IRF4-BLOC1S5: the first rearrangement gene identified in TEMPI syndrome

TEMPI syndrome is a rare multisystem disorder characterized by telangiectasias, elevated erythropoietin and erythrocytosis, monoclonal gammopathy, perinephric fluid collections and intrapulmonary shunting, first described by Sykes and reported in the literature in a total of 29 patients.[1] Regimens based on plasma cell-directed drugs have shown to be effective,[2] suggesting a pathogenic role of the monoclonal antibody or the monoclonal plasma cell in the syndrome. It has been hypothesized that autoimmune or oxygen sensing disorders mediated by aberrant monoclonal antibodies or their fragments are involved in the pathogenesis of TEMPI syndrome.[2,3] However, no convincing evidence has been identified. Professor Hu has reported the duplication of 22q11.23, where the gene of macrophage migration inhibitory factor (MIF) is located, in one TEMPI syndrome case and high expression of MIF was observed in three TEMPI syndrome patients, suggesting a possible role of MIF in the pathophysiology of TEMPI syndrome,[4] which still needed further study to verify these findings as recurrent or episodic. In this study, we report a novel fusion gene, *IRF4-BLOC1S5*, that drives the expansion of a plasma cell clone in a TEMPI syndrome patient. The patient exhibited a favorable response to a bortezomib-based regimen with decreased IRF4-BLOC1S5 expression, consistent with *in vitro* sensitivity data. This report unveils a previously unreported fusion gene between *IRF4* and *BLOC1S5*, offering a pioneering insight into the molecular mechanism implicated in TEMPI syndrome and opening avenues for targeted therapeutic strategies.

A 75-year-old female, with an 8-year history of diffuse telangiectasia, was referred to our hospital in July 2019 with unexplained erythrocytosis and exertional dyspnea. The physical examination corroborated the presence of widespread telangiectasia (Figure 1A). The laboratory investigations revealed elevated hemoglobin (156 g/L), serum erythropoietin (1,035 mIU/mL), and immunoglobulin A-λ monoclonal protein (10.96 g/L). Bone marrow aspirates indicated 13% monoclonal plasma cells, expressed with $CD38^{+}$, $CD138^{+}$, $CD19^{-}$, $CD56^{+}$, $CD117P^{+}$, $CD27^{dim}$, $CD81^{dim}$, $CD45^{dim}$. Imaging confirmed perinephric fluid collections, and 99mTc macro-aggregated albumin scintigraphy detected intrapulmonary shunting (Figure 1A). The patient did not exhibit signs and symptoms of hypercalcemia, renal failure, anemia, and bone lesion, excluding multiple myeloma. Molecular testing showing that *BCR/ABL* translocation and mutations of *JAK2*V617F*, JAK2* exon 12*, CALR,* and *MFL* were negative, excluding myeloproliferative neoplasms. Therefore, a diagnosis of TEMPI syndrome was made.

In order to identify the genetic abnormalities in this patient, we performed whole-genome sequencing and transcriptome analysis. This study was approved by the Ethics Committee of Ruijin Hospital. Informed consent was obtained from the patient. Whole-genome sequencing of paired tumor ($CD138^{+}$ plasma cells) and normal samples revealed a 7.6-Mb somatic inversion on chromosome 6 in the region 6p25.3-6p24.3 (Figure 1B; *Online Supplementary Figure S1*). Bulk RNA sequencing (RNA-seq) showed that this inversion caused fusion of *exon 1-5* of *IRF4* to the *3'UTR* of *BLOC1S5*, which generated a short N-terminal-truncated IRF4 protein. Western blotting confirmed that the corresponding truncated IRF4 protein was only expressed in the $CD138^{+}$ plasma cells of this patient, but not in the $CD138^{-}$ cells of this patient (Figure 1C). Furthermore, the truncated IRF4 protein was also not detectable in the multiple myeloma (MM) samples and cells from normal donors (Figure 1D, E). This specificity in expression substantiates the potential role of the truncated IRF4 in the TEMPI syndrome pathogenesis of this patient.

In order to decipher the role of the truncated-IRF4 protein in the aberrant plasma cell transcriptional program, single-cell RNA-seq (scRNA-seq) was performed, incorporating data from eight healthy controls to construct normal bone marrow cell types, particularly the scarce plasma cell population. A notable 11.2-fold expansion of the plasma cell population, with high expression of the rearranged IRF4-BLOC1S5, was observed in the patient (Figure 2A, B, D; *Online Supplementary Figure S2*). Furthermore, incorporating B-cell antigen receptor (BCR) sequence information into the scRNA-seq analysis revealed that this uniquely expanded plasma cluster expressed one specific immunoglobulin clonotype, sharing the same third complementarity determining region (CDR3) sequence (Figure 2B, C). The results indicated that this abnormally expanded cluster shared characteristics of one common B-cell origin. Differentially expressed genes (DEG) in plasma cells between this patient and healthy controls were enriched in the KEGG pathways, such as ribosome and protein processing in endoplasmic reticulum (ER) (Figure 2E, F), implicating that highly expressed truncated IRF4 contributed to the production of monoclonal immunoglobulin and the survival and expansion of monoclonal plasma cells.

In order to further dissect the contribution of the *BLOC1S5* moiety to the pathogenic role of the truncated *IRF4*, we mapped the chromatin occupancy of H3K27 acetylation (H3K27ac) on the *BLOC1S5* gene locus and found that high H3K27ac signals were enriched on the *3'UTR* of *BLOC1S5*, i.e., the genomic locus fused to the IRF4 gene (*Online Supplementary Figure S3*).[5] The results suggested that the

inversion did merely truncate *IRF4*; it also repositioned the regulatory elements of the *BLOC1S5* gene in proximity to the truncated *IRF4*, thereby triggering its overexpression. In order to determine the most appropriate treatment regimen for this patient, we constructed plasma cell lines with the ectopic expression of the *IRF4-BLOC1S5* fusion. The cell models were used to test the sensitivity of the fusion-expressing cells to various therapeutic agents commonly used in TEMPI syndrome, such as bortezomib and lenalidomide. Interestingly, we found that cells with the ectopic expression of IRF4-BLOC1S5 demonstrated sensitivity to bortezomib but showed resistance to lenalidomide (Figure 3A-E). This finding was further corroborated by the recent literature, which reported a similar resistance pattern in a multiple myeloma cell line with a similar truncated IRF4 formed by an *IRF4-BTN3A3* inversion mutation.[6] Based on the *in vitro* sensitivity data and the patient's age and overall health status, a decision was made to initiate a bortezomib-based treatment regimen for this patient. After four cycles of bortezomib and dexamethasone (BD) treatment, the patient achieved a complete response with a negative minimal residual disease (MRD) status, undetectable level of the serum M protein and normalization of free light-chain K/λ ratio, hemoglobin and erythropoietin level (Figure 3F-I). The symptoms were completely relieved, with the disappearance of the

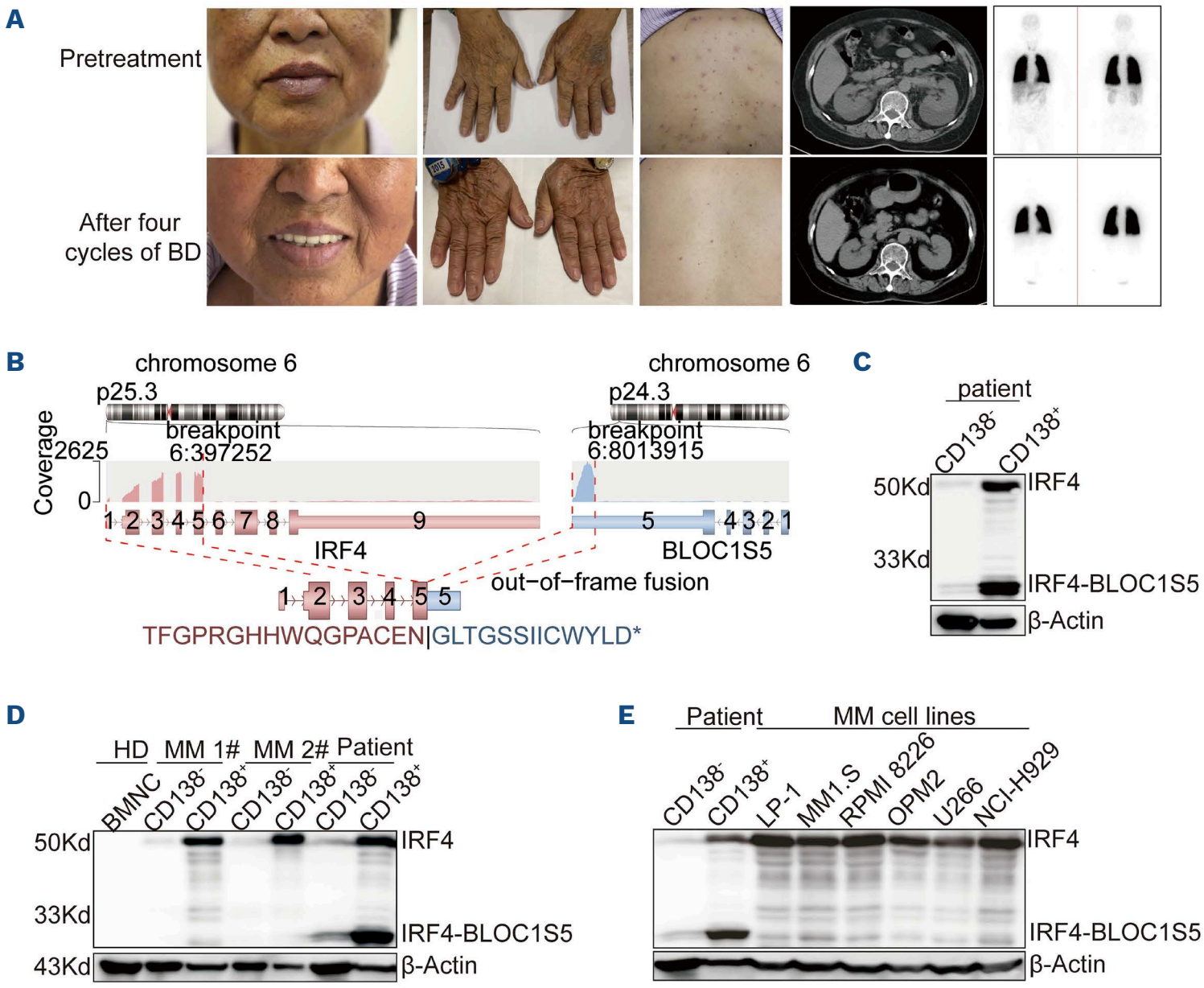

Figure 1. Identification of *IRF4-BLOC1S5* formed by chromosome inversion in TEMPI syndrome. (A) Clinical photographs of the TEMPI syndrome patient before and after bortezomib and dexamethasone (BD) treatment. The patient had typical clinical manifestations of the TEMPI syndrome, with diffuse telangiectasias on her face, hands, and back, and perinephric fluid collection and intrapulmonary shunting. These symptoms were relieved after 4 cycles of BD treatment. Cycle 1 and 2: bortezomib 1.3 mg/m^2 and dexamethasone 40 mg on day (D) 1, 4, 8, 11. Cycle 3: bortezomib 1.0 mg/m^2 and dexamethasone 40 mg on D1, 4, 8, 11. Cycle 4: bortezomib 1.0 mg/m^2 and dexamethasone 40 mg on D1, 4. (B) The schematic diagram of the rearrangement gene *IRF4-BLOC1S5*. An inversion on chromosome 6 in the region 6p25.3-6p24.3 was detected by whole-genome sequencing. The resulting transcript included the *exon 1-5* of *IRF4* and *3'UTR* of *BLOC1S5* and encoded a short N-terminal-truncated IRF4 protein, verified by RNA sequencing. (C) Expression of the truncated-IRF4 protein in CD138$^+$ cells of the patient. The CD138$^+$ cells were obtained from the bone marrow mononuclear cells (BMNC) with CD138 microbeads. The truncated-IRF4 protein was detected in CD138$^+$ cells of the patient. (D, E) Expression of the truncated-IRF4 protein in CD138$^+$ cells of multiple myeloma (MM) samples. Compared with the BMNC of the healthy donor and CD138$^+$ cells from MM patients and cell lines, the truncated-IRF4 was specifically expressed in CD138$^+$ cells of the patient.

cyanosis, telangiectasias, and perinephric-fluid collection and significant amelioration of intrapulmonary shunting (Figure 1A). Furthermore, the expression of IRF4-BLOC1S5 was significantly decreased after the BD treatment and during the clinical follow-up (up to 18 months) (Figure 3J), suggesting that *IRF4-BLOC1S5* could act as a biomarker for TEMPI syndrome treatment and prognosis.

IRF4 plays essential roles in the transition from B cells to plasma cells, orchestrating class-switch recombination and somatic hypermutation.[7-9] IRF4 activates genes essential for plasma cell identity and effector function, including *CD138*, immunoglobulin heavy/light chains, and *XBP1*.[10] The high expression of IRF4, necessary for the survival of MM plasma cells, is associated with a poor prognosis[11-13] and is often elevated due to *IgH/IRF4* rearrangements.[14] The truncated-IRF4 protein, a product of this fusion, emerges as a significant factor in the monoclonal gammopathy characteristic of TEMPI syndrome.

The exclusive expression of truncated-IRF4 in the patient's abnormal plasma cells not only offers insights into disease mechanisms but also presents potential therapeutic avenues. Targeting the aberrant protein or pathways it affects

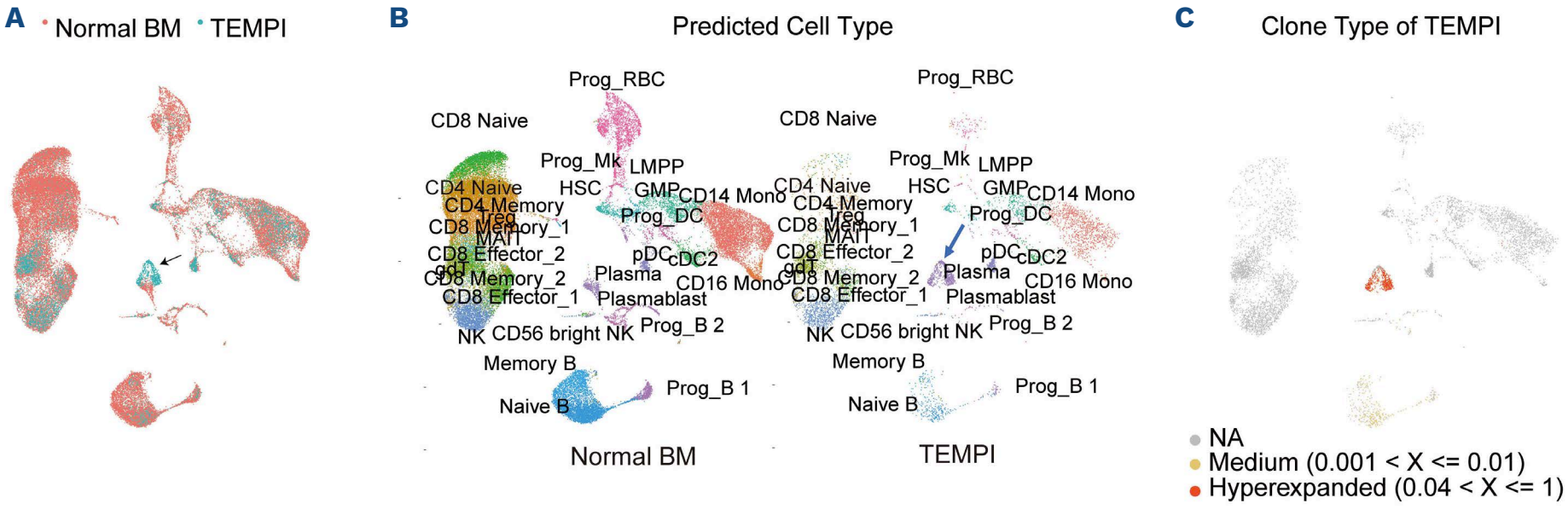

Figure 2. Strong expansion of the monoclonal plasma cell population accompanied by high expression of IRF4-BLOC1S5. (A) Cluster of cell population specially expanded in the bone marrow mononuclear cells (BMNC) of the patient. The UMAP plot integrated BMNC from normal donors and those from the patient of the TEMPI syndrome. The arrow points to a cluster of cells that was limited to the patient. (B) Plasma cells was particularly expanded in the patient. The same UMAP plot as is in (A) was separated, and the different cell clusters were identified by cell markers. The arrow points to the specifically expanded cell cluster which was mapped to plasma cells. (C) Plasma cells from the patient showed hyper-expansion and monoclonality. The UMAP plot of the patient was analyzed by B-cell antigen receptor (BCR) sequence. The color gradient corresponds to the expansion. (D) Percent of plasma cells occupied in BMNC of normal donors and the patient of the TEMPI syndrome. (E) Differentially expressed genes (DEG) in plasma cells. Comparison of the RNA profiling of plasma cells from normal donors and the patient by single-cell RNA sequencing. The DEG are shown in the dot plot. (F) Enriched KEGG pathways of DEG. The DEG from panel (E) were analyzed for functional enrichment analysis. The dot size represents the number of genes associated with a specific term. The dot color represents the adjusted *P* value.

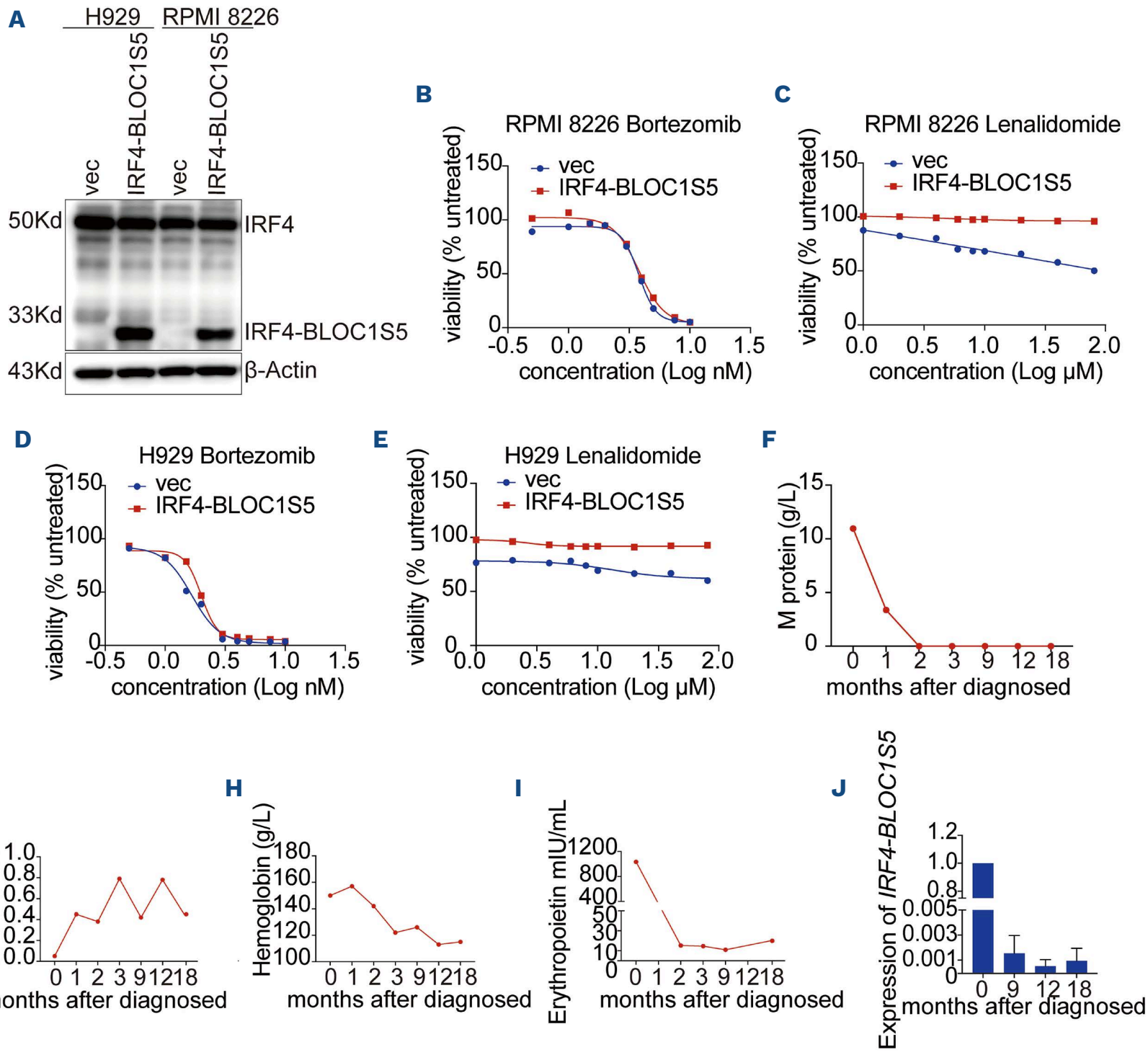

Figure 3. The TEMPI syndrome patient with high IRF4-BLOC1S5 expression was sensitive to bortezomib treatment. (A) The exogenous IRF4-BLOC1S5 fusion protein was overexpressed in the NCI-H929 and RPMI 8226 cell line. (B, C) RPMI 8226 cells overexpressing IRF4-BLOC1S5 were treated with bortezomib 0, 0.5, 1, 1.5, 2, 3, 4, 5, 7.5, 10 nM for 2 days and lenalidomide 0, 1, 2, 4, 6, 8, 10, 20, 40, 80 μM for 5 days. (D, E) NCI-H929 cells overexpressing IRF4-BLOC1S5 were treated with bortezomib 0, 0.5, 1, 1.5, 2, 3, 4, 5, 7.5, 10 nM for 2 days and lenalidomide 0, 1, 2, 4, 6, 8, 10, 20, 40, 80 μM for 5 days. CCK-8 assay was applied to detect the cell viability. (F-I) Trend of serum M-protein, free light-chain K/λ ratio, hemoglobin and erythropoietin of the TEMPI patient after bortezomib and dexamethasone treatment. (J) Expression level of IRF4-BLOC1S5 of the patient in clinical follow-ups. Quantitative real-time polymerase chain reaction was performed to monitor the expression level of *IRF4-BLOC1S*. *IRF4-BLOC1S5* was significantly decreased after bortezomib treatment. vec: Vector.

could pave the way for more effective treatments for TEMPI syndrome. The patient's positive response to bortezomib, a proteasome inhibitor, suggests that therapies targeting protein degradation pathways might be particularly promising. While this case report sheds light on possible molecular mechanisms driving TEMPI syndrome, further studies are essential. Investigating the prevalence of the *IRF4-BLOC1S5* fusion in other TEMPI patients, exploring its functional implications in cellular models or mouse models, and assessing the therapeutic potential of targeting this fusion are all critical future directions.

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Disclosures

No conflicts of interest to disclose.

Contributions

HY, KW, RBR, JML, and YLW designed the research. JL, MZ, ZLZ, ZF, MYJ and XYZ performed experiments. QY, WBX, CYW, CJY, and CW recruited the patient and did the follow-up. MZ, JL, KW, and HY analyzed the results. JL, MZ, KW, and HY wrote the paper.

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Data-sharing statement

The sequence data reported in this paper have been deposited in National Genomics Data Center, China National Center for Bioinformation/Beijing Institute of Genomics, Chinese Academy of Sciences (BioProject: PRJCA022841) that are publicly accessible at https://ngdc.cncb.ac.cn/.

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