaluation of thrombocytopenia (Figure 1). An initial workup revealed what was thought to be an incidental IgA λ and IgG κ monoclonal gammopathy of undetermined significance (MGUS), and the decision was made to proceed with observation. However, after developing anemia and progressive thrombocytopenia, further workup was initiated, including imaging that revealed mild mediastinal and bilateral axillary adenopathy and prominent splenomegaly. A bone marrow biopsy was performed in June 2017 that showed an atypical lymphoid population positive for B-lymphoid markers CD19 and CD20, negative for CD5 and CD23, and λ light chain restricted. Next-generation sequencing of the bone marrow aspirate using an in-house gene panel revealed a *TP53* I195S mutation with a variant allele frequency of 0.06 and loss of *PTEN*.[1] Though uncommon, adenopathy can occur in SMZL, and while splenic B-cell lymphoma unclassifiable was also considered,[2,3] the combination of an aberrant immunophenotype, cytopenias, and significant splenomegaly made SMZL the most likely diagnosis. However, given the absence of symptoms, therapy was deferred at that time.

Approximately 3 months later, surveillance imaging for his melanoma suggested metastatic disease, which was subsequently confirmed by biopsy of a right lung nodule. Pembrolizumab monotherapy every 3 weeks was recommended to treat his melanoma. At the time, he had also developed worsening and symptomatic splenomegaly (22.9 cm), intermittent drenching night sweats, fatigue, and ongoing cytopenias, suggesting the need for SMZL therapy. However, given that the metastatic melanoma was thought to be the more immediately threatening malignancy, treatment of the SMZL was deferred in order to initiate melanoma therapy. On the day he initiated treatment with pembrolizumab in September 2017 his laborarory results were notable for a white blood cell count (WBC) of 26 K/uL, of which 69% were lymphocytes, hemoglobin of 11.1 g/dL, and a platelet count of 60 K/uL.

Within 3 months of starting pembrolizumab, the melanoma metastases had significantly decreased in size. Interestingly, a substantial response was also observed in his SMZL: spleen size was reduced to 14.0 cm, WBC was 5.9 K/uL, absolute lymphocyte count was 0.84 K/uL, hemoglobin was 13.8 g/dL, and the B-symptoms had largely disappeared, although his platelets remained at 60. His pembrolizumab treatment course was complicated by type I diabetes mellitus, which was considered an immune-related adverse event requiring an inpatient admission for diabetic ketoacidosis that was effectively managed. Later in his course he was admitted for sepsis, which was thought to be unrelated to treatment. At the last documented follow-up, he had received 35 cycles of pembrolizumab, the melanoma was in complete remission, the spleen size was normal, and his blood counts had remained stable, with the only abnormality being persistent thrombocytopenia, which had improved, but remained in the 90-105 range. To date he has not received any therapy specifically directed to the SMZL.

After obtaining Instituational Review Board approval, genomic DNA was isolated from peripheral blood mononuclear cells (DNEasy Kit, Qiagen, Hilden, Germany). Hybrid capture was performed on the genomic DNA samples followed by library preparation with unique molecular identifiers using a custom bait set from Twist Bioscience (San Francisco, CA, USA). Sequencing was performed on the Illumina platform (Illumina, San Diego, CA, USA) and after deduplication and consensus sequence calling, mutations were identified using Varscan 2.2.3, and annotated using Annovar. Mutations were scored based on allele frequency, strand bias differential, local noise and mapping quality, and frequency in known single nucleotide polymorphism (SNP) databases. These variants were visually inspected in Integrated Genome Viewer (Broad Institute, Cambridge, MA, USA). Bone marrow staining was performed using antibodies to PD-L1 (Clone E1L3N, Cell Signaling Technology, Danvers, MA, USA) and BSAP (Clone PAX5, BD Biosciences, San Jose, CA, USA).

We had banked a pre-treatment peripheral blood mononuclear cell (PBMC) sample just prior to pembrolizumab initiation, and after observing this dramatic response of therapy-naive SMZL to PD-1 blockade, we obtained another PBMC sample approximately 6 months later. Genomic DNA was extracted from the samples and error-corrected deep sequencing using unique molecular identifiers was performed on a panel of genes that are recurrently mutated in hematologic malignancies. With this technology we are able to detect the presence of mutations to a variant allele frequency (VAF) as low as 0.003.[4] Prior to pembrolizumab initiation, the same *TP53* I195S mutation identified in the diagnostic bone marrow biopsy was again observed, this time with an elevated VAF of 0.78, consistent with the predominance of lymphocytes at the time of sample acquisition and the enrichment of lymphoid DNA from PBMC.[5] Remarkably, after 6 months of pembrolizumab treatment, the mutation was undetectable in the blood, suggesting a deep molecular response of the SMZL. The raw mutation calls visualized in Integrated Genome Viewer are shown in Figure 2A.

We also examined the clinical sequencing that was performed on the melanoma biopsy specimen using another custom panel.[6] The sequencing data reported two separate *TP53* mutations: I195S (VAF 0.29) and G105V (VAF 0.13). The *TP53* I195S mutation was therefore blood-derived, whereas the G105V was tumor derived. This conclusion is also consistent with the finding that the average VAF among all mutations identified in the tumor was 0.11, close to the VAF of the G105V mutation but less than half of that of the I195S mutation (Figure 2B). The "contamination" of solid tumor sequencing by somatic mutations present in blood cells is an increasingly recognized phenomenon, can complicate interpretation of these data, and may have important prognostic and therapeutic implications.[7-10]

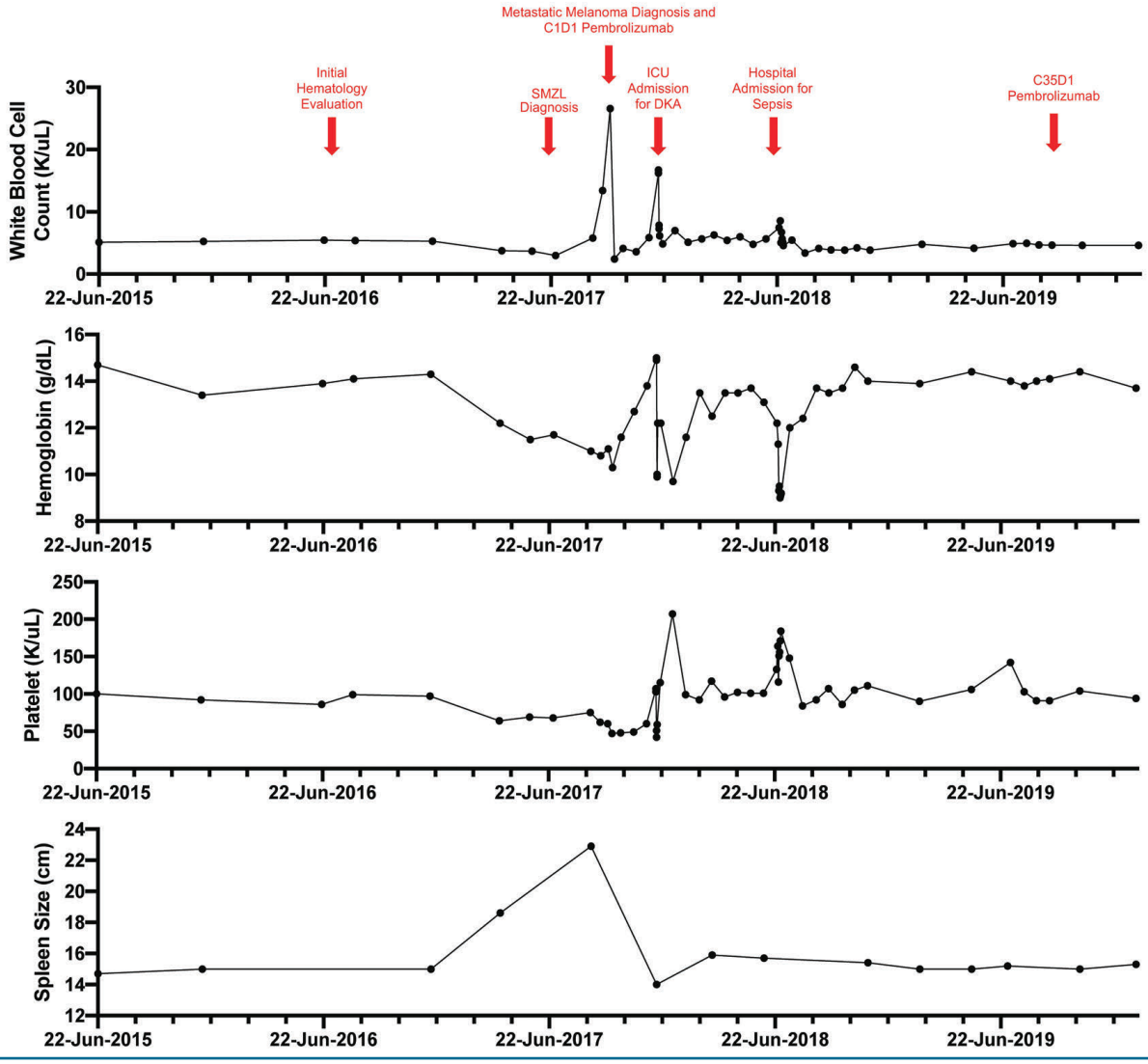

Figure 1. Blood counts and spleen size changes over time. Shown are the trends of white blood cell count, hemoglobin, platelet count, and spleen size across the clinical course of the patient. The red arrows at the top indicate significant events that occurred during this time.

In addition to eliminating his symptoms, normalizing the spleen size, lymphocytosis, and anemia, the response to pembrolizumab was both rapid and durable. This striking response was associated with the disappearance of the *TP53* I195S mutation and raises parallels to the concept of minimal residual disease and its implications in hematologic diseases. While we cannot rule out that sequencing of a bone marrow sample may have identified the mutation, the SMZL cells clearly were circulating (as evidenced by the lymphocytosis and sequencing data in the pre-treatment sample), and there is evidence to suggest high correlation between clonal mutations identified in the bone marrow and bloodstream.[11] The persistent thrombocytopenia after treatment was of uncertain etio-logy but presumed to be immune-mediated in nature due to the pembrolizumab therapy.

Finally, to gain insight into the mechanism of activity against the SMZL, we stained the bone marrow biopsy specimen for BSAP to highlight the B-cell population and PD-L1. Consistent with the striking response to pembrolizumab, the abnormal B-cell population uniformly expressed PD-L1 (Figure 2C). In contrast, the surrounding myeloid and erythroid cells did not appear to be PD-L1 positive and there was no obvious local inflammatory infiltrate.

There are limited data on the use of checkpoint inhibitors for the treatment of MZL, and we are not aware of any study reporting the use of these agents either as frontline therapy or specifically in SMZL. One patient with MZL was treated in a phase Ib study of nivolumab for relapsed or refractory hematologic malignancies but the results for this individual were grouped with other lymphoma subtypes and are therefore unavailable.[12] There is an ongoing trial of pembrolizumab alone or with idelalisib or ibrutinib for relapsed or refractory lymphomas that allows for MZL, but the full results have not been reported.[13] Furthermore, patients enrolled in clinical trials have generally already received numerous therapies and are thus likely more immune suppressed, in contrast to this case where the patient was therapy-naive. While our data suggest that frontline PD-1 blockade may be efficacious for the treatment of SMZL, we recognize that established frontline therapy options in this disease, in particular rituximab, are already effective

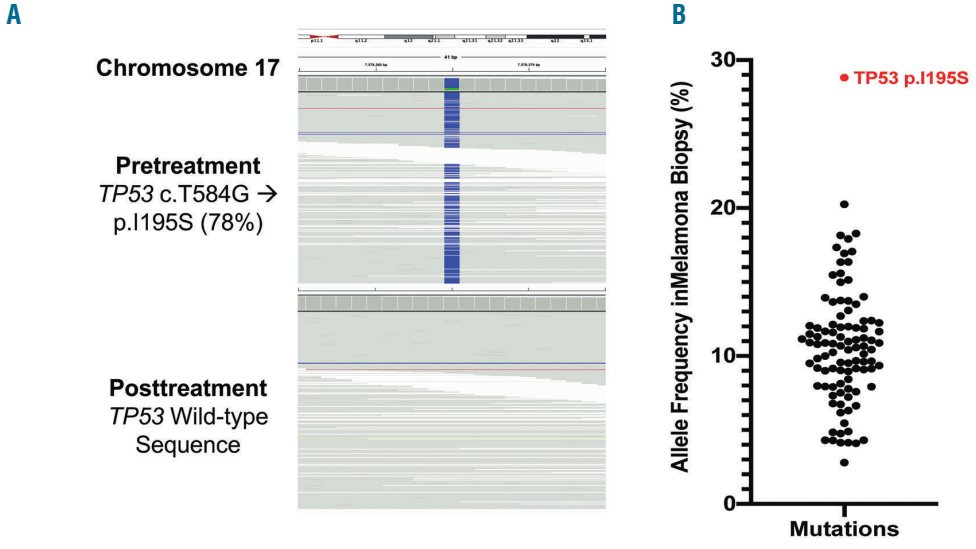

Figure 2. Identification and quantification of *TP53* I195S mutation. (A) The raw mutation calls visualized in Integrated Genome Viewer (IGV, Broad Institute, Cambridge, MA) are shown for the pretreatment (top) and post-treatment (bottom) blood samples. The T584G DNA mutation that corresponds to the protein change I195S was present in 78% of the reads in the pretreatment sample but absent in the post-treatment sample. (B) Variant allele frequency of missense, nonsense, and frameshift mutations identified in the metastatic melanoma biopsy. The *TP53* I195S mutation identified in the blood is highlighted in red. (C) Diagnostic bone marrow biopsy specimen stained with B-cell marker BSAP (red) and PD-L1 (brown).

and well-tolerated. Our data do suggest that studying PD-1 blockade in earlier lines of therapy for relapsed or refractory disease, particularly prior to utilizing chemotherapy, may be more likely to affect a therapeutic response due to the potential to harness a more intact immune system.

In this patient, the SMZL population expressed PD-L1, suggesting a direct T-cell mediated effect of pembrolizumab. The role of the tumor microenvironment in response to PD-1 blockade remains an area of active study, and despite the relative paucity of publications looking at checkpoint blockade in MZL, there does seem to be significant biological variability between and within different types of MZL.[14,15] For example, in one study of 54 SMZL, PD-L1 positive histiocytes and dendritic cells were found in 75% of the tumors but the Pax-5 tumor cells themselves were uniformly PD-L1 negative.[16] However, in clinical studies of patients with NHL, expression of PD-L1 does not always predict response to PD-1 blockade.[12,13] These observations further raise the potential role of the tumor microenvironment in mediating the response to these and other immunotherapies. Our findings and these data provide biological rationale for the use of PD-1 blockade in this setting.

TP53 missense mutations are common in B-NHL, including at the known hotspots R175H and R248Q. The *TP53* I195S missense mutation identified in this case occurs in the DNA binding domain of *TP53* where most of the hotspot mutations occur.[17] In a prior publication from our group we showed that mutations at position I195 generally, and I195S specifically, confer dominant negative activity on P53.[18] In one study that extracted SMZL cases from 14 different studies, *TP53* was the third most commonly mutated gene and present in 15% of cases.[19] While the identification of a *TP53* mutations does not provide diagnostic information, *TP53* mutations in SMZL are associated with a worse prognosis and overall survival, further highlighting the dramatic response in this patient.[20]

Taken together, this case report highlights the potential for PD-1 directed therapy in the frontline setting for marginal zone lymphoma, the potential value of molecular analysis in identifying residual disease after treatment, and the importance of considering hematopoietic mutations when interpreting solid tumor sequencing data. We believe that a prospective study of PD-1 blockade early in

the therapeutic paradigm for marginal zone lymphoma is warranted.

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Contributions: PGM, ASS, CJG, and MSD designed and conceived the study. MM, SH, EB, OP and WW collected the blood and bone marrow samples and processed the samples. PGM, ASS, CJG, and MSD.analyzed and interpreted the data. PGM, ASS and MSD drafted the manuscript. MSD and BLE obtained funding and supervised the study.

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