

An inflammatory T-cell-stromal axis contributes to hematopoietic stem/progenitor cell failure and clonal evolution in human myelodysplastic syndrome

Corresponding Author: Professor Marc Raaijmakers

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

In this manuscript, the authors established single-cell transcriptional taxonomy of human lower-risk MDS. They revealed that BM stromal cells undergo inflammatory remodeling independently of genetic lesions. They identified that a subset of CD8+ T cells is responsible for stromal inflammation through TNF-receptor signaling that eventually drives clonal expansion and disease progression.

The results are globally interesting and novel.

The manuscript is well-written and clear.

However, the manuscript is very descriptive and lacks functional validation studies.

The scRNA-seq experiments were well performed but not validated by BM biopsy analysis or another type of analysis, as reported below. The reviewer thinks that further validation by multiple immune-fluorescence may add value to the study. Particularly, it may give an indication of the interactions between BM cells. While this reviewer is familiar with tools such as CellPhoneBD, the reviewer believes these are only predictive tools that need further validation.

Moreover, the cell-based composition of the T cell compartment in BM needs to be validated by flow cytometry analysis. Expression analysis often may be wrong because some antigens may be expressed at a lower level. Surface marker validation is needed.

The second part of the study (Figure 6) is more convincing and of potential interest.

Fig 7. The author should demonstrate functionally that MDS cells are resistant to stromal inflammatory signaling (by performing the same experiments shown in Fig 6 using LR-MDS with a very high tumoral burden; VAF > 40%)

Reviewer #2

(Remarks to the Author)

This manuscript provides important insights into the role of inflammation in the pathogenesis of myelodysplastic syndromes (MDS). The authors show that inflammatory remodeling of the bone marrow microenvironment is a common feature of MDS and that this may contribute to clonal evolution and leukemic progression. The identification of the SIGHT gene signature may be useful for prognostication and therapeutic targeting in MDS.

I have three specific comments:

It is important to show that the IBMSC score is not a surrogate for disease heterogeneity. Please provide more data to address whether the BMSC score is associated with the mutational landscape in MDS. For example, it would be interesting to know if there is an association between the IBMSC score and the number of mutations in SF3B1, TET2, and ASXL1.

It would be important to assess the expression of Granzyme B and Perforin-1 in the IFNG+ EM CD8+ T-cell subset. Please provide data on the expression of these genes by RNA-seq and flow cytometry.

Please provide a rationale for the selection of the 9 genes that comprise the SIGHT-P score. Please include data showing that the expression of these genes is correlated with the IBMSC score.

Minor point:

The issue of inflammatory microenvironment in the progression of myeloid neoplasms has been previously investigated and some axes involving stromal cells and persistent immune stimulation have been explored (e.g. Tripodo C et al Cancer Res 2017)

Reviewer #3

(Remarks to the Author)

The authors have conducted a comprehensive study using single-cell sequencing data from human MDS patients, with an emphasis on stromal cells, which are well-represented in their dataset. This is further corroborated by bulk RNA sequencing data from a larger cohort of MDS patients, demonstrating alterations in inflammatory markers. Through potential interactions with effector memory (EM) CD8+ T cells, the study links stromal inflammation to the hematopoietic compartment, providing more understanding of the cellular dynamics within MDS. While the role of inflammatory cytokines in contributing to clonal expansion is not a novel concept, the detailed mapping of cell types involved and their interactions adds value to the field. The authors introduce a stromal inflammation signature termed SIGHT and validate its impact on leukemic transformation using both their data and external patient datasets.

Overall, the manuscript is well-written and presents the findings in a logical and accessible manner. The experiments are well designed, leveraging primarily human samples, which are challenging to obtain and analyze, particularly stromal cells. The insights derived are valuable and contribute to the understanding of MDS pathogenesis.

Questions:

The authors concentrate their analysis on low-risk MDS. I assume this is because the patients are from a clinical trial focusing on that group. It would be appropriate for the authors to comment in the discussion as to whether other groups would be envisioned to have different biology. Do the authors find different inflammatory markers in more advanced MDS?

Prior studies have shown distortions in the abundance of specific stromal cell populations with myeloid disease models in mice. How do the data here compare?

Referring to mutant cells interchangeably as 'clonal' is a bit confusing, particularly when referring to lymphoid or stromal cells. It seems that the authors uniformly mean 'mutant' since other clonal markers are not examined; it would be good to clarify that as clonal selection may be ongoing on the microenvironment side but just not measured.

The authors should consider softening the conclusion that the inflammatory microenvironment as a 'driver' of the phenotype. The data supporting this are largely correlative apart from the in vitro culture of normal HSPC. If such systems could show an alteration in the competitive growth of mutant and wild type cells, the conclusion of causation would be more supported.

The authors report increased Annexin 1a expression in stromal cells within the MDS microenvironment. Does targeting Annexin 1a in the co-culture system alter the impact on normal HSPC? Since drugs are available that alter production of Annexin-1, showing its relevance to the disease phenotype would enhance the translational potential of the study.

In Figure 1A, the term "lymphoid" is used in the sorting strategy, yet no lymphoid markers are depicted. As the population likely includes lymphoid cells (as shown by scRNA-seq), it would be more precise to label this fraction as "non-myeloid" unless specific lymphoid markers were used during sorting.

Figure 2C: The use of blue and green colors makes certain elements difficult to distinguish. Adjusting the color palette or employing distinct patterns would improve readability.

Figure 5H: The authors mention protein synthesis in the text, but it is not in the figure. How do the authors conclude about protein synthesis? For dense panels, emphasizing targets of interest using annotations would enhance ease of comprehension.

Reviewer #4

(Remarks to the Author)

The manuscript proposed by Chen & Bian et al. to Nature Communications presents an informative study. The authors have collected valuable scRNA-seq data of human LR-MDS and provide a comprehensive classification of the cell types in bone marrow stromal niches. They explored the potential sources, characteristics, and impact of inflammatory responses in bone marrow stromal niches and validated their findings through experiments. Furthermore, based on differentially expressed genes, they proposed prognosis-related scores, the iBMSC score and the SIGHT-P score. This is an impressive and important work. We do have the following concerns:

1. The BMSC-2 subpopulation shown in Figure 2c is a very important cell subpopulation in the manuscript. The iBMSC score is also defined by the genes upregulated in this cell population. However, there is still room for further in-depth exploration of the characteristics of the BMSC-2 subpopulation. Firstly, are these cells significantly different from other subpopulations? According to the results shown in Figure 2e, the BMSC-3 subpopulation also exhibits high expression of

inflammatory responses and TNFa signaling. The GSEA results shown in Figure S1c may be due to the other subpopulations in the 'other BMSC clusters' masking the BMSC-3 subpopulation's signal due to their numerical dominance. We recommend that the authors use dotplots or other forms to display the marker genes of these BMSC subpopulations to facilitate viewing of the unique characteristics of the BMSC-2 subpopulation, such as one or two specifically highly expressed genes. Additionally, the current scRNA-seq data in the manuscript only includes 4 NBM samples and 5 LRMDs samples. We recommend that authors use other public data or new data to verify the widespread existence and proportional changes of the BMSC-2 subpopulation.

2. The 165 upregulated genes of the BMSC-2 subpopulation shown in Figure 3a, which make up the iBMSC score gene set, are not provided. Additionally, we think that the information presented in the left panel of Figure 3a is limited and could be omitted.

3. In Figure 4a and Figure S2d, it can be observed that IFNG and TNF are expressed in both CD8+ T cells and NK cells. Therefore, NK cells have the potential to contribute to the interactions of the IFNG signaling pathway. We are curious whether NK cells are also one of the sources of the inflammatory remodeling of BMSCs. We suggest that the authors further explore the potential involvement of NK cells to enhance the comprehensiveness of the discussion.

4. The authors used correlation analysis and studies in mice to demonstrate the impact of stromal inflammation on HSPCs. However, from the interaction results presented in Figure S2a, it appears that CD8+ T cells also engage in inflammation-related interactions with HSPCs. Therefore, we are curious whether the changes in the transcriptional status and function of HSPCs are solely induced by stromal inflammation. Do CD8+ T cells or NK cells directly participate in this process?

5. SIGHT-P is a very important gene set. We would like the authors to provide the regularization parameters used for the cross-validation penalty regression in the Methods section, which would help the reproduction of the results.

Other comments:

1. Figure 1b lacks readability. It is recommended to enlarge the figure and avoid overlapping text.
2. There are no legends in Figure 1d.
3. There are no legends in the left panel of Figure 2e. In the violin plot of Figure 2e, is the difference between groups significant?
4. In the violin plot showing the expression of IL7 in Figure 2g, 'padj' is labeled, which represents the adjusted p-value. Is the p-value here adjusted?
5. Line 216: 'FOXOP3+CTLA4+ regulatory T cells' should be written as 'FOXP3+CTLA4+ regulatory T cells'.
6. Line 585: 'AddGeneScore' should be written as 'AddModuleScore'.
7. 'RNAseq' should be written as 'RNA-seq'.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

The authors have answered my questions.
This is a novel and important study

R#1's mediating comments for missing R#2:

I think that the authors responded to all concerns of Rev #2, thus I have accepted the MS

Reviewer #3

(Remarks to the Author)

The authors have responded appropriately to all my concerns.

Reviewer #4

(Remarks to the Author)

My reviewer points have been well addressed. I have one comment. Details of the deconvolution conducted on the BMSC LR-MDS bulk RNA-seq dataset should be supplemented in the Materials and Methods section.

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First of all, we would like to thank all reviewers for their kind remarks and thoughtful comments. Their efforts are really appreciated and we believe that addressing the comments has further substantially improved the quality of the paper. Here, we will address the reviewer's comments on a point-to-point basis:

Reviewer #1 (Remarks to the Author):

In this manuscript, the authors established single-cell transcriptional taxonomy of human lower-risk MDS. They revealed that BM stromal cells undergo inflammatory remodeling independently of genetic lesions. They identified that a subset of CD8+ T cells is responsible for stromal inflammation through TNF-receptor signaling that eventually drives clonal expansion and disease progression. The results are globally interesting and novel. The manuscript is well-written and clear.

We thank the reviewer for these kind comments

However, the manuscript is very descriptive and lacks functional validation studies. The scRNA-seq experiments were well performed but not validated by BM biopsy analysis or another type of analysis, as reported below. The reviewer thinks that further validation by multiple immune-fluorescence may add value to the study. Particularly, it may give an indication of the interactions between BM cells. While this reviewer is familiar with tools such as CellPhoneBD, the reviewer believes these are only predictive tools that need further validation. Moreover, the cell-based composition of the T cell compartment in BM needs to be validated by flow cytometry analysis. FACS on T cells Expression analysis often may be wrong because some antigens may be expressed at a lower level. Surface marker validation is needed.

We thank the reviewer for these valuable comments. In the revised version of the paper, in order to validate the (predictive) scRNAseq data, we have:

1. Performed multi-parameter *in situ* immune-fluorescence on bone marrow sections of low-risk (LR) *SF3B1*-mutated MDS to reveal very close anatomical proximity between a subset of CD8 T-cells and bone marrow stromal cells (p8, Fig. 4b).
2. Performed multi-parameter flow-cytometry on primary LR-MDS samples vs. NBM to validate the transcriptional data at the protein level, confirmed relative expansion of the CD8+ T EM (CCR7-CD45RA-) subset with high expression of CD69 in LR-MDS (p9, Fig. 4h-i and Fig. S2f), without relative expansion of the CD8+ TEMRA subset (CCR7-CD45RA+) and without an increase of expression in Granzyme B and Perforin-1 in comparison to the normal bone marrow (p9, Fig. S2f).

The second part of the study (Figure 6) is more convincing and of potential interest. Figure 7. The author should demonstrate functionally that MDS cells are resistant to stromal inflammatory signaling (by performing the same experiments shown in Figure 6 using LR-MDS with a very high tumoral burden; VAF> 40%)

We thank the reviewer for this excellent suggestion. In the revised version of the paper, we have indeed performed the suggested experiment, demonstrating that *SF3B1*-mutated HSPCs are relatively resistant to stromal-inflammation induced repression. Co-culture of CD34+ HSPCs from *SF3B1*-mutated MDS marrows with a high VAF (approximately 40%) (in comparison to CD34+ cells from normal bone marrow) demonstrated relative resistance to the stromal-inflammation-induced reduction in HSPC cell numbers (p13, Fig. 7e).

Reviewer #2 (Remarks to the Author):

This manuscript provides important insights into the role of inflammation in the pathogenesis of myelodysplastic syndromes (MDS). The authors show that inflammatory remodeling of the bone marrow microenvironment is a common feature of MDS and that this may contribute to clonal evolution and leukemic progression. The identification of the SIGHT gene signature may be useful for prognostication and therapeutic targeting in MDS.

We thank the reviewer for these kind words.

I have three specific comments:

It is important to show that the IBMSC score is not a surrogate for disease heterogeneity. Please provide more data to address whether the BMSC score is associated with the mutational landscape in MDS. For example, it would be interesting to know if there is an association between the IBMSC score and the number of mutations in SF3B1, TET2, and ASXL1.

We thank the reviewer for this question. In Fig. 3e and 3f, it is demonstrated that the degree of stromal inflammation is not associated with the mutational landscape in LR-MDS, at least not significantly so in the available dataset (n=45). There was also no relationship between the number of driver mutations and the degree of inflammation, supporting the notion that stromal inflammation seems independent from the genetic landscape. This has been added to the revised manuscript text (p7-8).

It would be important to assess the expression of Granzyme B and Perforin-1 in the IFNG⁺ EM CD8⁺ T-cell subset. Please provide data on the expression of these genes by RNA-seq and flow cytometry.

In the revised version of the manuscript we have:

1.included gene expression of *Granzyme B* and *Perforin-1* in the IFNG⁺ EM CD8⁺ T-cell subset, demonstrating low/absent expression of these genes in this population and a lack of increase in LR-MDS in comparison to NBM (Fig. S2e)

2.performed multi-parameter flow-cytometry on primary LR-MDS samples vs. NBM to validate the transcriptional data at the protein level, confirmed relative expansion of the CD8⁺ T EM (CCR7⁻ CD45RA⁻) subset with high expression of CD69 and Granzyme K in LR-MDS (Fig. 4h-i and Fig. S2f), without relative expansion of the CD8⁺ TEMRA subset (CCR7⁻CD45RA⁺) and without an increase of expression in Granzyme B and Perforin-1 in comparison to the normal bone marrow (Fig. S2f).

Please provide a rationale for the selection of the 9 genes that comprise the SIGHT-P score. Please include data showing that the expression of these genes is correlated with the IBMSC score.

The Sight-P (the 9-gene signature) is generated using a machine learning method (Lasso regression) with genes expressed in hematopoietic cells with high correlation with stromal inflammation (SIGHT) as input. This has now been more clearly indicated in the revised paper (p14). As described in the paper, SIGHT genes were identified based on the following criteria: 1, differentially expressed in human HSPCs in MDS vs. NBM (bulk-RNAseq); 2, differentially expressed in murine HSPCs co-cultured with OP9-IKK2 vs.OP9-EV.

SIGHT-P indeed accurately correlated with the extent of BMSC inflammation as indicated by the Spearman correlation with iBMSC scores ($\rho=0.72$, $\text{padj}<0.0001$) (p14 and Fig. 8b,c)

Minor point:

The issue of inflammatory microenvironment in the progression of myeloid neoplasms has been previously investigated and some axes involving stromal cells and persistent immune stimulation have been explored (e.g. Tripodo C et al Cancer Res 2017)

We would like to thank the reviewer for bringing this interesting paper to our attention. In the revised discussion we have referred to it, in the context of our findings. (p16, reference [71])

Reviewer #3 (Remarks to the Author):

The authors have conducted a comprehensive study using single-cell sequencing data from human MDS patients, with an emphasis on stromal cells, which are well-represented in their dataset. This is further corroborated by bulk RNA sequencing data from a larger cohort of MDS patients, demonstrating alterations in inflammatory markers. Through potential interactions with effector memory (EM) CD8⁺ T cells, the study links stromal inflammation to the hematopoietic compartment, providing more understanding of the cellular dynamics within MDS. While the role of inflammatory

cytokines in contributing to clonal expansion is not a novel concept, the detailed mapping of cell types involved and their interactions adds value to the field. The authors introduce a stromal inflammation signature termed SIGHT and validate its impact on leukemic transformation using both their data and external patient datasets.

Overall, the manuscript is well-written and presents the findings in a logical and accessible manner. The experiments are well designed, leveraging primarily human samples, which are challenging to obtain and analyze, particularly stromal cells. The insights derived are valuable and contribute to the understanding of MDS pathogenesis.

We thank the reviewer for these kind words.

Questions:

The authors concentrate their analysis on low-risk MDS. I assume this is because the patients are from a clinical trial focusing on that group. It would be appropriate for the authors to comment in the discussion as to whether other groups would be envisioned to have different biology. Do the authors find different inflammatory markers in more advanced MDS?

This is an interesting point raised by the reviewer. Although we have not been able to perform stromal sequencing in higher-risk MDS patients, the data in Figure S8 indicate that stromal inflammation (reflected by SIGHT-P) progressively increases with progression of the disease, reflected by the IPSS score. This is in line with the proposed contribution of stromal inflammation to clonal evolution/leukemic progression, although qualitative alterations of the inflammatory repertoire cannot be excluded. In the revised version of the manuscript, we have commented on this in the 'discussion'(p16).

Prior studies have shown distortions in the abundance of specific stromal cell populations with myeloid disease models in mice. How do the data here compare?

As discussed in our paper, the stromal cells captured in our study are most likely the human equivalent of murine LEPR⁺ sinusoid BMSCs that support hematopoiesis, making our data a bit challenging to compare with 'stromal' findings in murine models of myeloid neoplasms. Nevertheless, our findings seem consistent with data in mice with myeloid malignancies indicative of inflammatory remodeling and loss of HSPC supportive capacity (Rebezzo et al 2019, 10.1038/s41375-018-0350-3; Abdul-Aziz et al, 2019, 10.1182/blood-2018-04-845420; Baryawno et al 2019; doi: 10.1016/j.cell.2019.04.040).

Referring to mutant cells interchangeably as 'clonal' is a bit confusing, particularly when referring to lymphoid or stromal cells. It seems that the authors uniformly mean 'mutant' since other clonal markers are not examined; it would be good to clarify that as clonal selection may be ongoing on the microenvironment side but just not measured.

The reviewer raises an interesting and very valid point. In the revised version of the paper, we refer to the cells carrying a *SF3B1* mutation in the section 'clonal hematopoietic stem cells display resistance against stromal niche induced inflammatory stress and reside predominantly in a transcriptional 'high-output' state' as 'mutant' cells, rather than 'clonal' cells to address the point.

The authors should consider softening the conclusion the the inflammatory microenvironment as a 'driver' of the phenotype. The data supporting this are largely correlative apart from the in vitro culture of normal HSPC. If such systems could show an alteration in the competitive growth of mutant and wild type cells, the conclusion of causation would be more supported.

This is very well taken. In order to address this important point, in the revised version of the manuscript, we have performed experiments assessing the relative suppressive effect of the inflamed stroma on normal versus *SF3B1*-mutant HSPCs, providing further support to the view that mutated cells are relatively resistant to the suppressive effect of inflammatory stromal cells (p13 and Fig. 7e). Co-culture of CD34⁺ HSPCs from *SF3B1*-mutated MDS marrows with a high VAF (approximately 40%) (in comparison to CD34⁺ cells from normal bone marrow) demonstrated relative resistance to the stromal-inflammation-induced reduction in cell numbers. Nevertheless,

and also appreciating the notion that CD8 T-cells are unlikely to be the only driver of stromal inflammation, we have adapted the title from 'driving' to 'contribute to'.

The authors report increased Annexin 1a expression in stromal cells within the MDS microenvironment. Does targeting Annexin 1a in the co-culture system alter the impact on normal HSPC? Since drugs are available that alter production of Annexin-1, showing its relevance to the disease phenotype would enhance the translational potential of the study.

This is an interesting point raised by the reviewer. We have performed preliminary experiments in this direction, suggesting that Annexin a1 expression in stromal cells may indeed suppress HSPC growth and erythroid differentiation in co-culture experiments. The data, however, is preliminary and we would reasonably consider this data beyond the scope of the current manuscript (and hope the reviewer will concur)

In Figure 1A, the term "lymphoid" is used in the sorting strategy, yet no lymphoid markers are depicted. As the population likely includes lymphoid cells (as shown by scRNA-seq), it would be more precise to label this fraction as "non-myeloid" unless specific lymphoid markers were used during sorting.

We thank the reviewer for pointing this out and have corrected the term to 'non-myeloid' in the revised version of the paper.

Figure 2C: The use of blue and green colors makes certain elements difficult to distinguish. Adjusting the color palette or employing distinct patterns would improve readability.

We have increased visibility of BMSC subpopulations by adding dash-lines in the UMAP.

Figure 5H: The authors mention protein synthesis in the text, but it is not in the Figure. How do the authors conclude about protein synthesis? For dense panels, emphasizing targets of interest using annotations would enhance ease of comprehension.

We thank the reviewer for pointing this out. In the revised version of the manuscript we have omitted the term protein synthesis (on p. 10) and annotated targets of interest in Fig. 5h.

Reviewer #4 (Remarks to the Author):

The manuscript proposed by Chen & Bian et al. to Nature Communications presents an informative study. The authors have collected valuable scRNA-seq data of human LR-MDS and provide a comprehensive classification of the cell types in bone marrow stromal niches. They explored the potential sources, characteristics, and impact of inflammatory responses in bone marrow stromal niches and validated their findings through experiments. Furthermore, based on differentially expressed genes, they proposed prognosis-related scores, the iBMSC score and the SIGHT-P score. This is an impressive and important work.

We thank the reviewers for these kind words.

We do have the following concerns:

1. The BMSC-2 subpopulation shown in Figure 2c is a very important cell subpopulation in the manuscript. The iBMSC score is also defined by the genes upregulated in this cell population. However, there is still room for further in-depth exploration of the characteristics of the BMSC-2 subpopulation. Firstly, are these cells significantly different from other subpopulations? According to the results shown in Figure 2e, the BMSC-3 subpopulation also exhibits high expression of inflammatory responses and TNFa signaling. The GSEA results shown in Figure S1c may be due to the other subpopulations in the 'other BMSC clusters' masking the BMSC-3 subpopulation's signal due to their numerical dominance. We recommend that the authors use dotplots or other forms to display the marker genes of these BMSC subpopulations to facilitate viewing of the unique characteristics of the BMSC-2 subpopulation, such as one or two specifically highly expressed genes.

We thank the reviewers for this suggestion. In addition to providing all differentially expressed transcripts in the LR-MDS BMSC-2 subset in Table S2, in Fig. S1d and S1e of the revised manuscript we now show that the top5 differentially expressed genes in BMSC-2 vs. all other clusters in LR-MDS is not expressed in any other cluster, including BMSC-3.

Additionally, the current scRNA-seq data in the manuscript only includes 4 NBM samples and 5 LRMDs samples. We recommend that authors use other public data or new data to verify the widespread existence and proportional changes of the BMSC-2 subpopulation.

Unfortunately, there are no other public available datasets that represent the transcriptome of BMSCs in MDS. To further gauge the estimated abundance of BMSC-2 in LR-MDS versus the NBM, we have now performed cibersort deconvolution on the BMSC LR-MDS bulk-RNAseq dataset (n=45 for LR-MDS) through using the scRNAseq dataset as reference (Fig. S1f), supporting the view that relative expansion of the BMSC-2 cluster is a common finding in LR-MDS.

2. The 165 upregulated genes of the BMSC-2 subpopulation shown in Figure 3a, which make up the iBMSC score gene set, are not provided. Additionally, we think that the information presented in the left panel of Figure 3a is limited and could be omitted.

We thank the reviewer for this comment and now the 165 genes list has been added to Table S2 and shown in Figure 3a. We believe the left panel in Figure 3a may help to intuitively grasp the right panel and propose to maintain it.

3. In Figure 4a and Figure S2d, it can be observed that IFNG and TNF are expressed in both CD8+ T cells and NK cells. Therefore, NK cells have the potential to contribute to the interactions of the IFNG signaling pathway. We are curious whether NK cells are also one of the sources of the inflammatory remodeling of BMSCs. We suggest that the authors further explore the potential involvement of NK cells to enhance the comprehensiveness of the discussion.

We thank the reviewer for this comment. Although the upregulation of TNF and IFNG in NK cells does not meet statistical significance in differential gene expression analyses, the potential involvement of NK cells has been addressed in the revised discussion. To further appreciating the notion that CD8 T-cells are unlikely to be the only driver of stromal inflammation, we have adapted the title from 'driving' to 'contribute to'.

4. The authors used correlation analysis and studies in mice to demonstrate the impact of stromal inflammation on HSPCs. However, from the interaction results presented in Figure S2a, it appears that CD8+ T cells also engage in inflammation-related interactions with HSPCs. Therefore, we are curious whether the changes in the transcriptional status and function of HSPCs are solely induced by stromal inflammation. Do CD8+ T cells or NK cells directly participate in this process?

This point is well taken. We cannot completely exclude direct inflammatory interactions between ancillary cells in the bone marrow (including CD8+ T-cells and NK cells), as this was not the subject of our study, in which we have focused on the strongest predicted interaction based on the scRNAseq data (between CD8+ T-cells and stromal niches). This point has been addressed in the revised discussion.

5. SIGHT-P is a very important gene set. We would like the authors to provide the regularization parameters used for the cross-validation penalty regression in the Methods section, which would help the reproduction of the results.

We thank the reviewers for bringing this up. In the revised version of the manuscript, we provide the regularization parameters used for the cross-validation penalty regression in the Methods section.

Other comments:

1. Figure 1b lacks readability. It is recommended to enlarge the Figure and avoid overlapping text.

Figure 1b has been corrected accordingly

2. There are no legends in Figure 1d.

Legends were added to this figure

3. There are no legends in the left panel of Figure 2e. In the violin plot of Figure 2e, is the difference between groups significant?

A legend has been added to Figure 2e. There is a significant difference ($p < 0.001$) between BMSC-2 and all other clusters.

4. In the violin plot showing the expression of IL7 in Figure 2g, 'padj' is labeled, which represents the adjusted p-value. Is the p-value here adjusted?

The legend has been corrected, significance is defined by adjusted p-value.

5. Line 216: 'FOXP3+CTLA4+ regulatory T cells' should be written as 'FOXP3+CTLA4+ regulatory T cells'.

It is corrected accordingly, thanks for pointing it out.

6. Line 585: 'AddGeneScore' should be written as 'AddModuleScore'.

It is corrected accordingly, thanks for pointing it out.

7. 'RNAseq' should be written as 'RNA-seq'.

It is corrected accordingly, thanks for pointing it out.

Reviewer #1 (Remarks to the Author):

The authors have answered my questions. This is a novel and important study.

R#1's mediating comments for missing R#2:

I think that the authors responded to all concerns of Rev #2, thus I have accepted the MS.

We sincerely thank the reviewer#1 and #2 for the positive assessment and supportive comments on the novelty and importance of our study.

Reviewer #3 (Remarks to the Author):

The authors have responded appropriately to all my concerns.

We appreciate the acknowledgement that our revisions have addressed the concerns raised by Reviewer #3.

Reviewer #4 (Remarks to the Author):

My reviewer points have been well addressed. I have one comment. Details of the deconvolution conducted on the BMSC LR-MDS bulk RNA-seq dataset should be supplemented in the Materials and Methods section.

We would like to thank the kind remarks and thoughtful comments of Reviewer#4. We have supplemented the “details of the deconvolution conducted on the BMSC LR-MDS bulk RNA-seq dataset” in Methods section and its reference accordingly, on page 24(reference 89).