

## **Challenging the Concept of Functional High-Risk Myeloma through Transcriptional and Genetic Profiling**

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## KEY POINTS

1. True functional high-risk MM is very uncommon when comprehensive clinical and molecular profiling is applied.
2. High-risk GEP with standard-risk genetics conferred high early relapse rates, advocating for broader access to transcriptional diagnostics.

## ABSTRACT

Functional high-risk (FHR) multiple myeloma (MM) is defined as an unexpected, early relapse (ER) of disease in the absence of baseline molecular or clinical risk factors (RF), making FHR MM inherently dependent on which RFs were assessed at diagnosis, but also on what treatment patients received. To establish the true incidence of FHR, we analysed uniformly treated, transplant-eligible (TE) patients from the UK NCRI Myeloma-XI trial that had been profiled for IMS/IMWG defined high-risk cytogenetic aberrations (HRCA) and the SKY92 gene expression HR signature (GEP-HR). 135 TE MyXI patients meeting these criteria were studied, with a median follow-up of 88 months. 25 patients (18.5%) experienced ER, defined as relapse <18 months from maintenance randomization post-autologous stem-cell transplantation. Hereof, 15 (60%) were classified as IMS/IMWG-HR at diagnosis, of whom 8 were also GEP-HR. Another 6 patients were GEP-HR only and would have been missed by IMS/IMWG-HR. Among 4 patients with both IMS/IMWG- & GEP-standard risk (SR), 2 had isolated HR markers at diagnosis, leaving only 2 patients (8% of ER; 1.5% of all) truly meeting all FHR criteria. The combination of IMS/IMWG-HR and GEP-HR profiling identified 84% of ER, and differentiated long-term outcome across all 135 patients: co-occurring IMS/IMWG-HR and GEP-HR was associated with very short overall survival compared to the absence of both (HR=13.1, 95%-CI: 6.5–26.1, P<0.0001), followed by GEP-HR only (HR=5.1, 95%-CI: 2.4–11.1, P<0.0001) and IMS/IMWG-HR only (HR=3.2, 95%-CI: 1.6–6.2, P=0.0007). Our results support more comprehensive baseline diagnostic profiling to identify those at risk of ER upfront.

## INTRODUCTION

Frontline therapies for multiple myeloma (MM) have advanced significantly over the past two decades, especially in transplant-eligible (TE) newly diagnosed patients (NDMM) <sup>1</sup>. Combination induction regimens including immunomodulatory drugs (IMiDs, e.g., thalidomide-based Cereblon degraders), proteasome inhibitors (PIs), and anti-CD38 antibodies, followed by high-dose melphalan and autologous stem cell transplantation (ASCT), have made treatment responses the norm rather than the exception <sup>2,3</sup>.

Lenalidomide (Len) maintenance post-ASCT has been shown to improve both progression-free survival (PFS) and overall survival (OS), leading to its global approval <sup>4</sup>. With the loss of patent exclusivity, Len use has expanded worldwide, including in lower-income countries <sup>2</sup>. However, not all patients benefit equally; some relapse early (ER) and are classified as having high-risk multiple myeloma (HRMM). While many HRMM cases show molecular (mRF) or clinical risk factors (cRF) at diagnosis, others, termed functional high-risk (FHR) MM, relapse unexpectedly within 24 months despite lacking such markers <sup>5,6</sup>. FHR may account for one-third to half of all ER cases <sup>7-10</sup>, raising concerns about the reliability of current risk stratification and highlighting challenges in implementing early, risk-adapted therapies, as seen in OPTIMUM/MUKnine and GMMG-CONCEPT trials <sup>11-13</sup>.

Estimates of FHR prevalence have often come from cohorts with non-uniform treatment or incomplete molecular data. Until 2025, the IMWG definition of HRMM considered only t(4;14), t(14;16), and del(17p) as mRF, which are commonly used in registrational trials. The same was true before the second revision (R2-ISS) of the revised international staging system (R-ISS); the former still excluding t(14;16) as mRF. The updated IMS/IMWG definition now incorporates del(17p), TP53 mutations and biallelic del(1p32) as HR features; it furthermore classifies as HR those cases in which t(4;14), t(14;16), t(14;20), monoallelic del(1p32) and/or gain(1q21) co-occur <sup>14</sup>. But neither IMS/IMWG nor R2-ISS consider the validated and independent prognostic role of transcriptional, or gene expression, profiling (GEP) <sup>15,16</sup>, despite growing evidence supporting its additional clinical utility <sup>17,18</sup>. To better estimate FHR MM prevalence under current diagnostic standards, we analysed a uniformly treated, comprehensively profiled sub-group from the UK NCRI Myeloma XI (MyXI) trial, using certified diagnostic tests.

## **MATERIAL AND METHODS**

### **Patients**

A subgroup of 135 TE NDMM patients from the UK NCRI MyXI phase 3 trial was selected for analysis. All patients met the following inclusion criteria: (i) complete data for all high-risk cytogenetic abnormalities (HRCAs) defined by IMS/IMWG-HR<sup>14</sup>, including t(4;14), t(14;16), t(14;20), gain(1q21), del(1p32), and del(17p); (ii) an available SKY-92 gene expression profile (GEP); and (iii) randomisation to Len maintenance post-ASCT. We refer to gain(1q21) as gain(1q) and del(1p32) as del(1p) throughout the manuscript. The design and main outcomes of MyXI have been previously reported<sup>4</sup>. ER was defined as disease progression within 18 months of post-ASCT maintenance randomisation, corresponding to relapse within 24 months of diagnosis. Depth of response and progression were assessed according to the International Myeloma Working Group (IMWG) criteria. PFS was measured from the time of maintenance randomisation to progression or death from any cause, and OS was calculated from the same timepoint to death from any cause.

The Oxfordshire Research Ethics Committee approved the study and written informed consent was obtained for all patients included within the MyXI (MREC 17/09/09, ISRCTN49407852).

### **Samples and molecular profiling**

MM cells were isolated from bone marrow (BM) aspirate samples at diagnosis and enriched to >95% purity using immune-magnetic cell sorting with anti-CD138 antibodies (Miltenyi Biotec, Bergisch Gladbach, Germany). RNA and DNA were extracted with either RNA/DNA mini kit or Allprep kits (QIAGEN), following the manufacturers' protocols. Cytogenetic abnormalities were centrally assessed in BM samples using multiplexed ligation-dependent probe amplification (MLPA; P425-B1 MM probemix; MRC Holland, Amsterdam, The Netherlands) for copy number aberrations and a TC-classification-based multiplex qRT-PCR, as previously described and validated against FISH<sup>4,19-22</sup>. Targeted next-generation sequencing (NGS) of TP53 coding regions was performed for 11 ER cases lacking translocations or copy number aberrations using the UKAS15189-accredited RMH200 Haemonc panel (Clinical Genomics Laboratory, Royal Marsden Hospital), in accordance with NHS England guidelines.

## Statistical methods

Statistical analyses were performed using RStudio (v2024.12.0). Categorical variables were compared using chi-squared or Fisher's exact tests, and continuous variables, with the Wilcoxon rank-sum test. Survival outcomes were assessed using Kaplan-Meier estimates and log-rank tests, implemented via 'survival', 'survminer' and 'ggplot2' packages. Multivariate Cox proportional hazards models were used to estimate hazard ratios (HRs) and associated 95% confidence intervals (CIs). All tests were two-sided and *P*-values < 0.05 were considered significant. Sankey plots were generated using the 'ggplot2' and 'ggalluvial' packages. Oncoplots were coded with the packages 'ComplexHeatmap' and 'circlize'.

## RESULTS

### Patient and treatment characteristics

Of 897 MyXI patients with available genetic information, 552 underwent ASCT and maintenance randomization. Amongst these 135 met all inclusion criteria including available GEP data and were included in this study (**Supplementary Table 1**). By design, all 135 patients received ASCT and were randomised to Len maintenance. Median follow-up from randomisation was 88.3 months (IQR 47.3–104).

Of these 135 patients, 25 (18.5%) experienced ER (**Figure 1A**). Among the 110 non-ER patients, 66 experienced a late relapse at a median of 52.3 months (IQR 30.7–70.3), compared to a median of 9.8 months (IQR 4.5–16.3) in the ER-group. Baseline clinical and treatment characteristics of ER and non-ER groups are summarized in **Table 1**. As per inclusion criteria, extended genetic profiles were available for all 135 patients, with the frequency of individual lesions consistent with previously reported MyXI data (**Table 1**)<sup>4</sup>.

### Early relapse and baseline risk factors

To estimate the extent of FHR, defined as ER with absence of any baseline mRF or cRF, we analysed the 25 patients who experienced ER. Using the IMS/IMWG-HR defined mRF, 12 patients (48%) were classified as HR, including 7 with  $\geq 2$  HRCAs and 5 with  $\geq 3$  HRCAs. Incorporating the cRF b2m, 2 additional cases without genetic aberrations were classified as HR. We additionally performed targeted NGS of TP53 for the 11 ER patients classified IMS/IMWG-standard risk (SR). One ER patient without translocations or copy number

aberrations carried an isolated clonal, pathogenetic TP53 mutation (codon 309 C>A; variant allele frequency (VAF) 0.58). This patient also showed elevated LDH as a cRF at diagnosis, bringing the total to 15 IMS/IMWG-HR (60%) (**Figure 1B**). Amongst these 15 IMS/IMWG-HR patients, 8 also met GEP-HR criteria - all having gain(1q), 6 (75%) with del(1p), and 4 (50%) with  $\geq 3$  HRCAs. Of the remaining 10 IMS/IMWG-SR patients, 6 were GEP-HR at diagnosis, including 4 with a single HRCA (**Figure 1B**). Of the final 4 patients with neither IMS/IMWG-HR nor GEP-HR features, 2 had isolated HRCAs (one with t(4;14) and one with gain(1q)). While these cases did not meet IMS/IMWG-HR criteria, they do not qualify as FHR due to the presence of at least one mRF. Applying a strict FHR definition only 2 patients (8% of ER; 1.5% of the total cohort) met criteria. Notably, both harboured a t(11;14) translocation (**Figure 1B**).

### **Predictive value of baseline risk factors**

Given the importance of early risk identification for timely intervention, we assessed the sensitivity and specificity of updated classifiers for ER in context of this MyXI subgroup in receipt of ASCT and Len maintenance. Analyses were based on IMS/IMWG-defined HRCA alone, since TP53 mutation status was not available for all 135 patients. On this basis, both IMS/IMWG-HR and GEP-HR identified 14 of the 25 ER cases, yielding a sensitivity of 56% for each (**Supplementary Table 2**). Although these groups partially overlapped, nearly half (48%) of ER cases were flagged by only one of the two methods, suggesting that IMS/IMWG-HR and GEP-HR capture distinct aspects of HR biology.

Across all 135 patients, GEP-HR demonstrated greater specificity for ER (83%, 95% CI: 74.3–89.3) than IMS/IMWG-HR (68%, 95% CI: 58.6–76.7). When patients with elevated b2m but no mRF were excluded from the IMS/IMWG-HR group, specificity improved to 76.4%, though sensitivity dropped to 48%. Redefining ER as relapse within 24 months of ASCT (instead of 18 months post maintenance randomisation) did not increase the sensitivity of either classifier (both remained at 58.3%) but improved the specificity of GEP-HR to 88% (**Supplementary Table 2**). Combining GEP-HR and/or IMS/IMWG-HR into a single risk group raised sensitivity for ER to 80%, with a specificity of 62%. When applying the 24-month definition, sensitivity and specificity were 80.6% and 66.7%, respectively (for ER < 24 months: 80.6% sensitivity, 66.7% specificity). To further explore the complementary value of these classifiers, we defined four risk categories: (i) Dual High-Risk: IMS/IMWG-HR and GEP-HR; (ii) GEP-

HR only: GEP-HR with IMS/IMWG-SR; (iii) IMS/IMWG-HR only: IMS/IMWG-HR with GEP-SR and (iv) Standard-Risk (SR): IMS/IMWG-SR & GEP-SR.

### **Survival outcome**

Based on these findings and the extended follow-up in MyXI, we evaluated survival outcomes beyond the binary ER classification across the four defined risk groups. Relative to IMS/IMWG-SR & GEP-SR (median PFS: 93.0 months), PFS was poorest for patients with IMS/IMWG-HR & GEP-HR (median PFS: 22.1 months, hazard ratio (HR) = 5.9, 95% CI: 3.3–10.6,  $P < 0.0001$ ), followed by GEP-HR (median PFS: 19.6 months, HR = 4.1, 95% CI: 2.1–8.2,  $P < 0.0001$ ) and IMS/IMWG-HR (median PFS: 46.5 months, 2.4, 95% CI: 1.4–3.9,  $P = 0.0013$ ) (**Figure 2A**). Risk discrimination was consistent and even more pronounced for OS. Patients with dual HR had the shortest OS (median: 31.9 months; HR = 13.1, 95% CI: 6.5–26.1,  $P < 0.0001$ ), followed by GEP-HR only (median: 53.2 months; HR = 6.4, 95% CI: 3.1–13.2,  $P < 0.0001$ ) and IMS/IMWG-HR only (median: 82.8 months; HR = 2.8, 95% CI: 1.4–5.8,  $P = 0.004$ ) (**Figure 2B**).

Interestingly, patients classified as IMS/IMWG-HR solely based on b2m elevation but with no mRF ( $n = 10$ ) had a median OS of nearly 8 years (92.3 months, 95% CI: 34.6 – NA), which did not differ significantly from the SR group ( $P = 0.23$ , **Supplementary Figure 1A and B**).

### **Composition and overlap of risk classifiers**

Comprehensive molecular profiling allowed assessment of the contribution of individual lesions to the updated IMS/IMWG-HR classification and their overlap with GEP-HR (**Figure 3A**). The concordance between HRCAs count and the individual risk classifiers IMS/IMWG, GEP status, and R-ISS is illustrated in **Supplementary Figures 2A–C**. Among IMS/IMWG-HR patients, 71% had  $\geq 2$  HRCAs. Elevated b2m without renal impairment accounted for 12% ( $n=10$ ) of IMS/IMWG-HR classifications. A total of 14 patients (10%) were classified as GEP-HR without meeting IMS/IMWG-HR criteria (**Figure 3A**). Of these, 12 had a single HRCA: gain(1q) ( $n=6$ ), t(4;14) ( $n=4$ ), t(14;20) ( $n=1$ ), and del(1p) ( $n=1$ ). Del(1p) was present in 19 patients (14%), with 68% also GEP-HR. Most del(1p) cases co-occurred with gain(1q) (90%), del(17p) (37%), or t(4;14) (21%). Four cases (21%) had biallelic deletions, all with  $\geq 2$  HRCAs (**Figure 3B**). Among IMS/IMWG-SR patients, over half had at least one risk feature: 44% had a single HRCA, 16% were GEP-HR, and 13% had elevated b2m with renal impairment

(creatinine >106 mmol/L). The most common single abnormality in this group being gain (1q) (61%), followed by t(4;14) (29%) and t(14;16) (10%). Isolated gain(1q) was also found in 43% of GEP-HR cases lacking additional IMS/IMWG-HR features, although most gain(1q) tumours were not GEP-HR. Co-occurrence of HR features and their association with patient outcome is shown in **Figure 4**.

## DISCUSSION

In this study, we used a uniformly treated and comprehensively profiled cohort of transplant-eligible patients with NDMM to assess the prevalence and characteristics of FHR disease. True FHR MM, here defined as ER in the absence of both mRF and cRF, proved to be relatively rare in our analysis. Prior estimates of FHR prevalence appear inflated in settings where baseline diagnostic assessments were incomplete, emphasizing the importance of comprehensive risk profiling at diagnosis. Notably, in line with most authors<sup>23</sup>, we consider as true FHR only those patients with complete absence of baseline mRF and cRF, as these patients pose the biggest diagnostic challenge, rather than using the term synonymously for ER.

Our results indicate that ER is significantly enriched among patients classified as high-risk by GEP, which emerged as one of the most specific predictors of ER. Reliance on IMS/IMWG-defined risk alone, even though updated criteria are markedly more sensitive, may still miss around 10% of NDMM patients at risk of ER, who can be identified by GEP. We also demonstrate that incorporating GEP can isolate an ultra-HR subset of patient as a specific target group for new, innovative treatment approaches. Therefore, our data supports the argument for routine incorporation of GEP-HR assessment into frontline diagnostics, consistent with recent findings<sup>17,18</sup>. Historically, limited access to tumour RNA has constrained the use of GEP, but RNA is increasingly obtainable as a by-product of DNA extraction for next-generation sequencing (NGS) workflows, in line with recent updates to IMS/IMWG risk assessment guidelines.

Importantly, combining IMS/IMWG-HR and SKY92-based GEP-HR identified 84% of patients with ER, and captured a broader population with consistently inferior PFS and OS. This combined approach offers a strong foundation for early identification of HR patients and

timely deployment of intensified treatment strategies, such as those explored in the OPTIMUM/MUKnine and GMMG-CONCEPT protocols. Moreover, since most HRMM trials are single-arm studies, prospectively stratifying the HR cohort by GEP status could provide a valuable benchmark, enable external comparisons and increase the validity of outcome assessments.

Nonetheless, our findings underscore a trade-off between sensitivity and specificity in ER prediction. This highlights the role of non-biological factors, such as treatment adherence, tolerability, or real-world drug exposure, that are not captured in baseline diagnostics. For example, challenges with oral therapy like Len maintenance may contribute to relapse risk independently of MM biology<sup>24</sup>. Moreover, conventional mRF and cRF may not fully capture disease heterogeneity or predict response to therapies designed to exploit plasma cell vulnerabilities as described by Boise et al<sup>25</sup>. Interestingly, the two remaining FHR cases we identified harboured t(11;14), a translocation associated with a more B-cell-like transcriptional program<sup>26</sup>, as well as plasma cell leukaemia, which may impact responsiveness to plasma cell-targeting therapies. The biological implications of this phenotype remain to be established but suggest a need for more tailored therapeutic strategies. Several risk factors now recognized as clinically relevant, such as circulating tumour plasma cells, extramedullary disease, and functional imaging abnormalities, were not assessed in the MyXI trial, which started in 2011. The incorporation of advanced imaging modalities such as whole-body MRI could further refine risk prediction by identifying early signs of relapse that escape standard diagnostics<sup>27,28</sup>.

The strengths of our study include a long follow-up, consistent treatment with a widely accessible standard of care (ASCT followed by Len maintenance), and robust profiling using validated diagnostic platforms. Including only patients with complete genetic profiling data could, in theory, introduce selection bias. However, in the OPTIMUM/MUKnine trial we successfully obtained very similar complete cytogenetic and GEP information in nearly 90 % of all-comer NDMM patients through central screening, with comparable results in terms of frequency of genetic and GEP risk markers<sup>29</sup>, rendering clinically significant selection bias in the current study unlikely. Future validation of our results in an external cohort would be desirable but is currently limited by the lack of readily available clinical-trial datasets providing also diagnostic/clinical GEP profiling, uniform treatment, and long-term follow-up. Another limitation is the absence of anti-CD38 monoclonal antibody therapy in the MyXI treatment regimen, although recent data suggest that while these agents improve outcomes in HR patients,

particularly in those with multiple HRCAs, they do not eliminate the adverse prognosis <sup>30,31</sup>. A further constraint is the incomplete data on *TP53* mutation status, which were available for only a subset of patients. However, our cohort had complete information on del(17p), and isolated pathogenic *TP53* point mutations, especially those that would meet diagnostic NGS reporting threshold, are rare in NDMM. Nevertheless, the finding of a clonal isolated *TP53* point mutation in our ER group underpins their potential clinical relevance and supports their inclusion in the updated IMS/IMWG HR definition.

Finally, although the addition of GEP testing adds to upfront diagnostic expense, the cost of a single, one-off assay is still modest relative to repeated cycles of contemporary standard anti-myeloma therapy. Addition of GEP profiling to standard diagnostics has been found to be cost-effective in breast cancer (e.g., Oncotype DX) and is reimbursed in public healthcare systems like the NHS. In the context of a growing body of evidence demonstrating differential clinical meaning of MRD test results for SR vs HR patients and its potential impact on treatment decision making, e.g. with respect to ongoing vs timely limited treatment <sup>32,33</sup>, our results suggests that addition of baseline GEP diagnostics for MM can provide marked value for patients and healthcare systems, by improving the diagnosis of HR as well as of SR patients.

In conclusion, when comprehensive molecular and clinical diagnostics are applied, true FHR MM is uncommon. Our findings advocate for broader access to advanced diagnostic tools in both research routine and clinical practice, to enable more accurate risk stratification and inform treatment planning.

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## **AUTHORSHIP CONTRIBUTIONS**

MFK designed the study. SB analysed the data. MFK and SB wrote the manuscript. All contributing authors collected and curated data, revised and approved the manuscript.

## **CONFLICTS OF INTEREST**

D.A.C. received research funding from Celgene Corporation, Amgen, Merck Sharp, and Dohme. C.P. received consulting fees, honoraria, and travel support from Amgen, Celgene Corporation, Janssen, Sanofi, and Takeda Oncology. G.C. received consulting fees and honoraria for Janssen, Bristol Myers Squibb (BMS), Amgen, Takeda, Karyopharm Therapeutics, and Oncopeptides; honoraria from Jazz Pharmaceuticals; and research support from Takeda and Celgene. K.B. received consultancy fees and honoraria from Janssen, Celgene/BMS, Sanofi, and Takeda Oncology. F.E.D. received honoraria and consulting fees from Celgene/BMS, Takeda, Sanofi, GSK, Janssen, Oncopeptides, Amgen, and AbbVie. M.J. received honoraria from Janssen Oncology, Takeda, and Celgene/BMS and consulted for Janssen Oncology, Takeda, AbbVie, Sanofi, and Celgene/BMS. G.J.M. received consultancy fees, honoraria, and travel support from Amgen, Celgene Corporation, Janssen, Sanofi, and Takeda Oncology. R.O. received honoraria from Janssen Oncology, BeiGene, and AstraZeneca and consulting fees from BeiGene and Janssen Oncology. G.J. received research funding from Takeda and Celgene/BMS and honoraria for speaking from Takeda, Celgene/BMS, Amgen, Janssen, Sanofi, and Oncopeptides. M.D. owns stock in Abbingdon Health. M.F.K. received consultancy and honoraria from AbbVie; research funding from BMS; consultancy from GSK; research funding, consultancy, and honoraria from Janssen; and consultancy from

Karyopharm, Pfizer, Regeneron, and Takeda. None of the remaining authors have any disclosures to declare.

#### **DATA AVAILABILTY STATEMENT**

Data are available on request from the corresponding author, Martin F. Kaiser (martin.kaiser@icr.ac.uk). Only methodologically sound proposals whose proposed use of the data has been approved by the independent trial steering committee will be considered. Following approval, data requestors will need to sign a data access agreement.

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## TABLES

**Table 1: Clinical, laboratory and molecular characteristics of the study cohort.**

|   | ER group<br>N = 25 | Non-ER group<br>N = 110 | P-value       |
|---|--------------------|-------------------------|---------------|
| <b>Clinical and treatment characteristics</b> |                    |                         |               |
| Age, years (range)                            | 57.9 (44 - 69)     | 56.6 (28 - 69)          | 0.5           |
| Sex, male, n (%)                              | 18 (72)            | 74 (67)                 | 0.83          |
| WHO PS, median (range)                        | 1 (0-3)            | 1 (0-3)                 | 0.77          |
| B2-microglobulin, mg/l (range)                | 5.7 (2 - 17.5)     | 4.8 (1.6 - 20)          | 0.419         |
| > 5.5, n (%)                                  | 7 (28)             | 24 (22)                 | 0.58          |
| > 5.5 & creatine < 1.2mg/dl, n (%)            | 4 (16)             | 13 (12)                 |               |
| Unknown                                       | 8 (32)             | 31 (28)                 | NA            |
| Haemoglobin level, g/l (range)                | 99.4 (52 - 151)    | 107.3 (63 - 151)        | 0.06          |
| ISS, n (%)                                    |                    |                         |               |
| 1   | 1 (4)              | 21 (19)                 | <b>0.018*</b> |
| 2   | 9 (36)             | 24 (22)                 | 0.12          |
| 3   | 7 (28)             | 18 (16)                 | 0.59          |
| R-ISS, n (%)                                  |                    |                         |               |
| 1   | 0                  | 9 (8)                   | <b>0.024*</b> |
| 2   | 14 (56)            | 44 (40)                 | 0.08          |
| 3   | 3 (12)             | 10 (9)                  | 1             |
| R2-ISS, n (%)                                 |                    |                         |               |
| 1   | 0                  | 9 (8)                   | 0.21          |
| 2   | 1 (4)              | 38 (34.5)               | <b>0.03*</b>  |
| 3   | 12 (48)            | 53 (48)                 | 0.38          |
| 4   | 4 (16)             | 10 (9)                  | <b>0.02*</b>  |
| Unknown ISS / R-ISS / R2-ISS                  | 8 (32)             | 47 (43)                 | NA            |
| MM type, n (%)                                |                    |                         |               |
| IgG   | 15 (60)            | 62 (56)                 | 0.8           |
| IgA   | 7 (28)             | 31 (28)                 | 1             |
| LCO   | 2 (8)              | 13 (12)                 | 0.7           |
| IgM   | 1 (4)              | 0                       | 0.2           |
| IgD   | 0                  | 2 (2)                   | 1             |
| Unknown                                       | 0                  | 2 (2)                   | NA            |
| HDC/ASCT, n (%)                               | 25 (100)           | 110 (100)               | 1             |
| Response pre maintenance, n (%)               |                    |                         |               |
| CR  | 6 (24)             | 35 (32)                 | 0.63          |
| VGPR  | 15 (60)            | 64 (58)                 | 1             |
| PR  | 4 (16)             | 10 (9)                  | 0.29          |
| Len maintenance, n (%)                        | 25 (100)           | 110 (100)               | 1             |
| <b>Molecular characteristics</b>              |                    |                         |               |

|  |          |           |                    |
|--|----------|-----------|--------------------|
| Complete genetic panel, n (%)          | 25 (100) | 110 (100) | NA                 |
| HRD                                    | 10 (40)  | 56 (51)   | 0.45               |
| t(4;14) or t(14;16) or t(14;20), n (%) | 11 (44)  | 22 (20)   | <b>&lt; 0.01**</b> |
| t(11;14), n (%)                        | 5 (20)   | 18 (16)   | 0.89               |
| 1q+, n (%)                             | 14 (56)  | 43 (39)   | 0.09               |
| Gain 1q, n (%)                         | 11 (44)  | 32 (29)   |                    |
| Amp1q, n (%)                           | 3 (12)   | 11 (10)   |                    |
| Del1p, n (%)                           | 6 (24)   | 13 (12)   | 0.2                |
| Hemizygous deletion of 1p              | 4 (16)   | 11 (10)   |                    |
| Homozygous deletion of 1p              | 2 (8)    | 2 (2)     |                    |
| Del17p, n (%)                          | 5 (20)   | 11 (10)   | 0.29               |
| Hemizygous deletion of 17p             | 5 (20)   | 11 (10)   |                    |
| Homozygous deletion of 17p             | 0        | 0         |                    |
| Number of HRCA, n (%)                  |          |           |                    |
| 0 HRCA                                 | 7 (28)   | 50 (45.5) | 0.12               |
| 1 HRCA (single hit)                    | 6 (24)   | 38 (34.5) | 0.35               |
| 2 or more HRCA (double hit)            | 12 (48)  | 22 (20)   | <b>&lt; 0.01**</b> |
| 3 or more HRCA (triple hit)            | 5 (20)   | 6 (5)     | <b>0.03*</b>       |
| IMS/IMWG-HR, n (%)*                    |          |           |                    |
| Standard risk                          | 11 (44)  | 76 (69)   | <b>0.03*</b>       |
| High risk                              | 14 (56)  | 34 (31)   |                    |
| GEP-HR (SKY-92 signature), n (%)       |          |           |                    |
| Standard risk                          | 11 (44)  | 91 (82.7) | <b>&lt; 0.01**</b> |
| High risk                              | 14 (56)  | 19 (17.3) |                    |
| IMS/IMWG- & GEP-SR*                    | 5 (20)   | 68 (62)   | <b>0.01*</b>       |
| IMS/IMWG- & GEP-HR*                    | 8 (32)   | 11 (10)   |                    |

PS, Performance Status; MM, Multiple Myeloma; LCO, Light chain only; ISS, international staging system; HDC/ASCT, High-dose chemotherapy/autologous stem cell transplantation; CR, complete response; VGPR, very good partial response; PR, partial response; HRCA, high-risk cytogenetic abnormalities; GEP, gene expression profiling; IMS, International Myeloma Society; IMWG, International Myeloma Working Group; NA, not applicable. \* Note: IMS/IMWG-HR in this table is defined exclusively by cytogenetic aberrations and does not consider TP53 mutation status, which was only assessed in 11 patients, with 1 patient found to harbour a TP53 mutation (see Figure 1).

## FIGURES LEGENDS

**Figure 1: (A) Study overview:** Functional high-risk (HR) multiple myeloma (MM) in 135 Myeloma-XI trial patients was determined using advanced molecular profiling, including HRCA per updated IMS/IMWG-HR criteria and GEP-HR based on the SKY92 signature. **(B) Risk profiles of early relapse (ER) patients.** Barplot illustrating the composition of four risk groups based on IMS/IMWG-HR and GEP-HR classification (SR, IMS/IMWG-HR, GEP-HR, IMS/IMWG-HR & GEP-HR) in the 25 patients with ER. SR, standard risk; HRCA, high-risk cytogenetic abnormalities; GEP, gene expression profiling; IMS, International Myeloma Society; IMWG, International Myeloma Working Group; ASCT, autologous stem cell transplantation; Lena, Lenalidomide; PCL, plasma cell leukaemia; CTPC, circulating tumour plasma cells.

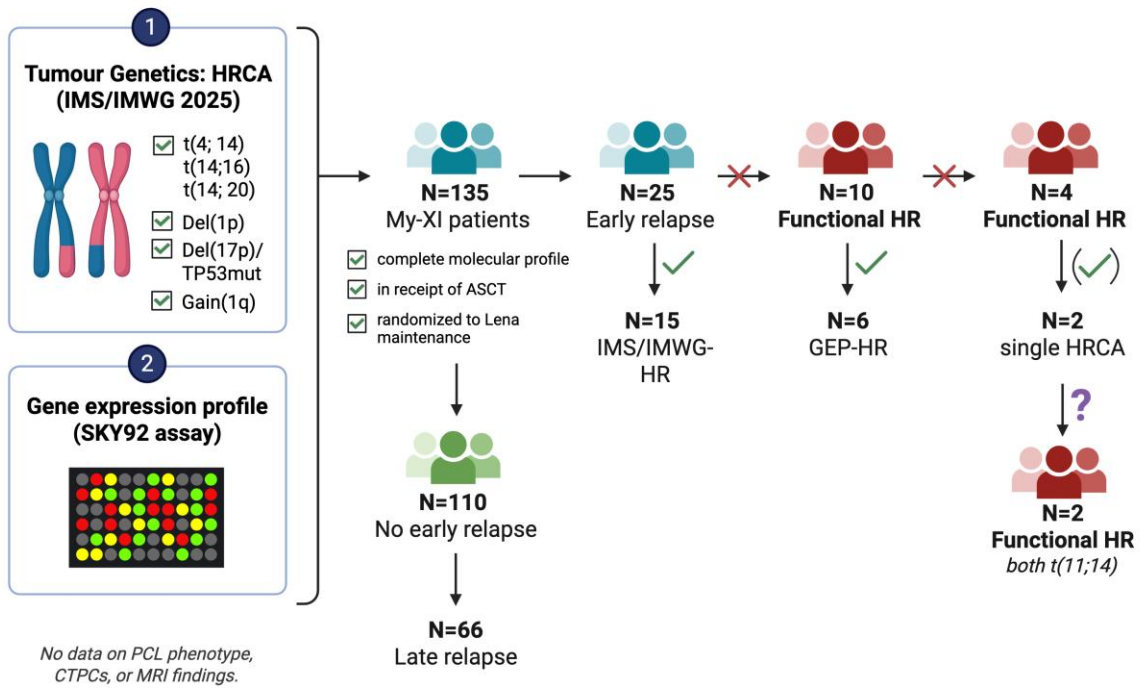
**Figure 2: Kaplan–Meier survival estimates for the entire study cohort (n = 135).** **(A) Progression-free survival and (B) overall survival** stratified by IMS/IMWG-HR and GEP-HR classification (SR, IMS/IMWG-HR, GEP-HR, IMS/IMWG-HR & GEP-HR). X-axis: time since maintenance randomization (months), y-axis: survival probability, risk tables: number of patients at risk. Log-rank *P*-values are shown. IMS, International Myeloma Society; IMWG, International Myeloma Working Group; GEP, gene expression profiling; SR, standard risk; HR, high risk.

**Figure 3: (A) Sankey plot illustrating the composition and contribution of individual risk factors to the updated IMS/IMWG-HR classification.** Six groups are displayed based on combinatorial risk features, including HRCA count, GEP signature, and isolated b2m elevation. Elevated b2m is subdivided by creatinine levels below or above the ULN, according to the IMS/IMWG-HR definition. Note that one patient with GEP-HR carried an isolated del(17p) and therefore also classified as IMS/IMWG-HR. Please note that TP53 mutation status was not included in this analysis, as only 11 patients in the study had available information. **(B) Co-occurrence of HR genetic features in patients harbouring del(1p).** Each row represents one of 19 individual patients, while columns indicate the presence (grey) or absence (white) of a HR feature, including IMS/IMWG-defined HRCAs and GEP-HR. Biallelic del(1p) are highlighted in dark red. HRCA, high-risk cytogenetic abnormalities; IMS, International Myeloma Society; IMWG, International Myeloma Working Group; GEP, gene expression profiling; b2m, beta 2 microglobulin; SR, standard risk; HR, high risk; ULN, upper limit of normal.

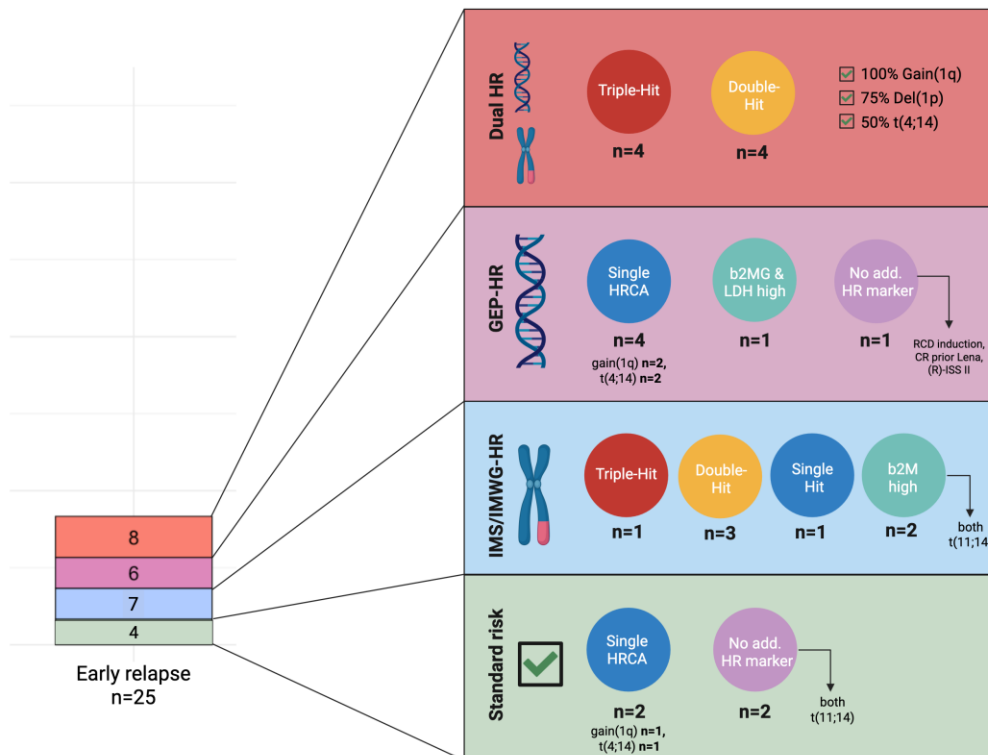
**Figure 4: Oncoplot illustrating HR features at an individual patient level (n = 135),** including IMS/IMWG-defined HRCAs, GEP-HR, t(11;14), and elevated b2m or LDH. ER as in progression free survival (PFS, defined < 18 months or < 24 months) and overall survival (OS) events (at 48 months and 60 months) are shown in red, while no event displayed in grey. Co-occurrence of  $\geq 2$  HR features appear in dark blue, single HR features in light blue, and no HR feature in white. Translocation t(11;14) is separately listed in green. Elevated laboratory parameters (b2m, LDH) are marked in pink. Please note that TP53 mutation status was not included in this analysis, as only 11 patients in the study had available information HRCA, high risk cytogenetic abnormalities; IMS, International Myeloma Society; IMWG, International Myeloma Working Group; GEP, gene expression profiling; ER, early relapse; b2m, beta 2 microglobulin; LDH, lactate dehydrogenase.

# FIGURES

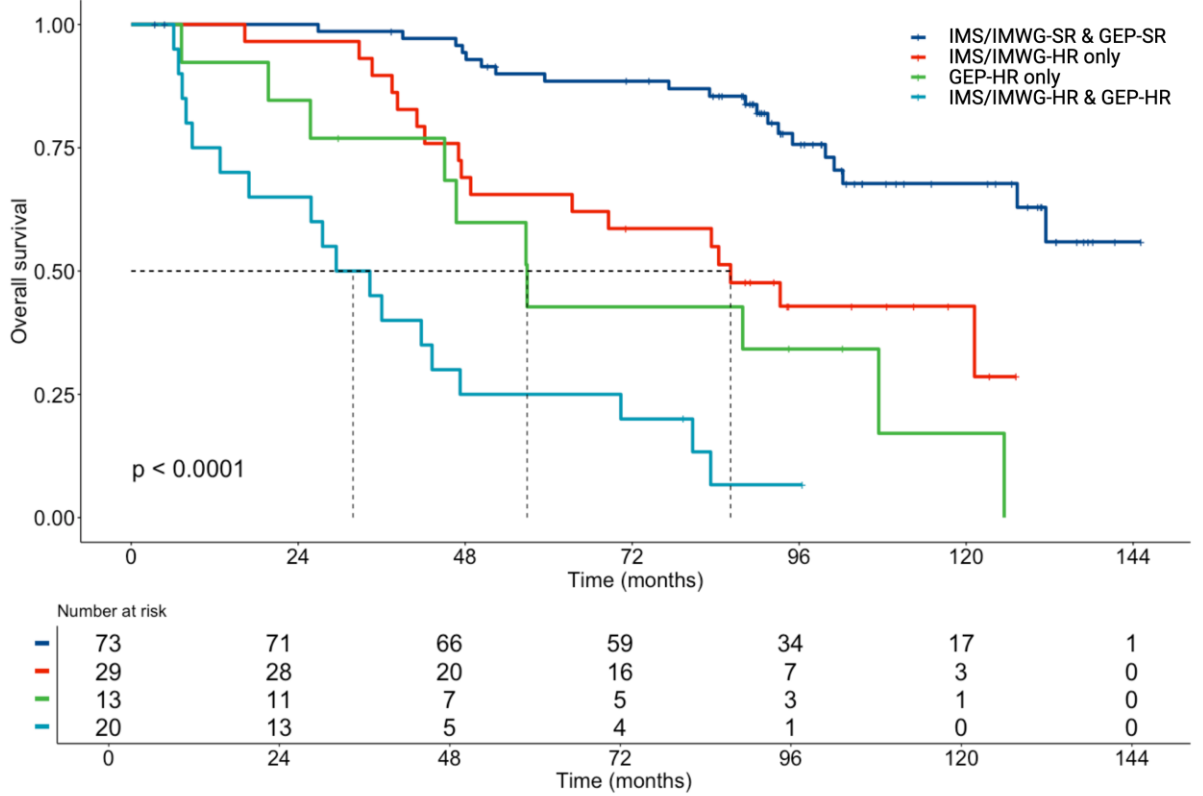
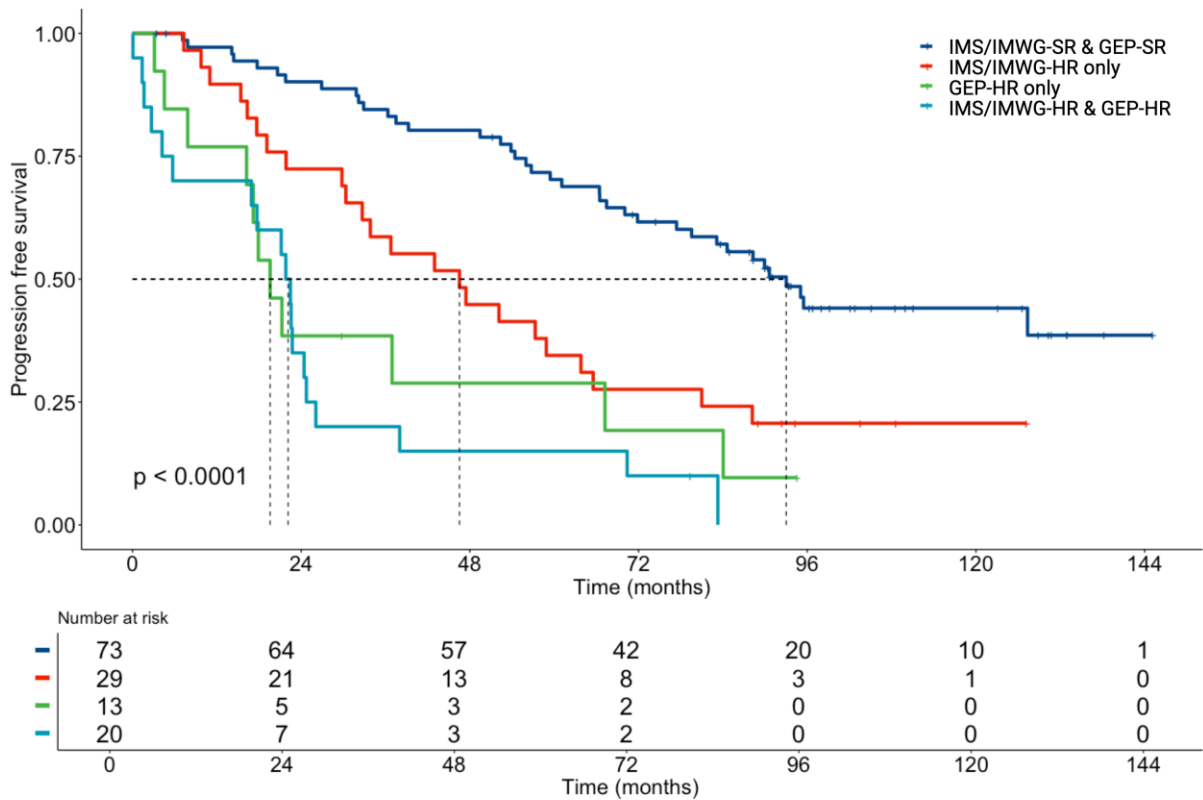
## Figure 1A



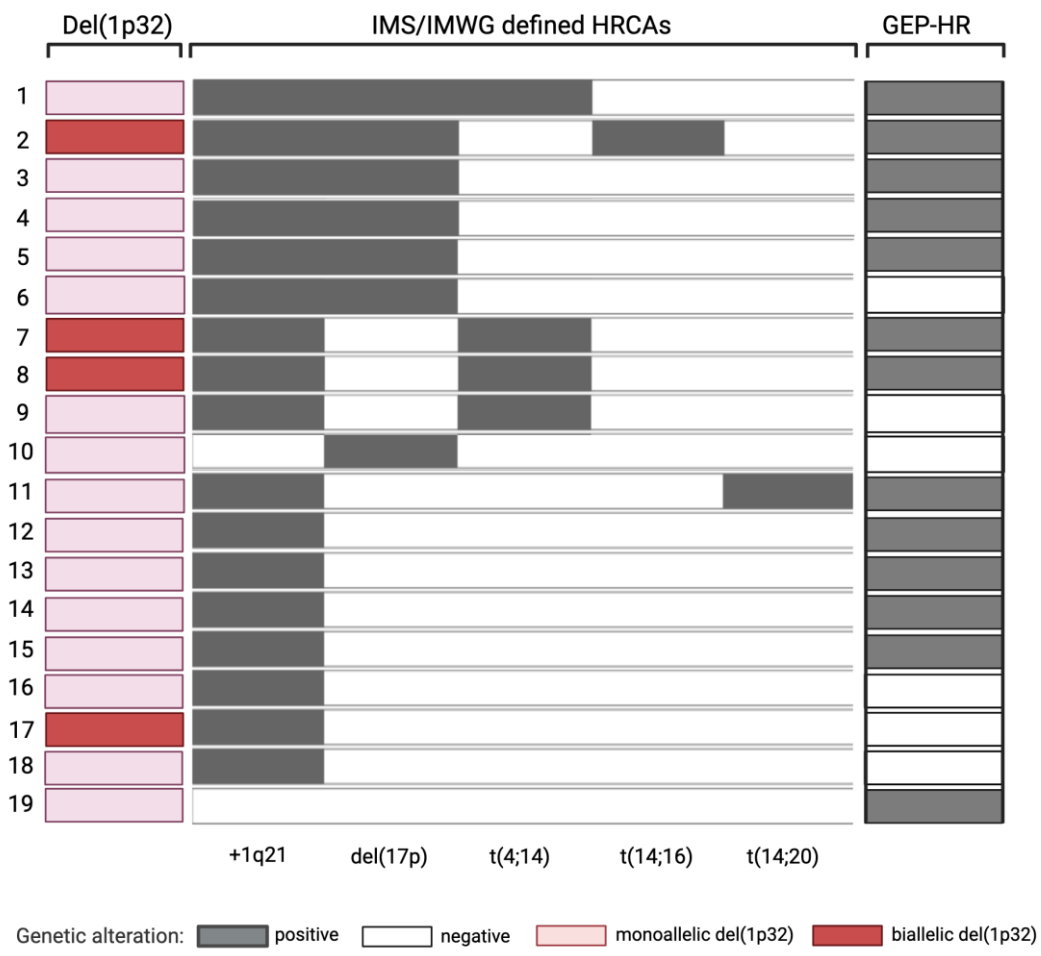
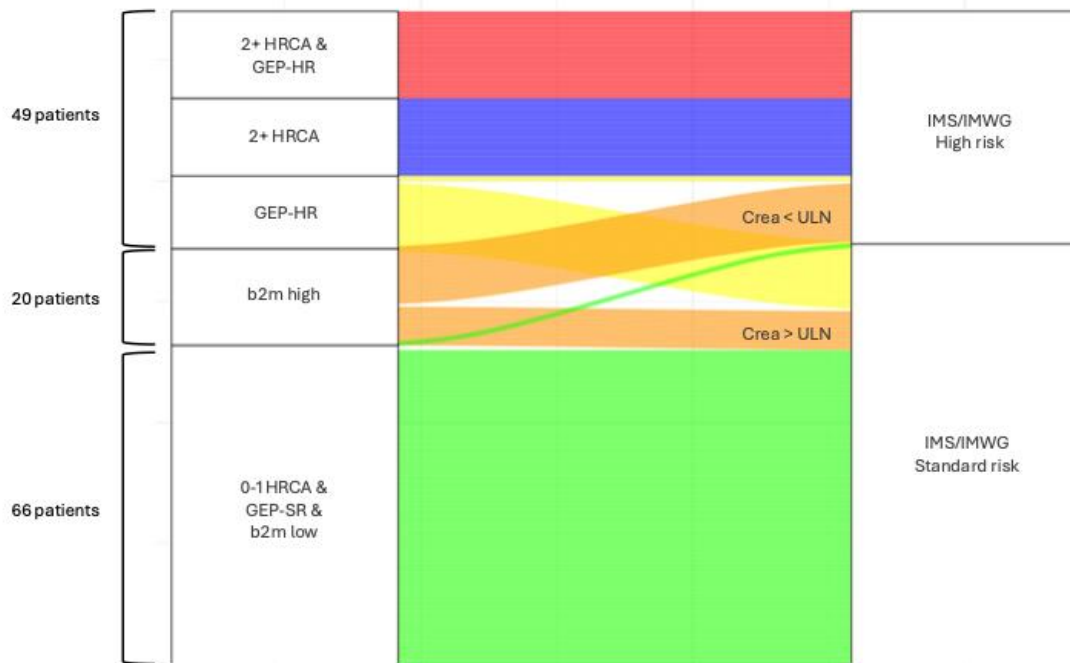
## Figure 1B



**Figure 2A and 2B**



**Figure 3A and 3B**



**Figure 4**

