Shared Immune Features Correspond to High Risk Multiple Myeloma across Multiple Human Subtypes and Murine Models

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BACKGROUND

- Despite new therapies, ~20% of myeloma patients relapse early and die within 1–2 years.
- The 2025 IMWG genomic staging defines four high-risk (HR) groups: TP53 loss, IgH translocations + 1q gain/1p loss, double-hit chr 1, and high β_2-microglobulin with normal creatinine.
- The myeloma microenvironment (MM-TME) may have a critical role in disease emergence and treatment outcomes. Therefore, understanding the contributions of the MM-TME in high-risk scenarios is essential to optimize and refine patient risk stratification and management.
- In this study, we combined single-cell RNA-seq from newly diagnosed patients with immunocompetent mouse models to uncover lesion-specific immune programs driving aggressive disease.

METHODS

Patient selection and study design

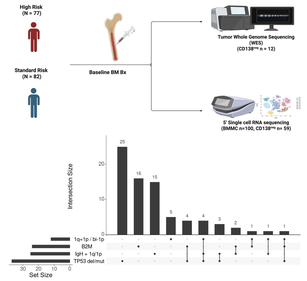
- Bone Marrow samples were collected at diagnosis from 159 NDMM patients, including 77 high-risk (**HR**) and 82 standard-risk (**SR**) patients.
- High-risk was defined by the presence of specific alterations:
- **HR Group 1**: TP53 deletion (17p13 del) and/or TP53 mutation;
- **HR Group 2**:
  - Bi-allelic deletion of 1p (1p32)
  - Gain of 1q (1q21) and deletion of 1p (1p32)
  - Either gain of 1q or deletion of 1p in combination with one of the following translocations: t(4;14) (NSD2), t(14;16) (MAF), or t(14;20) (MAFB)
- **HR Group 3**: B2M ≥ 5.5 mg/L with Creatinine < 1.2 mg/dL

Immunophenotyping by 5' single cell RNA-seq

- Single cell RNA sequencing was performed to analyze transcriptional changes in MM cells and in the MM-TME.
- Samples were filtered based on total cell count (> 1000 cells), total unique molecular identifiers (UMIs) count (> 500 UMIs), and mitochondrial gene expression.
- Our dataset was comprised of 879,588 BM cells, categorized into 63,432 myeloid cells, 60,558 NK cells, 409,675 T cells, 47,113 B cells, and 180,292 plasma cells.
- Downstream analysis was performed using R packages Seurat, CellphoneDB, SCENIC, InferCNV and SComatic.

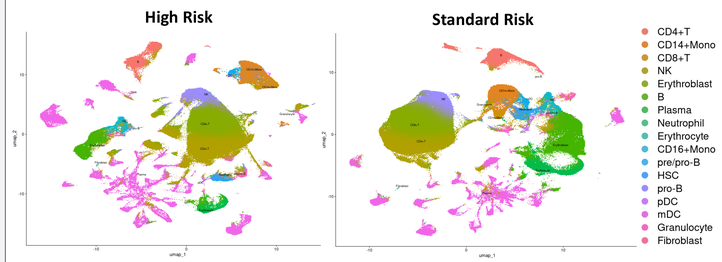
RESULTS

1. Study Design Schematic and Table of Patient Characteristics



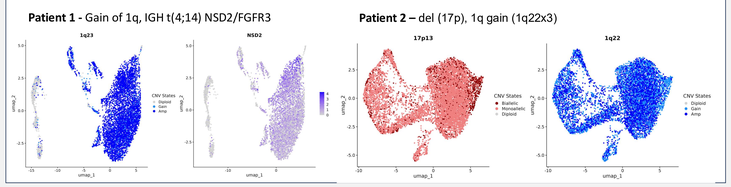
Clinical Variables	High Risk	Standard Risk
Total number of patients	77	82
Age (median)	66	64
Gender		
Male	45.45%	59.76%
Female	54.55%	49.25%
Myeloma Subtype		
Heavy chain	84.42%	84.15%
Light chain	15.58%	15.85%
High Risk Subgroups		
17p del/ TP53 mutation	48.05%	
+1q + (t(4;14), t(4;20), t(14;16))	32.47%	
B2M/Creatinine ratio	31.17%	
ISS I	25.97%	39.02%
ISS II	25.97%	23.17%
ISS III	38.96%	13.41%

2. Single Cells are Categorized into Distinct Clusters Based on Their Gene Expression Profiles.

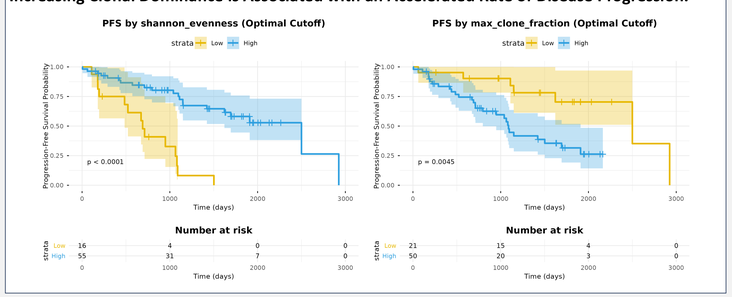


3. High-Risk Lesions are Identified at the Single-Cell Level through Integrated Tumor Analysis.

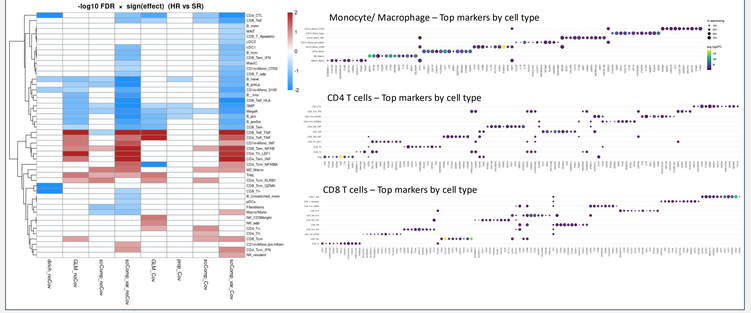
Patient 1 - Gain of 1q, IGH t(4;14) NSD2/FGFR3 **Patient 2 –** del (17p), 1q gain (1q22x3)



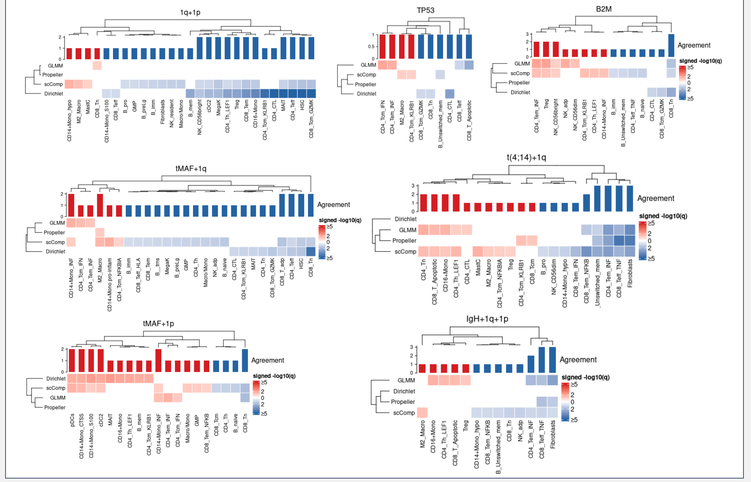
4. High Clonal Evenness is Predictive of Extended Progression-free Survival (PFS), whereas Increasing Clonal Dominance is Associated with an Accelerated Rate of Disease Progression.



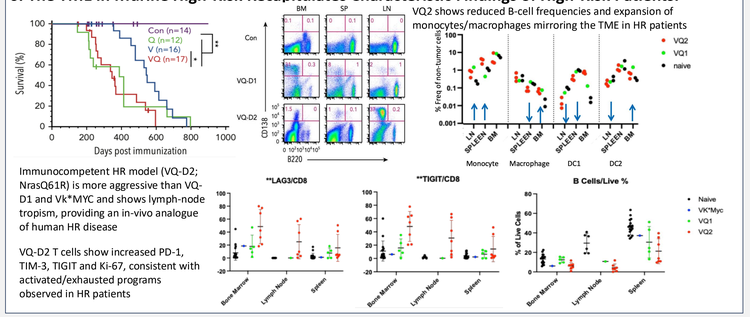
4. High-risk Patients Exhibit a Significantly Higher Frequency of Myeloid and T Cell Subsets Characterized by a Chronic Inflammatory Transcriptional Signature.



5. High Risk Subcategories are Characterized by Enrichment or Depletion of Distinct Tumor Microenvironment Cell Populations.



6. The TME in Murine High-Risk Recapitulates Characteristic Findings of High-Risk Patients.



CONCLUSIONS

- Our findings indicate that HR patients have a higher frequency of myeloid and T cell subsets associated with chronic inflammations (such as CD4 T effector TNF+ , CD8 T effector TNF+ and CD14 monocytes IFN+), and a lower frequency of B cell populations (such a B naïve, B memory and immature B cells) ($p < 0.05$), which were also identified in VQ2 High Risk Multiple Myeloma murine models.
- Low Shannon evenness and high clonal fraction serve as adverse prognostic markers, demonstrating a significant correlation with shorter Progression-Free Survival (PFS) in high-risk patients.

CLINICAL RELEVANCE

- Understanding the interplay between high-risk alterations and the MM-TME can improve patient risk stratification and lead to better treatment outcomes for HR MM.
- Single-cell analysis identified a depletion of Antigen-Presenting Cell (APC) populations and CD8 T cell subsets in the HR Cohort, which may result in a less effective cell-mediated immune response in HR MM.

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