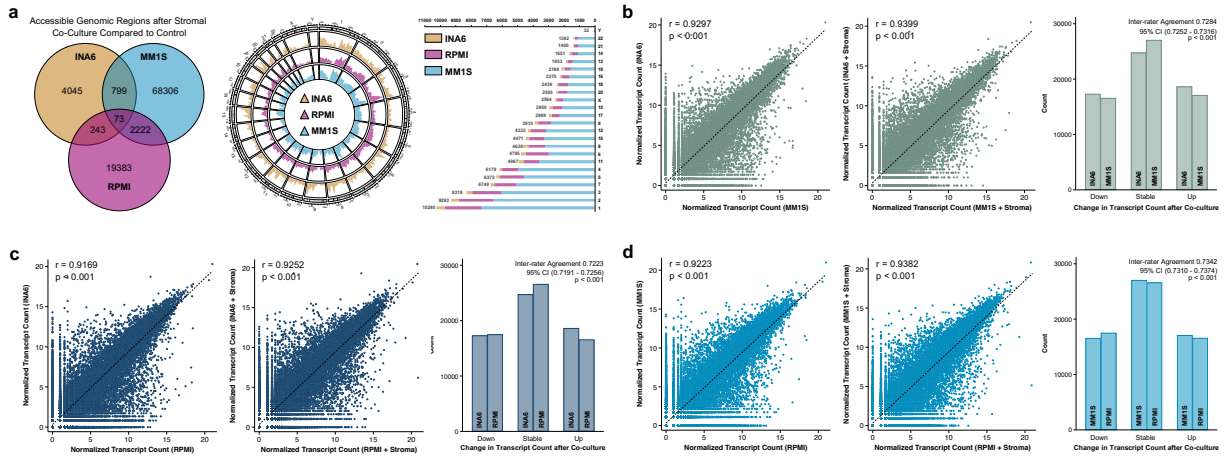
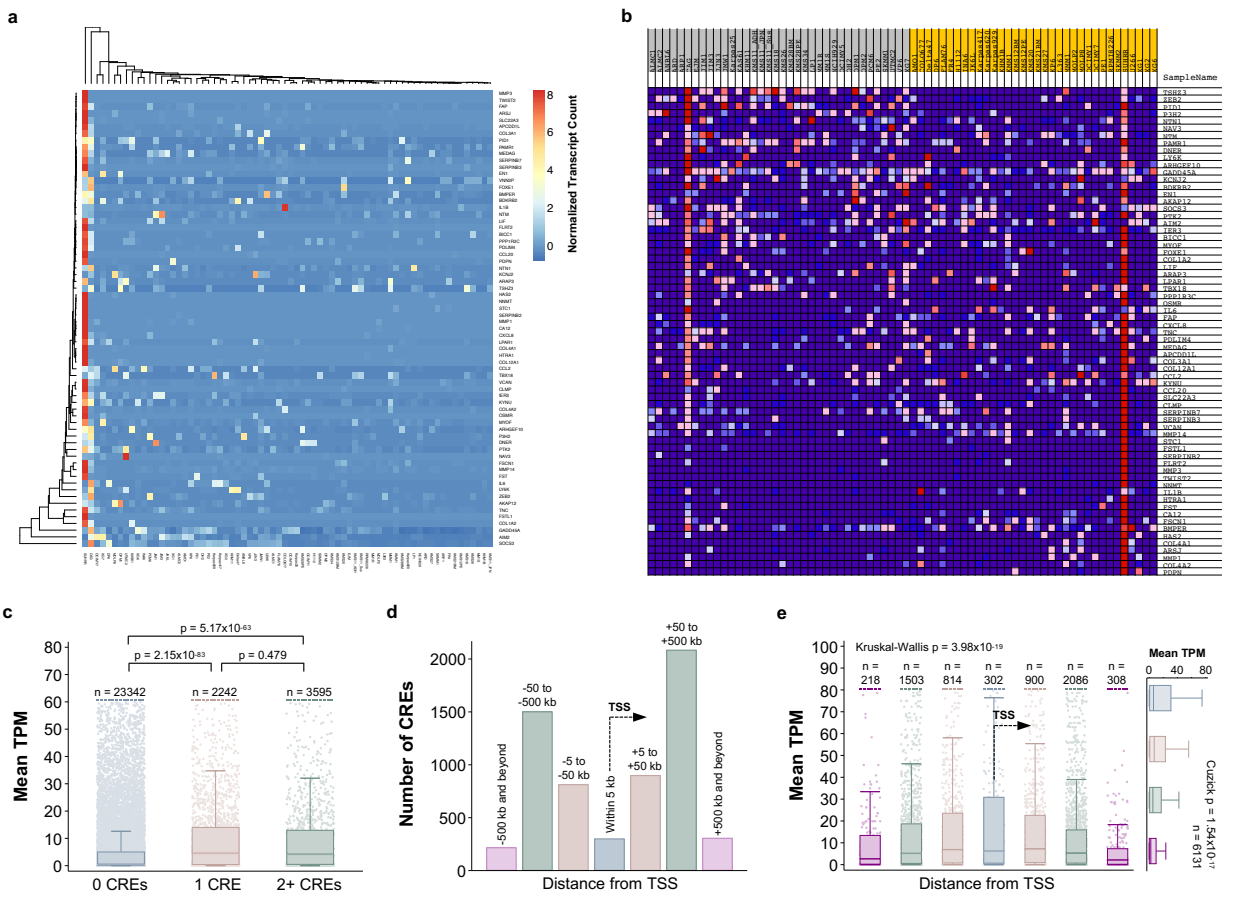
## SUPPLEMENTARY FIGURE 1



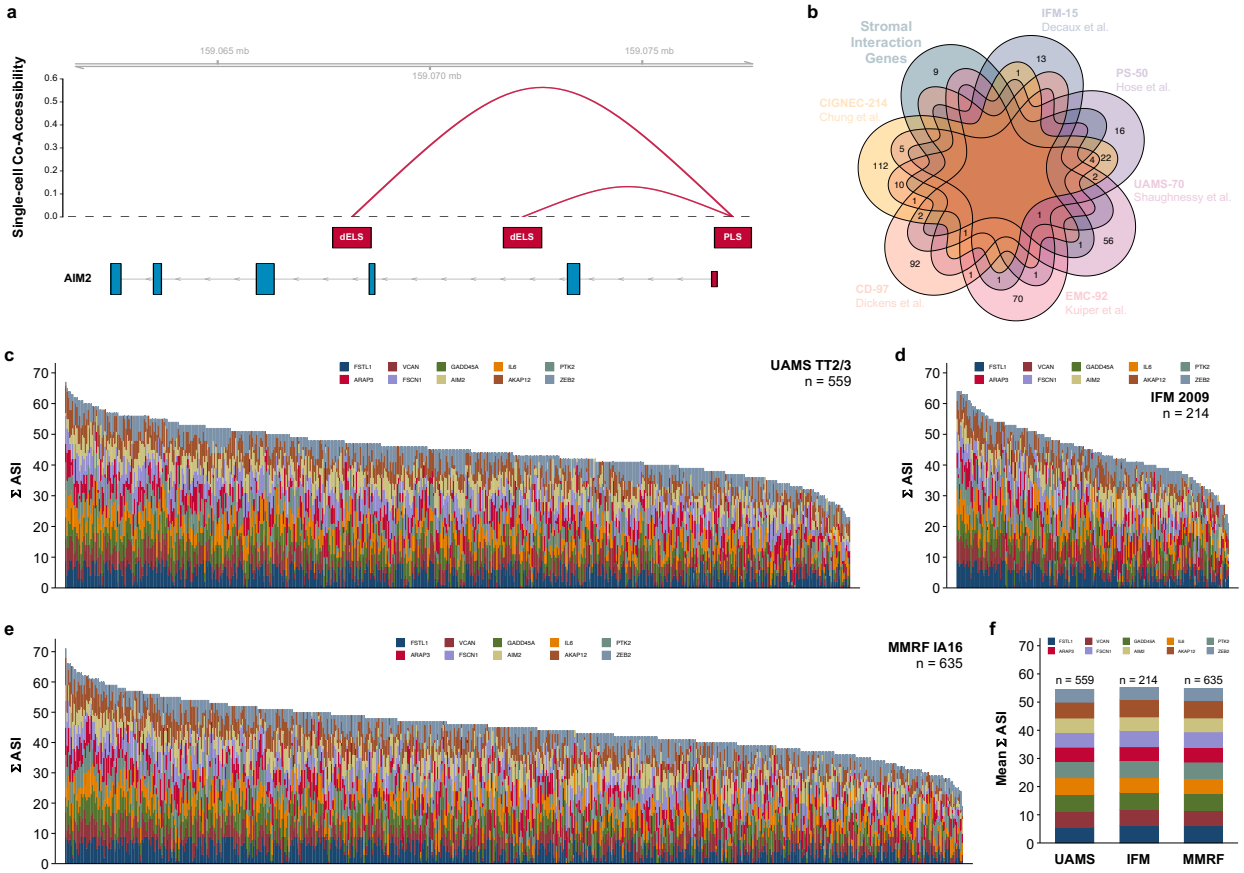

**Supplementary Figure 1** Gene expression and chromatin accessibility of individual myeloma cell lines in response to stromal co-culture. **a**, Euler diagram (left panel), circular area plots (middle panel), and bar graphs (right panel) showing the localization and overlap of the genomic regions with gained accessibility after stromal co-culture stratified by myeloma cell line. **b**, Scatter plots showing the pairwise correlation between INA6 and MM1S gene expression at baseline (left panel) and after stromal co-culture (middle panel). Bar graphs showing the agreement of direction in gene expression between INA6 and MM1S after stromal co-culture (right panel). **c**, Scatter plots showing the pairwise correlation between INA6 and RPMI gene expression at baseline (left panel) and after stromal co-culture (middle panel). Bar graphs showing the agreement of direction in gene expression between INA6 and RPMI after stromal co-culture (right panel). **d**, Scatter plots showing the pairwise correlation between MM1S and RPMI gene expression at baseline (left panel) and after stromal co-culture (middle panel). Bar graphs showing the agreement of direction in gene expression between MM1S and RPMI after stromal co-culture (right panel). The pairwise correlation coefficients in 1b-d represent Pearson correlation coefficients for all transcripts (n = 60675).

## SUPPLEMENTARY FIGURE 2




**Supplementary Figure 2** Baseline myeloma cell line gene expression and additional analyses of enhancer effects on the expression of the predicted target genes. **a**, Heatmap showing the baseline gene expression of the 68 stroma-induced genes in 70 myeloma cell lines. **b**, Gene set enrichment analysis of the 68 stroma-induced genes stratified by the presence (gray) and absence (yellow) of high-risk IGH translocations among the 70 myeloma cell lines (Enrichment Score -0.57, p = 0.644). High-risk IGH translocations were defined as the presence of t(4;14), t(14;16), or t(14;20). **c**, Box and strip plots demonstrating the association between the presence of CREs with altered chromatin accessibility after stromal co-culture and increased expression of their predicted target genes (Mann-Whitney-U test). There was no dosage effect (i.e. the presence of more than one CRE was not associated with a further increase in gene expression). **d**, Bar graphs showing the distribution of associated CREs with the vast majority being located outside the promoter regions of the predicted target genes. **e**, Box and strip plots showing the expression of the predicted target genes stratified by location and direction of the associated CRE (upstream *versus* downstream and by distance). There was a decrease in the expression of the predicted target genes as distance increased (Kruskal-Wallis test). Data are presented as standard Tukey boxplots (with the box encompassing Q1 to Q3, the median denoted as a central horizontal line in the box, and the whiskers covering the data within ±1.5 IQR in 2e).

## SUPPLEMENTARY FIGURE 3




**Supplementary Figure 3** Single-cell co-accessibility studies and the contribution of each of the 10 prognostically significant stromal interaction genes to the summary measure Σ ASI. **a**, Connection plot showing the positive single-cell co-accessibility between the *AIM2* promoter region and two intronic distal enhancers predicted to govern *AIM2* expression. **b**, Venn diagram showing the limited, one-gene, overlap of the 10 selected stromal interaction genes with the established high-risk gene expression classifiers in myeloma. **c**, Bar graphs demonstrating the absolute contribution of each individual gene to the Σ ASI score in the derivation cohort (n = 559). **d**, Bar graphs demonstrating the absolute contribution of each individual gene to the Σ ASI score in the first validation cohort (n = 214). **e**, Bar graphs demonstrating the absolute contribution of each individual gene to the Σ ASI score in the second validation cohort (n = 635). **f**, Bar graphs showing the mean contribution of each individual gene to the Σ ASI score among patients considered ASI+ (Σ ASI ≥ 50) in the three patient populations (n = 1408).

## REGRESSION MODELS

Gene expression data and progression-free survival outcomes from UAMS TT2/3 (GSE24080) were used for feature selection by employing automated model selection procedures (forward and backward selection with a p-value threshold of 0.200). Forward and backward selection included 13 (**Supplementary Table 1**) and 17 (**Supplementary Table 2**) genes associated with progression-free survival, respectively. The 10 genes selected by both methods (**Supplementary Table 3**) were selected for further study and were used to calculate the 10-gene classifier (**Supplementary Table 4**).

**Supplementary Table 1** Multivariable-adjusted proportional hazards regression for progression-free survival (forward selection) in UAMS TT2/3 (GSE24080, n = 559)

| Gene | HR (95% CI) | p-value |
|---|---|---|
| *GADD45A* | 0.82 (0.71 - 0.94) | 0.005 |
| *MMP14* | 0.52 (0.24 - 1.10) | 0.085 |
| *AIM2* | 1.33 (1.12 - 1.58) | 0.001 |
| *FSCN1* | 0.67 (0.42 - 1.07) | 0.093 |
| *IL1B* | 2.12 (1.08 - 4.16) | 0.030 |
| *FSTL1* | 0.49 (0.28 - 0.85) | 0.011 |
| *AKAP12* | 0.66 (0.46 - 0.93) | 0.018 |
| *VCAN* | 0.64 (0.42 - 0.97) | 0.034 |
| *IL6* | 1.21 (1.01 - 1.46) | 0.043 |
| *PTK2* | 1.28 (0.95 - 1.73) | 0.109 |
| *FLRT2* | 0.28 (0.07 - 1.08) | 0.065 |
| *ARAP3* | 1.97 (0.90 - 4.30) | 0.088 |
| *ZEB2* | 2.89 (0.65 - 12.83) | 0.163 |

HR = Hazard Ratio | CI = Confidence Interval

**Supplementary Table 2** Multivariable-adjusted proportional hazards regression for progression-free survival (backward selection) in UAMS TT2/3 (GSE24080, n = 559)

| Gene | HR (95% CI) | p-value |
|---|---|---|
| *AIM2* | 1.37 (1.16 - 1.62) | < 0.001 |
| *BICC1* | 2.07 (1.19 - 3.61) | 0.010 |
| *FSTL1* | 0.45 (0.25 - 0.80) | 0.006 |
| *PTK2* | 1.24 (0.91 - 1.68) | 0.168 |
| *TBX18* | 0.19 (0.02 - 2.12) | 0.178 |
| *AKAP12* | 0.65 (0.46 - 0.93) | 0.017 |
| *ARAP3* | 1.72 (0.76 - 3.86) | 0.190 |
| *FSCN1* | 0.68 (0.41 - 1.12) | 0.131 |
| *HAS2* | 0.63 (0.39 - 1.02) | 0.058 |
| *VCAN* | 0.65 (0.43 - 0.99) | 0.043 |
| *VNN3* | 0.50 (0.18 - 1.44) | 0.200 |
| *GADD45A* | 0.81 (0.70 - 0.93) | 0.003 |
| *MMP3* | 0.37 (0.11 - 1.25) | 0.109 |
| *IL6* | 1.22 (1.01 - 1.46) | 0.038 |
| *ZEB2* | 3.48 (0.76 - 15.91) | 0.108 |
| *HTRA1* | 1.53 (0.89 - 2.61) | 0.121 |
| *FOXE1* | 1.81 (0.80 - 4.11) | 0.154 |

HR = Hazard Ratio | CI = Confidence Interval

**Supplementary Table 3** Multivariable-adjusted proportional hazards regression for progression-free survival in UAMS TT2/3 (GSE24080, n = 559) using the 10 genes included by both forward and backward selection

| Gene | HR (95% CI) | p-value |
|---|---|---|
| *GADD45A* | 0.80 (0.70 - 0.91) | 0.001 |
| *AIM2* | 1.29 (1.10 - 1.51) | 0.002 |
| *FSCN1* | 0.50 (0.32 - 0.78) | 0.002 |
| *FSTL1* | 0.46 (0.27 - 0.77) | 0.003 |
| *AKAP12* | 0.68 (0.48 - 0.96) | 0.029 |
| *VCAN* | 0.69 (0.46 - 1.01) | 0.058 |
| *IL6* | 1.20 (1.00 - 1.45) | 0.051 |
| *PTK2* | 1.29 (0.97 - 1.71) | 0.081 |
| *ARAP3* | 1.60 (0.81 - 3.15) | 0.173 |
| *ZEB2* | 5.09 (1.29 - 20.14) | 0.020 |

HR = Hazard Ratio | CI = Confidence Interval

**Supplementary Table 4** Univariable proportional hazards regression for progression-free survival in UAMS TT2/3 (GSE24080, n = 559) using the 10-gene classifier (score) resulting from the genes included by both forward and backward selection

| Gene | HR (95% CI) | p-value |
|---|---|---|
| Score | 1.06 (1.04 - 1.08) | < 0.001 |

HR = Hazard Ratio | CI = Confidence Interval

Since progression-free survival outcomes were used for the derivation of the 10-gene classifier, the independent prognostic significance of the dichotomized 10-gene classifier for overall survival was first confirmed in the derivation patient population (**Supplementary Table 5**). The dichotomized 10-gene classifier was then validated in two independent patient populations (**Supplementary Table 6**, **Supplementary Table 7**). To increase power, the two validation patient populations were pooled to test the independence of the dichotomized 10-gene classifier of the UAMS-70 and EMC-92 classifier (**Supplementary Table 8**, **Supplementary Table 9**).

**Supplementary Table 5** Multivariable-adjusted proportional hazards regression for overall survival in UAMS TT2/3 (GSE24080, n = 559) using the dichotomized 10-gene classifier (ASI+)

| Gene | HR (95% CI) | p-value |
|---|---|---|
| Age | 1.02 (1.00 - 1.03) | 0.044 |
| Sex | 1.11 (0.81 - 1.51) | 0.512 |
| ISS | 1.69 (1.36 - 2.11) | < 0.001 |
| LDH | 2.40 (1.71 - 3.37) | < 0.001 |
| ASI+ | 1.79 (1.32 - 2.42) | < 0.001 |

HR = Hazard Ratio | CI = Confidence Interval | ISS = International Staging System | LDH = Elevated Lactate Dehydrogenase | No evidence for violations of the proportional hazards assumption (p = 0.294)

**Supplementary Table 6** Multivariable-adjusted proportional hazards regression for overall survival in IFM 2009 (n = 152) using the dichotomized 10-gene classifier (ASI+)

| Gene | HR (95% CI) | p-value |
|---|---|---|
| Age | 1.00 (0.96 - 1.04) | 0.901 |
| Sex | 0.84 (0.48 - 1.47) | 0.539 |
| ISS | 1.26 (0.85 - 1.86) | 0.246 |
| HRT | 3.01 (1.43 - 6.32) | 0.004 |
| Del(17p) | 2.79 (1.41 - 5.50) | 0.003 |
| Gain(1q) | 2.23 (1.25 - 3.99) | 0.007 |
| Del(1p) | 0.89 (0.49 - 1.62) | 0.702 |
| ASI+ | 2.04 (1.16 - 3.60) | 0.013 |

HR = Hazard Ratio | CI = Confidence Interval | ISS = International Staging System | HRT = High-risk IGH Translocation | No evidence for violations of the proportional hazards assumption (p = 0.228)

**Supplementary Table 7** Multivariable-adjusted proportional hazards regression for overall survival in MMRF IA16 (n = 447) using the dichotomized 10-gene classifier (ASI+)

| Gene | HR (95% CI) | p-value |
|---|---|---|
| Age | 1.04 (1.02 - 1.06) | < 0.001 |
| Sex | 1.47 (1.04 - 2.08) | 0.028 |
| ISS | 1.47 (1.18 - 1.83) | 0.001 |
| LDH | 1.92 (1.26 - 2.92) | 0.002 |
| HRT | 2.01 (1.32 - 3.08) | 0.001 |
| Del(17p) | 1.42 (0.88 - 2.28) | 0.154 |
| Gain(1q) | 1.51 (1.08 - 2.11) | 0.017 |
| ASI+ | 1.55 (1.11 - 2.17) | 0.010 |

HR = Hazard Ratio | CI = Confidence Interval | ISS = International Staging System | LDH = Elevated Lactate Dehydrogenase | HRT = High-risk IGH Translocation | No evidence for violations of the proportional hazards assumption (p = 0.225)

**Supplementary Table 8** Multivariable-adjusted proportional hazards regression for overall survival in IFM 2009 and MMRF IA16 (n = 723) using the dichotomized 10-gene classifier (ASI+)

| Gene | HR (95% CI) | p-value |
|---|---|---|
| Population | 0.74 (0.41 - 1.31) | 0.299 |
| Age | 1.03 (1.02 - 1.05) | < 0.001 |
| Sex | 1.45 (1.12 - 1.88) | 0.005 |
| ISS | 1.50 (1.26 - 1.77) | < 0.001 |
| HRT | 1.60 (1.16 - 2.20) | 0.005 |
| del(17p) | 2.51 (1.35 - 4.66) | 0.004 |
| UAMS-70 | 2.60 (1.88 - 3.58) | < 0.001 |
| ASI+ | 1.33 (1.02 - 1.73) | 0.038 |

HR = Hazard Ratio | CI = Confidence Interval | Population = MMRF versus IFM | ISS = International Staging System | HRT = High-risk IGH Translocation | No evidence for violations of the proportional hazards assumption (p = 0.932)

**Supplementary Table 9** Multivariable-adjusted proportional hazards regression for overall survival in IFM 2009 and MMRF IA16 (n = 723) using the dichotomized 10-gene classifier (ASI+)

| Gene | HR (95% CI) | p-value |
|---|---|---|
| Population | 0.74 (0.41 - 1.31) | 0.300 |
| Age | 1.03 (1.02 - 1.05) | < 0.001 |
| Sex | 1.39 (1.07 - 1.80) | 0.013 |
| ISS | 1.54 (1.30 - 1.81) | < 0.001 |
| HRT | 1.37 (0.97 - 1.91) | 0.071 |
| del(17p) | 2.58 (1.39 - 4.77) | 0.003 |
| EMC-92 | 2.73 (1.95 - 3.83) | < 0.001 |
| ASI+ | 1.38 (1.06 - 1.79) | 0.017 |

HR = Hazard Ratio | CI = Confidence Interval | Population = MMRF versus IFM | ISS = International Staging System | HRT = High-risk IGH Translocation | No evidence for violations of the proportional hazards assumption (p = 0.877)

We were interested in investigating the association between the presence of adverse stromal interactions and the development of therapeutic resistance beyond progression-free survival outcomes. We examined response to therapy with novel agents at 3 months after treatment initiation to remove the potential confounding effect of high-dose chemotherapy (given as conditioning in those patients undergoing autologous hematopoietic stem cell transplantation as part of their first-line treatment). Defining PD/SD by IMWG unified response criteria as “early therapeutic resistance”, we tested the hypothesis that the dichotomized 10-gene classifier is associated with this lack of early response to treatment. Considering all first-line regimens, the sample size allowed multivariable-adjusted modeling (**Supplementary Table 10**). Considering only patients receiving first-line VRd, the sample size was too limited for extensive modeling and we therefore fit univariable models for each potential predictor separately (**Supplementary Table 11**).

**Supplementary Table 10** Multivariable-adjusted unconditional logistic regression for early therapeutic resistance to any first-line regimen (PD/SD by IMWG unified response criteria at 3 months) in MMRF (n = 396) using the dichotomized 10-gene classifier (ASI+)

| Gene | OR (95% CI) | p-value |
|---|---|---|
| Age | 1.02 (1.00 - 1.05) | 0.091 |
| Sex | 0.92 (0.52 - 1.62) | 0.774 |
| ISS | 0.92 (0.64 - 1.33) | 0.676 |
| LDH | 1.02 (0.43 - 2.40) | 0.959 |
| HRT | 0.68 (0.26 - 1.75) | 0.423 |
| Del(17p) | 0.55 (0.20 - 1.53) | 0.253 |
| Gain(1q) | 1.16 (0.63 - 2.13) | 0.633 |
| UAMS-70 | 0.51 (0.17 - 1.50) | 0.221 |
| EMC-92 | 1.79 (0.63 - 5.08) | 0.277 |
| ASI+ | 2.45 (1.36 - 4.41) | 0.003 |

OR = Odd Ratio | CI = Confidence Interval | ISS = International Staging System | LDH = Elevated Lactate Dehydrogenase | HRT = High-risk Translocation | Good calibration (Hosmer-Lemeshow p = 0.893) and acceptable discrimination (C = 0.646)

**Supplementary Table 11** Univariable unconditional logistic regression for early therapeutic resistance to first-line VRd (PD/SD by IMWG unified response criteria at 3 months) in MMRF (n = 211) using the dichotomized 10-gene classifier (ASI+)

| Gene | OR (95% CI) | p-value |
|---|---|---|
| Age | 1.03 (0.98 - 1.07) | 0.243 |
| Sex | 0.96 (0.42 - 2.18) | 0.916 |
| ISS | 1.19 (0.69 - 2.06) | 0.524 |
| HRT | 0.58 (0.13 - 2.65) | 0.483 |
| Del(17p) | 0.82 (0.18 - 3.84) | 0.806 |
| Gain(1q) | 1.19 (0.45 - 3.14) | 0.723 |
| Del(1p) | 0.62 (0.14 - 2.81) | 0.536 |
| UAMS-70 | 0.30 (0.04 - 2.32) | 0.247 |
| EMC-92 | 1.03 (0.98 - 1.07) | 0.243 |
| ASI+ | 2.62 (1.14 - 6.03) | 0.023 |

OR = Odd Ratio | CI = Confidence Interval | ISS = International Staging System | HRT = High-risk Translocation

We then showed that the development of early therapeutic resistance translated into inferior progression-free survival among patients receiving first-line VRd in MMRF IA16 (n = 211) and in IFM 2009 (n = 214). The models below demonstrate that the presence of adverse stromal interactions predict progression-free survival independent of the responder status (depth of response). Since the best response to treatment is unknown at baseline it potentially represents a time-varying covariate, which makes the interpretation of coefficients from standard proportional hazards models (**Supplementary Table 12**, **Supplementary Table 13**) difficult. Instead of engaging in more complicated modeling of time-varying covariates, we show here that the effect of adverse stromal interactions on progression-free survival remains consistent among responders (PR or better). This suggests that the prognostic significance of adverse stromal interactions is not just due to identifying the non-responders (**Supplementary Table 14**, **Supplementary Table 15**).

**Supplementary Table 12** Multivariable-adjusted proportional hazards regression for progression-free survival in IFM 2009 (first-line VRd only, n = 214) using the dichotomized 10-gene classifier (ASI+)

| Gene | HR (95% CI) | p-value |
|---|---|---|
| PD / SD | 1.00 (reference) | |
| PR | 0.03 (0.01 - 0.11) | < 0.001 |
| VGPR | 0.02 (0.01 - 0.06) | < 0.001 |
| CR / sCR | 0.01 (0.00 - 0.02) | < 0.001 |
| ASI+ | 1.73 (1.25 - 2.40) | 0.001 |

HR = Hazard Ratio | CI = Confidence Interval | PD = Progressive Disease | SD = Stable Disease | PR = Partial Response | VGPR = Very Good Partial Response | CR = Complete Response | sCR = Stringent Complete Response | No evidence for violations of the proportional hazards assumption (p = 0.668)

**Supplementary Table 13** Multivariable-adjusted proportional hazards regression for progression-free survival in MMRF IA16 (first-line VRd only, n = 211) using the dichotomized 10-gene classifier (ASI+)

| Gene | HR (95% CI) | p-value |
|---|---|---|
| PD / SD | 1.00 (reference) | |
| PR | 0.29 (0.09 - 1.02) | 0.053 |
| VGPR | 0.12 (0.03 - 0.38) | < 0.001 |
| CR / sCR | 0.01 (0.02 - 0.18) | < 0.001 |
| ASI+ | 1.66 (1.13 - 2.44) | 0.010 |

HR = Hazard Ratio | CI = Confidence Interval | PD = Progressive Disease | SD = Stable Disease | PR = Partial Response | VGPR = Very Good Partial Response | CR = Complete Response | sCR = Stringent Complete Response | No evidence for violations of the proportional hazards assumption (p = 0.806)

**Supplementary Table 14** Univariable proportional hazards regression for progression-free survival in IFM 2009 (first-line VRd only, responders only, n = 208) using the dichotomized 10-gene classifier (ASI+)

| Gene | HR (95% CI) | p-value |
|---|---|---|
| ASI+ | 1.56 (1.13 - 2.17) | 0.007 |

HR = Hazard Ratio | CI = Confidence Interval | No evidence for violations of the proportional hazards assumption (p = 0.110)

**Supplementary Table 15** Univariable proportional hazards regression for progression-free survival in IFM 2009 (first-line VRd only, responders only, n = 206) using the dichotomized 10-gene classifier (ASI+)

| Gene | HR (95% CI) | p-value |
|---|---|---|
| ASI+ | 1.84 (1.25 - 2.71) | 0.002 |

HR = Hazard Ratio | CI = Confidence Interval | No evidence for violations of the proportional hazards assumption (p = 0.287)

To test the hypothesis that the presence of adverse stromal interactions is associated with disease dissemination we used the (1) definite development of new bone lesions or soft tissue plasmacytomas and (2) the definite increase in the size of existing bone lesions or soft tissue plasmacytomas as a composite endpoint. We fit both univariable (**Supplementary Table 16**) and multivariable-adjusted models (**Supplementary Table 17**).

**Supplementary Table 16** Univariable proportional hazards regression for the development or progression of bone lesions or soft tissue plasmacytomas (MMRF IA16, n = 622) using the dichotomized 10-gene classifier (ASI+)

| Gene | HR (95% CI) | p-value |
|---|---|---|
| ASI+ | 2.24 (1.51 - 3.31) | < 0.001 |

HR = Hazard Ratio | CI = Confidence Interval | No evidence for violations of the proportional hazards assumption (p = 0.983)

**Supplementary Table 17** Multivariable-adjusted proportional hazards regression for the development or progression of bone lesions or soft tissue plasmacytomas (MMRF IA16, n = 442) using the dichotomized 10-gene classifier (ASI+)

| Gene | HR (95% CI) | p-value |
|---|---|---|
| Age | 1.00 (0.98 - 1.03) | 0.699 |
| Sex | 1.54 (0.93 - 2.55) | 0.092 |
| ISS | 1.21 (0.89 - 1.65) | 0.213 |
| LDH | 1.33 (0.67 - 2.63) | 0.413 |
| HRT | 0.78 (0.35 - 1.74) | 0.550 |
| Del(17p) | 1.23 (0.58 - 2.58) | 0.590 |
| Gain(1q) | 2.01 (1.23 - 3.27) | 0.005 |
| ASI+ | 2.18 (1.35 - 3.51) | 0.001 |

HR = Hazard Ratio | CI = Confidence Interval | ISS = International Staging System | HRT = High-risk IGH Translocation | No evidence for violations of the proportional hazards assumption (p = 0.212)