Clinical and Experimental Medicine (2025) 25:37
https://doi.org/10.1007/s10238-025-01561-x

RESEARCH

Decreased STING predicts adverse efficacy in bortezomib regimens and poor survival in multiple myeloma

Yang Liu[1] · Yu Zhao[1,2] · Bo Li[3] · Xiaomin Chen[1] · Hao Xiong[1] · Chunlan Huang[1,2]

Received: 29 November 2024 / Accepted: 6 January 2025

Abstract

Purpose STING (stimulator of interferon genes) is involved in viral and bacterial defense through interferon pathway and innate immunity. Increased susceptibility to infection is a common manifestation of multiple myeloma (MM). Thus, we aimed to explore the clinical significance and possible mechanism of STING in MM.

Materials and methods Immunohistochemistry and qPCR were used to detect STING expression in the bone marrow of MM patients, and flow cytometry was used to detect the amount of intracellular STING. All data were analyzed with clinical characteristics.

Results STING expression was remarkably reduced in MM tissues compared to normal tissues and was not associated with stage. Multivariate analysis identified STING as an independent prognostic factor in MM patients ($P=0.001$). In the bortezomib-containing regimens, patients with low STING expression were more difficult to achieve remission. A model incorporating STING and m-SMART significantly improved the predictive accuracy of overall survival in bortezomib regimens (AUC, 0.511 to 0.630, $P=0.044$). Bortezomib efficacy has been reported to correlate with activated immunity, but the low expression group manifested as immune apathy. Although baseline characteristics showed intergroup differences in infection, the low expression group had an increased proportion of bacterial infections (1.7-fold) and a prolonged duration of antibiotic/antifungal medication (3.55 additional days); these patients were accompanied by a decreased neutrophil-to-lymphocyte ratio (NLR) and rarely activated neutrophils and leukocytes. The intracellular STING ratio was also defective in neutrophil-dominated leukocytes.

Conclusion Our study revealed that STING had a strong association with bortezomib and could serve as a potential target for immunotherapy in multiple myeloma.

Keywords Multiple myeloma · STING · Bortezomib · Immunity · Prognosis

Introduction

Multiple myeloma remains the second most common tumor in the hematopoietic system and is largely incurable [1]. During the course of repeated chemotherapy, infection is an important cause of early death in MM patients. The immune system has been shown to be involved in the pathogenesis and progression of MM, from immune induction and response processes, creating a microenvironment favorable for malignant proliferation of MM cells [2], suppressing anti-tumor immunity, and promoting dysregulation of MM proliferation in an inflammatory state [3]. This suggests that the inflammatory state may influence the prognosis of MM patients.

Stimulator of interferon genes (STING) is a linker protein located in the endoplasmic reticulum of various immune

Yang Liu and Yu Zhao have contributed equally to this work.

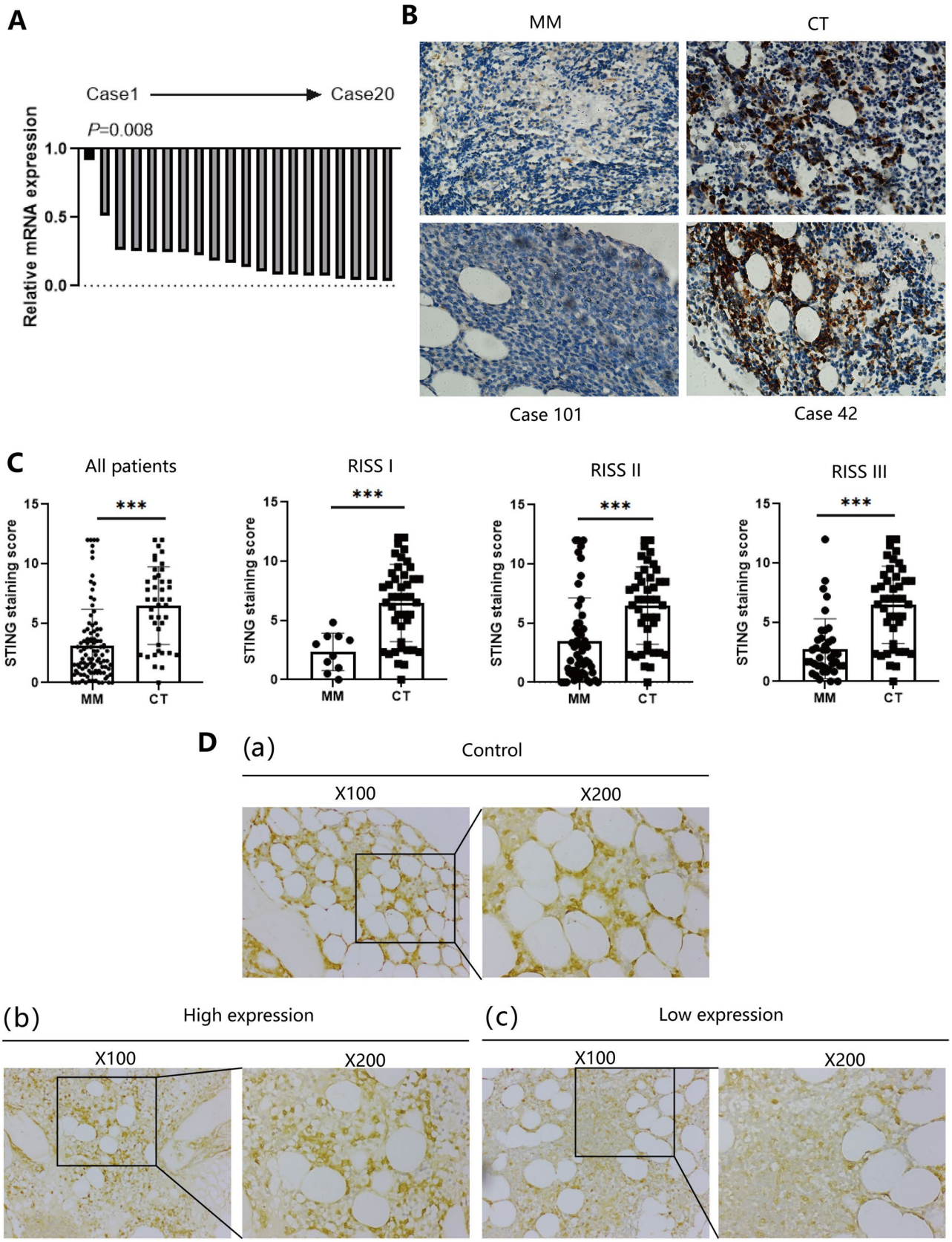
✉ Chunlan Huang
huangchunlan@swmu.edu.cn

[1] Stem Cell Immunity and Regeneration Key Laboratory of Luzhou, The Affiliated Hospital, Southwest Medical University, Luzhou, Sichuan, China

[2] Department of Hematology, The Affiliated Hospital, Southwest Medical University, Luzhou, China

[3] Department of Intensive Care Unit, The Affiliated Hospital, Southwest Medical University, Luzhou, Sichuan, China

Published online: 14 January 2025


A
Case1
Case20
P=0.008
Relative mRNA expression
1.0
0.5
0.0
B
MM
CT
Case 101
Case 42
C
All patients
RISS I
RISS II
RISS III
STING staining score
***
MM
CT
D
(a)
Control
X100
X200
(b)
High expression
X100
X200
(c)
Low expression
X100
X200

◂**Fig. 1** STING expression is down-regulated in NDMM. The STING mRNA level of NDMM ($n=20$) and healthy tissues ($n=3$) was evaluated by PCR (normal samples level normalized to 1), $P=0.008$. **B** Typical IHC staining of STING in NDMM and normal tissues (100X). **C** Mean density of immunohistochemical tests in different stages of NDMM patients ($n=101$) and healthy samples ($n=42$). ns $P>0.05$,*$P\leq0.05$, **$P\leq0.01$, ***$P\leq0.001$. **D** Representative staining of normal tissues and high or low expression of STING in MM bone marrow, staining with reduced bluing time to more clearly assess STING expression

cells and is encoded by TMEM173. As an important intracellular pattern recognition receptor, it was initially found to play a role in the perception of invasion by exogenous microorganisms (viruses, bacteria, etc.), activation of specific immunity, induction of interferon (IFN) release [4], and a key point of anti-tumor immunity that could reflect the overall state of the immune system. In recent years, several studies have highlighted its potential value in tumor immunity, including gastric, nasopharyngeal and lung cancers [5–7], as well as its important role in bone formation [8, 9]. A study obtained MM tumor regression by injection of a STING agonist [10]. In recent years, it has been reported that bortezomib, the main drug for MM treatment, induces MM-specific immunity through the cGAS/STING pathway and type I interferon production [11]. However, there are no clinical reports of STING in MM and its clinical role remains largely unknown.

Considering the relevance of MM pathogenesis to immune and bone homeostasis, STING may be involved in MM progression and prognosis. We aimed to evaluate the expression and clinical significance of STING in MM and to provide an experimental basis for therapy.

Material and methods

Inclusion and exclusion criteria

Patients with multiple myeloma who were regularly visit, excluded from immune system disorders, and cooperated with follow-up met the inclusion criteria, while those who did not were excluded.

Attrition

Patients with loss of information and missing treatment sessions were sentenced to attrition. In this study, 1 patient was missing information on RISS stage, 4 could not be assessed for m-SMART criteria, 5 had no lactate dehydrogenase, and 11 had no blood cell analysis. During the course of treatment, 7 patients were lost to follow-up for treatment information and survival.

Patient samples and information

101 newly diagnosed multiple myeloma (NDMM) and 4 relapsed/refractory multiple myeloma (RRMM) were enrolled in the study. Patient names are coded with numbers, and information was kept independently by the investigator. Clinical and pathological features, Revised International Staging System (RISS), Mayo Stratification of Myeloma and Risk-adapted Therapy (m-SMART) risk stratification, fluorescence in situ hybridization (FISH), Eastern Cooperative Oncology Group (ECOG) score, CPIS, IPS scores, treatments, efficacy, and other relevant clinical information were obtained from hospital records. Fluorescence in situ hybridization (FISH) analysis was performed on unsorted plasma cells from bone marrow samples identified using cytoplasmic immunoglobulin staining using the following probes: RB1 (13q deletion), TP53, D13S319 (deletion of the D13S319 region at 13q14), 1q21, MYC, IgH and against a single IGH rearrangement $t(11;14)$, $t(4;14)$. Patients are diagnosed and evaluated for response in agreement with IMWG criteria, and basic information is provided in Table S1 of supplementary materials.

Use IMWG-recommended antibiotics with levofloxacin and acyclovir as prophylaxis/treatment when patients receiving proteasome inhibitors are assessed to be at increased risk for infection (e.g., agranulocytosis) or when patients already have an infection at the time of their first visit. Antibiotics were not considered for otherwise ineligible patients.

The use of human tissue samples and clinical data was approved by the research ethics committee, and informed consent was obtained from all subjects and/or their legal guardian(s).

Real-time PCR

Total RNA was purified from bone marrow using TRIzol (TIANGEN), and then reversed transcription and quantitative PCR from ReverTraAce® qPCR kit and SYBR® Green Realtime PCR Master Mix (Toyobo, Japan). The primers used were as follows: Human STING, sense:CCAGAG CACACTCTCCGGTA, anti-sense:CGCATTTGGGAG GGAGTAGTA; Human TBK1, sense:TGGGTGGAATGA ATCATCTACGA, anti-sense: GCTGCACCAAAATCT GTGAGT;Human IRF3, sense: AGAGGCTCGTGATGG TCAAG,anti-sense:AGGTCCACAGTATTCTCCAGG; Human IFN-α, sense:GCTTGGGATGAGACCCTCCTA, anti-sense:CCCACCCCCTGTATCACAC;Human GAPDH, sense: GGAGCGAGATCCCTCCAAAAT, anti-sense:GGC TGTTGTCATACTTCTCATGG.

Table 1 STING expression and baseline characteristics in NDMM patients

Factors	*N*	STING expression		X^2	*P* $\alpha=0.05$
		Low $n=45$	High $n=56$		
Gender				0.012	0.912
Male	50	22	28		
Female	51	23	28		
Age(years)				0.113	0.737
< 65	61	28	33		
≥ 65	40	17	23		
R-ISS stage				1.728	0.422
I	5	1	4		
II	68	31	37		
III	25	13	12		
m-SMART 3.0				0.984	0.321
Standard-risk	41	21	20		
High-risk	56	23	33		
Immunophenotype				1.826	0.768
IgG	62	25	37		
IgA	20	11	9		
IgD	6	3	3		
Light chain type	11	6	5		
Others	2	1	1		
Infection				**4.071**	**0.044**
Yes	43	23	20		
No	49	16	33		
Objective response					
< PR	36	19	17	1.011	0.315
≥ PR	57	24	33		
Outcome				**4.672**	**0.031**
Death	45	26	19		
Survival	48	17	31		

RISS Revised International Staging System; *m-SMART* Mayo Stratification of Myeloma and Risk-adapted Therapy; Diagnosis of infection is made by a combination of patient symptoms, definitive imaging, microbiologic culture and testing of body fluids, and infectious indicators such as procalcitonin and C-reactive protein

Statistically significant values are defined in bold

Immunohistochemistry

Bake overnight at 60 ℃, soak 3 times in fresh xylene, 10 min each time, wait until the surrounding background of the specimen is clear and transparent; clean with 100-95-75% gradient alcohol, and then 5-3-5 min heating method strong antigen exposure, blocking to cut off endogenous peroxidase, add the TMEM173/STING polyclonal antibody (Proteintech, No. 19851-1-AP) or CD138(Abcam,No.ab714) after 1:150 dilution, and then add an appropriate amount of secondary antibody. After DAB visualizing, differentiation, bluing, and dehydration, add neutral resin to mount the slide and observe under microscope to take pictures. IHC staining score was assessed by two independent researchers who were blinded to the patients' clinicopathological data. The score for the extent of the IHC-stained area was set as 0 for < 5%; 1 for 5–25%; 2 for 26–50%; 3 for 51–75%; and 4 for 76–100% of tumor cells stained. The score for IHC intensity was also scaled as 0 for no IHC signaling, 1 for weak, 2 for moderate, and 3 for strong. The final score used in the analysis was calculated by multiplying the extent score and intensity score, with a series of results ranging from 0 to 12. STING values less than or equal to 1.75 were considered as the low expression, based on receiver operating characteristic (ROC) analysis of MM staining.

In the analysis of overlap rate of CD138 + and STING +, the staining results of bone marrow sections of the same patients were selected and the overlap rate was assessed by two independent researchers who were unaware of patients' clinicopathological data.

Flow cytometry

For extracellular staining of immunomarkers, we lysed erythrocytes from bone marrow fluid using BD FACS™ Lysing Solution to obtain single nucleated cells and stained them with different combinations of antibodies: leukocytes (CD11b +), M2 macrophages (CD11b + CD206 +), NK cells (CD3-CD56 +), and MM cells (CD138 +). For intracellular staining, we performed intracellular STING (Abcam, No.ab239074; Beyotime, A0423) staining after membrane rupture (invitrogen, 00-5523-00) according to the manufacturer's protocol. Fluorescence data were collected by the FACSCanto II system (BD Biosciences) and analyzed with FlowJo software.

Statistical analysis

SPSS 22.0 (IBM Corporation, Armonk, New York, USA) and GraphPad Prism 5 (GraphPad Software, La Jolla, California, USA) were used for analysis. Chi-square test was used to assess the relationship between clinical variables and tow expression groups (*P* value was 0.1). Correlation analysis was performed using Pearson's test. The Mann–Whitney *U* test was used to make comparisons between the two groups (if the data does not conform to a normal distribution). Kaplan–Meier curves were used to determine overall survival, and log-rank tests were used to compare survival curves between subgroups. Independent associations between progression-free survival (PFS), overall survival (OS) and assessed clinicopathological predictors were assessed by multivariate Cox proportional hazards regression models.

The expression level of STING was determined by ROC analysis, with the mean density as the test variables and death/survival as the state variables. The point

corresponding to the maximum of the area under the curve (AUC) was used as the segmentation, above which was considered to be high expression, and the opposite was low expression. Specific information for the ROC curves can be found in Table S2 and Fig. S1 of supplementary materials.

Meanwhile, ROC analysis was used to assess the accuracy of the model in predicting overall survival. The model was established based on logistic regression, and the model used the formula Risk Score = intercept + (estimated regression coefficient*item), and PONV = 1/(1 + e^(-risk score)). Nomogram and C-index were performed by Sangerbox, a free online platform for data analysis (http://www.sangerbox.com/tool). The results were considered to be significant at $P < 0.05$.

Results

The expression of STING is decreased in newly diagnosed multiple myeloma

Relative STING mRNA expression analysis of 20 NDMM bone marrow specimens showed that 95% were significantly reduced ($P = 0.008$, Fig. 1A). Immunohistochemistry was used to detect the level of STING in 101 bone marrow pathology sections of NDMM and 42 controls. STING was predominantly located in the cytoplasm, highly expressed in control bone marrow and down-regulated in NDMM ($P < 0.001$, Fig. 1B). Furthermore, we did not find a more significant decrease of STING in the RISS II-III subgroup than in the RISS I (Fig. 1C), indicating that the reduction of STING was not associated with the progression of multiple myeloma.

Baseline characteristics and correlation with STING expression in multiple myeloma patients

Referring to previous studies [5], NDMM patients were divided into high or low expression group according to the ROC curve (Table S2). The staining details of the two groups are compared in Fig. 1Da–c, and the differences in baseline characteristics were analyzed by chi-square in Table 1. The difference between immunophenotype and infection was statistically significant ($P < 0.05$), and there was no correlation between age, gender, stage, stratification, ECOG score, or objective response. IgG (60.4%) and IgA (19.8%) dominated the immunophenotype; 59.7% of IgG was concentrated in the STING high expression group. First-line treatment included hematopoietic stem cell transplantation, and the regimen of bortezomib + thalidomide/lenalidomide + dexamethasone, with some patients receiving the regimens of lenalidomide + dexamethasone, bortezomib + cyclophosphamide + dexamethasone, as appropriate. Due to economic deprivation, the majority of patients do not opt for transplantation; they will continue the effective regimen consolidation for 2–4 courses of maintenance regimen of lenalidomide or combined bortezomib after 4–6 courses of initial treatment.

Trends in STING expression during treatment course

STING expression was downregulated in the same patients at new diagnosis and relapse phase compared to healthy samples (healthy sample levels normalized to 1), while expression increased during the treatment (Fig. 2B). No significant difference in STING expression between treatment regimens. The re-decrease of STING in MM patients after treatment suggested possible recurrence.

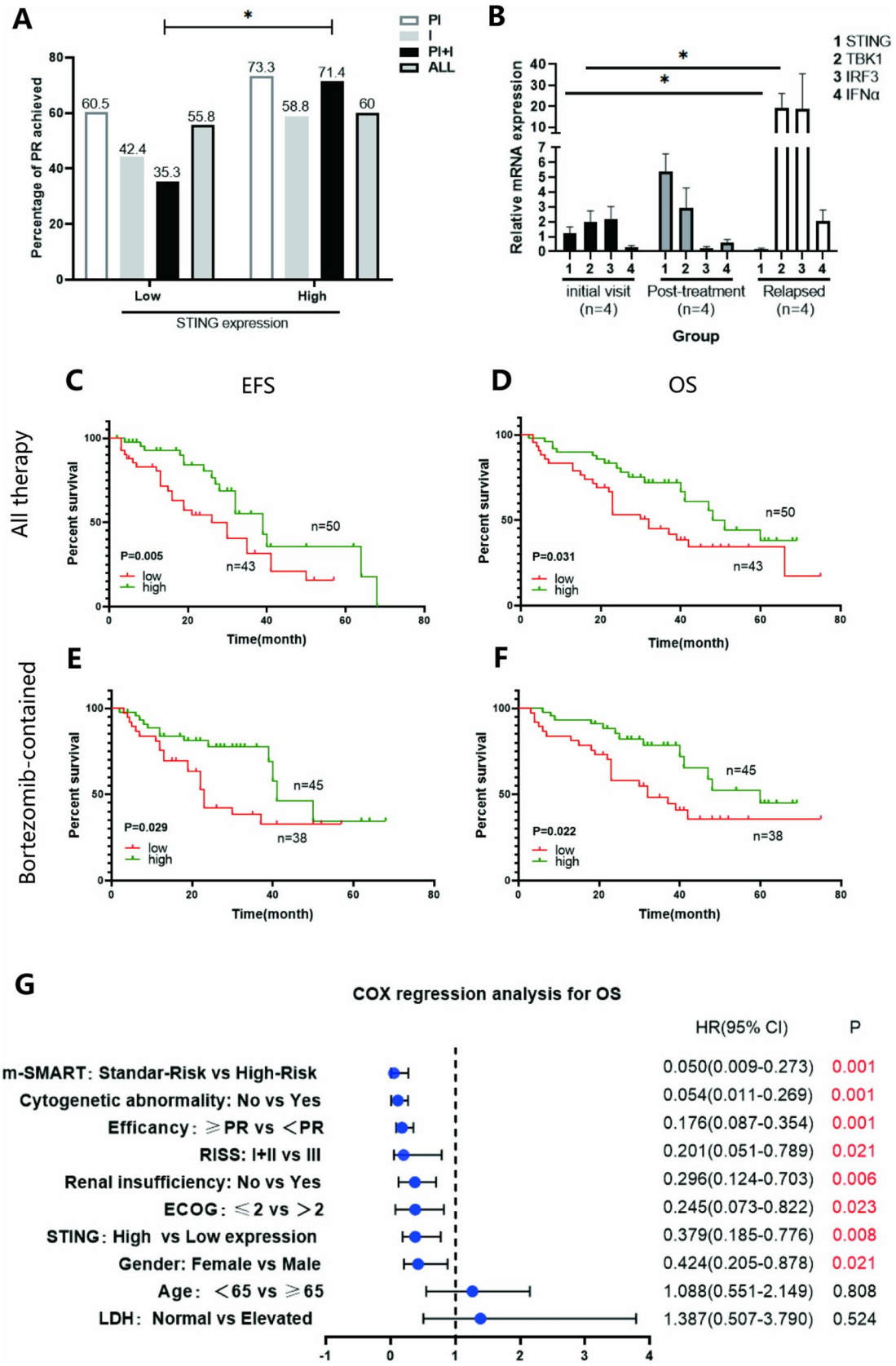
Low STING expression associated with adverse treatment response and survival in bortezomib-based regimen

Treatment data were available for 93 patients, of whom 83 (89.2%) received a bortezomib-based regimen, 35 (37.6%) received an IMiD-based regimen, and 31 (33.3%) received the bortezomib + IMiD combination. Among patients who received bortezomib combined with thalidomide, the probability of achieving partial remission (PR) was higher in the STING high-expression group (71.4% vs. 35.3%, $P < 0.05$, Chi-square test, Fig. 2A). In contrast, in patients treated with IMiD or bortezomib alone, there was no significant difference in the PR rate (IMiD, 58.3% vs. 42.9%; bortezomib: 73.3% vs. 60.5%, $P > 0.05$). This illustrates that a decreased STING is more difficult to achieve remission in regimens containing bortezomib.

Furthermore, in patients on the bortezomib regimen, we observed an association of the low STING expression group with poor progression-free survival (PFS) and overall survival (OS) (Fig. 2E, F). The low-expression group showed a cliff drop in PFS from 81.4 to 40% at 20 months, whereas the high-expression group experienced this at 40 months. Compared with data from all regimens, bortezomib significantly prolonged the time to recurrence in patients with high-expression STING (24.75 to 35.69 months) during the 20–40 months, whereas no bortezomib-specific drug advantage was observed in OS.

Association of STING expression with overall survival in multiple myeloma

The median follow-up for the entire cohort was 29 ± 19.72 months after diagnosis, and 1-, 3- and 5-year survival rates were 85.9%, 32.1% and 9.8%, respectively. Kaplan–Meier curves were used to show the relationship between STING expression and survival in NDMM patients with different treatment regimens. Patients in the low STING


A
PI
I
PI+I
ALL
Percentage of PR achieved
60.5
42.4
35.3
55.8
73.3
58.8
71.4
60
Low
High
STING expression
B
1 STING
2 TBK1
3 IRF3
4 IFNα
Relative mRNA expression
initial visit (n=4)
Post-treatment (n=4)
Relapsed (n=4)
Group
C
EFS
All therapy
Percent survival
P=0.005
low
high
n=50
n=43
Time(month)
D
OS
P=0.031
n=50
n=43
E
Bortezomib-contained
P=0.029
n=45
n=38
F
P=0.022
n=45
n=38
G
COX regression analysis for OS
HR(95% CI)
P
m-SMART: Standar-Risk vs High-Risk 0.050(0.009-0.273) 0.001
Cytogenetic abnormality: No vs Yes 0.054(0.011-0.269) 0.001
Efficancy: ⩾PR vs <PR 0.176(0.087-0.354) 0.001
RISS: I+II vs III 0.201(0.051-0.789) 0.021
Renal insufficiency: No vs Yes 0.296(0.124-0.703) 0.006
ECOG: ⩽2 vs >2 0.245(0.073-0.822) 0.023
STING: High vs Low expression 0.379(0.185-0.776) 0.008
Gender: Female vs Male 0.424(0.205-0.878) 0.021
Age: <65 vs ⩾65 1.088(0.551-2.149) 0.808
LDH: Normal vs Elevated 1.387(0.507-3.790) 0.524

◂**Fig. 2** Decreased expression of STING predicts adverse objective response rates and survival. **A** Differences in partial remission (PR) rate of chemotherapy regimens in high/low expression groups. *PI* proteasome inhibitor; *I* immunomodulatory drug; *ALL* all therapy, $*P<0.05$. **B** STING and relative pathway in MM patients at different treatment stages. $n=4$, healthy samples as a control,$*P<0.05$. **C**–**F** Progression-free survival (PFS) and overall survival (OS) in different treatment regimens (bortezomib-contained, all therapy) were examined by Kaplan–Meier analysis of relatively high or low STING expression. **G** Multivariate Cox analysis to screen for independent prognostic factors in NDMM patients

expression group had adverse PFS and OS (Fig. 2C–F). The survival rate in the low expression group decreased 1.55-fold compared to the high expression group (39.5% vs. 62%), including an 8.4% decrease in the first year.

Prognostic factors in multiple myeloma patients

Further multivariate analyses are shown in Fig. 2G, gender is female (HR,0.424;95%CI,0.205–0.878;$P=0.021$), ECOG score of 1–2 (HR,0.245; 95%CI,0.073–0.822; $P=0.023$), m-SMART for standard-risk (HR,0.050;95%CI, 0.009–0.273; $P=0.001$), without renal insufficiency (HR,0.296;95%CI, 0.124–0.703; $P=0.006$) or cytogenetic abnormality (HR,0.054;95%CI, 0.011–0.269; $P=0.001$), RISS stage (HR,0.201;95%CI,0.051–0.789; $P=0.021$), efficacy ≥ PR (HR,0.176; 95%CI,0.0876–0.354; $P=0.001$), and high STING expression (HR,0.379;95%CI, 0.185–0.776; $P=0.008$) were identified as positive and independent factors that might affected overall survival of multiple myeloma.

Improved predictive accuracy of mortality risk and overall survival combined with STING expression

To establish a more accurate survival prediction model for multiple myeloma, STING expression was combined with the m-SMART stratification to construct a nomogram to integrate and quantify these prognostic factors. A multivariate analysis combining m-SMART and STING expression demonstrated the accuracy and predictive value of statistical analysis (Fig. 3A). Our data showed that patients with low expression of STING increased the risk of death. Calibration plots show that the histograms of OS at both 1 year and 3 year have good predictive value, with higher similarity at 3 year (Fig. 3B). Kaplan–Meier curves were obtained for high- and low-risk according to the score of each subgroup (Fig. 3C). The results indicate that the model provides a good stratification of survival in MM patients.

Furthermore, to validate the effectiveness for predicting survival of bortezomib-containing regimens, we used ROC curves to compare the sensitivity and specificity of m-SMART, STING expression, and joint models for this population (Table S3). The joint model had significant value (AUC, 0.630; 95% CI, 0.507–0.753; $P=0.044$) in predicting OS of bortezomib regimens compared with m-SMART (AUC, 0.511; $P=0.757$) or STING expression alone (AUC, 0.623; $P=0.057$) (Fig. 3D). The C-index similarly showed this trend (Fig. 3E). According to these findings, STING enhanced the survival prediction value in MM patients and, moreover, considerably increased the predictive accuracy of m-SMART stratification for bortezomib-containing therapy.

Low STING expression group combined with elevated infection risk

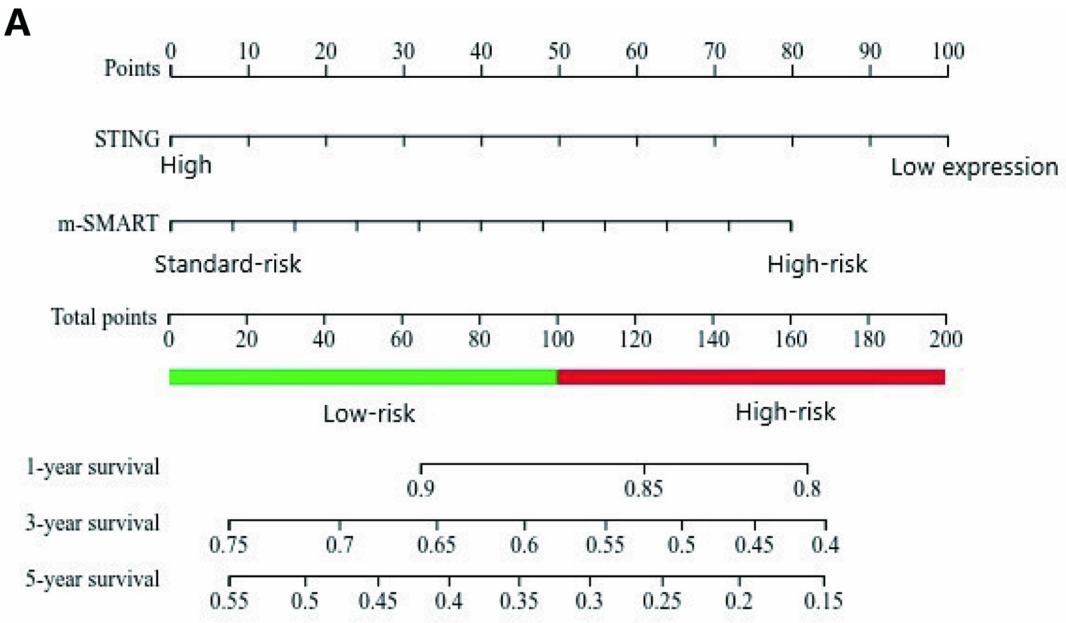
We further explored the reasons for the poor prognosis of low STING expression. The efficacy of bortezomib has been found to correlate with the immune response as well as with infections, and infection is an important cause of death in patients with MM [12]. In this study, we observed that NDMM patients with reduced STING (low expression) were more susceptible to bacterial infections. During 4 courses of chemotherapy, the low expression group had a 1.7-fold higher likelihood to develop bacterial infections (55% vs 32.2%, $P=0.024$, Fig. 4A), and they used antibiotics/antifungal drugs for 3.55 days longer on average (Table 2).

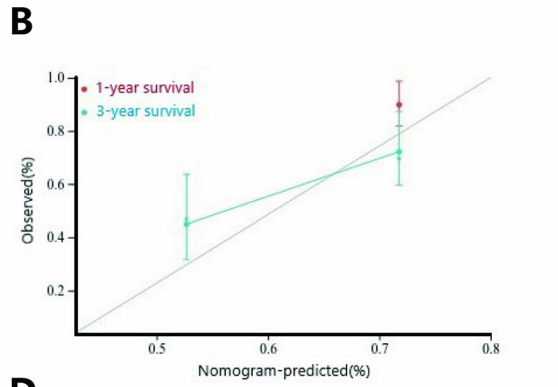
Patients with reduced STING exhibit granulocyte inactivation

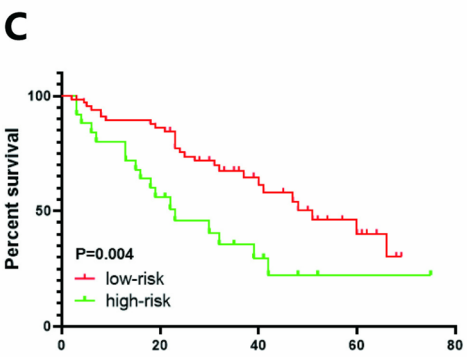
Bacterial infections are mainly associated with the granulocyte lineage; we further compared the leukocyte and neutrophil counts among two groups. Low-expression group did not show a significant elevation of granulocytes (the elevation only accounted for 8.6%), while the neutrophil–lymphocyte ratio (NLR) also decreased (Fig. 4B, C). These findings suggested that the leukocytes and neutrophils were difficult to be activated in the inflammatory state of MM patients and manifest as immune apathy or immune escape.

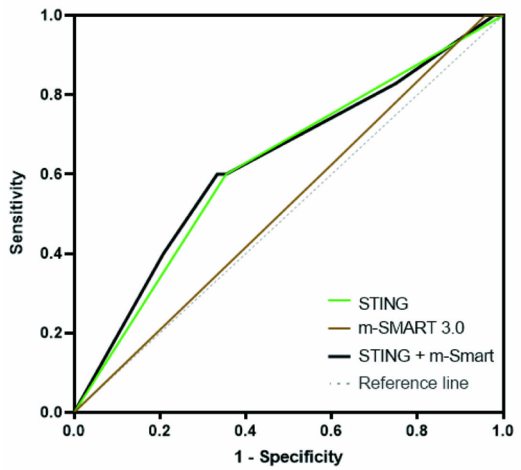
Cellular distribution of STING expression

We would like to explore which part of the process changed from normal to high and finally low expression, leading to immune apathy. We first clarified that STING reduction is mainly in tumor cells or microenvironment. By detecting the positivity of CD138 and STING at the same site by IHC, we visually compared the consistency of the positive areas to get the overlap rate (CD138 + STING +), there was no difference between MM patients and the normal group (6.81 ± 7.35 vs. 12.78 ± 10.34, $P=0.12$, Fig. 4D, F). Flow cytometry detection of intracellular STING levels in CD138 cells supported









	AUC	95%CI	P
STING	0.623	0.500-0.746	0.057
m-SMART	0.511	0.394-0.646	0.757
STING+SMART	0.630	0.507-0.753	0.044

E

Predictive Accuracy of Three Prognositic Models	
Models	C-index
STING	0.672
m-SMART	0.552
STING+m-SMART	0.792
C-index, Harrell's concordance index	

◂**Fig. 3** STING combined with m-SMART for better prediction of survival in MM patients. **A** Nomogram was used to quantify the combined effect of proven prognostic factors on 1-, 3- and 5-year overall survival. **B** Calibration plot of the nomogram for 1-year and 3-year survival rate. 5 year was not shown due to insufficient numbers of patients. **C** Patients were divided into two groups based on the total score of 0–100 and 101–200 in the nomogram, which were low and high risk subgroups. Kaplan–Meier analysis was used to assess the correlation between risk subgroups and overall survival. **D** The predictive accuracy of STING, m-SMART and joint models for OS in bortezomib regimens was compared by ROC curves. **E** The predictive accuracy of the three models was compared through the C-index

these results (Fig. 4E). The results showed that STING reduction is distributed in the tumor microenvironment.

Considering the close association of STING with bacterial infection, we further examined STING expression in the granulocyte lineage. Flow cytometry was used to detect intracellular STING in CD11b + (labeling monocytes, macrophages, neutrophils, NK cells) cells and the components contained therein, and we obtained neutrophil level by subtracting macrophage and NK cells from CD11b + cells. In MM patients, there was a significant loss of STING in CD11b + cells (54.19% vs. 68.17%, $P = 0.022$, Fig. 4F, G), but not in the NK or macrophages (Fig. S2 of supplementary material). We got the STING levels within neutrophils in MM patients were significantly lower than in control tissues, which showed that the distribution of STING reduction was predominantly in neutrophils.

Discussion

STING induces innate immunity to microorganism and genes; it has been extensively studied since the discovery of its participation in the detection of DNA fragments. Many kinds of infection-induced tumors, such as nasopharyngeal carcinoma, breast cancer and gastric cancer, can be negatively regulated by this innate immune pathway via the activation of STING signaling [5, 6]. Multiple myeloma presents a high risk of susceptibility. A study from Sweden reported that the hazard ratio for infection in MM patients versus controls was 7.1 (95% CI 6.8 to 7.4), and after the first year of diagnosis the ratio increased to 11.6 (95% CI 10.6 to 12.7) [13]. The increased risk of susceptibility may be related to modulation of the DNA sensing pathway. Our study is the first report to clarify the significance of STING in prognostic value in multiple myeloma patients and, importantly, we identified the association between STING and the clinical efficacy of bortezomib. The results also suggested that MM patients combined with an increased risk of bacterial infection, which may possibly due to inactivated granulocytes containing deficient STING expression.

In terms of efficacy and survival, it was more difficult for patients with reduced STING to benefit from a bortezomib-containing regimen. This is consistent with the findings of *Annamaria Gull*a [11] that bortezomib triggers immunogenic cell death (ICD) as well as induces MM-specific immunity mediated through the cGAS/STING pathway and type I interferon production. In our study, bortezomib efficacy was significantly correlated with STING expression. Although the causal relationship cannot be determined whether low STING expression is more difficult to be effective for bortezomib, or the inability of bortezomib to up-regulate STING expression results in poor efficacy, we reveal the association and hypothesize that a treatment regimen of STING agonists in combination with bortezomib might lead to deeper remission in MM patients.

The current commonly used prognostic assessment system for MM patients, m-SMART, has been updated since the Mayo Clinic in 2018 to include stage and gene mutations [14]. However, its ability to distinguish patient subgroups is limited due to tumor heterogeneity and host-related factors. Therefore, identification of new molecules in tumor cells that are associated with tumorigenesis will contribute to the understanding of myeloma progression. Gordon Cook et al. developed the UK Myeloma Research Alliance Risk Profile (MRP) through 2 large clinical studies (NCRI-XI and MRC-IX) in 2019 [15], which included C-reactive protein concentration as a prognostic variable and affirmed the value of indicators of infection as prognostication. Given the strong association between STING and infection immunity, we found that combining STING improved the predictive accuracy of m-SMART for survival, particularly in those who used the bortezomib regimen. The combined model highlighted the predictive value of survival in such subgroups.

Interestingly, we did not find the predictive value for STING in thalidomide or lenalidomide, which revealed that IMiDs may interfere with STING expression, at least to some extent. However, it did not affect the overall survival advantage of high expression subgroup of patients.

In our study, we identified granulocytes difficult to activate in MM patients. Neutrophils (NEUs) dominate the early inflammatory response, and these immune cells rely on NOX2-catalyzed NADPH to generate large amounts of reactive oxygen species (ROS) to kill invading pathogens, a process also known as the oxidative burst. Current studies have shown that many physiological functions of neutrophils, such as phagocytosis and the formation of neutrophil extracellular traps (NETs), are dependent on the oxidative burst [16]. It has been found that ROS production promoted the cellular release of mtDNA, which led to the activation of mtDNA-cGAS-STING signaling [17]. It can be obtained that activation of neutrophils leads to a rise in STING expression.

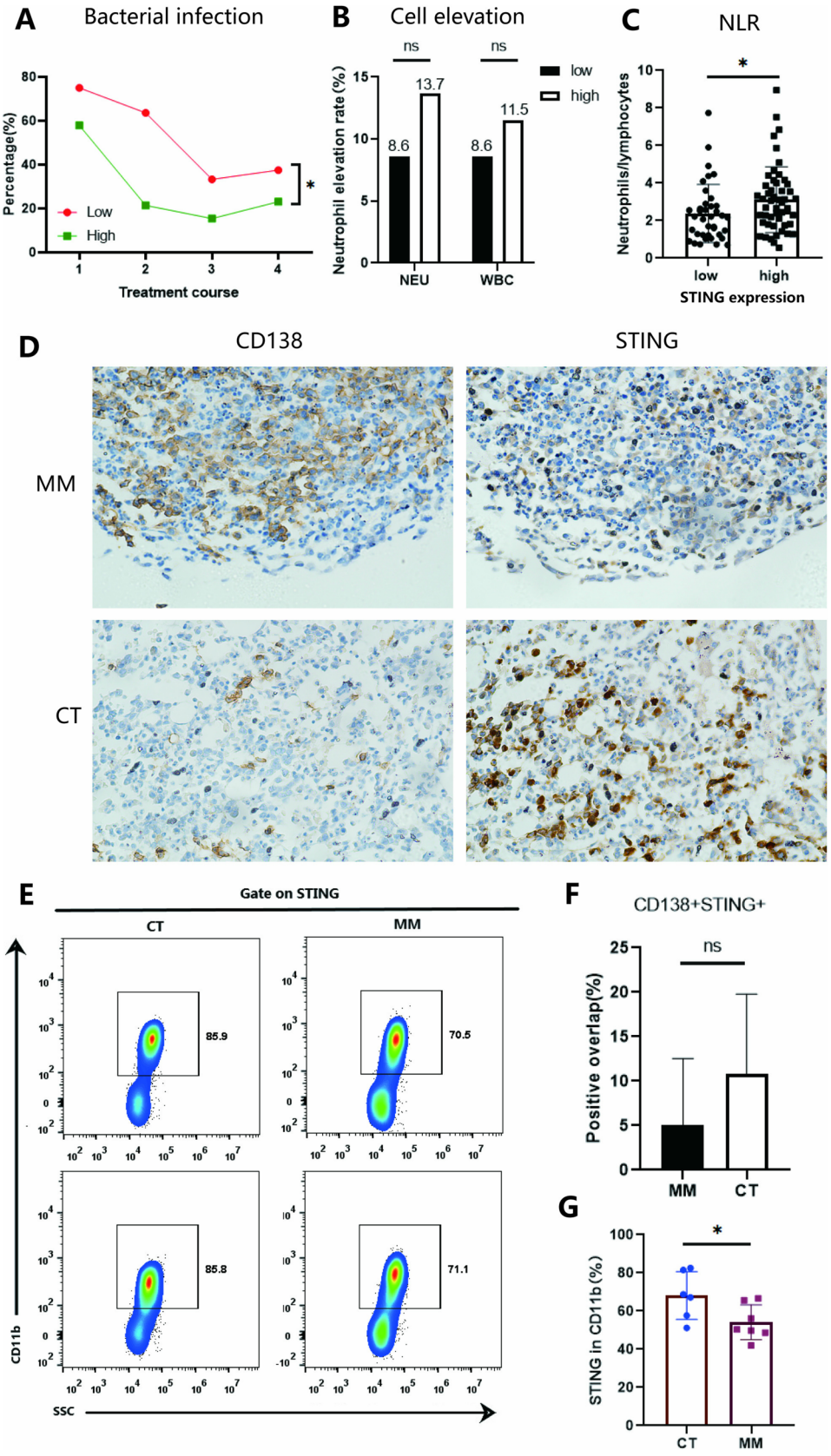


Fig. 4 Cellular distribution of STING expression. **A** Bacterial infections in STING relative high and low expression groups during treatment courses, $^{*}P < 0.05$; **B** Neutrophil and leukocyte activation ratio(%) in STING subgroups, and cell counts above the upper limit were defined as activation, ns $P > 0.05$; **C** Neutrophil-to-lymphocyte count ratio (NLR) in STING subgroups,$^{*}P < 0.05$; **D** Overlap of CD138 + and STING + positivity was manually assessed by IHC; **E** Representative flow cytometric analysis of intracellular STING of CD11b cells in multiple myeloma and normal tissues. Plots were gated on STING + cells. CT, control; MM, multiple myeloma; **F** Percentage overlap of CD138 + and STING + expression. Data are shown as mean ± SD; ns $P > 0.05$; **G** Intracellular STING levels in CD11b + cells. Data are shown as mean ± SD;$^{*}P < 0.05$, ns $P > 0.05$

Table 2 Infection statistics during treatment courses in NDMM patients with different STING expressions

Initial visit, before chemotherapy				*P*
Factors	STING expression		X^2	
	High	Low		
Fungal infections rate (%)	16.9	27.5	0.884	0.347
Bacterial infection rate (%)	32.2	55.0	5.106	**0.024**
Antibiotic usage (%)	32.2	55.0	5.106	**0.024**
Duration of antibiotics/antifungal drugs(average day)	4.47 ± 7.92	8.02 ± 9.51	–	**0.036**

Statistically significant values are defined in bold

And we detected a decrease in intracellular STING levels in neutrophil-dominated granulocytes, consistent with neutrophils being difficult to be activated. This also emphasizes that restoration of STING expression in the microenvironment during treatment may represent activation of tumor and infection immunity. At the same time, due to the severe immunosuppression in MM patients, more attention should be paid to the prophylactic use of antibiotics to avoid early death from pre/post-chemotherapy infections.

STING was detected in both the tumor and the interstitium of MM patients. At the same time, we found a tendency overlap some areas of high STING expression and CD138 positivity, suggesting that STING may have different expression and roles in MM malignant plasma cells. Overall, the profound molecular roles of STING signaling in multiple myeloma remains far from being fully elucidated and needs to be further explored.

In conclusion, response (PR or better) is difficult to achieve in patients with low STING expression treated with bortezomib-based regimens, showing poorer overall survival and higher early mortality. A more accurate early prognostic prediction model could be formulated by combining STING expression with the m-SMART system. Accordingly, STING may become a new biomarker for MM treatment and prognosis, and targeting it might provide a novel avenue for immunotherapy of MM.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10238-025-01561-x.

Acknowledgements The authors thank all the patients involved in this study.

Author contributions All authors have read and approved the manuscript. Conceptualization was done by Y.L. and C.H. Collection of tissue samples and clinical information was performed by Y.L. and Y.Z. Material preparation and immunohistochemistry were performed by Y.L., Y.Z., B.L., and X.C. The data collection and analysis were performed by Y.L., Y.Z., H.X., and B.L. Writing, review and editing were done by Y.L. and C.H. All authors read and approved the final manuscript.

Funding This study was supported by Luzhou Municipal Government—Southwest Medical University Cooperation Application Foundation (Grant No. 2023LZXNYDJ045) and Academic Research Projects of Southwest Medical University (Grant No. 2024ZKY040).

Data availability All Figures and Tables in the manuscript, as well as data generated or analyzed in the study, are available in Supplementary Materials.

Declarations

Conflict of interest The authors declare no competing interests.

Ethical approval and consent to participate This study was approved by the Institutional Review Board of Southwest Medical University.

References

1. Kumar SK, et al. Multiple myeloma. Nat Rev Dis Primers. 2017;3:17046.
2. Kawano Y, et al. Multiple myeloma and the immune microenvironment. Curr Cancer Drug Targets. 2017;17(9):806–18.
3. Weitzmann MN. Bone and the immune system. Toxicol Pathol. 2017;45(7):911–24.
4. Su T, et al. STING activation in cancer immunotherapy. Theranostics. 2019;9(25):7759–71.
5. Song S, et al. Decreased expression of STING predicts poor prognosis in patients with gastric cancer. Sci Rep. 2017;7:39858.
6. Zhang CX, et al. STING signaling remodels the tumor microenvironment by antagonizing myeloid-derived suppressor cell expansion. Cell Death Differ. 2019;26(11):2314–28.
7. Della CC, et al. STING pathway expression identifies NSCLC with an immune-responsive phenotype. J Thorac Oncol. 2020;15(5):777–91.
8. Baum R, et al. STING contributes to abnormal bone formation induced by deficiency of DNase II in mice. Arthritis Rheumatol. 2017;69(2):460–71.
9. Chen X, et al. STING inhibition accelerates the bone healing process while enhancing type H vessel formation. FASEB J. 2021;35(11):e21964.
10. Tang CH, et al. Agonist-mediated activation of STING induces apoptosis in malignant B cells. Cancer Res. 2016;76(8):2137–52.
11. Gulla A, et al. Bortezomib induces anti-multiple myeloma immune response mediated by cGAS/STING pathway activation. Blood Cancer Discov. 2021;2(5):468–83.
12. Garcia DVSJ, et al. HLC pair suppression as a risk factor for bacterial bloodstream infections and early mortality in newly

diagnosed intact immunoglobulin multiple myeloma patients. Front Oncol. 2021;11:599532.
13. Friman V, et al. Secondary immunodeficiency in lymphoproliferative malignancies. Hematol Oncol. 2016;34(3):121–32.
14. Gonsalves WI, et al. Utilization of hematopoietic stem cell transplantation for the treatment of multiple myeloma: a mayo stratification of myeloma and risk-adapted therapy (mSMART) consensus statement. Bone Marrow Transpl. 2019;54(3):353–67.
15. Cook G, et al. A clinical prediction model for outcome and therapy delivery in transplant-ineligible patients with myeloma (UK myeloma research alliance risk profile): a development and validation study. Lancet Haematol. 2019;6(3):e154–66.
16. Zatti M, Rossi F. Early changes of hexose monophosphate pathway activity and of NADPH oxidation in phagocytizing leucocytes. Biochim Biophys Acta. 1965;99(3):557–61.
17. Liu Z, et al. XBP1 deficiency promotes hepatocyte pyroptosis by impairing mitophagy to activate mtDNA-cGAS-STING signaling in macrophages during acute liver injury. Redox Biol. 2022;52:102305.