

## ORIGINAL PAPER

## Haematological Malignancy – Clinical

# Enhancing risk stratification and treatment decision in multiple myeloma with SKY92 gene expression profiling in real-world data

Noa Biran<sup>1</sup>  | Binod Dhakal<sup>2</sup>  | Ruben Niesvizky<sup>3</sup> | Suzanne Lentzsch<sup>4</sup> |  
 Divaya Bhutani<sup>4</sup>  | John T. McKay<sup>5</sup> | David H. Vesole<sup>1,6</sup> | Ajay Nooka<sup>7</sup>  |  
 Barry Paul<sup>8</sup>  | Parameswaran N. Hari<sup>2</sup> | Silvia D'Ambrosi<sup>9</sup>  | Rowan Kuiper<sup>9</sup> |  
 Martin van Vliet<sup>9</sup> | David Siegel<sup>1</sup> | Saad Z. Usmani<sup>10</sup> | Frits van Rhee<sup>11</sup>

<sup>1</sup>Myeloma Division, Hackensack University Medical Center, Hackensack, New Jersey, USA

<sup>2</sup>Division of Hematology and Oncology, Department of Medicine, Medical College of Wisconsin, Milwaukee, Wisconsin, USA

<sup>3</sup>Division of Hematology & Medical Oncology, Weill Cornell Medicine/New York Presbyterian Hospital, New York, New York, USA

<sup>4</sup>Division of Hematology and Oncology, Columbia University Medical Center, New York, New York, USA

<sup>5</sup>Atrium Health Wake Forest Baptist Medical Center, Winston-Salem, North Carolina, USA

<sup>6</sup>Lombardi Comprehensive Cancer Center, Medstar Georgetown Medical Center, Washington, District of Columbia, USA

<sup>7</sup>Department of Hematology and Medical Oncology, Winship Cancer Institute, School of Medicine, Emory University, Atlanta, Georgia, USA

<sup>8</sup>Department of Hematologic Oncology & Blood Disorders, Levine Cancer Institute, Atrium Health/Wake Forest Baptist, Charlotte, North Carolina, USA

<sup>9</sup>SkylineDx, Rotterdam, The Netherlands

<sup>10</sup>Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, New York, USA

<sup>11</sup>Myeloma Center, Winthrop P. Rockefeller Cancer Institute, University of Arkansas for Medical Sciences, Little Rock, Arkansas, USA

**Correspondence**

Noa Biran, Myeloma Division, Hackensack University Medical Center, Hackensack, NJ, USA.

Email: [noa.biran@hmn.org](mailto:noa.biran@hmn.org)

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SkylineDx

**Summary**

Over the years, numerous prognostic markers for multiple myeloma (MM) risk classification have been identified; however, their variability can lead to inconsistent clinical interpretations. Gene expression profiling (GEP) signatures, such as SKY92, offer a more accurate method for patient stratification. The PROspective Observational Multiple Myeloma Impact Study (NCT02911571) aimed to validate SKY92's prognostic performance using real-world data and assess its impact on risk classification and treatment decisions compared to conventional markers. In a study of 251 newly diagnosed MM patients, physicians completed questionnaires to capture risk classification, hypothetical treatment plans and their confidence in those plans before and after unblinding SKY92 results. Poor concordance was observed between initial clinical risk assessment (iCRA) and SKY92 results (high risk: 51% iCRA vs. 28% SKY92, Cohen's  $\kappa=0.21$ ). SKY92 showed superior performance in identifying high-risk patients, leading to better predictions of progression-free survival and overall survival ( $p \leq 0.0001$ ) than traditional risk markers. Unblinding SKY92 results led to hypothetical treatment revisions for 50% of patients ( $p < 0.001$ ) and increased physicians' confidence in treatment decisions for 40% of cases. These findings support SKY92's prognostic value in identifying high-risk MM patients, outperforming traditional risk markers and demonstrating the potential added value of its integration into clinical practice for more personalized risk assessment.

**KEY WORDS**

gene expression profiling, molecular hematology, multiple myeloma, prognostic

## INTRODUCTION

MM is a haematological malignancy mainly affecting plasma cells (PCs) in the bone marrow.<sup>1</sup> MM comprises around 1% of all cancer and 14% of blood-related cancers.<sup>2,3</sup> It primarily affects the elderly, with a median diagnosis age of 69 years.<sup>2</sup> Consequently, the prevalence of MM is anticipated to increase due to the ageing population worldwide.<sup>4</sup> Its heterogeneity is reflected by a diverse clinical and molecular landscape, correlating with the prognosis of the patients and resulting in survival ranging from a few months to over 10 years.<sup>5</sup> Thanks to novel therapies, the survival of newly diagnosed multiple myeloma (NDMM) patients has significantly increased in the last decade.<sup>6</sup> Despite recent advancements, some patients, termed high risk (HR), derive limited benefit from modern therapies. The definition of HR has evolved over the years, leading to various interpretations regarding the characterization of HR disease features. Despite various validated risk stratification systems, there is no standardized method for assessing risk in MM. Establishing what clinical or molecular data should be routinely gathered in practice for the identification of HR patients is crucial for effectively defining baseline risk. This would aid in understanding prognosis and treatment response, resulting in more informed risk-based therapy decisions, including which patients may benefit from participating in clinical trials.

In addition to traditional staging systems and chromosomal aberrations associated with outcome, gene expression profiling (GEP) based on the genetic profile of myeloma cells has emerged as a valuable tool for risk stratification in MM. Guidelines, such as those from the National Comprehensive Cancer Network (NCCN), recognize GEP as a valuable tool to identify HR MM patients, including those who may not be identified through conventional risk classification methods.<sup>7-9</sup>

The SKY92 molecular signature is based on the GEP of 92 genes derived from bone marrow PCs. This prognostic model classifies patients into two groups: HR, characterized by poorer survival outcomes, and standard risk (SR). SKY92 can accurately predict the prognosis for NDMM and relapsed/refractory multiple myeloma patients for both PFS and OS.<sup>10</sup> Since its discovery in 2012, SKY92 has been independently validated across 16 patient cohorts, comprising a total of 3339 patient cases including both retrospective studies and clinical trials.<sup>11</sup>

Building on these findings, a prospective multicentre study (PROspective Observational Multiple Myeloma Impact Study [PROMMIS]; NCT02911571) was designed to validate SKY92's prognostic performance for PFS and OS using real-world data, and to compare SKY92 with other classification methodologies to determine its added value in clinical practice.

## METHODS

### Study design

PROMMIS trial (NCT02911571) is an observational, prospective, multi-centre study involving nine US hospitals (Table S1). Inclusion criteria were adults ( $\geq 18$  years) with

confirmed MM per International Myeloma Working Group (IMWG) criteria, suitable for systemic therapy with an immunomodulatory drug and/or proteasome inhibitor, and who had received  $\leq 8$  weeks of initial treatment.<sup>12</sup> Exclusion criteria were an Eastern Cooperative Oncology Group (ECOG)<sup>13</sup> performance status over 3, active pregnancy or failure to meet the bone marrow sample's quality criteria as defined by the MMprofiler's instruction for use (IFU) (File S1).

The study protocol was approved by the institutional review boards of participating centres and all participants provided written informed consent.

The study has been designed with the following objectives:

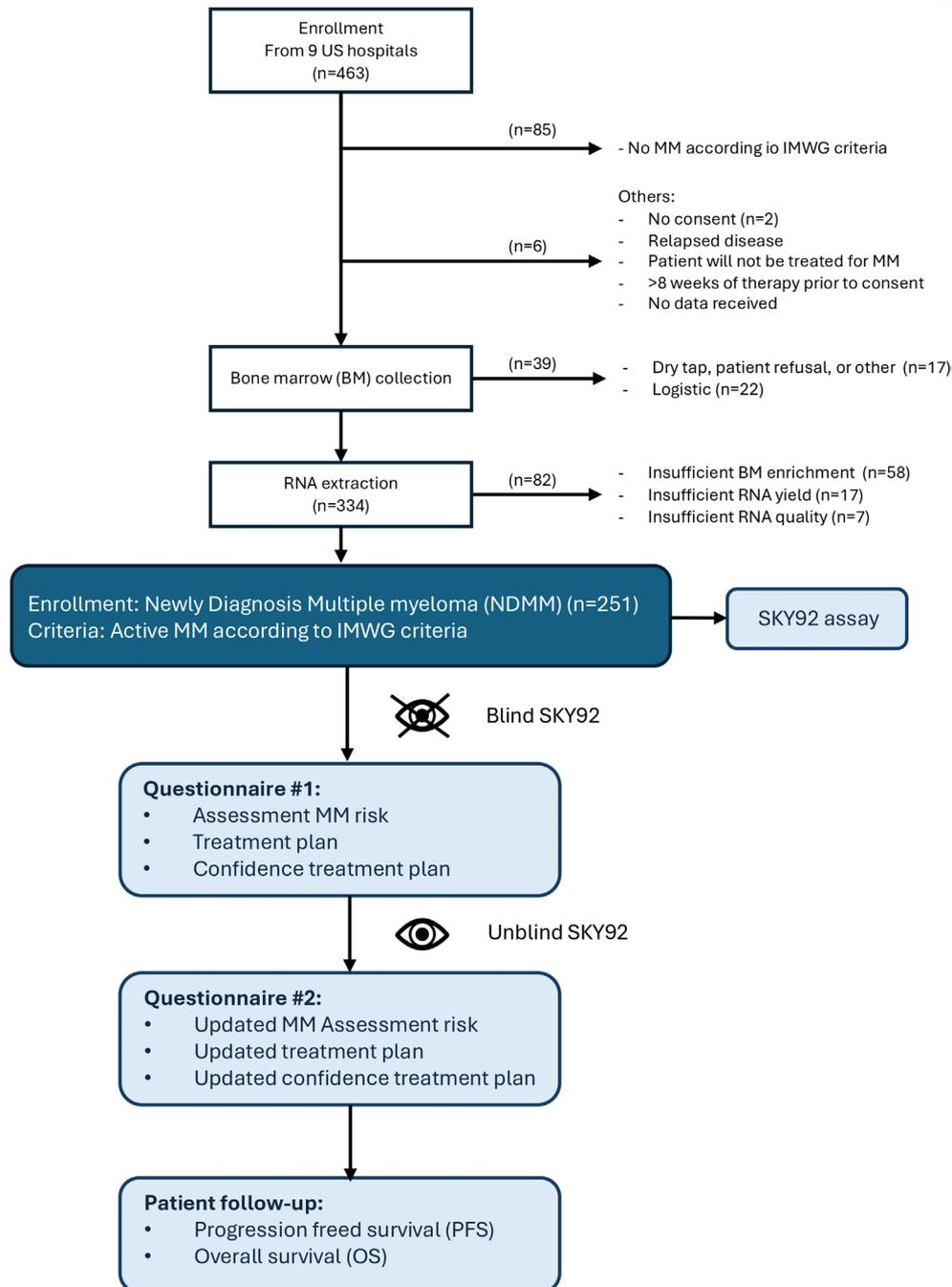
1. To prospectively validate the prognostic performance of the SKY92 classifier for PFS and OS, evaluating both survival outcomes using conventional stratification methods and comparing them with the impact of SKY92 on clinical decision-making.
2. To evaluate the impact of SKY92 on physician decisions regarding
  - Patient risk classification.
  - Intended patient treatment plan.
  - Clinicians' confidence in their proposed treatment plan.

Physicians were asked to complete a questionnaire for each patient both before and after unblinding the SKY92 classification (Figure 1; File S1). In the pre-unblinding questionnaire, the physician stated (1) their initial clinical risk assessment (iCRA) reflecting the disease risk in their routine clinical practice; (2) provided their proposed treatment plan; (3) and indicated their confidence in the proposed treatment plan. Following the unblinding of the SKY92 classifications, the physicians completed a second questionnaire that repeated the same questions (final clinical risk assessment—fCRA). The details of the questionnaires are outlined in the File S1 and Biran et al.<sup>14</sup> By comparing the responses from the two questionnaires, we quantified the impact of the SKY92 classification on risk assessment, treatment plans and physician confidence in their clinical decisions. It should be noted that any changes in the treatment plan post-unblinding of SKY92 were hypothetical and intended solely for the purposes of this study.

Clinical and pathological characteristics of each patient were documented through electronic clinical report forms. Cytogenetic aberration analyses were performed according to the protocols and guidelines of the laboratory at each participating hospital or their referral laboratory. In this study, gain 1q21 is defined as the presence of three or more copies of the 1q21 region.

### SKY92

Bone marrow sample workup was performed either at one of the local reference laboratories (Hackensack University Medical Center, Columbia University Medical Center, Versiti Blood Center of Wisconsin and Carolinas Pathology Group) or at SkylineDx's CAP/CLIA laboratory in San Diego.



**FIGURE 1** CONSORT Diagram of the PROMMIS trial analysis. IMWG, International Myeloma Working Group; MM, multiple myeloma.

SKY92 classifications were obtained using the MMprofiler assay (SkylineDx), following the manufacturer's instructions. Briefly, fresh bone marrow aspirates, collected in heparin or ethylene-diaminetetraacetic acid tubes, were subjected to Ficoll density gradient centrifugation, and the PC-enriched buffy coat layer was isolated for further analysis. PCs were further isolated using CD138+ immunomagnetic beads (EasySep™; Stem Cell Technologies) and preserved in RNeasy Lysis Buffer (RLT) buffer. Samples were included for analyses only if they contained  $\geq 80\%$  CD38+ cells, as assessed by fluorescence-activated cell sorting.

RNA was extracted using a DNA/RNA AllPrep kit (QIAGEN; Hilden) according to the manufacturer's instructions. Extracted RNA underwent assessment for concentration, quality and purity using Nanodrop. A minimum of 100 ng total RNA was used as test input. RNA was retrotranscribed to cDNA, and fragmented cRNA was then mixed with hybridization reagents. The mix was hybridized to a U133 Plus 2.0 GeneChip (Thermo Fisher) and imaged using a GCS3000Dx2 scanner (Thermo Fisher).

Samples failing to meet the quality control criteria outlined in the MMprofiler's IFU were excluded from further

analysis. The SKY92 scores were calculated as previously described.<sup>10</sup>

## Statistical analysis

PFS and OS were defined as the time from diagnosis until event, that is, progression or death from any cause, respectively. Patients who had not experienced the event by the end of the study period or had been lost to follow up were censored at the last known event-free time point. The median follow-up was estimated using the reverse Kaplan–Meier method.<sup>15</sup> All computational analyses were done in R4.4.0. The association between risk status and outcome was assessed by Cox proportional hazards models using the ‘*coxph*’ function and checked for violation of the proportional hazard assumption by the ‘*cox.zph*’ function. Kaplan–Meier survival estimates were determined by the ‘*survfit*’ function. Survival analyses were performed using the survival package (v3.7.0).

The proportion of patients whose treatment plans changed after unblinding SKY92 was evaluated using a two-sided exact binomial test. The principal investigators were aligned in considering 15% to be the acceptable predefined threshold of clinical relevance.

## RESULTS

### Patient enrolment and clinicopathologic characteristics

From February 2018 to August 2021, 463 patients from nine institutes were enrolled in the PROMMIS study (Figure 1). Of these, 85 did not meet the IMWG criteria and were consequently excluded. Additionally, 127 patients were excluded from the trial; 82 due to low bone marrow purity or insufficient RNA quality to proceed with the workup for the SKY92 classifier and 45 due to various reasons such as withdrawal of consent, logistical issues during bone marrow collection, or other. Ultimately, 251 patients were enrolled and used in this interim analysis. The median age was 66 years (range: 35–95), with males representing 62% of the cohort. Based on R-ISS staging, 31% of patients were classified as stage I, 58% as stage II and 11% as stage III. Cytogenetics were assessed for all but one patient, showing 16% with del(17p), 9.6% with t(4;14), 3.6% with t(14;16), 22% with t(11;14), 53% with del(13q) and 41% with gain(1q21) (Table S2).<sup>16</sup> The median follow-up for all 242 patients is 44 months (95% confidence interval [CI]: 43–46).

### Risk classification for MM patients through clinical parameters

Before unblinding the SKY92 results, physicians conducted the iCRA, based on the clinical parameters used in their

clinic. This process classified 51% (127/251) of patients as HR and 49% (124/251) as SR. HR patients' proportion varies significantly across hospitals, ranging from 10% to 63% ( $p < 0.001$ ; Figure S1A).

A strong association was observed between iCRA and R-ISS ( $p < 0.001$ ) and its components t(4;14) ( $p = 0.003$ ), t(14;16) ( $p = 0.036$ ), del(17p) ( $p < 0.001$ ),  $\beta$ 2-microglobulin ( $p = 0.002$ ), albumin ( $p = 0.006$ ) and LDH ( $p = 0.034$ ), as well as for gain(1q21) ( $p < 0.001$ ; Table S2).

Patients within the iCRA-HR group demonstrated shorter PFS compared to the SR group, with a hazard ratio and 95% CI of 1.65 [1.13–2.42] ( $p = 0.01$ ). However, no significant difference in OS was observed (hazard ratio: 1.87, [0.93–3.78],  $p = 0.08$ ; Figure 2A). The 3-year survival rates for PFS in the two risk groups were HR: 50% [41–60] and SR: 66% [57–75], and for OS, they were HR: 84% [77–91] and SR: 92% [86–97].

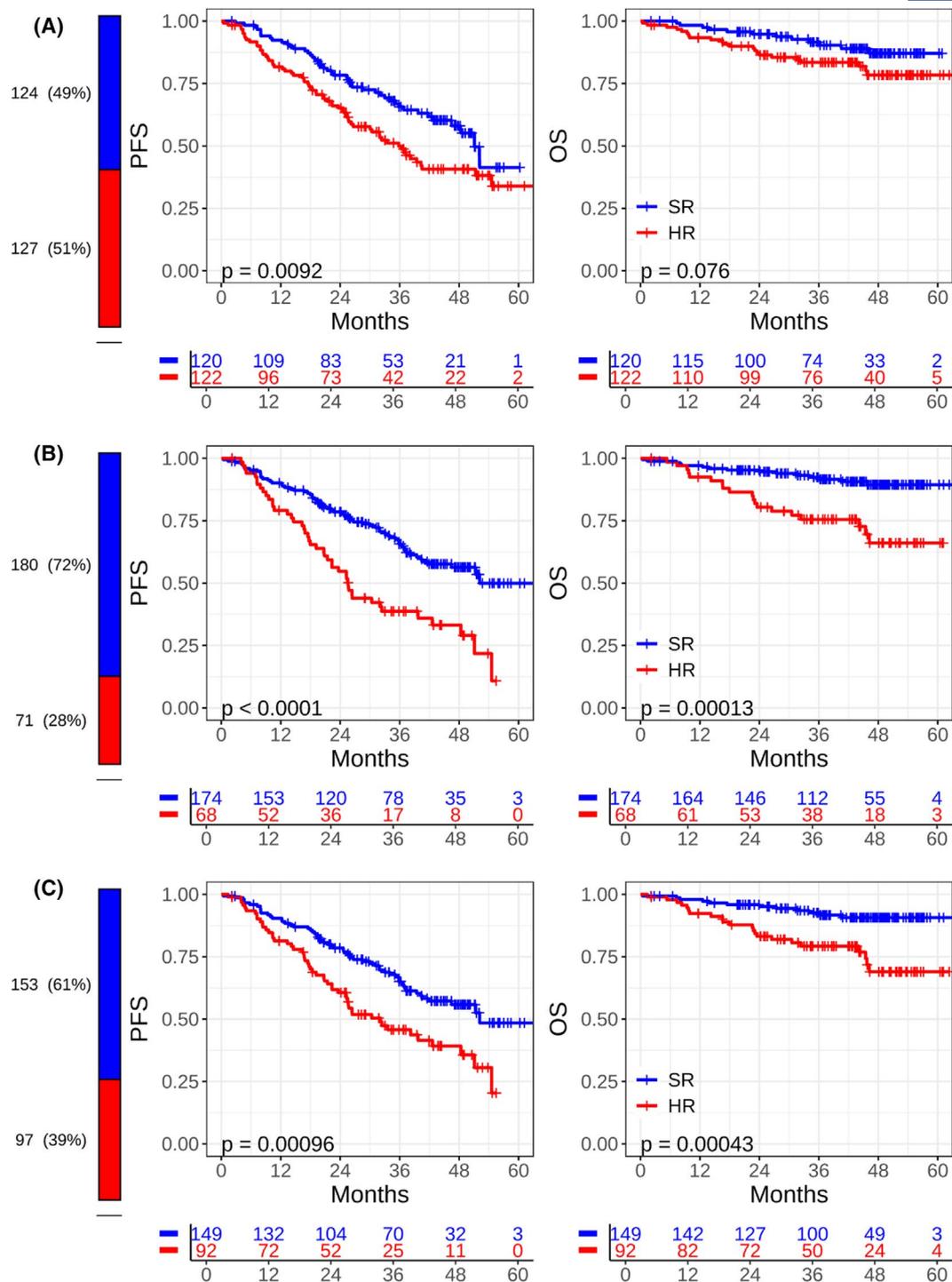
Furthermore, no significant prognostic value was found based on individual cytogenetic abnormalities (Figure S2) or their co-occurrence (Figure S3), with the only exception of gain(1q21), which was associated with shorter PFS and OS. Notably, its prognostic significance for PFS was exclusive to patients aged 65 years and older (Figure S4).

### SKY92 enhances identification of HR MM patients

SKY92 identified 28% (71/251) of patients with a SKY92-HR profile and 72% (180/251) with a SKY92-SR profile (Figure 2B). In contrast to iCRA, SKY92 demonstrated a significant difference between the two risk groups in terms of both PFS (hazard ratio: 2.30, [1.57–3.37],  $p < 0.001$ ) and OS (hazard ratio: 3.45, [1.75–6.80],  $p < 0.001$ ). The 3-year survival rates and 95% CI's were HR: 39% [28–53] and SR: 66% [58–74] for PFS and HR: 76% [66–87] and SR: 92% [88–97] for OS. Interestingly, while the proportion of iCRA-HR differs significantly between sites, no such statistical variability was seen for SKY92 (Figure S1B).

A strong discrepancy is observed between iCRA and SKY92 risk classification. Specifically, a 40% of patients (iCRA-SR: 9%; 22/251, and iCRA-HR: 31%; 78/251) have been differently classified by SKY92 compared to iCRA (Figure S5A). By comparing both assessments, we found that patients identified as HR by both methods (20%, 49/251) exhibited significantly shorter PFS and OS compared to those classified as SR by both methods or as SR by at least one of the approaches (PFS: hazard ratio: 3.37, [2.07–5.48],  $p < 0.001$  and OS: hazard ratio: 4.29, [1.89–9.72],  $p < 0.001$ ) (Figure S5B). The 3-year survival rates and 95% CI's for PFS between the two risk groups were 28% [17–45] for iCRA\_SKY92-HR and 67% [57–78] for iCRA\_SKY92-SR, and for OS, they were 69% [56–84] for iCRA\_SKY92-HR and 92% [87–98] for iCRA\_SKY92-SR.

Consistent with previous studies, SKY92 is independently prognostic from cytogenetic aberrations (Table 1). Patients with a SKY92 HR profile consistently had significantly shorter PFS compared to those with an SR profile, regardless



**FIGURE 2** Survival for (A) iCRA, (B) SKY92 and (C) fCRA. Shown are risk distributions by the bar plots, and Kaplan–Meier plots for progression-free survival (PFS) (left panel) and overall survival (OS) (right panel), with a log-rank  $p$ -value for high risk (HR; red) versus standard risk (SR; blue).

of cytogenetic status (Figure 3). Specifically, SKY92 SR patients showed no significant PFS variation with or without cytogenetic aberrations. SKY92 HR patients also generally had shorter PFS, except for those with gain(1q21). Among patients with gain(1q21), only those with a SKY92 HR profile had notably shorter PFS compared to other subgroups (Figure 3D; Table S3).

### Integration of SKY92 into R-ISS

Correlation suggests that the physician's decision on risk classification is also influenced by R-ISS (Table S2). Information on R-ISS status was available for 92% (230/251) of patients. Among them, 31% (71/230) were classified as R-ISS I, 58% (133/230) as R-ISS II and 11% (26/230) as R-ISS III. The 3-year

**TABLE 1** Multivariate Cox model for progression-free survival (PFS) and overall survival (OS) including SKY92 together with cytogenetic markers and R-ISS. Markers t(4;14) and del(17) for PFS, and del(13q) for OS, had to be excluded due to violation of the proportional hazards assumption.

Characteristic	PFS			OS		
	HR	95% CI	p-Value	HR	95% CI	p-Value
SKY92	1.80	1.16, 2.80	0.009	2.55	1.19, 5.46	0.016
del(17p)	0.98	0.55, 1.76	>0.9	0.54	0.19, 1.57	0.3
t(14;16)	0.55	0.17, 1.79	0.3	0.28	0.04, 2.15	0.2
gain(1q)	1.45	0.95, 2.23	0.086	1.77	0.82, 3.81	0.14
del(13q)	0.91	0.60, 1.39	0.7			
t(4;14)				0.23	0.05, 1.01	0.051
R-ISS						
I	—	—		—	—	
II	1.56	0.95, 2.56	0.080	6.48	1.50, 28.0	0.012
III	2.18	1.05, 4.53	0.036	19.0	3.88, 93.4	<0.001

Abbreviations: CI, confidence interval; HR, hazard ratio.

PFS rates and 95% CI's per R-ISS stage were I: 72% [61–85], II: 53% [45–63] and III: 39% [23–67], and correspondingly for OS: I: 97% [93–100], II: 86% [80–93] and III: 63% [45–87] (Figure 4A; Table S4A).<sup>17</sup> No statistical variability has been observed on R-ISS risk distribution between sites (Figure S1C). Integrating R-ISS staging with SKY92 classification subdivided patients into three risk group categories: low-risk (LR) 27% (63/230), intermediate-risk (IR) 47% (109/230) and HR 25% (58/230) (Figure 4B). Consistent with previous studies, R-ISS and SKY92 hold independent prognostic value (Table 1). This combined classification method increased the number of HR patients when compared to the R-ISS alone (25% vs. 11%), while maintaining its prognostic value. The HR group exhibited worse survival compared to the largest risk group (i.e. IR) with PFS—hazard ratio: 2.10 [1.36–3.23],  $p < 0.001$  and OS—hazard ratio: 3.38 [1.64–6.97],  $p < 0.001$ . In addition, the stratification was extended with a group of LR patients additional to the SKY92, with a favourable survival relative to the IR group (PFS—hazard ratio: 0.68, [0.40–1.17],  $p = 0.2$  and OS—hazard ratio: 0.28 [0.06–1.25],  $p = 0.1$ ) (Table S4B). Observed 3-year survival rates for PFS were LR: 74% [62–87], IR: 60% [51–71], HR: 36% [25–52] and for OS were LR: 97% [92–100], IR: 91% [85–97], HR: 70% [59–84].

### Impact of SKY92 on physician risk assessment for MM patients

Upon unblinding the SKY92 results, physicians re-assessed the risk of each patient into a fCRA. They indicated 39% of patients (97/250) as fCRA-HR and 61% (153/250) as fCRA-SR (Figures 2C and 5A). For one patient, the fCRA was not disclosed.

The classifications for fCRA and SKY92 aligned for 90% (224/250) of the patients, whereas it aligned for 71% (177/250) with the iCRA. Specifically, all SKY92-HR patients (71/71) were classified as fCRA-HR and 85% (153/179) of SKY92-SR patients were considered fCRA-SR. When examining the PFS and OS of the remaining 26 (24 with follow-up

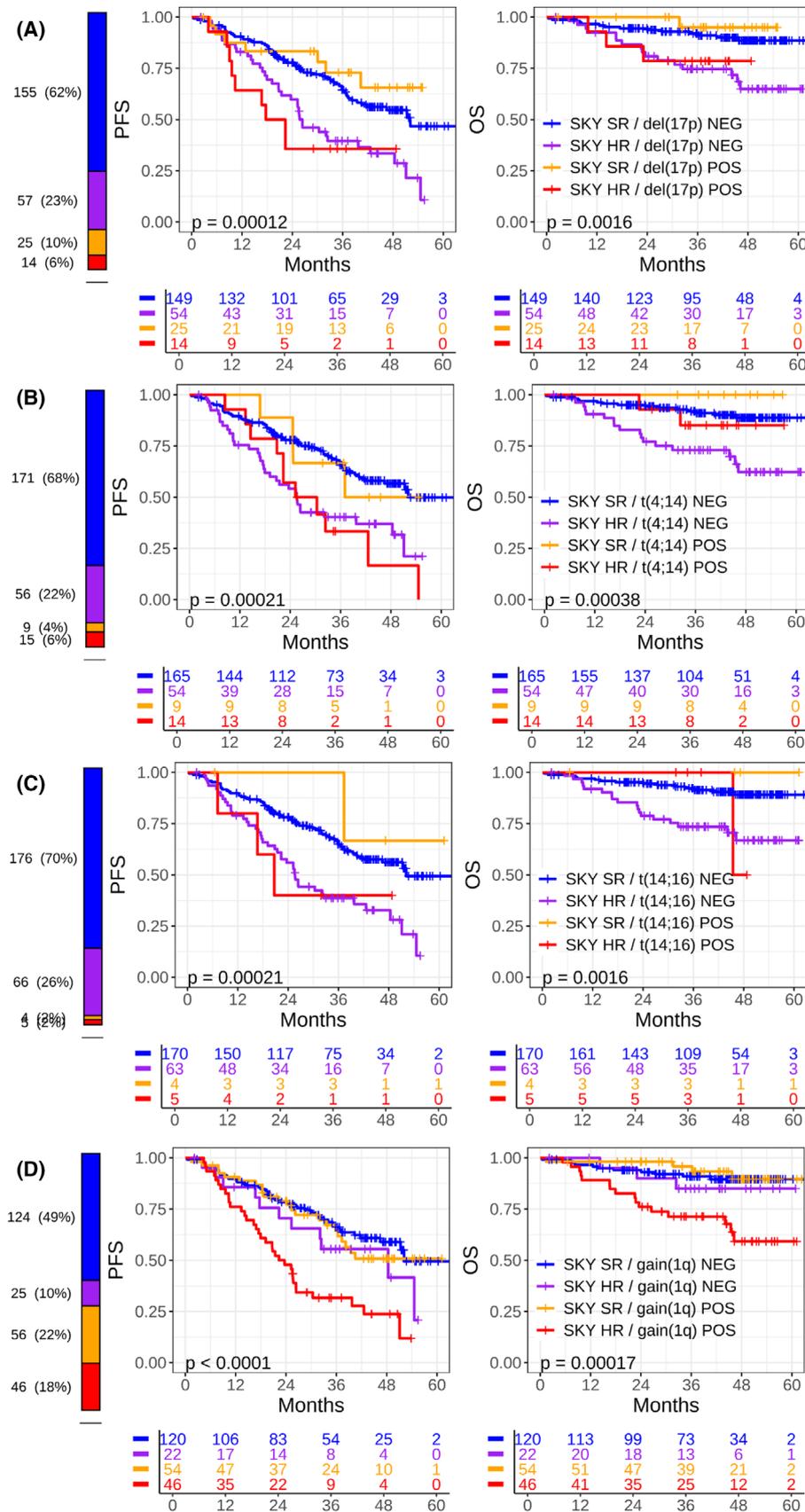
data) SKY92-SR within the fCRA-HR patients, relative to the SKY92-SR within the fCRA-SR group, both groups aligned with an SR profile, such that there was no statistically significant difference observed for PFS (hazard ratio: 1.13 [0.52–2.48],  $p = 0.80$ ) and OS (hazard ratio: 0.55 [0.15–1.95],  $p = 0.40$ ). However, relative to the patients classified as HR by both SKY92 and fCRA, a significantly longer PFS (hazard ratio: 0.39, [0.2–0.9],  $p = 0.02$ ) and OS (hazard ratio: 0.48, [0.1–1.6],  $p = 0.2$ ; Figure S6) was observed.

### SKY92's influence on physician treatment plans

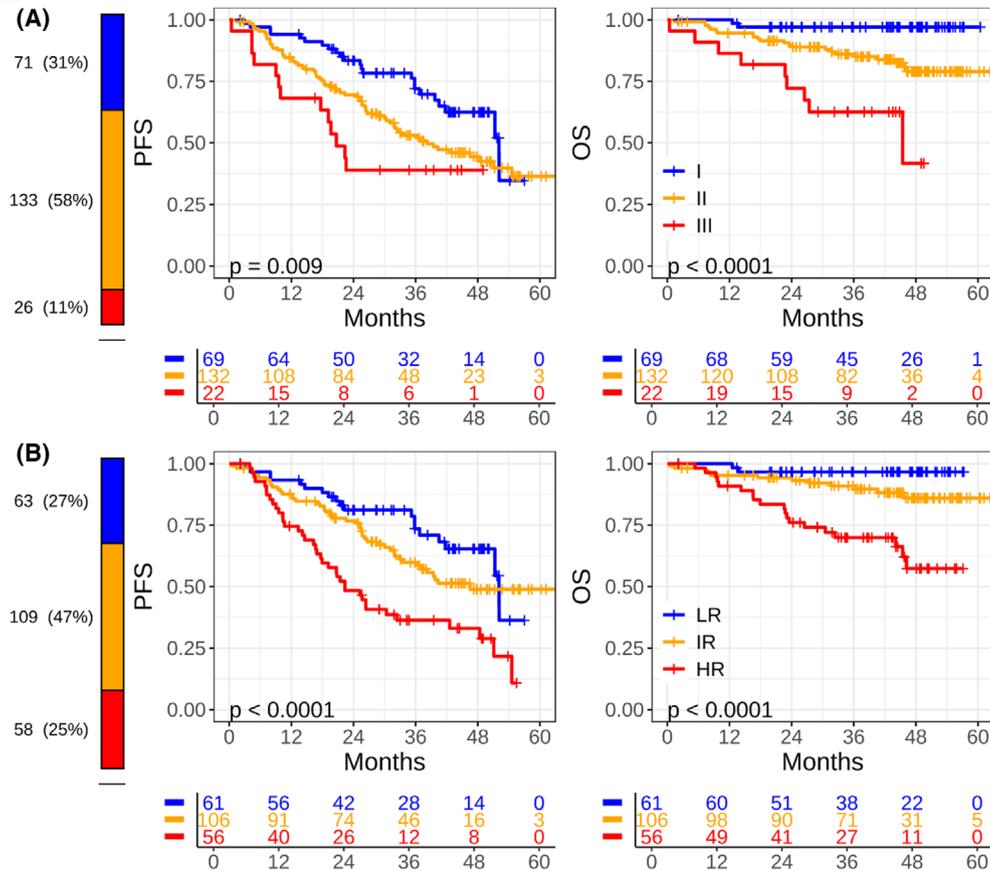
The changes in the risk classification of patients following the unblinding of SKY92 classifications have also prompted corresponding adjustments in their hypothetical treatment plans. Of 246 patients whose treatment plans were evaluated, incorporating the SKY92 into clinical decision-making would have impacted the hypothetical treatment plans for 50% (123/246) of the cases, exceeding the predefined threshold for clinical relevance of 15% ( $p < 0.001$ , Figure 5).

For 73 patients, the inclusion of SKY92 would have resulted in either intensifying or reducing the treatment regimen. Specifically, for 21 of 22 (96%) patients initially classified as iCRA-SR but re-classified as fCRA-HR in accordance with SKY92, their treatment approach would have involved an intensification of the treatment regimen (Figure 5E). On the other hand, 47 of 51 (94%) patients initially classified as iCRA-HR who were re-classified as fCRA-SR would have ideally undergone a reduction of their treatment intensity, in line with the risk downregulation (Figure 5C). For an additional five patients, physicians reported SKY92 would have influenced treatment decisions, potentially leading to considering the use of alternative drug combinations or different approaches to transplantation even without an altered risk status.

For the remaining (50/123) patients, physicians indicated that SKY92 results were useful in confirming their treatment strategy.



**FIGURE 3** Discrepancy between SKY92 and the cytogenetic (cyto) markers del(17p) (A), t(4;14) (B), t(14;16) (C) and gain(1q) (D). Shown are risk distributions by the bar plots, and Kaplan Meier plots for progression-free survival (PFS) (left panel) and overall survival (OS) (right panel), stratified by SKY92-standard risk (SR) with (yellow) or without (blue) cyto-high risk (HR) feature, and SKY92-HR with (red) or without cyto-HR feature (purple). The log-rank  $p$ -value is given.



**FIGURE 4** Shown are risk distributions by the bar plots, and Kaplan–Meier plots for R-ISS (A) and the SKY92 + R-ISS combination (B), for progression-free survival (PFS) (left panel) and overall survival (OS) (right panels). Patients are stratified by the R-ISS stage I (low-risk [LR]: blue), II (intermediate-risk [IR]: yellow) or III (high risk [HR]: red), and the log-rank  $p$ -value is given.

## Boosting physician confidence in patients' treatment plans through SKY92

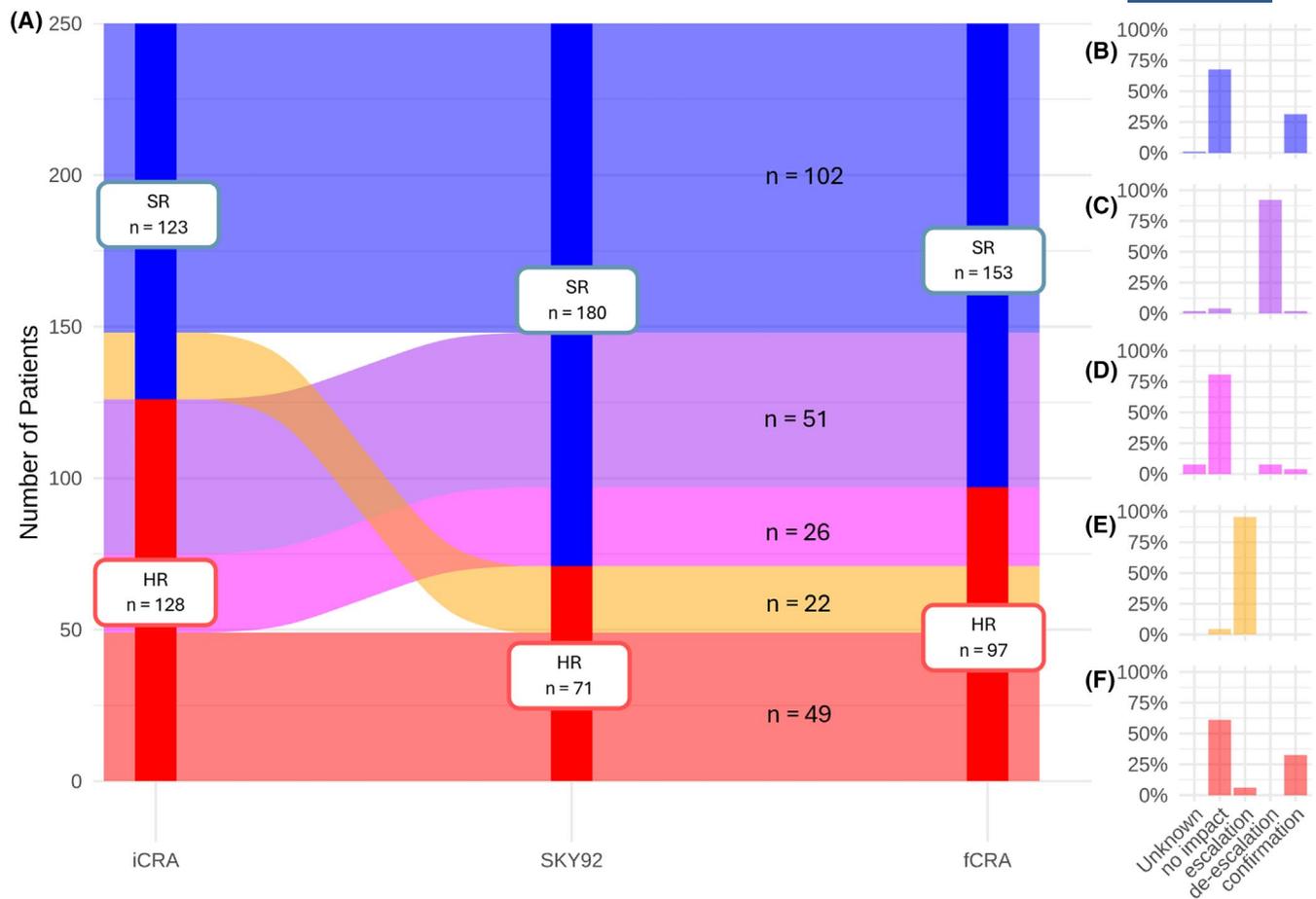
The clinicians were asked to indicate their level of confidence in the treatment plan for each patient, both before and after consulting the SKY92 results (Figure 6A). A higher level of confidence was reported in their assessment after incorporating the SKY92 classification into their evaluation in 40% (100/247) of cases versus lower confidence for 3% (7/247; Figure 6B). For the remaining patients, no change in confidence was reported. The SKY92 information increased their confidence levels whether by confirming the patient's risk status as indicated by iCRA (64/100, 64%) or by indicating a different risk status compared to iCRA (46/100, 46%).

Conversely, for the seven patients whose physicians experienced a decrease in confidence in their assessment after the unblinding of SKY92, most of them (5/7) received a different risk status based on SKY92 compared to the one determined previously. Overall, consulting SKY92 resulted in 95% of physicians stating they were 'confident' or 'strongly confident' in the risk assessment and treatment decision, compared to 77% prior to SKY92 unblinding (Figure 6A).

## DISCUSSION

The definition of HR in MM patients has undergone a dynamic evolution over the recent years, driven by continuous research and refinement of diagnostic and therapeutic methods. As a result, various clinical biomarkers and staging systems have been identified for prognosis.<sup>18–20</sup> However, this heterogeneity can complicate decision-making processes and lead to differing interpretations of clinical data among healthcare professionals, resulting in inconsistency in defining HR patients in practical and clinical trial applications.<sup>21</sup>

The gene expression-based signature SKY92 has emerged as a standardized and precise tool for predicting the prognosis of MM patients.<sup>10,11</sup> This prospective multicentre trial confirmed the prognostic utility of SKY92 to accurately identify HR MM patients for both PFS and OS. In contrast, iCRA shows limited prognostic performance, exhibiting only minimal difference between HR and SR groups for PFS and no significant difference for OS at the time of the analysis. Survival analysis revealed that only one out of the nine hospitals identified HR patients based on iCRA with significantly shorter PFS and OS compared with SR patients. The limited prognostic value of iCRA may be due to its reliance on cytogenetic



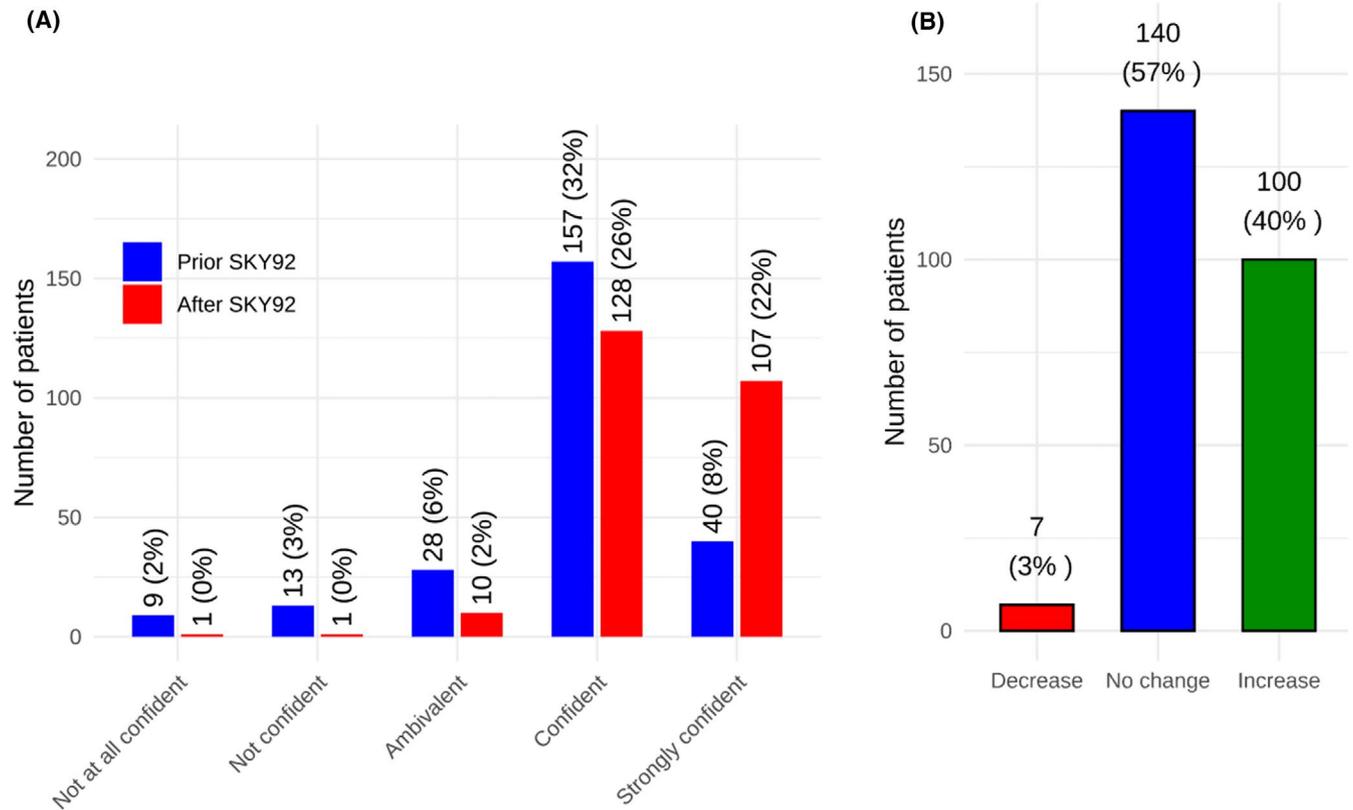
**FIGURE 5** Alluvial plot (A) showing the number of patients (vertical axis) per classification time point (horizontal axis), and treatment impact per stratum (B–F). In chronological order, patients are assessed according to the initial clinical risk assessment (iCRA), the SKY92 classifications are unblinded and clinicians perform the final clinical risk assessment (fCRA). Patients receiving the same classification over the three time points are assigned into the same stratum (by colour). HR, high risk; SR, standard risk.

abnormalities, which also show poor prognostic value and reliability in these data. Indeed, individual cytogenetic abnormalities or their co-occurrence showed no prognostic value, with the sole exception of the 1q21 abnormality. This might be attributed to the lack of standardized cut-off thresholds used to define a positive result for cytogenetic abnormalities, suggesting the need for more standardized methodologies to ensure accurate assessment of the patients' status. This may also be partially related to the relatively small sample size for certain cytogenetic abnormalities in this cohort.

Inconsistencies in risk classification were observed, extending beyond cytogenetic features and clinical parameters, like R-ISS, highlighting a lack of consensus among physicians on HR definitions. We observed a high variability in physicians' risk classification (iCRA-HR) across sites, ranging from 10% to 63%. Initially, we could not rule out that this disparity might result from variations in the demographics of patients referred to each site. However, SKY92-based classification provided a different insight, consistently showing a stable proportion of HR patients across sites. Therefore, the variability of iCRA-HR may be attributed to overinterpretation of factors rather than differences in tumour biology. This highlights the potential influence of subjective assessment in clinical practice

and underscores the importance of objective biomarkers like SKY92 for standardizing HR patient identification. Integrating SKY92 into clinical practice may help identify true HR patients who could benefit from intensified therapy or enrolment in clinical trials tailored to high-risk MM. At the same time, SKY92 can reduce the overestimation of risk in patients who may not require aggressive treatment, minimizing unnecessary toxicity associated with intensive therapy.

Consistent with previous studies, SKY92 has shown independent prognostic value from cytogenetic aberrations. Of 152 patients with a HR profile based on either cytogenetics (t(4;14), t(14;16), del17p, gain(1q21)) or SKY92-HR, only 38% exhibited both markers. Interestingly, 9% had an SKY92-HR profile without cytogenetic HR markers, aligning with the NCRI Myeloma XI trial (ISRCTN49407852), where approximately 10% HR patients were SKY92-positive but lacked chromosomal HR markers.<sup>22</sup> This discrepancy suggests the potential complementary use of these markers. However, combining SKY92 with HR cytogenetic aberrations such as del17p, t(4;14) and t(14;16) did not provide additional prognostic value for patients already assessed with SKY92 in our data, suggesting that SKY92 has superior discriminatory performance in identifying HR patients. The only



**FIGURE 6** Changes in treatment confidence before and after unblinding of the SKY92, showing the absolute patient number based on questionnaire answers (A). Changes in treatment confidence before and after unblinding of the SKY92 (B).

exception was observed in patients with gain(1q21), where a diploid 1q21 status might lessen the poor prognosis associated with SKY92-HR. While not statistically significant, this trend suggests a potential modifying effect of gain(1q21) on SKY92, warranting further study. Additionally, the prognostic impact of the 1q21 aberration varies with age: patients over 65 had worse outcomes than younger patients, possibly due to better fitness or treatment response in the younger group. Additionally to cytogenetics, the R-ISS is a widely used method to determine risk in MM. Prior studies have demonstrated that integrating SKY92 with R-ISS enhances predictive accuracy compared to using either marker independently.<sup>17</sup> Our study shows that the combined SKY92-R-ISS system identifies a significantly larger HR group (25%) compared to R-ISS alone (11%) without diminishing the prognostic value for PFS and OS. Although PFS and OS rates were similar between the SKY92-R-ISS system and R-ISS alone, the combined approach's broader HR patient identification suggests that it could enhance the detection of patients otherwise missed by R-ISS alone. This underscores the potential clinical utility of combining SKY92 with R-ISS for more comprehensive MM risk assessment.

One of the main aims of this study was to explore whether SKY92 could offer additional guidance to physicians for better risk stratification of MM patients. Physicians showed an increasing preference for SKY92 in their final assessments, with fCRA aligning with SKY92 in 90% of cases, compared to just 71% for the iCRA. Of the 10% (26/250) of patients where fCRA did not match SKY92, survival more closely

resembled the SR group as suggested by SKY92, indicating that SKY92 provides a more accurate prognosis.

Unblinding SKY92 classifications resulted in adjustments to hypothetical treatment plans for 50% of patients ( $p < 0.001$ ). Moreover, consultation of SKY92 significantly increased physicians' confidence for 40% of the patients. This heightened confidence is crucial in the complex and heterogeneous clinical practice of MM, where physicians must interpret extensive and continually evolving data. Overall, these findings underscore SKY92's substantial influence on clinical decision-making and its role in enhancing treatment planning.

However, the PROMMIS study is a non-interventional trial without a control group, which limits its ability to provide conclusive evidence on whether these observed changes in treatment plans significantly impact or benefit patient survival. This limitation emphasizes the need for future research focused on designing specific interventional trials aimed at determining optimal treatment strategies, particularly for HR patients.

Recent studies showed that early diagnosis of HR MM, combined with risk-adapted stratification for first-line therapy, can significantly improve patient outcomes.<sup>23–25</sup> An example of such an approach is the OPTIMUM trial, which assessed the effectiveness of intensive modern therapies in patients with ultra-high-risk multiple myeloma (uHRMM). The molecular screening protocol which combines genetic and GEP successfully provided complete results for 88% of patients, with a median turnaround time of 2 weeks. This treatment path led to a significant improvement in PFS and

an early trend for OS benefit for uHRMM over conventional management.<sup>23</sup>

The findings advocate for change in the existing model of care. As suggested in the recent Good Practice Paper from the BSH,<sup>26</sup> such change should include the implementation of accessible, comprehensive diagnostics, including GEP, to provide a more personalized clinical approach for HR patients.

The addition of anti-CD38 monoclonal antibodies to standard triplet drug combinations through quadruplet induction and consolidation strategies has significantly improved patient survival. However, despite these advancements, the prognosis of uHRMM patients remains suboptimal. A major challenge in developing robust evidence and strong clinical recommendations for HR and uHRMM patients is the inconsistency in risk definitions across different trials. Therefore, establishing a general consensus on risk status in MM patients is crucial. Given the multitude of prognostic factors identified over the years, it is essential to integrate these factors into a cohesive framework for better prognostication of MM patients, as suggested in some recent publications.<sup>21,27</sup>

In conclusion, this study validates the prognostic value of the SKY92 classifier in identifying HR MM patients in a prospective clinical trial, demonstrating superior accuracy compared to iCRA and other conventional HR markers. The incorporation of SKY92 into clinical practice could significantly enhance physicians' ability to stratify risk and optimize treatment strategy for MM patients.

#### AUTHOR CONTRIBUTIONS

S.L., D.S., S.U., P.N.H. and R.N. were responsible for the study design. N.B., B.D., R.N., S.L., J.T.M., D.H.V., A.N., B.P., P.N.H., D.S., S.U. and F.v.R. performed the research. Pritish K. Bhattacharyya and D.P. Dash performed SKY92 analysis, and Sena Zümürütçü critically reviewed all QC data and provided technical assistance to the sites. S.D. and R.K. analysed and interpreted the data and wrote the manuscript. All authors participated in data interpretation and critical appraisal of the manuscript and approved the final manuscript.

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#### CONFLICT OF INTEREST STATEMENT

B.D. received research funding from Acrelx, Sanofi, Carsgen, C4 therapeutics; consultancy and speaker bureau for Janssen, Karyopharm and Pfizer, and consultancy for

Bristol Myers Squibb, Genentech and Regeneron. R.N. is a consultancy and received research funding from Takeda, Amgen, BMS, Janssen, Karyopharm and GSK. S.L. is a speaker bureau for Peerview, Clinical Care Options (COO), RedMed, Aptitude, Bio Ascend and Medscape; consultancy for Pfizer, Regeneron, Janssen, GSK, Sanofi, BMS, Karyopharm, Angitia, Alexion, Takeda, Adaptive; owns stock options in Magenta and Poseida and patents and royalties in Caelum Bioscience; received research funding from Sanofi and Zentalis. D.H.V. is a speaker bureau for BMS, Amgen, Takeda, Janssen, Karyopharm and Sanofi. A.N. received honoraria and membership on an entity's Board of Directors or advisory committees from Adaptive Biotechnologies, AstraZeneca, Cellectar Biosciences, K36 Therapeutics, ONK Therapeutics, Sanofi, Sebia and additional funding from Amgen, Bristol-Myers Squibb, GSK, Janssen, Pfizer, Takeda; received research funding from Aduro Biotech, Arch Oncology, Cellectis, Genentech, Karyopharm, Kite Pharma and Merck. B.P. received membership on an entity's board of directors or advisory committees from Adaptive Biotechnologies, AstraZeneca, Cellectar Biosciences, K36 Therapeutics, ONK Therapeutics, Sanofi, Sebia and additional funding from Amgen, Bristol-Myers Squibb, GSK, Janssen, Pfizer, Takeda; received research funding from Aduro Biotech, Arch Oncology, Cellectis, Genentech, Karyopharm, Kite Pharma and Merck. B.P. received membership on an entity's board of directors or advisory committees from Regeneron Pharmaceuticals, Johnson & Johnson, and AbbVie Inc. received research funding from Bristol-Myers Squibb. P.N.H. is currently employed at Obsidian Therapeutics. S.D. is currently employed at SkylineDx. R.K. is currently employed at SkylineDx. M.v.V. is currently employed at SkylineDx. D.S. received honoraria from Sebia, Envision Pharma, Pfizer, Merck, BMS, Envision Pharma, K36 Therapeutics, Roche, Prothena and Sanofi; holds stock options in COTA. S.Z.U. is a consultancy for Gracell, Pfizer, Genentech, EdoPharma, Oncopeptides, SecuraBio and additionally received research funding from Johnson & Johnson, Bristol-Myers Squibb-Celgene, Abbvie, Sanofi, Amgen, GSK, SeaGen, SkylineDx and Takeda; received research funding from Gilead, Array Biopharma, Merck and Pharmacyclics. F.v.R. is a consultancy for EUSA Pharma, GSK, Janssen, Karyopharm, Takeda and received research funding from Janssen Pharmaceuticals and BMS. The remaining authors declare no competing financial interests.

#### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

#### ETHICS STATEMENT

The protocol has been written and the study was conducted according to the ICH Harmonized Tripartite Guideline for Good Clinical Practice.

## PATIENTS CONSENT STATEMENT

The study protocol was approved by the institutional review boards of participating centres and all participants provided written informed consent.

## CLINICAL TRIAL REGISTRATION

The PROMMIS study is registered on ClinicalTrials.gov under the identifier NCT02911571.

## ORCID

Noa Biran  <https://orcid.org/0000-0003-0693-4202>

Binod Dhakal  <https://orcid.org/0000-0002-4377-9742>

Divaya Bhutani  <https://orcid.org/0000-0002-5482-7543>

Ajay Nooka  <https://orcid.org/0000-0003-4165-6869>

Barry Paul  <https://orcid.org/0000-0003-2702-8225>

Silvia D'Ambrosi  <https://orcid.org/0000-0002-7742-2215>

## REFERENCES

- van de Donk NWCJ, Pawlyn C, Yong KL. Multiple myeloma. *Lancet*. 2021;397:410–27.
- Padala SA, Barsouk A, Barsouk A, Rawla P, Vakiti A, Kolhe R, et al. Epidemiology, staging, and management of multiple myeloma. *Med Sci*. 2021;9:3.
- Huang J, Chan SC, Lok V, Zhang L, Xu W, Zheng ZJ, et al. The epidemiological landscape of multiple myeloma: a global cancer registry estimate of disease burden, risk factors, and temporal trends. *Lancet Haematol*. 2022;9:e670–e677.
- Palumbo A, Bringhen S, Ludwig H, Dimopoulos MA, Bladé J, Mateos MV, et al. Personalized therapy in multiple myeloma according to patient age and vulnerability: a report of the European Myeloma Network (EMN). *Blood*. 2011;118:4519–29.
- Cliff ERS, Mohyuddin GR. Overall survival as a primary end point in multiple myeloma trials. *Nat Rev Clin Oncol*. 2022;19:565–6.
- Kumar SK, Rajkumar SV, Dispenzieri A, Lacy MQ, Hayman SR, Buadi FK, et al. Improved survival in multiple myeloma and the impact of novel therapies. *Blood*. 2008;111:2516–20.
- mSMART. Mayo stratification for myeloma and risk-adapted therapy. 2024.
- Quach H, Prince HM. Clinical practice guideline multiple myeloma. Myeloma Australia; 2022. [Cited 2022 Jun 1]. Available from: [https://myeloma.org.au/wp-content/uploads/2022/09/MSAG\\_Myeloma-Clinical-Practice-Guideline-2022\\_Final-1.pdf](https://myeloma.org.au/wp-content/uploads/2022/09/MSAG_Myeloma-Clinical-Practice-Guideline-2022_Final-1.pdf)
- Kumar SK, Callander NS, Adekola K, Anderson LD, Baljevic M, Baz R, et al. NCCN guidelines Version 1.2025 multiple myeloma NCCN guidelines. 2024. [Cited 2024 Sep 17]. Available from: <https://www.nccn.org/home/member->
- Kuiper R, Broyl A, de Knegt Y, van Vliet MH, van Beers EH, van der Holt B, et al. A gene expression signature for high-risk multiple myeloma. *Leukemia*. 2012;26:2406–13.
- Cerchione C, Usmani SZ, Stewart AK, Kaiser M, Rasche L, Kortüm M, et al. Gene expression profiling in multiple myeloma: redefining the paradigm of risk-adapted treatment. *Front Oncol*. 2022;12:820768.
- Rajkumar SV, Dimopoulos MA, Palumbo A, Blade J, Merlini G, Mateos MV, et al. International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. *Lancet Oncol*. 2014;15:e538–e548.
- Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. *Am J Clin Oncol*. 1982;5:649–55.
- Biran N, Dhakal B, Lentzsch S, Siegel D, Usmani SZ, Rossi A, et al. Gene expression profiling impacts treatment decision making in newly diagnosed multiple myeloma patients in the prospective PROMMIS trial. *EJHaem*. 2021;2:375–84.
- Schemper M, Smith TL. A note on quantifying follow-up in studies of failure time. *Control Clin Trials*. 1996;17:343–6.

- Cardona-Benavides IJ, de Ramón C, Gutiérrez NC. Genetic abnormalities in multiple myeloma: prognostic and therapeutic implications. *Cells*. 2021;10:336.
- Kuiper R, Zweegman S, van Duin M, van Vliet MH, van Beers EH, Dumee B, et al. Prognostic and predictive performance of R-ISS with SKY92 in older patients with multiple myeloma: the HOVON-87/NMSG-18 trial. *Blood Adv*. 2020;4:6298–309.
- Wallington-Beddoe CT, Mynott RL. Prognostic and predictive biomarker developments in multiple myeloma. *J Hematol Oncol*. 2021;14:151.
- Palumbo A, Avet-Loiseau H, Oliva S, Lokhorst HM, Goldschmidt H, Rosinol L, et al. Revised international staging system for multiple myeloma: a report from International Myeloma Working Group. *J Clin Oncol*. 2015;33:2863–9.
- D'Agostino M, Cairns DA, Lahuerta JJ, Wester R, Bertsch U, Waage A, et al. Second revision of the international staging system (R2-ISS) for overall survival in multiple myeloma: a European myeloma network (EMN) report within the HARMONY project. *J Clin Oncol*. 2022;40:3406–18.
- Rees MJ, D'Agostino M, Leyboldt LB, Kumar S, Weisel KC, Gay F. Navigating high-risk and ultrahigh-risk multiple myeloma: challenges and emerging strategies. *Am Soc Clin Oncol Educ Book*. 2024;44:e433520.
- Shah V, Sherborne AL, Johnson DC, Ellis S, Price A, Chowdhury F, et al. Predicting ultrahigh risk multiple myeloma by molecular profiling: an analysis of newly diagnosed transplant eligible myeloma XI trial patients. *Leukemia*. 2020;34(11):3091–6. <https://doi.org/10.1038/s41375-020-0750-z>
- Kaiser MF, Hall A, Walker K, Sherborne A, de Tute RM, Newnham N, et al. Daratumumab, cyclophosphamide, bortezomib, lenalidomide, and dexamethasone as induction and extended consolidation improves outcome in ultra-high-risk multiple myeloma. *J Clin Oncol*. 2023;41:3945–55.
- Leyboldt LB, Tichy D, Besemer B, Hänel M, Raab MS, Mann C, et al. Isatuximab, carfilzomib, lenalidomide, and dexamethasone for the treatment of high-risk newly diagnosed multiple myeloma. *J Clin Oncol*. 2024;42:26–37.
- Touzeau C, Perrot A, Hulin C, Manier S, Macro M, Chretien ML, et al. Daratumumab, carfilzomib, lenalidomide, and dexamethasone induction and consolidation with tandem transplant in high-risk newly diagnosed myeloma patients: final results of the phase 2 study IFM 2018-04. *Blood*. 2023;142:207.
- Kaiser M, Pratt G, Bygrave C, Bowles K, Stern S, Jenner M, et al. Diagnosis and initial treatment of transplant-eligible high-risk myeloma patients: a British Society for Haematology/UK myeloma society good practice paper. *Br J Haematol*. 2024;205:833–9. <https://doi.org/10.1111/bjh.19623>
- Rees MJ, Kumar S. High-risk multiple myeloma: redefining genetic, clinical, and functional high-risk disease in the era of molecular medicine and immunotherapy. *Am J Hematol*. 2024;99(8):1560–75. <https://doi.org/10.1002/ajh.27327>

## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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