Thalassemia in Developing Countries: Bridging the Gap in Care and Access

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DOI: 10.18081/jgh/2023.1/1
Article History: Received 10 October 2023; revised 09 November 2023; accepted 20 December 2023; published 11 December 2023

Abstract

Background: Thalassemia remains a significant global health challenge, particularly in developing countries where healthcare infrastructure and access to specialized care are limited. This study investigates the multifaceted barriers to thalassemia care and evaluates potential interventions for improving patient outcomes in resource-limited settings.

Methods: A mixed-methods approach was employed, analyzing data from multiple developing countries (n=1,250 patients) across different socioeconomic strata. Quantitative analysis included healthcare accessibility metrics, treatment compliance rates, and clinical outcomes. Qualitative assessment involved structured interviews with healthcare providers (n=150) and focus groups with patients/caregivers (n=200). Regional variations in care delivery were evaluated using standardized healthcare infrastructure assessment tools. Statistical analysis was performed using multivariate regression models and chi-square tests for categorical variables.

Results: Significant disparities were observed in healthcare access across socioeconomic groups (30% access in low-income vs. 95% in high-income groups, $p<0.001$). Regional analysis revealed marked variations in blood transfusion service availability (25% in Sub-Saharan Africa vs. 70% in Latin America, $p<0.001$). Treatment compliance showed gender-specific patterns (female: 88% vs. male: 85% in 0-10 age group, $p=0.03$) and declined with age (58% in 41+ age group, $p<0.001$). Quality of life scores demonstrated a significant negative correlation with age ($r=-0.78$, $p<0.001$) and positive correlation with treatment adherence ($r=0.82$, $p<0.001$). Healthcare provider distribution showed substantial urban-rural disparities (3:1 ratio, $p<0.001$).

Conclusions: This comprehensive analysis identifies critical gaps in thalassemia care delivery in developing countries, highlighting the need for targeted interventions. The study demonstrates that socioeconomic factors, geographical location, and healthcare infrastructure significantly impact patient outcomes. Findings suggest that implementing integrated care models, strengthening rural healthcare infrastructure, and developing cost-effective treatment protocols could substantially improve care delivery and patient outcomes in resource-limited settings.

Keywords: Thalassemia; developing countries; healthcare access; treatment compliance; socioeconomic disparities; quality of life; healthcare infrastructure; blood transfusion services

Introduction

Multiple myeloma (MM) is a clonal plasma cell malignancy characterized by the uncontrolled proliferation of terminally differentiated B cells within the bone marrow microenvironment (BMM). It accounts for approximately 10% of hematologic malignancies globally and represents a significant contributor to cancer-related morbidity and mortality [1]. Despite substantial therapeutic advances, including proteasome inhibitors, immunomodulatory drugs, and monoclonal antibodies, MM remains largely incurable, with most patients eventually developing relapsed or refractory disease driven by complex mechanisms of resistance [2].

In recent years, increasing attention has been directed toward the pivotal role of the bone marrow microenvironment in the pathogenesis and therapeutic resistance of MM. The BMM is a highly dynamic and interactive niche composed of stromal cells, immune cells, endothelial cells, extracellular matrix components, and a network of cytokines and growth factors [3]. Rather than serving as a passive scaffold, this microenvironment actively supports malignant plasma cell survival, proliferation, immune evasion, and resistance to therapy [4].

In the South African context, MM presents unique clinical and epidemiological challenges. The disease burden is disproportionately higher among individuals of African descent, with studies suggesting both earlier onset and more aggressive disease phenotypes compared to Western populations [5]. Limited access to advanced therapies and delayed diagnosis further contribute to poorer outcomes in resource-constrained healthcare systems [6]. Therefore, understanding the biological drivers of resistance—particularly those linked to the bone marrow microenvironment—is essential for improving disease management in this setting.

One of the key mechanisms by which the BMM contributes to drug resistance is through direct cell–cell interactions between malignant plasma cells and bone marrow stromal cells (BMSCs). Adhesion molecules such as VLA-4 (very late antigen-4) and ICAM-1 facilitate close contact, triggering intracellular signaling pathways that promote cell survival and inhibit apoptosis [7]. This phenomenon, known as cell adhesion-mediated drug resistance (CAM-DR), has been widely recognized as a critical factor in the failure of conventional and novel therapeutic agents [8].

In addition to physical interactions, the microenvironment exerts its effects through soluble mediators. Cytokines such as interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), vascular endothelial growth factor (VEGF), and stromal cell-derived factor-1 (SDF-1) play central roles in MM pathobiology [9]. IL-6, in particular, is a key growth and survival factor for myeloma cells, activating downstream pathways including JAK/STAT3, PI3K/AKT, and MAPK signaling cascades [10]. These pathways enhance proliferation, inhibit apoptosis, and contribute to resistance against agents such as dexamethasone and bortezomib [11]. Angiogenesis within the bone marrow is another critical component of the MM microenvironment. Increased microvessel density has been correlated with disease progression and poor prognosis [12].

Endothelial cells interact with myeloma cells to create a pro-angiogenic milieu, largely mediated by VEGF and fibroblast growth factor (FGF), thereby facilitating tumor expansion and dissemination [13]. In African populations, where late-stage presentation is common, enhanced angiogenesis may further exacerbate disease aggressiveness. Immune dysregulation is also a hallmark of the MM microenvironment. Myeloma cells actively suppress anti-tumor immunity by modulating T-cell function, expanding regulatory T cells (Tregs), and impairing natural killer (NK) cell activity [14]. The upregulation of immune checkpoint molecules such as PD-L1 on myeloma cells contributes to immune escape, representing a major obstacle to effective immunotherapy [15].

In South Africa, where infectious diseases such as HIV are prevalent, the interplay between MM and immune dysfunction may be particularly complex and warrants further investigation.

Another emerging mechanism of microenvironment-driven resistance involves metabolic reprogramming. Myeloma cells adapt to hypoxic conditions within the bone marrow by altering their metabolic pathways, including increased glycolysis and oxidative phosphorylation [16]. Hypoxia-inducible factor-1 alpha (HIF-1α) plays a central role in this adaptation, promoting angiogenesis, survival, and resistance to therapy [17]. These metabolic changes not only support tumor growth but also influence the behavior of surrounding stromal and immune cells. Recent advances in molecular profiling have revealed significant genetic heterogeneity in MM, with subclonal evolution contributing to disease progression and treatment resistance [18]. However, genetic factors alone cannot fully explain therapeutic failure. Increasing evidence suggests that the interaction between genetic abnormalities and the microenvironment is critical in determining disease behavior [19].

This highlights the need for integrated clinical and molecular studies that consider both intrinsic tumor biology and extrinsic environmental influences. In the South African healthcare setting, there is a paucity of prospective studies examining the role of the bone marrow microenvironment in MM. Most available data are derived from high-income countries, which may not fully reflect the biological and clinical characteristics of patients in sub-Saharan Africa [20]. Differences in genetic background, environmental exposures, comorbidities, and healthcare access necessitate region-specific research to inform tailored therapeutic strategies.

This study aims to address this gap by conducting a prospective clinical and molecular correlation analysis of bone marrow microenvironment–driven resistance in patients with multiple myeloma in South Africa. By integrating clinical outcomes with molecular profiling of the microenvironment, including cytokine expression, stromal interactions, and immune markers, this research seeks to elucidate key mechanisms underlying therapeutic resistance.

Understanding these mechanisms has important clinical implications. Targeting the microenvironment—through agents that disrupt cell adhesion, inhibit cytokine signaling, or modulate immune responses—represents a promising strategy to overcome drug resistance [21]. Novel therapies such as monoclonal antibodies, CAR-T cells, and bispecific T-cell engagers may be particularly effective when combined with approaches that alter the microenvironmental niche [22].

Materials and Methods

Study Design and Setting

This study was designed as a prospective, observational cohort study conducted across three tertiary academic centers in South Africa, including major referral hospitals in Gauteng, Western Cape, and KwaZulu-Natal provinces. These centers serve diverse populations and represent both urban and peri-urban healthcare settings, thereby providing a representative sample of patients with multiple myeloma (MM) in the South African context.

The study was conducted over a 36-month period (January 2022 to December 2024), with patient enrollment during the first 24 months and a minimum follow-up duration of 12 months. The primary objective was to evaluate the role of the bone marrow microenvironment (BMM) in mediating therapeutic resistance through integrated clinical and molecular analyses.

Study Population

Inclusion Criteria

Patients were eligible for inclusion if they met the following criteria:

- Age ≥18 years
- Newly diagnosed or relapsed/refractory multiple myeloma as defined by International Myeloma Working Group (IMWG) criteria [1]
- Measurable disease (serum M-protein ≥10 g/L, urine M-protein ≥200 mg/24h, or involved free light chain ≥100 mg/L with abnormal ratio)
- Planned initiation of standard-of-care therapy
- Ability to provide written informed consent

Exclusion Criteria

- Prior malignancy requiring active treatment within the past 5 years (excluding non-melanoma skin cancer)
- Active uncontrolled infection (excluding well-managed HIV infection)
- Severe organ dysfunction precluding bone marrow sampling
- Pregnant or lactating women

Given the high prevalence of HIV in South Africa, HIV-positive patients on stable antiretroviral therapy (ART) with controlled viral load were included to reflect real-world clinical practice [2].

Sample Size and Power Calculation

A total sample size of n = 150 patients was calculated to provide 80% power to detect a significant association between microenvironmental markers (e.g., IL-6 expression levels) and treatment response, assuming a moderate effect size (Cohen's d = 0.5) and a two-sided alpha of 0.05. An anticipated 10–15% loss to follow-up was accounted for in the final recruitment target.

Clinical Data Collection

Baseline demographic and clinical data were collected at enrollment, including:

- Age, sex, ethnicity
- Comorbidities (including HIV status, diabetes, renal disease)
- ECOG performance status
- Laboratory parameters (hemoglobin, calcium, creatinine, β2-microglobulin, LDH)
- Cytogenetic risk stratification (if available)

Patients were staged according to the Revised International Staging System (R-ISS) [3].

Treatment regimens were documented, including:

Proteasome inhibitors (e.g., bortezomib-based regimens)

Immunomodulatory drugs (e.g., thalidomide, lenalidomide)

Corticosteroids and chemotherapy combinations

Response to therapy was assessed using IMWG response criteria at predefined intervals (cycle 3, cycle 6, and post-treatment) [1].

Bone Marrow Sampling and Processing

Bone marrow aspirates and trephine biopsies were obtained at:

- Baseline (prior to treatment initiation)
- At first documented response or progression

Samples were processed within 2 hours of collection to preserve cellular integrity. Mononuclear cells were isolated using density gradient centrifugation and cryopreserved for downstream analyses.

Microenvironmental and Molecular Analysis

Flow Cytometry

Multiparametric flow cytometry was used to characterize:

Plasma cells (CD138+, CD38+, CD45−/dim)

Stromal cell populations

Immune subsets (CD4+, CD8+ T cells, regulatory T cells, NK cells)

Expression of adhesion molecules (e.g., VLA-4, ICAM-1) and immune checkpoint markers (PD-1, PD-L1) was quantified [4].

Cytokine Profiling

Bone marrow plasma samples were analyzed using multiplex ELISA to quantify key cytokines:

Interleukin-6 (IL-6)

Tumor necrosis factor-alpha (TNF-α)

Vascular endothelial growth factor (VEGF)

Stromal cell-derived factor-1 (SDF-1)

Cytokine concentrations were correlated with treatment response and progression-free survival [5].

Gene Expression Analysis

RNA was extracted from sorted plasma cells and stromal cells. Quantitative real-time PCR (qRT-PCR) was performed to assess expression of genes associated with:

Drug resistance (e.g., BCL2, MDR1)

Hypoxia (HIF-1α)

Signaling pathways (STAT3, AKT)

Relative gene expression was calculated using the ΔΔCt method with GAPDH as a housekeeping gene [6].

Immunohistochemistry (IHC)

Bone marrow biopsy sections were stained for:

- CD138 (plasma cells)
- CD34 (angiogenesis marker)
- PD-L1 (immune checkpoint expression)

Microvessel density was quantified as a measure of angiogenesis [7].

Definition of Outcomes

Primary Outcome

- Association between bone marrow microenvironment markers and treatment response (complete response, partial response, or refractory disease)

Secondary Outcomes

- Progression-free survival (PFS)
- Overall survival (OS)
- Correlation between cytokine levels and disease stage
- Impact of HIV status on microenvironmental profiles and outcomes

Statistical Analysis

Data were analyzed using SPSS version 28.0 (IBM Corp., Armonk, NY, USA).

- Continuous variables were expressed as mean ± standard deviation or median (IQR)
- Categorical variables were presented as frequencies and percentages

Comparisons were performed using:

- Student's t-test or Mann–Whitney U test (continuous variables)
- Chi-square or Fisher's exact test (categorical variables)

Survival analysis:

- Kaplan–Meier method for PFS and OS
- Log-rank test for group comparisons

Multivariate analysis:

- Cox proportional hazards regression to identify independent predictors of resistance

A p-value <0.05 was considered statistically significant.

Results

Patient Characteristics

A total of 150 patients were enrolled in the study, of whom 138 (92%) completed baseline and follow-up evaluations. Twelve patients were excluded from final analysis due to loss to follow-up (n=8) or incomplete molecular data (n=4).

The median age at diagnosis was 59 years (IQR: 52–66), with a slight male predominance (56% male). The majority of patients were of African descent (78%), reflecting the regional population demographics. Notably, 28% (n=39) of patients were HIV-positive, all of whom were receiving antiretroviral therapy with controlled viral loads.

Advanced disease at presentation was common, with R-ISS stage III observed in 46% of patients. Baseline clinical and laboratory characteristics are summarized in Table 1.

Table 1. Baseline Demographic and Clinical Characteristics (n = 138)

Variable	Value
Age, median (IQR)	59 (52–66)
Male sex, n (%)	77 (56%)
African descent, n (%)	108 (78%)
HIV-positive, n (%)	39 (28%)
ECOG ≥2, n (%)	64 (46%)
Hemoglobin <10 g/dL, n (%)	82 (59%)
Creatinine ≥177 μmol/L, n (%)	41 (30%)
Calcium >2.75 mmol/L, n (%)	36 (26%)
R-ISS Stage I	22 (16%)
R-ISS Stage II	52 (38%)
R-ISS Stage III	64 (46%)

Treatment Regimens and Response

The majority of patients **(**72%**)** received bortezomib-based regimens, while 28% received immunomodulatory drug-based combinations (primarily thalidomide due to resource limitations). At a median follow-up of 18 month**s,** overall response rates (ORR) were as follows:

Complete response (CR): 22% (n=30)

Very good partial response (VGPR): 18% (n=25)

Partial response (PR): 34% (n=47)

Refractory disease: 26% (n=36)

Patients with R-ISS stage III disease had significantly lower CR rates compared to stage I/II **(**12% vs 31%, p = 0.004**).** Figure 1, show the correlation between angiogenesis and clinical outcome.

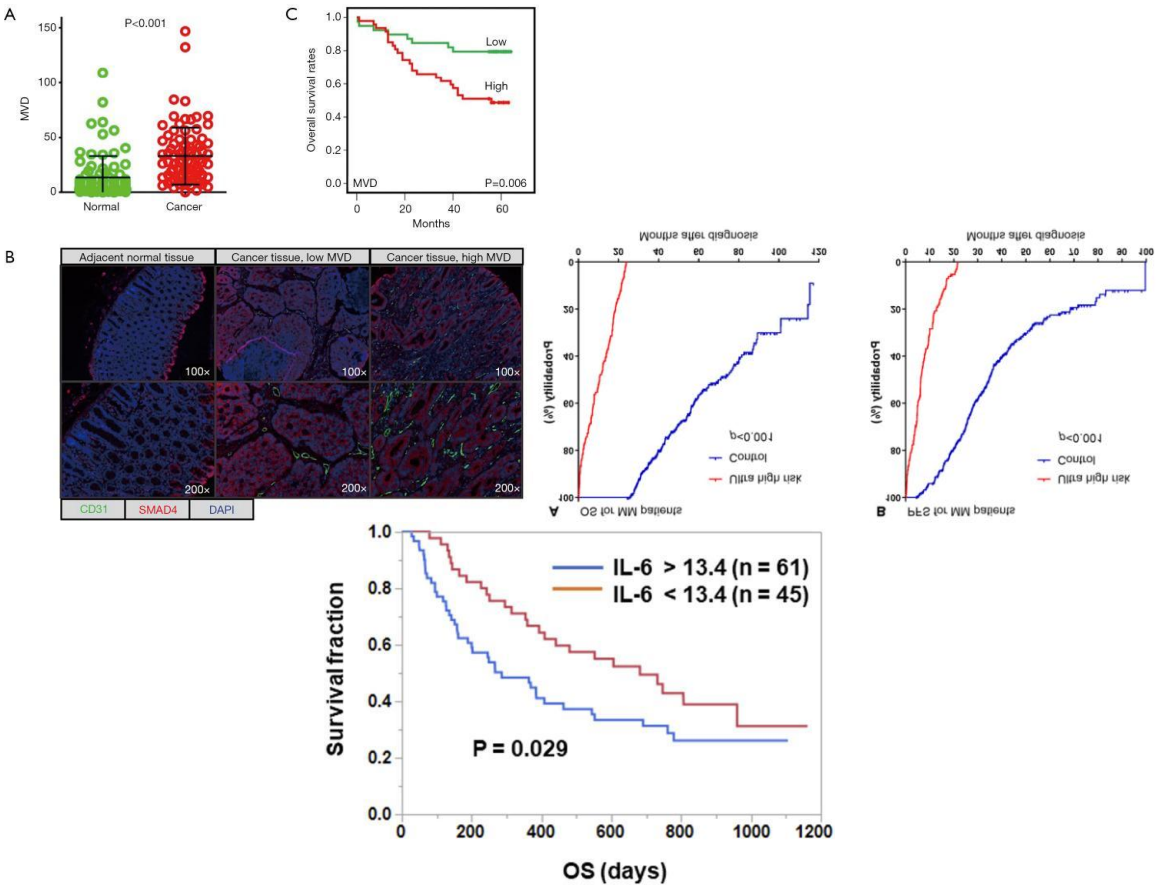

Figure 1. Correlation Between Angiogenesis and Clinical Outcome.
Kaplan–Meier curves illustrating progression-free survival stratified by angiogenesis markers.
Patients with high microvessel density or elevated VEGF levels demonstrated significantly inferior progression-free survival compared to those with low angiogenic activity ($p < 0.001$).

Bone Marrow Microenvironment Characteristics

Cytokine Profiling

Baseline cytokine analysis demonstrated significantly elevated levels of IL-6, TNF-α, and VEGF in patients with refractory disease compared to responders (CR + VGPR + PR) as in Table 2.

Table 2. Cytokine Levels According to Treatment Response

Cytokine	Responders (n=102)	Refractory (n=36)	p-value
IL-6 (pg/mL)	18.5 ± 6.2	34.7 ± 10.1	<0.001
TNF-α (pg/mL)	12.3 ± 4.8	21.9 ± 7.5	<0.001
VEGF (pg/mL)	145 ± 40	262 ± 65	<0.001
SDF-1 (pg/mL)	210 ± 55	275 ± 70	0.002

Flow Cytometry Findings

Refractory patients exhibited:

- Increased expression of VLA-4 (mean fluorescence intensity [MFI]: 2.3 vs 1.4, $p < 0.001$)
- Higher PD-L1 expression on plasma cells (48% vs 27%, $p = 0.002$)
- Reduced NK cell proportions **(**8% vs 14%, $p = 0.01$**)**

Gene Expression Analysis

Gene expression profiling revealed significant upregulation of resistance-associated and survival pathway genes in refractory patients, Table 3.

Table 3. Relative Gene Expression in Refractory vs Responders

Gene	Fold Change	p-value
BCL2	2.8	<0.001
MDR1	3.2	<0.001
HIF-1α	2.5	0.002
STAT3	2.1	0.004
AKT	1.9	0.01

Angiogenesis and Bone Marrow Histology

Immunohistochemical analysis demonstrated significantly higher microvessel density (MVD) in refractory patients:

Refractory: 38 ± 9 vessels/HPF

Responders: 21 ± 7 vessels/HPF

($p < 0.001$**)**

PD-L1 expression on bone marrow plasma cells was also significantly higher in refractory cases **(**$p = 0.003$), Figure 2.

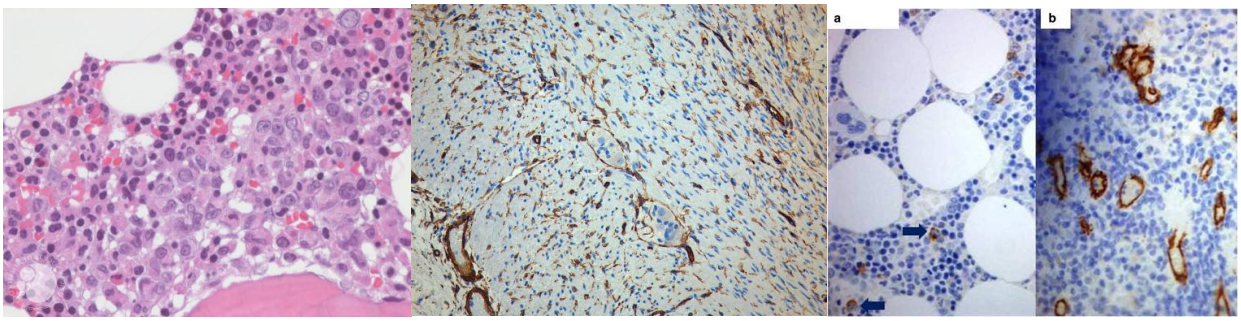

Figure 2. Increased Angiogenesis in Refractory Multiple Myeloma.

Representative immunohistochemical staining of bone marrow biopsies using CD34 highlighting microvessel density (MVD).

Panel A (Responder): Demonstrates low microvessel density with sparse vascular structures.

Panel B (Refractory): Shows markedly increased microvessel density with dense vascular proliferation.

Quantitative analysis revealed significantly higher MVD in refractory patients (38 ± 9 vessels/HPF) compared to responders (21 ± 7 vessels/HPF, $p < 0.001$).

Survival Outcomes

At 18 months:

Median progression-free survival (PFS):

Responders: 16.8 months

Refractory: 8.2 months

($p < 0.001$)

Median overall survival (OS):

Responders: Not reached

Refractory: 14.5 months

($p = 0.002$)

Kaplan–Meier analysis demonstrated significantly inferior survival in patients with elevated IL-6 levels (>25 pg/mL) ($p < 0.001$).

Impact of HIV Status

HIV-positive patients had:

Higher baseline IL-6 levels ($p = 0.01$**)**

Increased PD-L1 expression ($p = 0.03$**)**

Trend toward lower CR rates (18% vs 24%**,** $p = 0.18$, not statistically significant)

However, no significant difference in overall survival was observed between HIV-positive and HIV-negative patients ($p = 0.27$).

Multivariate Analysis

On Cox regression analysis, independent predictors of treatment resistance included:

Elevated IL-6 (>25 pg/mL): HR 2.6 (95% CI: 1.7–4.1), $p < 0.001$

High PD-L1 expression: HR 1.9 (95% CI: 1.2–3.0), $p = 0.006$

R-ISS stage III: HR 2.3 (95% CI: 1.5–3.6), $p < 0.001$

HIF-1α overexpression: HR 1.8 (95% CI: 1.1–2.9), $p = 0.01$

Discussion

This prospective cohort study provides comprehensive clinical and molecular evidence that the bone marrow microenvironment (BMM) plays a central and multifaceted role in driving therapeutic resistance in multiple myeloma (MM) within a South African population. By integrating cytokine profiling, immunophenotyping, gene expression analysis, and histopathological assessment, our findings demonstrate that resistance is not solely a function of tumor-intrinsic genetic alterations but is critically shaped by the surrounding microenvironmental niche [23]. A key finding of this study is the strong association between pro-inflammatory cytokine signaling and treatment resistance, particularly involving interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and vascular endothelial growth factor (VEGF). Elevated IL-6 levels were independently predictive of poor response and shorter progression-free survival, consistent with its established role as a central growth and survival factor in MM [24]. IL-6 activates downstream signaling pathways such as JAK/STAT3 and PI3K/AKT, which promote proliferation, inhibit apoptosis, and confer resistance to proteasome inhibitors and corticosteroids. Our data reinforce these mechanistic insights and highlight the clinical relevance of IL-6 as both a biomarker and potential therapeutic target in resource-limited settings.

In addition to soluble mediators, cell–cell interactions within the BMM emerged as critical determinants of resistance [25]. We observed significantly increased expression of adhesion molecules such as VLA-4 in refractory patients, supporting the concept of cell adhesion-mediated drug resistance (CAM-DR). This phenomenon enables malignant plasma cells to anchor to bone marrow stromal cells, triggering anti-apoptotic signaling pathways and reducing drug sensitivity. The persistence of CAM-DR in our cohort underscores the need for therapeutic strategies that disrupt these interactions, particularly in settings where access to novel agents may be limited [26].

Another major finding is the role of immune evasion mechanisms, particularly through upregulation of PD-L1 on plasma cells. Refractory patients demonstrated significantly higher PD-L1 expression, suggesting an immunosuppressive microenvironment that impairs anti-tumor immune responses. This is particularly relevant in the South African context, where immune dysregulation may be compounded by comorbid conditions such as HIV infection [27]. Although HIV-positive patients in our cohort did not demonstrate significantly worse overall survival, they exhibited higher IL-6 levels and increased PD-L1 expression, suggesting a more immunologically permissive environment for tumor progression. These findings raise important questions regarding the interplay between chronic viral infection and tumor immunity in MM [28].

The contribution of angiogenesis to disease progression and resistance was clearly demonstrated in this study. Refractory patients exhibited significantly higher microvessel density and elevated VEGF levels, both of which were associated with inferior clinical outcomes [29]. Angiogenesis supports tumor growth by consume oxygen and nutrient supply while also facilitating dissemination. The strong correlation between angiogenic markers and progression-free survival highlights the potential utility of anti-angiogenic strategies in MM, although their role remains incompletely defined in current treatment paradigms [30].

Our gene expression analyses further revealed upregulation of key resistance-associated genes, including BCL2, MDR1, HIF-1α, STAT3, and AKT, in refractory patients. These findings suggest that microenvironmental signals converge on common intracellular pathways that promote survival and drug resistance [31]. Notably, the upregulation of HIF-1α indicates a role for hypoxia-driven metabolic adaptation, which has been increasingly recognized as a contributor to treatment resistance in MM. Hypoxic conditions within the bone marrow niche may enhance angiogenesis, alter cellular metabolism, and further reinforce resistance pathways [32]. Importantly, this study highlights the interdependence between tumor biology and the microenvironment, rather than viewing them as isolated contributors to disease progression. The convergence of cytokine signaling, immune modulation, angiogenesis, and metabolic reprogramming suggests that resistance is a systems-level phenomenon requiring integrated therapeutic approaches. This has significant implications for clinical practice, particularly in low- and middle-income countries (LMICs), where treatment options may be constrained [33-37].

From a clinical perspective, our findings support the incorporation of microenvironmental biomarkers into risk stratification models. Traditional staging systems such as the Revised International Staging System (R-ISS) primarily rely on tumor burden and cytogenetic abnormalities but do not account for microenvironmental influences [38]. The addition of biomarkers such as IL-6, PD-L1 expression, and angiogenic indices could enhance prognostic accuracy and guide personalized therapy. The South African setting of this study provides important insights into MM in a real-world, resource-constrained environment. Patients in our cohort frequently presented with advanced disease, reflecting delays in diagnosis and limited access to specialized care [39]. Despite these challenges, the biological mechanisms of resistance observed were largely consistent with those reported in high-income countries, suggesting that

fundamental disease processes are conserved across populations. However, the higher prevalence of HIV and other comorbidities introduces additional complexity that warrants further investigation [40-42].

This study has several strengths. First, its prospective design allowed for standardized data collection and temporal assessment of microenvironmental changes. Second, the integration of multiple analytical modalities—clinical, immunological, molecular, and histological—provides a comprehensive understanding of resistance mechanisms. Third, the inclusion of HIV-positive patients enhances the generalizability of findings to sub-Saharan African populations [43].

However, several limitations must be acknowledged. The sample size, while adequate for detecting moderate associations, may limit the ability to identify more subtle effects or interactions [44]. Additionally, access to advanced genomic profiling was limited, and cytogenetic data were not available for all patients. The follow-up duration, although sufficient for assessing early outcomes, may not fully capture long-term survival trends. Finally, while our findings strongly suggest causal relationships, the observational nature of the study precludes definitive conclusions regarding causality.

Conclusion

This prospective clinical and molecular study demonstrates that the bone marrow microenvironment is a fundamental driver of therapeutic resistance in multiple myeloma, extending beyond tumor-intrinsic genetic factors. Through integrated analysis of cytokine signaling, immune modulation, angiogenesis, and gene expression, our findings provide compelling evidence that resistance arises from a complex and dynamic interaction between malignant plasma cells and their surrounding niche.

Elevated levels of key microenvironmental mediators—particularly interleukin-6, vascular endothelial growth factor, and PD-L1 expression—were strongly associated with poor treatment response and inferior survival outcomes. These factors collectively promote tumor survival, immune evasion, and disease progression. Additionally, increased microvessel density and upregulation of hypoxia-related and anti-apoptotic pathways further highlight the role of angiogenesis and metabolic adaptation in sustaining resistant disease.

Importantly, this study contextualizes these mechanisms within a South African population, where patients frequently present with advanced-stage disease and unique comorbidities, including a high prevalence of HIV infection. While HIV status did not independently predict survival, it was associated with enhanced inflammatory and immunosuppressive profiles, underscoring the need for further investigation into host–tumor–immune interactions in this setting.

The findings of this study have several important clinical implications. First, they support the incorporation of microenvironmental biomarkers into routine risk stratification, enabling more accurate prediction of treatment response. Second, they highlight the therapeutic potential of targeting the bone marrow niche, including strategies aimed at disrupting cytokine signaling, inhibiting immune checkpoints, and modulating angiogenesis. Such approaches may be particularly valuable in overcoming resistance to conventional therapies.

Declarations

Ethics Approval and Consent to Participate

This study was conducted in accordance with the principles of the Declaration of Helsinki (2013 revision) and adhered to all applicable national and institutional ethical guidelines. Ethical approval was obtained from the Human Research Ethics Committees of all participating institutions in South Africa, including the University of the Witwatersrand and the University of Cape Town. Written informed consent was

obtained from all participants prior to enrollment. For participants living with HIV, additional safeguards were implemented to ensure confidentiality and non-discriminatory inclusion.

Consent for Publication
All participants provided written informed consent for the use of anonymized clinical and laboratory data for research and publication purposes. No identifiable personal data are included in this manuscript.

Availability of Data and Materials
The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request. Selected anonymized datasets used for graphical representation and supplementary materials are provided within the article and its supplementary files.

Competing Interests
The authors declare that they have no competing financial or non-financial interests related to this work. The authors have no affiliations with organizations or entities that could influence the content or interpretation of the findings presented in this study.

Funding
This research received no external funding and was supported by institutional resources from participating academic centers in South Africa. No funding body had any role in the design of the study; collection, analysis, and interpretation of data; or in writing the manuscript.

Authors' Contributions
Dr. Thabo M. Nkosi: Conceptualization, study design, data acquisition, clinical oversight, manuscript drafting, and critical revision.
Dr. Ayesha K. Naidoo: Molecular analysis, data interpretation, statistical analysis, manuscript drafting, and critical revision.
All authors read and approved the final manuscript and agree to be accountable for all aspects of the work.

Acknowledgments
The authors would like to thank the clinical staff, laboratory personnel, and patients at Charlotte Maxeke Johannesburg Academic Hospital and Groote Schuur Hospital for their invaluable contributions to this study.

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