The specific transcriptional profile and clonal selection of monoclonal gammopathy of undetermined significance-like behavior predict an exceptionally favorable prognosis in multiple myeloma

Undetectable minimal residual disease (MRD) typically correlates with favorable survival in multiple myeloma (MM) and has been considered the surrogate endpoint in clinical trials.[1] However, one needs to acknowledge that some myeloma patients failed to achieve complete response (CR) or undetectable MRD after systematic treatment, but can achieve unexpected long-term disease control.[2-4] These patients may transition to an early disease state following treatment, displaying a stable clinical course of monoclonal gammopathy of undetermined significance-like (MGUS-like) behavior. Previous studies had also suggested these patients as MGUS-like phenotype due to their resemblance to MGUS, as observed through gene expression profiling[5] and flow cytometry[6-9] at diagnosis. Despite these observations, research on the identification and underlying mechanisms of MGUS-like behavior remains limited.

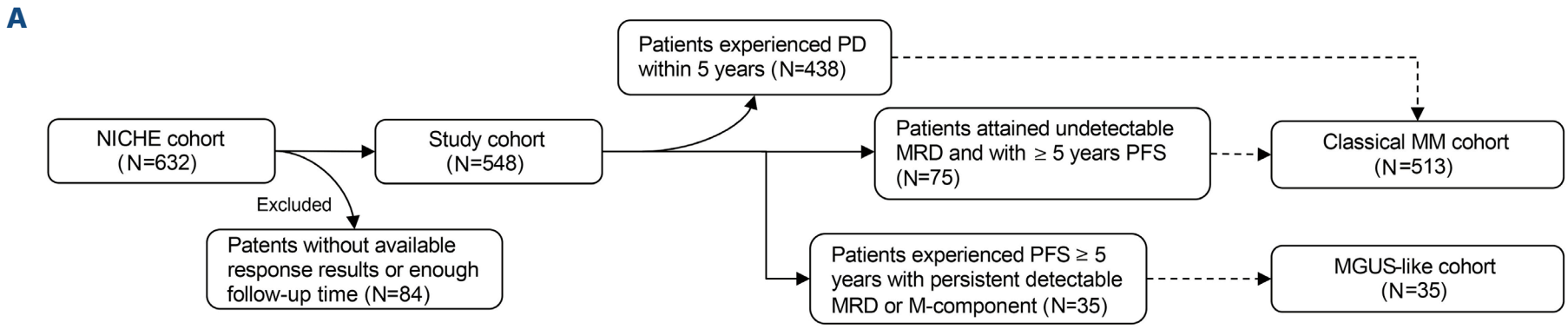
Herein, we retrospectively identified MGUS-like patients who attained long-term disease control (progression-free period ≥5 years), but still had persistent detectable MRD or M-protein, from the National Longitudinal Cohort of Hematological Diseases in China (NICHE, clinicaltrials.gov identifier: 04645199). Simultaneously, we conducted a comprehensive investigation covering clinical, transcriptomic, and genomic aspects to illuminate the underlying causes contributing to the distinctive MGUS-like clinical behavior. The study was approved by the Ethics Committee of the Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Science & Peking Union Medical College.

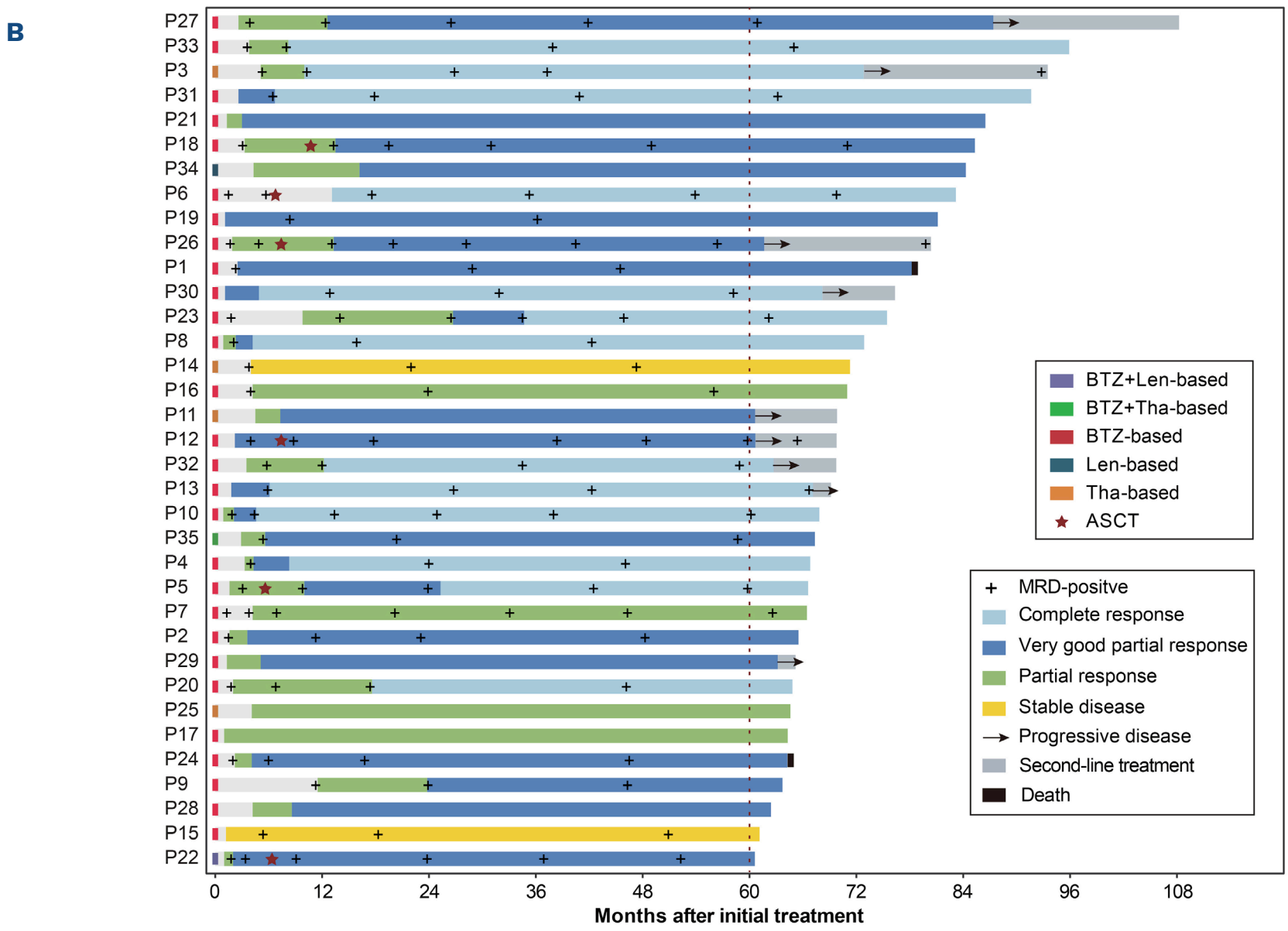
Minimal residual disease was detected by multiparameter flow cytometry (MFC) prior to 2017 or the EuroFlow method from 2018 onwards. To account for differences in MRD sensitivity (MFC: 0.01%, EuroFlow: 0.001%), MRD positivity was defined as ≥2x10^5 total events with ≥20 clonal plasma cell events (≥0.01%). Cytogenetic abnormalities followed previously reported definitions.[10] Progression-free survival (PFS) and overall survival (OS) were analyzed using the Kaplan-Meier method, with significance assessed by log-rank tests. P<0.05 was considered statistically significant.

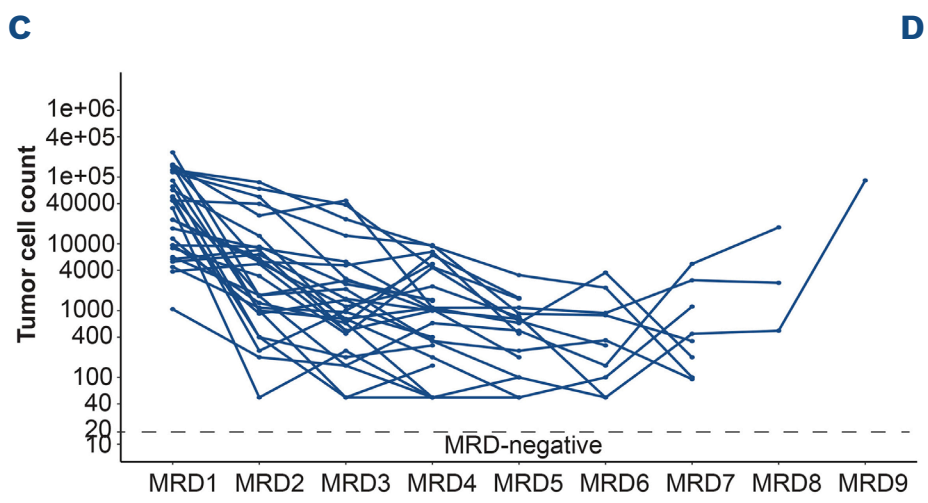
In our study cohort, 20.1% (110/548) of patients achieved more than five years disease remission, with the majority (68.2%, 75/110) achieving undetectable MRD. A small subset (6.4%, 35/548) exhibited MGUS-like behavior, while the remaining patients were classified as classical MM (Figure 1A). Among MGUS-like patients, 25 showed improved response, 13 achieved CR despite residual tumor cells, and 2 even maintained stable disease (Figure 1B). Most patients initially experienced a partial decrease in tumor burden, followed by sustained low tumor load throughout treatment (Figure 1C). Comparing our retrospective definition with the recently reported MGUS-like calculator10, we only observed an 8.6% (3/35) overlap (Figure 1D). The low concordance may stem from differences in study design, methods, and tumor heterogeneity.

Refering to clinical features, MGUS-like patients were featured by a lower proportion of International Staging System III (25.7% *vs*. 46.0%, P=0.022), anemia (42.9% *vs*. 66.3%, P=0.009) and bone lesions (71.9% *vs*. 90.3%, P=0.004), but a higher frequency of t(11;14) (33.3% *vs*. 15.1%, P=0.018) (*Online Supplementary Table S1*). Although MGUS-like patients tended to have fewer high-risk features, including elevated lactate dehydrogenase (LDH) (8.6% *vs*. 18.1%), Gain(1q) (32.4% *vs*. 50.0%), and HRCA (20.6% *vs*. 30.4%), although these differences are not statistically significant. Due to the study design, MGUS-like patients experienced significantly longer survival than the classical MM cohort (PFS: 87.4 *vs*. 34.0 months, P<0.001; OS: not reached *vs*. 72.3 months, P<0.001). However, within the MGUS-like cohort, PFS and OS did not differ significantly between those who achieved CR and those who did not (PFS: P=0.52; OS: P=0.26). These findings indicate that, for MGUS-like patients, a moderate treatment approach aimed at decreasing tumor burden and restoring an MGUS-like state[9] may be sufficient, rather than aggressive escalation to achieve MRD negativity.

To further explore the internal mechanisms of uncommon MGUS-like behavior, we performed transcriptional sequencing to compare the molecular features of tumor cells. We collected 10 CD138$^+$ bone marrow samples from MGUS-like patients and 30 randomly-selected CD138$^+$ samples from classical MM patients at diagnosis for transcriptional sequencing. Compared to classical MM, 329 genes were up-regulated and 141 down-regulated in MGUS-like patients (Figure 2A, B). Notably, up-regulated genes included B-cell lineage genes (*CD79A, CD79B*), osteogenesis-related genes (*MAST4, MBD2, PLXNB1*), and dormant myeloma cell-related genes (*VPREB3*). Gene Ontology (GO) enrichment analysis revealed upregulation of genes involved in cell-mediated immunity, cell adhesion, and immune cell activation (Figure 2C). Gene Set Enrichment Analysis (GSEA) identified







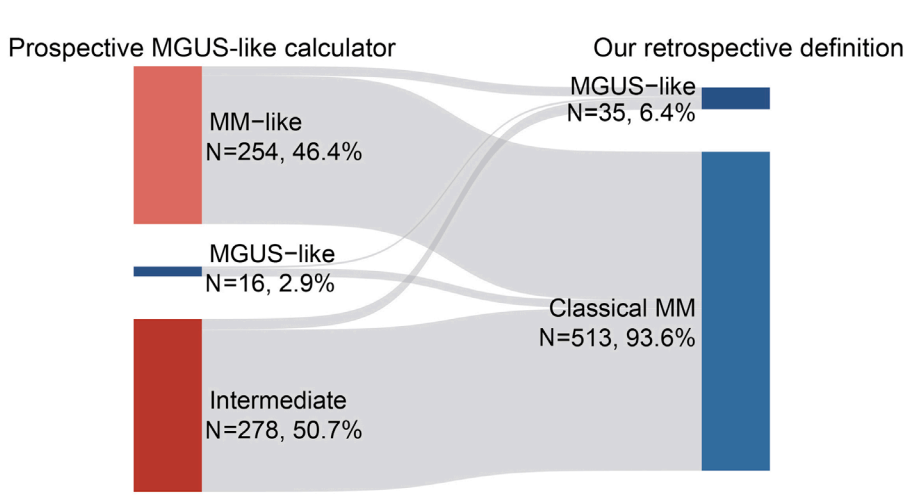


Figure 1. Identification of patients exhibiting monoclonal gammopathy of undetermined significance-like behavior. (A) Study design. (B) Treatment and response over time in these monoclonal gammopathy of undetermined significance-like (MGUS-like) patients. (C) Dynamic change in minimal residual disease (MRD) level over time, the X-axis (MRD 0, 1, 2 ...) refers to the MRD detection order. (D) The comparison and redistribution from a prospective MGUS-like calculator to our retrospective definition for MGUS-like behavior. ASCT: autologous stem cell transplantation; BTZ: bortezomib; Len: lenalidomide; MM: multiple myeloma; N: number; PD: progressive disease; PFS: progression-free survival; Tha: thalidomide.

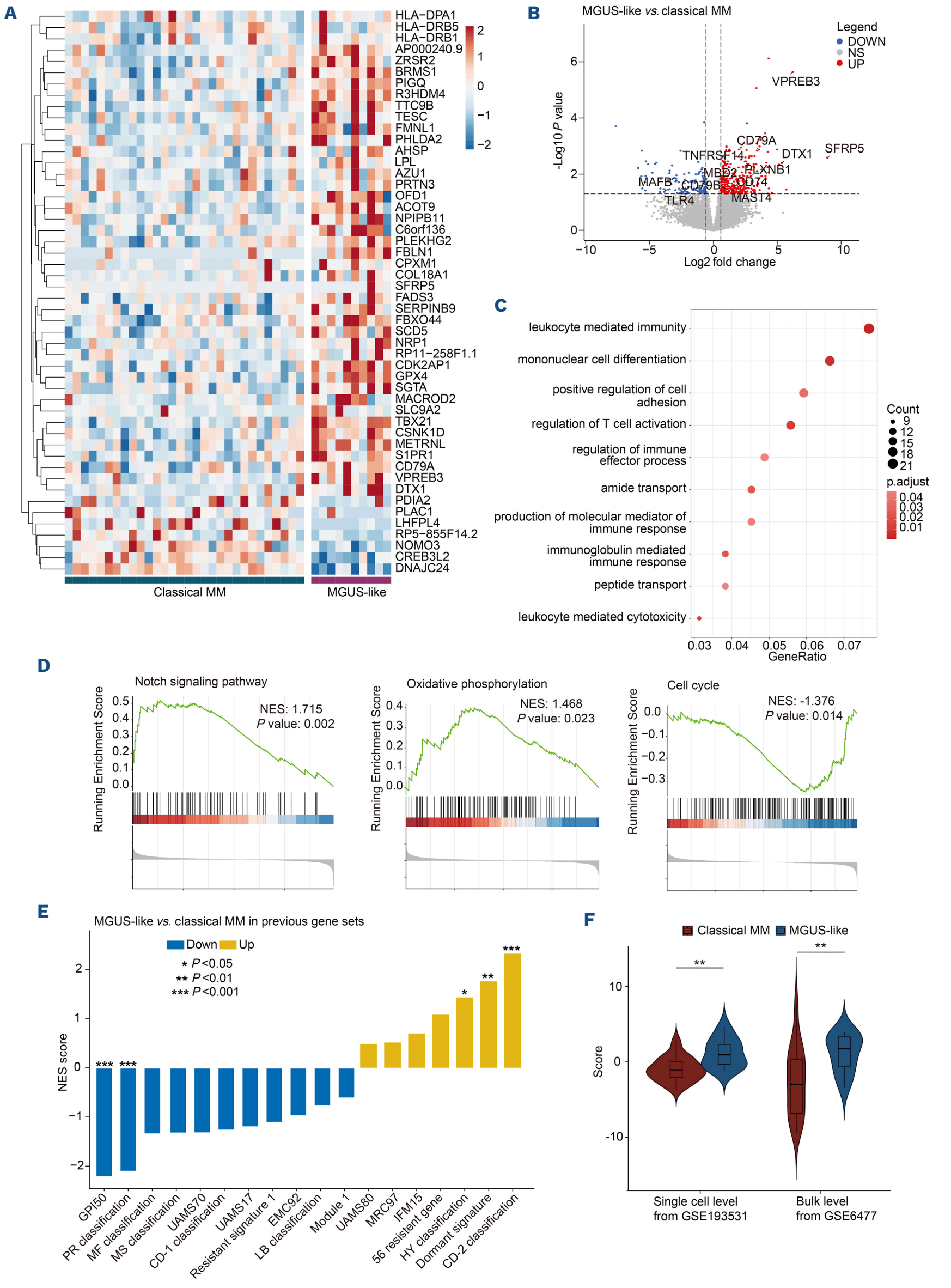


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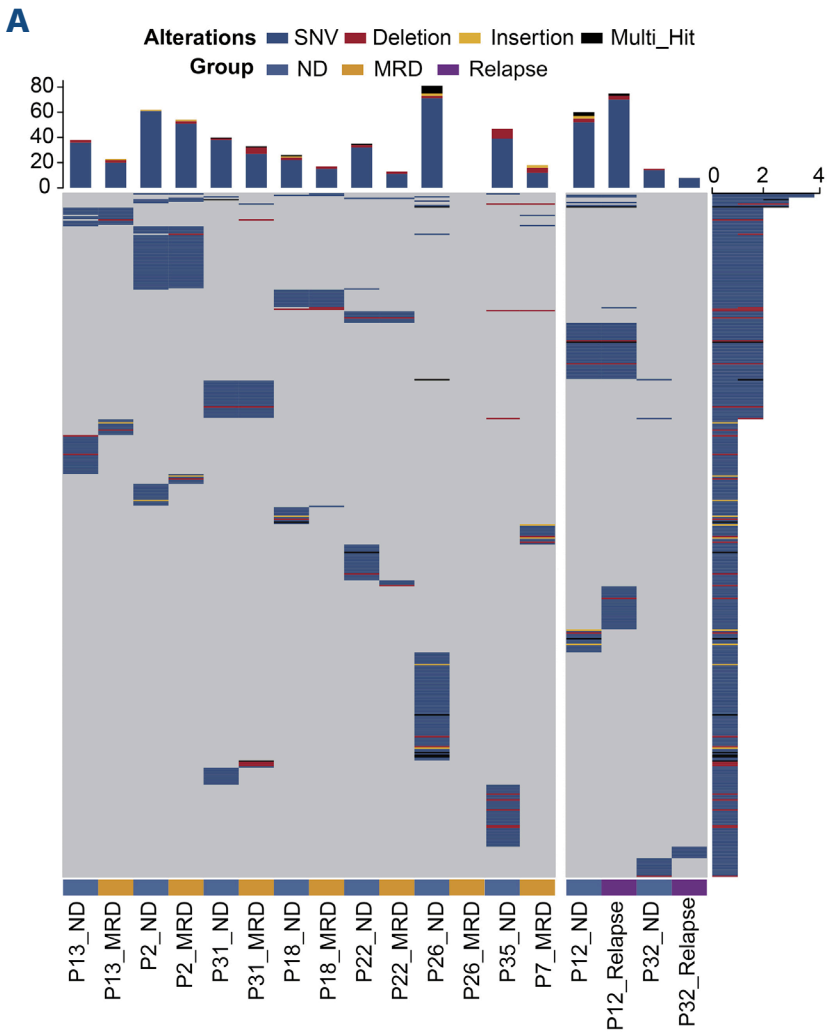
Figure 2. Specific molecular determinants of patients exhibiting monoclonal gammopathy of undetermined significance-like behavior. (A) Top 50 most variable genes between monoclonal gammopathy of undetermined significance-like (MGUS-like) and classical multiple myeloma (MM) cohort. (B) Gene expression profiling comparing differentially expressed genes between MGUS-like and classical MM (adjusted $P<0.05$ and $\log^2$ FC>0.58). *P* value calculated by the Wald test and corrected for multiple hypothesis test using the Benjamini-Hochberg method. (C) Gene ontology (GO) enrichment analysis showed that 18 biological process pathways found up-regulated in MGUS-like patients. *P* value was calculated by over-representation test based on hypergeometric distribution and corrected for multiple hypothesis test using the Benjamini-Hochberg method. (D) Gene set enrichment analysis (GSEA) between MGUS-like and classical MM cohort. *P* value was calculated using permutation test. (E) Barplot representing the enrichment degree of MGUS-like cohort in the context of previous proposed gene sets. *P* value was calculated by permutation test. (F) Violin plot representing the resemblance score of MGUS-like patients to MGUS at bulk and single cell level. *P* value was calculated by Student *t* test. Scores were calculated by the Viper algorithm.

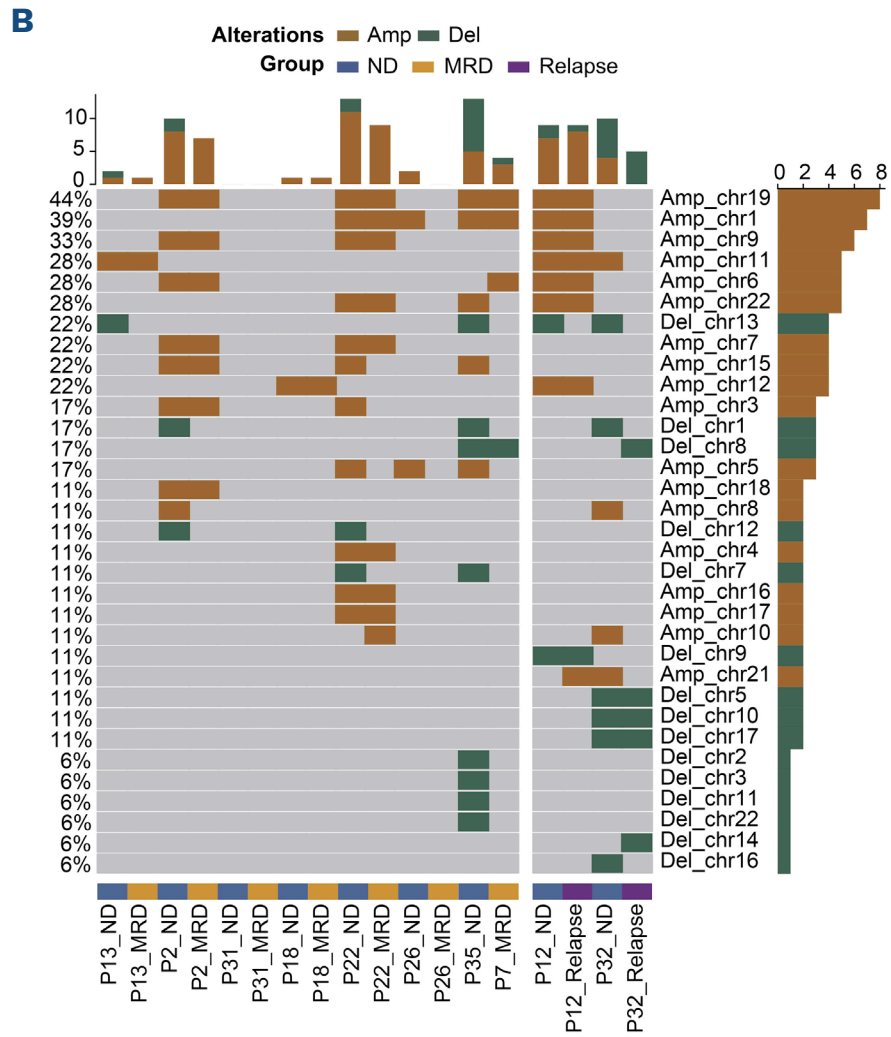
up-regulated pathways such as oxidative phosphorylation and NOTCH, alongside downregulation of cell cycle signal (Figure 2D). Compared to established predictive gene sets (*GPI150, UAMS17, UAMS70, UAMS80, EMC92, Module 1, MRC97, IFM15*, 56-resistent gene, dormant myeloma signature) and molecular subtypes described by Zhan *et al.*,[11] MGUS-like tumor cells significantly up-regulated the favorable CD-2 subtype while down-regulating the high-risk PR subtype (Figure 2E). Additionally, the dormant myeloma signature was up-regulated, while the gene proliferation index (GPI50) was down-regulated (Figure 2E). Comparing our transcriptional data with public MGUS datasets at bulk level[12] and single cell level,[13] as expected,[5] MGUS-like tumor cells closely resembled the earliest precursor conditions (MGUS) (Figure 2F). The indolent and low proliferative tumor cells surrounded by a favorable bone marrow immune environment may explain the surprisingly prolonged survival observed in the MGUS-like cohort.

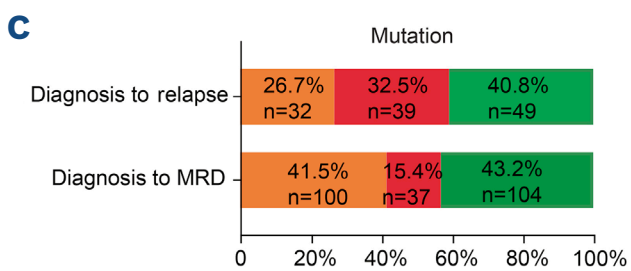
Using these differentially expressed genes (DEG), we identified a 44-gene signature with weighted scores (*Online Supplementary Table S2*) and determined the optimal cutoff using ROC curve analysis. For validation, we analyzed 761 patients from the prospective MMRF CoMMpass study (clinicaltrials.gov identifier: 01454297) with available clinical and transcriptional data. Based on the weighted score (>62.1), 15.4% (117/761) of patients were classified as MGUS-like high. Clinically, MGUS-like high patients had lower rates of anemia, thrombocytopenia, and renal dysfunction, but a higher incidence of abnormal LDH level compared to the MGUS-like low group (*Online Supplementary Figure S1A*). Despite receiving similar treatments, MGUS-like high patients showed significantly longer PFS and OS (*Online Supplementary Figure S1B, C*) but had a lower CR rate (35.6% *vs.* 51.6%, $P=0.027$). The positive prognostic impact remained independent of age, International Staging System (ISS) staging, LDH level, cytopenia, and treatment regimen, with a hazard ratio of 0.521 (95% CI: 0.302-0.900) for PFS and 0.336 (95% CI: 0.123-0.919) for OS (*Online Supplementary Figure S1D*). Notably, such MGUS-like high patients achieved favorable survival regardless of response depth, suggesting a potential MGUS-like status (*Online Supplementary Figure S1E, F*). However, due to limited follow-up and unavailable MRD data in the validation cohort, prediction of true MGUS-like behavior remains inconclusive.

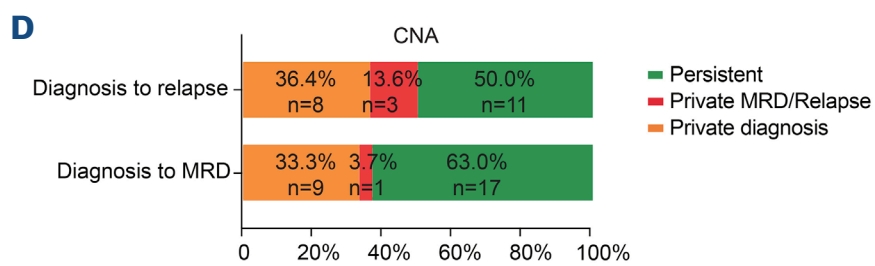
We characterized the genomic landscape of MGUS-like patients using whole-exome sequencing (WES) of paired tumor samples from diagnosis (N=9) and either MRD (N=7) or first relapse (N=2). All MRD samples had $\geq 10^{-3}$ tumor burden with ≥60% purity. In summary, 514 mutations and 49 copy number alterations (CNA) were detected (Figure 3A, B). While gene mutations varied between patients, intra-patient consistency across timepoints was observed. Potentially driver mutations[14,15] in genes such as *DIS3, EGR1, LTB, DDX3X,* and *DTX1*, disappeared at MRD stage, but *CCND1* and *WYH3* persisted. In 4 MGUS-like patients (P2, P13, P22, P26), driver mutations were lost at MRD. Interestingly, 15.4% (37/241) of new mutations emerged at MRD, significantly lower than at relapse (39/120, 32.5.%, $P<0.001$) (Figure 3C). CNA were more stable, with 63.0% retained at MRD, but significantly higher at relapse (Figure 3D). To better elucidate treatment pressure on MGUS-like behavior, we analyzed 16 longitudinal from 8 MGUS-like patients, clustering clonal evolution using Pyclone/CITUP algorithms. P26 failed to identify its clonal pattern due to minimal mutations at MRD. Five patients showed a consistent evolutionary pattern, where subclones underwent intertumoral selection under treatment pressure (Figure 3E, F). One patient displayed a similar clonal selection pattern from diagnosis to relapse, resembling the MRD phase, while P32 exhibited branching evolution (Figure 3G). No overlap gene mutations were found in stable major tumor clones from 6 patients with similar clonal selection patterns. These findings suggest that most MGUS-like patients retain an indolent primary clone at diagnosis, with minimal changes in their dominant tumor clone at MRD, despite undergoing complex subclonal selection under treatment pressure (Figure 3H).

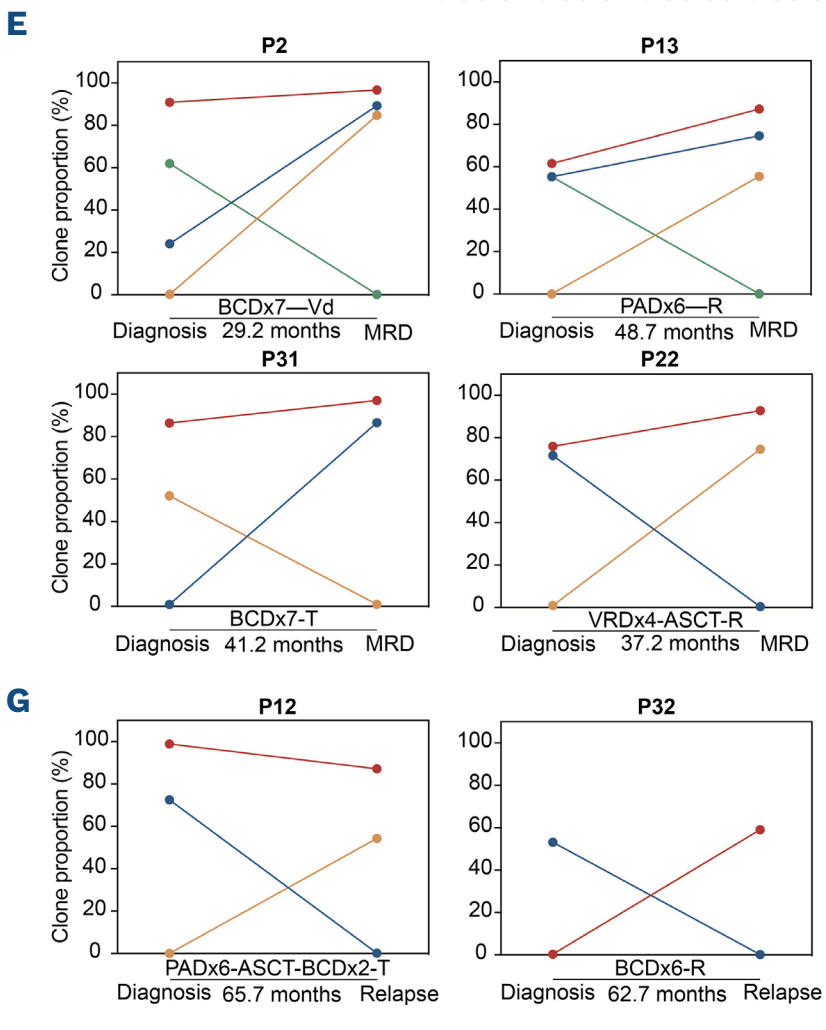
To our knowledge, this study is the first to employ deep sequencing and investigate clonal evolution to elucidate MGUS-like behavior. We retrospectively identified 6.4% of patients who, despite persistent residual tumor cells, experienced unexpected long-term remission (PFS ≥5 year). Multi-omics analysis revealed that their unique transcriptional and genomic features, along with intratumor clonal selection under treatment pressure, may contribute to this distinct clinical trajectory. Although undetectable MRD is typically linked to superior survival in MM, we confirmed that MGUS-like patients could defy the MRD-negative paradigm but achieve prolonged survival. These MGUS-like patients

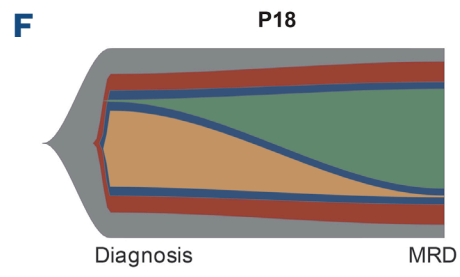












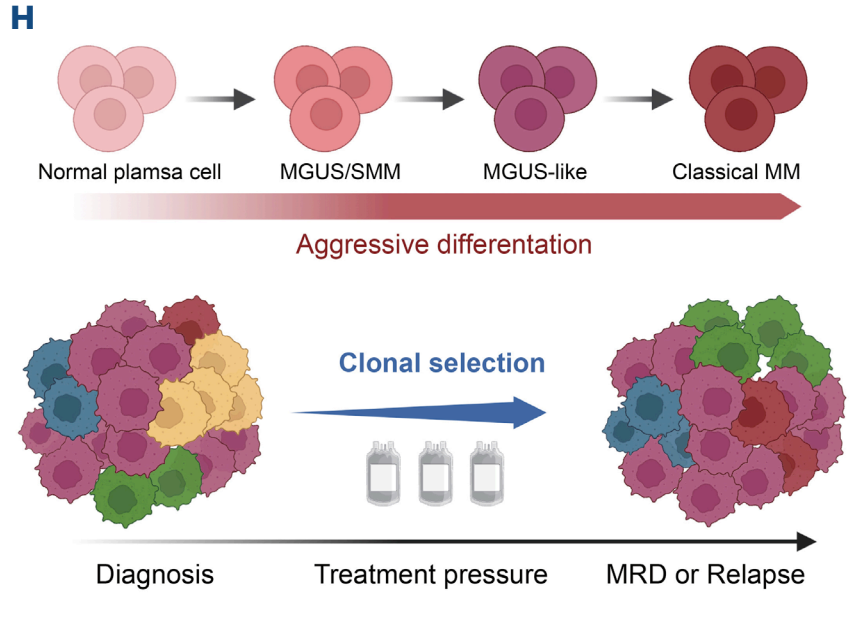


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Figure 3. The genomic landscape and clonal evolution of patients exhibiting monoclonal gammopathy of undetermined significance-like behavior. Oncoprint of genomic mutations (A) and copy number alterations (CNA) (B) in monoclonal gammopathy of undetermined significance-like (MGUS-like) patients at different timepoints. Number and frequency of shared *versus* private mutations (C) and CNA (D) in paired diagnostic and minimal residual disease (MRD) or relapse tumor cells. Line plots showing tumor cell clones evolution from diagnosis to MRD/relapse: (E) clonal selection pattern from diagnosis to MRD phase; (F) fish plot showing tumor clonal selection in P18; (G) clonal selection pattern from diagnosis to relapse. (H) Potential mechanism of MGUS-like behavior (created with BioRender.com). P: Patient ID.

exhibit several particular features previously reported in the literature.[6,8,9] but they do not always present with a conventional low-risk profile, making prediction at diagnosis challenging. Notably, we developed and validated a 44-gene signature that predicts MGUS-like status, associated with favorable prognosis regardless of response depth.

Despite these insights, small sample size, a retrospective design, and unavailable MRD data in the validation cohort limit generalizability. Further large-scale prospective validation is needed to refine risk stratification and assess whether MGUS-like patients may benefit from tailored, less-intensive treatment approaches rather than aggressive therapy escalation. Collectively, we identified a distinct myeloma subgroup with prolonged PFS despite persistent residual tumor, exhibiting MGUS-like status. Recognizing heterogeneous clinical trajectories in myeloma could help refine personalized treatment strategies.

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Disclosures

No conflicts of interest to disclose.

Contributions

WYa, CQ and GA analyzed data, interpreted results, and drafted the manuscript. JZ, JX, JC, YL, CD, TY and SZ collected data and performed patient follow-up. WS, SD, YX and GA acquired data and managed patients. MH, YX, DZ and WYu suggested revisions. MH, LQ, YC and GA designed the research and approved the final version.

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Data-sharing statement

All the raw data of RNA seq and WES have been deposited in the Genome Sequence Archive (GSA) for Human in the Big Data Center (https://ngdc.cncb.ac.cn/gsa-human/), under the accession number HRA006164.

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