RNA-Seq of newly diagnosed patients in the PADIMAC study leads to a bortezomib/lenalidomide decision signature

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Key points

- A seven-gene signature is derived which can identify myeloma patients who respond better to bortezomib- or to lenalidomide-based therapy.
- Treatment according to the signature is non-inferior to treatment with combined bortezomib, lenalidomide, and dexamethasone.

Abstract

Improving outcomes in multiple myeloma will not only involve development of new therapies, but better use of existing treatments. We performed RNA sequencing (RNA-Seq) on samples from newly diagnosed patients enrolled into the phase II PADIMAC (Bortezomib, Adriamycin, and Dexamethasone (**PAD**) Therapy for Previously Untreated Patients with Multiple Myeloma: **I**mpact of Minimal Residual Disease (MRD) in Patients with Deferred **A**S**C**T) study. Using synthetic annealing and the Large-Margin-Nearest-Neighbor algorithm, we developed and trained a seven-gene signature to predict treatment outcome. We tested the signature on independent cohorts treated with bortezomib- and lenalidomide-based therapies. The signature was capable of distinguishing which patients would respond better to which regimen. In the CoMMpass (relating **C**linical **o**utcomes in **M**ultiple **M**yeloma to **p**ersonal **ass**essment of genetic profile) dataset, patients who were treated correctly according to the signature had a better progression-free survival (median 20.1 months versus not reached; hazard ratio 0.40; confidence interval 0.23-0.72; p=0.0012) and overall survival (median 30.7 months versus not reached; hazard ratio 0.41; confidence interval 0.21-0.80; p=0.0049) than those who were not. Indeed, the outcome for these correctly treated patients was non-inferior to those treated with combined bortezomib, lenalidomide, and dexamethasone, arguably the standard of care in the United States, but not widely available elsewhere. The small size of the signature will facilitate clinical translation, thus enabling more targeted drug regimens to be delivered in myeloma.

Introduction

Multiple myeloma is a plasma cell neoplasm characterised by lytic bone lesions, hypercalcemia, renal impairment and bone marrow failure. Although outcomes have improved in recent years with the introduction of novel agents, the disease remains incurable and clinical responses display considerable heterogeneity.[1,2] Further improvements will not only come from introduction of new drugs but from better use of existing drugs. Younger, fitter patients are usually treated with a drug combination involving a proteasome inhibitor (PI) and/or an immunomodulatory drug (IMiD) followed by high-dose melphalan therapy with autologous stem cell transplant (ASCT). For transplant-ineligible patients, recent trial data suggest that the treatment of choice may be a combination of the PI, bortezomib (Velcade, Millennium Pharmaceuticals, Cambridge, MA), the IMiD, lenalidomide (Revlimid, Celgene, Summit, NJ), and dexamethasone (VRD).[3] However, this combination is expensive and is not funded in most countries outside the United States (US). Furthermore, for very frail patients, three-drug combinations may prove too toxic.

It is possible that treatment outcomes in myeloma might be improved by the application of precision medicine, i.e. the rational selection of drugs based on the biology of each patient's tumour. Several studies have demonstrated the potential of using transcriptomic data to derive prognostic information in myeloma.[4-6] Signatures can be usefully combined,[7] but are generally agnostic to treatment[4,5,8-12] and their main clinical utility is likely to be the identification of patients who may benefit from trials for high-risk disease. We sought to derive a signature that could predict responses to specific therapies.

The phase II study of Bortezomib, Adriamycin, and Dexamethasone (**PAD**) therapy for previously untreated patients with multiple myeloma: **I**mpact of minimal residual disease (**M**RD) in patients with deferred **A**S**C**T (PADIMAC) was designed to examine whether

patients with good responses to PAD could safely avoid upfront ASCT. We employed RNA-sequencing (RNA-Seq) on available good-quality RNA from enrolled patients and derived a training dataset from patients with sustained deep responses in the absence of ASCT. We thus generated a signature for predicting bortezomib-responsiveness in myeloma patients not receiving ASCT. When tested in independent datasets, the signature performed well, identifying patients who benefited from bortezomib-based treatment in the absence of an IMiD. Furthermore, when tested on lenalidomide-dexamethasone (RD) treated patients, the signature performed in a reciprocal fashion, suggesting that it could be used as a binary classifier to choose between bortezomib-based treatment and RD. Patients who had been treated correctly according to the signature classification had a superior survival to those who had not. Indeed, in the relating **C**linical **o**utcomes in **M**ultiple **M**yeloma to **p**ersonal **ass**essment of genetic profile (CoMMpass) dataset, correctly treated patients receiving either bortezomib-based therapy (without IMiD) or receiving RD (without bortezomib) had a non-inferior survival to those treated with VRD. This suggests that our signature could be employed to improve the safety and cost-effectiveness of myeloma therapy without compromising outcomes.

Materials and Methods

Sample accrual and processing and data generation

Sample accrual and RNA isolation

RNA of sufficient quality for RNA-Seq was available from 44 patients treated on the PADIMAC trial (ISRCTN03381785). The trial protocol is described in the Supplementary Materials. PADIMAC was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines and was approved by the NHS National Research Ethics Service. Participants provided written informed consent. Patient registration and trial

management were performed by the Cancer Research UK and University College London Cancer Trials Centre. All patients had newly diagnosed untreated myeloma, Eastern Co-operative Oncology Group performance status 0-3, and were eligible for ASCT. Total RNA was isolated using standard methodology, as described in the Supplementary Materials.

Identification of mutations and gene expression

Standard methods were used to identify mutations and determine gene expression. Detailed methodology is described in the Supplementary Materials and Table S1. Briefly, reads were mapped with TopHat[13] and aligned with Samtools.[14] Single nucleotide variants (SNVs) and small indels were identified using VarScan[15,16] and RNA fusions were identified using FusionCatcher.[17] Read counts were generated with the Rsubread package.[18,19] Raw and count level data have been uploaded to Gene Expression Omnibus (GEO), reference GSE116324. Differentially expressed genes were identified using DESeq2[20-22] and the Gage[23] and Pathview[24,25] packages were used for pathway analysis.

Machine learning

Selection of test datasets

Test RNA-Seq datasets were derived from CoMMpass (https://research.themmrf.org/). Microarray test sets were obtained for relapsed/refractory patients treated with bortezomib[26] (GEO reference GSE9782), plasma cell leukemia (PCL) patients treated with RD[27] (GSE39925), and newly diagnosed myeloma patients treated with PAD followed by ASCT[28] (GSE19784). We refer to these data as the Millennium, PCL, and HOVON/GMMG datasets, respectively.

Data pre-processing, training, validation, and testing

RNA-Seq counts were normalized and corrected for heteroscedasity according to published methods.[29-35] Potential signature genes were identified from the PADIMAC dataset by an

empirical Bayes method,[36] then selected as described using synthetic annealing [37] with an error rate determined by a support vector machine implemented from the e1071 package (https://cran.r-project.org/web/packages/e1071/index.html).

Signature assignments were made using the Large Margin Nearest Neighbors (LMNN) algorithm.[38] Performance within the PADIMAC dataset was checked by ten-fold cross-validation. For external testing, all PADIMAC data were used for training, with an initial 50:50 split into a training and internal validation set that was fixed for all testing. R and Matlab scripts replicating this process have been included with the Supplementary Materials. All the CoMMpass, Millennium, PCL, or HOVON/GMMG data were used for testing. To determine the robustness of the signature performance in each case, a form of permutation testing was used, as described in the Supplementary Materials.

Statistical considerations

Null and observed assignments were compared using the Mann-Whitney-Wilcoxon test. Survival was compared using the Cox proportional hazards model. P-values of 0.05 or less were considered significant.

Results

Initial assessment of PADIMAC data excludes a mutation-based classifier

We performed RNA-Seq on purified CD138+ plasma cells from a cohort of 44 patients treated on the PADIMAC trial. Clinical data are shown in Table S2. We first explored the possibility of using a mutation-based classifier for bortezomib-responsiveness. We identified fusion and SNV transcripts from the RNA-Seq data (Figures 1A and 1B and Tables S3 and S4). There were 0-8 fusions in each sample with a median of one (Figure 1A). Expected IgH-WHSC1 fusions were detected from t(4;14) patients (Table S3). There was a median of nine

SNVs per patient in coding regions, which is lower than seen in previous DNA sequencing studies.[39-42] This may reflect reduced expression from mutant alleles[43] as well as a failure to detect mutations in the furthest 5' regions of some genes. Nevertheless, we identified many of the known driver mutations in myeloma (Figure 1B and Table S4). Other known drivers were not mutated in our cohort, which may be related to sample size or may reflect lack of expression of mutated alleles.[43]

Overall, 45.5% of patients in the cohort achieved very good partial remission (VGPR) or better following PAD induction (Table S2). We defined a "bortezomib-good" group, namely patients who achieved a VGPR or better and who were progression-free at one year without ASCT (13/44; 29.5%). We termed the remaining patients "bortezomib-standard" (31/44; 70.5%). There were no associations between these groups and age, International Staging System (ISS), or myeloma type (Table S5). We also saw no significant associations between bortezomib-responsiveness and the presence of key cytogenetic, SNV, and translocation events (Table S6). There were trends towards significant associations between the bortezomib-good group and (a) the presence of any translocation and (b) the presence of a beta2-microglobulin translocation (Table S6). However, we did not feel that these associations were sufficiently strong for predicting clinical outcomes. We therefore turned to expression profiling.

Derivation of a seven-gene bortezomib-response signature

Expression of target genes known to be differentially expressed using microarray[44] and qPCR[45] technologies in the Translocation-Cyclin D (TC) classification was consistent with that previously described (Figure 1C),[44] confirming the utility of RNA-Seq for measuring relative gene expression in myeloma. We therefore proceeded to identify a gene signature for bortezomib-responsiveness. We ranked potential genes using synthetic annealing[37] (Figures

S1 and S2). Derived signatures comprising 4-11 genes performed better than permuted assignments in cross-validation of PADIMAC data using the LMNN algorithm (Figures S3 and S4; Matthews Correlation Coefficient (MCC) median 0.55 versus -0.045, Mann-Whitney U 0, p=0.00090, two-tailed, Figure S4A; F-measure median 0.67 versus 0.25, Mann-Whitney U 0, p=0.00090, two-tailed, Figure S4B). Of these signatures, the best performing was the seven-gene signature (Figure 2A). As training of the LMNN algorithm parameters involves splitting the training set into a training and internal validation set (Figure S2), we checked that the seven-gene signature was robust by performing multiple (n=100) training/validation splits and comparing performance with permuted assignments (n=100) during cross-validation. The observed assignments had higher MCC and F-measures than the null assignments (MCC median 0.50 versus 0.0054, Mann-Whitney U 8, $p=2.85x10^{-34}$, two-tailed, Figure 2B; F-measure median 0.64 versus 0.26, Mann-Whitney U 3, $p=2.42x10^{-34}$, two-tailed, Figure 2C), confirming that the signature performed well regardless of the training/validation split.

The genes comprising the signature are EMC9, FAM171B, PLEK, MYO9B, RCN3, FLNB, KIF1C (Table S7). We did not see enrichment of these genes within the pathway genesets from the Molecular Signatures Database[46-48] (http://software.broadinstitute.org/gsea/msigdb/annotate.jsp; C2 collection). However, at least three of the proteins (EMC9, RCN3, and KIF1C) are associated with the endoplasmic reticulum and three others (PLEK, MYO9B, and FLNB) interact with actin filaments. Furthermore, three genes (EMC9, MYO9B, and KIF1C) associate positively with proliferation in myeloma.[10] Despite the lack of objective pathway enrichment in our signature, supervised analysis of the RNA-Seq data as a whole did reveal pathways upregulated in the bortezomib-good patients (Table S8).

The seven-gene signature is predictive of outcome of bortezomib-treated patients in the independent CoMMpass dataset

To enable testing of our signature in an independent external dataset, we extracted RNA-Seq data from CoMMpass. We selected previously untreated patients who did not proceed to ASCT, as none of the bortezomib-good patients had received transplant. There were 147 such bortezomib-treated patients (who had received no IMiD), 40 RD patients, and 208 VRD patients for whom RNA-Seq data were available (Tables S9-S11). There were a few differences in clinical features between the groups. PADIMAC patients, being transplant-eligible, were younger than all the CoMMpass cohorts (Figures S5-S7). VRD-treated patients in CoMMpass were younger than the bortezomib-treated and RD cohorts (Figures S9 and S10). RD-treated patients had higher rates of del13 than PADIMAC or bortezomib-treated patients and a lower rate of t(4;14) than bortezomib-treated patients (figures S6 and S8). There were no differences in ISS stage between the groups (figures S5-S10).

We trained our seven-gene signature on the PADIMAC data and tested its ability to identify patients who would benefit from bortezomib-based therapy within CoMMpass (Figure S11). Patients who received bortezomib-based therapy and were assigned to the bortezomib-good group had a better progression-free survival (PFS) than those assigned to the bortezomib-standard group (Figure 2D; Table 1, row 1). The randomization seed for the PADIMAC training/validation split had been fixed prior to testing. To ensure that the predictive ability of the signature was robust, we performed multiple additional training/validation splits of the PADIMAC training set and compared the resulting assignments in the CoMMpass test set with permuted assignments that formed a null dataset. As expected for a robust signature, hazard ratios (HRs) for the predicted bortezomib-good patients were lower than the HRs from random predictions (Figure 2E; Table 2, row 1).

The seven-gene signature has reciprocal performance in RD-treated patients and has the potential to select therapy

To distinguish between the signature acting as a general predictor of good-prognosis disease and as a specific predictor of bortezomib-sensitive disease, we tested it in RD-treated patients. We reasoned that, if the signature were bortezomib-specific, the survival of RD-patients assigned to the bortezomib-good would be no better than those assigned to the bortezomib-standard groups. To our surprise, RD-treated patients assigned to the bortezomib-good group in fact had an inferior PFS to those assigned to the bortezomib-standard group (Figure 3A; Table 1, row 2). Whilst the difference was not significant, those assigned to the bortezomib-good group across repeated training/validation splits had consistently lower survival, with higher hazard ratios (HRs) than those obtained by permuting the assignments (Figure 3B; Table 2, row 2).

The implication of these findings is that patients predicted to do well with bortezomib by our signature do poorly when treated with RD and vice versa. Hence the seven-gene signature could be used as a binary classifier to rationally choose between bortezomib-based therapy and RD. To test this, we selected CoMMpass patients treated with bortezomib-based therapy or with RD and assigned each to a bortezomib-best or lenalidomide-best group. We then compared survival between those patients who received the predicted best treatment with those who did not. Patients who received the correct therapy had a superior PFS (Figure 3C; Table 1, row 3) and overall survival (OS; Figure 3D; Table 1, row 4). The incorrectly treated patients had a median PFS of 20.1 months and a median OS of 31.2 months, whereas the median PFS and OS were not reached for correctly treated patients. These predictions were again robust to the initial training/validation split of the PADIMAC dataset (Figure S12; Table 2, rows 3 and 4).

We excluded the possibility that the signature was acting as a surrogate for clinical features. We saw no association between signature assignment and key cytogenetic events (p=0.13; Fisher's exact test; Table S12) and in multivariate Cox regression, ideal treatment according to the signature retained significance for survival when age, ISS, or myeloma subtype were taken into account (Tables S13, rows 1 and 2).

Because of the finding of proliferative genes in our signature, we also wanted to check that it was not acting as a surrogate for the gene-expression based proliferation index (GPI50) signature[10] or other prognostic signatures. As these signatures have not, to our knowledge, been applied to RNA-Seq data previously, we first tested that they could be applied to the CoMMpass dataset. Indeed, when all of the GPI50, University of Arkansas for Medical Sciences (UAMS70), Erasmus University Medical Centre (EMC92), and Intergroupe Francophone du Myélome (IFM15) signatures[4-6,10] were applied to CoMMpass, the distribution of scores was similar to that seen in microarray datasets[5] (Figures S13A, S13C, S3E, and S13G). Furthermore, all signatures retained prognostic significance using thresholds equivalent to those previously published[5] (Figures S13B, S13D, S13F, S13H). Having established that these prognostic signatures were effective in RNA-Seq data, we examined whether there was any association between their assignments and the assignments of our seven-gene signature. None was seen (Table S14). Furthermore, receiving ideal treatment according to the seven-gene signature retained its prognostic significance even in a multivariate analysis including these signatures (Table S15).

A recent trial reported the superiority of VRD over RD in transplant-ineligible patients,[3] but VRD treatment is not currently funded widely outside the US. We wondered whether rationally selected therapy could be a cost-effective alternative to this gold-standard treatment. We first demonstrated that VRD was superior to unselected bortezomib-based treatment or RD in CoMMpass (Figure S14; Table 1, rows 5 and 6). We then compared the

survival of patients treated correctly according to our signature with the survival of all patients treated with VRD in CoMMpass. There was no statistically significant difference in OS (Figure 3E; Table 1, row 7) or PFS (Figure S15A; Table 1, row 8) between patients treated correctly with bortezomib or RD and those treated with VRD. This was also true in a multivariate analysis incorporating clinical features (Table S13, rows 3 and 4). We also compared the outcome of CoMMpass patients treated correctly with bortezomib according to the signature (without transplant) and all patients receiving bortezomib-based induction followed by ASCT. Interestingly, there was no significant difference in survival between the two groups (Figure S16; Table 1 rows 9 and 10), although there was weak evidence of an effect implying longer OS with transplant.

We hypothesized that the seven-gene signature should have minimal predictive ability in VRD-treated patients. As expected, we saw no difference between the outcomes for patients assigned to the bortezomib-best or lenalidomide-best groups when those patients were treated with VRD. This was true both for OS (Figure 3F; Table 1, row 11) and for PFS (Figure S15B; Table 1, row 12). This lack of predictive ability was also seen in multivariate analyses incorporating clinical features (Table S13, rows 5 and 6).

The seven-gene signature performs well in other independent datasets

We were keen to test how our signature would perform in other non-transplant settings, such as relapsed disease. However, being limited by the availability of publicly-available RNA-Seq data, we turned to microarray data. There were two suitable datasets available. One comprised samples from patients with relapsed-refractory myeloma treated with single-agent bortezomib[26] (the Millennium dataset). The second contained transcriptomic data from a small series of patients with PCL treated with RD[27] (the PCL dataset). We reasoned that, within the Millennium dataset, patients assigned to the bortezomib-best class should have a

better survival, whereas within the PCL dataset, those assigned to the lenalidomide-best class should have the superior outcome.

Signature assignments behaved as predicted. In the Millennium dataset the bortezomib-best group had a superior PFS (Figure 4A; Table 1, row 13) and OS (Figure 4B; Table 1, row 14) to the lenalidomide-best group. These results were robust to training/validation splits (Figure 4C; Table 2, rows 5 and 6). In the PCL dataset, those predicted to be in the lenalidomide-best group had a superior PFS (Figure 4D; Table 1, row 15) and OS (Figure 4E; Table 1, row 16) than patients assigned to the bortezomib-best group. Again, the signature was robust, with little influence from the training/validation split (Figure 4F; Table 2, rows 7 and 8).

The seven-gene signature loses predictive power in patients proceeding to ASCT

The bortezomib-good patients in the PADIMAC training set avoided ASCT because of their good response, according to trial protocol. We had thus far confined testing in external datasets to patients who had not had ASCT. We wondered whether the signature would retain its predictive power in patients proceeding to ASCT or whether transplant would overcome the survival differences between correctly and incorrectly treated patients. The HOVON-64/GMMG-HD4 phase III trial[49] compared patients with newly diagnosed myeloma treated with conventional chemotherapy versus those treated with PAD. Both groups of patients proceeded to ASCT.

We used our signature to make bortezomib-best and lenalidomide-best assignments in patients who had received PAD. We reasoned that, if the signature retained its predictive power in the ASCT setting, we would see superior survival in those patients assigned to the bortezomib-best group. This was not the case, however, and we saw no significant difference in either PFS (Figure 5A; Table 1, row 17) or OS (Figure 5B; Table 1, row 18) between the different signature assignments.

As a further check for the specificity of the signature, we also tested its predictive value in the dexamethasone-only arm. As anticipated, there was no difference in PFS between those patients predicted to be bortezomib-good and those predicted to be lenalidomide-good (Figure S17A). However, patients predicted to be bortezomib-good had a superior OS in this arm (Figure S17B). This is likely to be because patients receiving dexamethasone were eligible to receive cross-over bortezomib upon disease progression.

Discussion

If it can be realized, precision cancer medicine will benefit patients in terms of improved efficacy and reduced toxicity and will benefit society in terms of better management of limited drug budgets. Transcriptomics has considerable promise in this area.[50] There are signatures that predict for overall prognosis in cancer,[51-62] including myeloma,[4-12] and signatures that predict response to individual therapies.[63-67] However, we are not aware of any published signature that can be used to rationally select between different active cancer therapies. Remarkable improvements in myeloma outcome over recent years have been seen thanks to the introduction of multiple novel agents, but this has been associated with increasing costs of treatment.[68] Therefore, precision medicine is arguably of particular importance in this disease to help navigate through the increasing armamentarium of available therapies.

Herein, we describe the derivation and testing of a seven-gene signature that can be used to select between bortezomib-based or RD therapy in myeloma patients not undergoing ASCT. Patients treated correctly according to the signature in the CoMMpass dataset had a 69.7 months 3-year OS, similar to the outcome of patients treated with VRD, probably the current standard care for transplant-ineligible patients.[3] These comparisons have to be viewed with caution, as CoMMpass is not a clinical trial. Nevertheless, the OS seen in the VRD group

(74.5 months) is similar to that of the VRD-treated patients in the trial of Durie *et al*. (75.0 months).[3] Furthermore, there was no evidence that VRD-treated patients represented a poor-prognosis cohort in CoMMpass; there were no significant differences between the rates of poor-risk cytogenetics or ISS in the VRD group and the bortezomib- or RD-treated groups, and non-inferior survival was maintained in a multivariate model incorporating ISS.

If the outcome of rationally selected bortezomib- or lenalidomide-based therapy is equivalent to that of VRD, it would be important to consider why this might be. It may be that many patients treated with VRD are predominantly benefiting from just the bortezomib or the lenalidomide. Alternatively, it might be that any gains in combining the drugs are offset by increased toxicity, particularly in older or frailer patients. It is important to note that the reciprocal performance that we observe is an intrinsic property of the signature and not simply because bortezomib-sensitivity is automatically associated with lenalidomide-resistance (and vice versa). This is clearly not the case in clinical practice, nor is it consistent with the existence of multiple treatment-agnostic prognostic signatures in myeloma.

Although PADIMAC was a trial for transplant-eligible patients, the signature was trained on patients who had had a good response in the absence of ASCT. Therefore, our initial test set comprised patients who were transplant-ineligible (there were no datasets from transplant-eligible patients who did not proceed to transplant). Transplant-ineligible patients would be the most obvious to benefit following successful translation of the signature to the clinic. When tested in transplant-eligible patients who had received PAD and ASCT in the HOVON-64/GMMG-HD4 trial, the signature lost its predictive ability, implying that transplant can overcome the effect of receiving the "wrong" treatment. An interesting question would be whether receiving the correct predicted treatment without transplant is equivalent to ASCT. We saw no survival difference between transplant-ineligible patients treated correctly with bortezomib and all transplant-eligible patients treated with bortezomib

followed by ASCT. However, there was weak evidence of an effect suggesting better OS for patients who had a transplant.

There are limitations of our signature that need to be overcome before employment in a clinical trial. The signature assigns approximately one-quarter to one-third of patients to the bortezomib-best group and the remainder to the lenalidomide-best group by default. It may be that this larger group is heterogeneous, with some patients having poorer prognosis or multi-clonal disease and thus requiring VRD or the addition of other novel agents. Others may benefit equally from bortezomib- or lenalidomide-based treatments, regardless of signature assignment. Our signature was not capable of identifying these different groups (data not shown). Our external test cohorts were fairly small, owing to the lack of publicly-available and appropriate test datasets, and prospective validation of our signature will be needed. This will probably require the development of a quantitative PCR or focused sequencing panel, though the small number of genes in the signature means that this should be feasible. Finally, it is not clear to what extent our signature represents a drug effect or a class effect, because of the lack of publicly available test datasets involving patients treated with alternative PIs or IMiDs. Future data may become available from clinical trials in which expression profiling has been incorporated into the protocol.

We believe that our signature has the potential to move the myeloma field towards rational therapy decisions for transplant-ineligible patients in the future. It is essential that myeloma genomic datasets with relevant clinical outcome data continue to be made publicly available to allow refinement and prospective validation of these approaches. This will require the ongoing support of the myeloma research community.

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Authorship

Contributions

MAC, JS, JA, CR, RP, JJK, JH, and KW conceived the present study and performed data analysis. All authors contributed to data collection and writing of the manuscript.

Conflicts of interest

Alexion: honoraria (MK). Amgen: travel bursaries (GC), advisory role/consultancy (KY), honoraria (RP, GP, KY), speaker's bureau (GC), research (FW, GC, LCH, KY). Autolus: advisory role/consultancy (KY). Bayer: honoraria (JC). Celgene: travel bursaries (CR, RP, FW, GC, RGO), advisory role/consultancy (RGO), honoraria (GP, RGO), speaker's bureau (MJS, GC, GP), research (KY). Chugai: advisory role/consultancy (KY), research (KY).

Gilead: travel bursaries (JK), honoraria (MK, GP) speakers bureau (MK, GP). Janssen: travel bursaries (GC, GP), advisory role/consultancy (RP, MJS, GC, RGO, KY), honoraria (MAC, RP, MFQ, GC, GP, RGO, KY), speakers bureau (MJS, GC, GP, KY). Novartis: travel bursaries (AEV). Pfizer: travel bursaries (JC), honoraria (AEV), research (LCH). Sanofi: travel bursaries (GC), speaker's bureau (GC). Seattle Genetics: advisory role/consultancy (GC). Takeda: travel bursaries (MAC, GC, GP), advisory role/consultancy (RP, GC, KY), honoraria (MAC, RP, RGO, GC, GP, KY), speaker's bureau (GC, GP), research (GC).

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Tables

Row	Treatment (dataset)	Comparison	n1	n2	Median survival 1 (months)	Median survival 2 (months)	HR (CI)	p-value
1	Bortezomib-based (C)	Bortezomib-standard vs. bortezomib-good (PFS)	108	39	21.9	36.2	0.37 (0.17-0.81)	0.0063
2	RD (C)	Bortezomib-standard vs. bortezomib-good (PFS)	29	11	Not reached	18.8	2.35 (0.76-7.63)	0.16
3	Bortezomib-based or RD (C)	Incorrectly-treated vs. correctly-treated (PFS)	119	68	20.1	Not reached	0.40 (0.23-0.72)	0.0012
4	Bortezomib-based or RD (C)	Incorrectly-treated vs. correctly-treated (OS)	119	68	30.7	Not reached	0.41 (0.21-0.80)	0.0049
5	Bortezomib-based or RD or VRD (C)	All VRD vs. all non-VRD (PFS)	208	187	43.7	26.0	1.50 (1.06-2.13)	0.02
6	Bortezomib-based or RD or VRD (C)	All VRD vs. all non-VRD (OS)	208	187	Not reached	37.8	2.05 (1.34-3.13)	0.00084
7	Bortezomib-based or RD or VRD (C)	All VRD vs. non-VRD correctly-treated (OS)	208	68	Not reached	Not reached	1.12 (0.58-2.13)	0.74
8	Bortezomib-based or RD or VRD (C)	All VRD vs. non-VRD correctly-treated (PFS)	208	68	43.7	Not reached	0.86 (0.50-1.47)	0.57
9	Bortezomib-based +/- ASCT	Bortezomib correctly treated vs. ASCT (PFS)	47	39	36.6	36.2	1.16	0.75
10	Bortezomib-based +/- ASCT	Bortezomib correctly treated vs. ASCT (OS)	47	39	43.7	Not reached	3.10	0.08
11	VRD (C)	Lenalidomide-best vs. bortezomib-best (OS)	164	44	Not reached	Not reached	1.41 (0.69-2.90)	0.36
12	VRD (C)	Lenalidomide-best vs. bortezomib-best (PFS)	164	44	43.7	45.4	1.11 (0.62-1.92)	0.74
13	Bortezomib alone (M)	Lenalidomide-best vs. bortezomib-best (PFS)	135	38	4.14	4.77	0.66 (0.43-1.0)	0.04
14	Bortezomib alone (M)	Lenalidomide-best vs. bortezomib-best (OS)	148	40	15.2	25.8	0.57 (0.53-0.91)	0.01
15	RD (PCL)	Lenalidomide-best vs. bortezomib-best (PFS)	8	10	Not reached	1.0	Not defined*	2.52×10^{-5}
16	RD (PCL)	Lenalidomide-best vs. bortezomib-best (OS)	8	10	Not reached	12.5	Not defined*	0.0013
17	PAD/ASCT (H)	Lenalidomide-best vs. bortezomib-best (PFS)	84	57	26.6	31.8	0.91 (0.60-1.37)	0.63
18	PAD/ASCT (H)	Lenalidomide-best vs. bortezomib-best (OS)	85	58	Not reached	Not reached	0.73 (0.40-1.34)	0.30

Table 1 – Cox regression results for survival comparisons. All signature assignments were based on the seven-gene signature. Correctly-treated patients (rows 3-6) were those predicted by the signature as lenalidomide-best and who were treated with RD or those predicted as bortezomib-best and treated with bortezomib-based therapy. The datasets are CoMMpass (C), Millennium (M), plasma cell leukemia (PCL), or HOVON/GMMG (H). The numbers in the first group in the comparison (n1) and second group in the comparison (n2) are given. The hazard ratio (HR) is that of the second group versus the first group. CI – confidence interval. *The HR for the PCL group is not defined because all patients in group 2 progressed before any progressions in group 1 (row 11) or because there were no deaths in group 1 (row 12).

Row	Treatment (dataset)	Comparison	Median HR observed	Median HR null	Mann-Whitney U	p-value
1	Bortezomib-based (C)	Bortezomib-standard vs. bortezomib-good (PFS)	0.44	0.94	348	5.67×10^{-30}
2	RD (C)	Bortezomib-standard vs. bortezomib-good (PFS)	2.01	0.93	1526	1.91×10^{-17}
3	Bortezomib-based or RD (C)	Incorrectly-treated vs. correctly-treated (PFS)	0.48	0.92	185	5.44×10^{-32}
4	Bortezomib-based or RD (C)	Incorrectly-treated vs. correctly-treated (OS)	0.46	0.85	3	2.52×10^{-34}
5	Bortezomib alone (M)	Lenalidomide-best vs. bortezomib-best (PFS)	0.68	0.97	489	2.75×10^{-28}
6	Bortezomib alone (M)	Lenalidomide-best vs. bortezomib-best (OS)	0.53	1.00	108	5.67×10^{-33}
7	RD (PCL)	Bortezomib-best vs. lenalidomide-best (PFS)	0.24	0.83	1334	2.84×10^{-19}
8	RD (PCL)	Bortezomib-best vs. lenalidomide-best (OS)	0.20	0.96	1117	1.98×10^{-21}

Table 2 – Mann-Whitney-Wilcoxon results testing the robustness of the seven-gene signature to assignments across multiple (n=100) training/validation data splits. Each dataset is from CoMMpass (C), Millennium (M), plasma cell leukemia (PCL), or HOVON/GMMG (H). One hundred assignments were made in each dataset by the seven-gene signature following random training/validation splits (observed). Each of these assignments was then permuted to maintain assignment ratios (null). The performance of the observed and null assignments for predicting progression-free survival (PFS) and overall survival (OS) was compared in terms of hazard ratios (HRs) by the Mann-Whitney-Wilcoxon test. The hazard ratio (HR) is that of the second group versus the first group.

Figure Legends

Figure 1 – Fusion events, key single nucleotide variations (SNVs), and translocation-cyclin D (TC) classification of PADIMAC data. Each column represents a single sample. Samples are arranged into and color-coded by their TC classes (shown at the bottom). (A) Numbers of fusion events in each sample. (B) Key SNVs in each sample. (C) Expression of genes whose dysregulation is associated with TC classification in each sample.

Figure 2 – A seven-gene signature accurately predicts response to bortezomib-based therapy in PADIMAC and the independent CoMMpass datasets. (A) Matthews correlation coefficients (MCCs) and F-measures of bortezomib-good assignments by 4-11 gene signatures derived from synthetic annealing, following cross-validation within the PADIMAC dataset. (B) and (C) MCCs and F-measures of bortezomib-good assignments by the seven-gene signature following multiple (n=100) cross-validations within the PADIMAC dataset (Signature) compared to the MCC and F-measures of permuted assignments (Null). The p-values are those of the Wilcoxon-Mann-Whitney test, under the null hypothesis that the distributions of observed and null performances are the same. (D) Kaplan-Meier plot showing the progression-free survival of patients who received bortezomib-based therapy within CoMMpass (n=147) and who were predicted to benefit (n=39; broken line) or not (n=108; solid line) from bortezomib-based therapy by the seven-gene signature following training in PADIMAC. The p-value and hazard ratios (HRs) are those obtained from Cox regression analysis. (E) HRs for disease progression of bortezomib-good versus bortezomib-standard patients who received bortezomib-based therapy in CoMMpass. Predictions were made by the seven-gene signature, trained in PADIMAC, and followed repeated (n=100) training/validation splits (Signature). The HRs are compared with a null dataset of HRs obtained following permutations of the assignments (Null). The p-value is that of the

Wilcoxon-Mann-Whitney test, under the null hypothesis that the distributions of observed and null performances are the same.

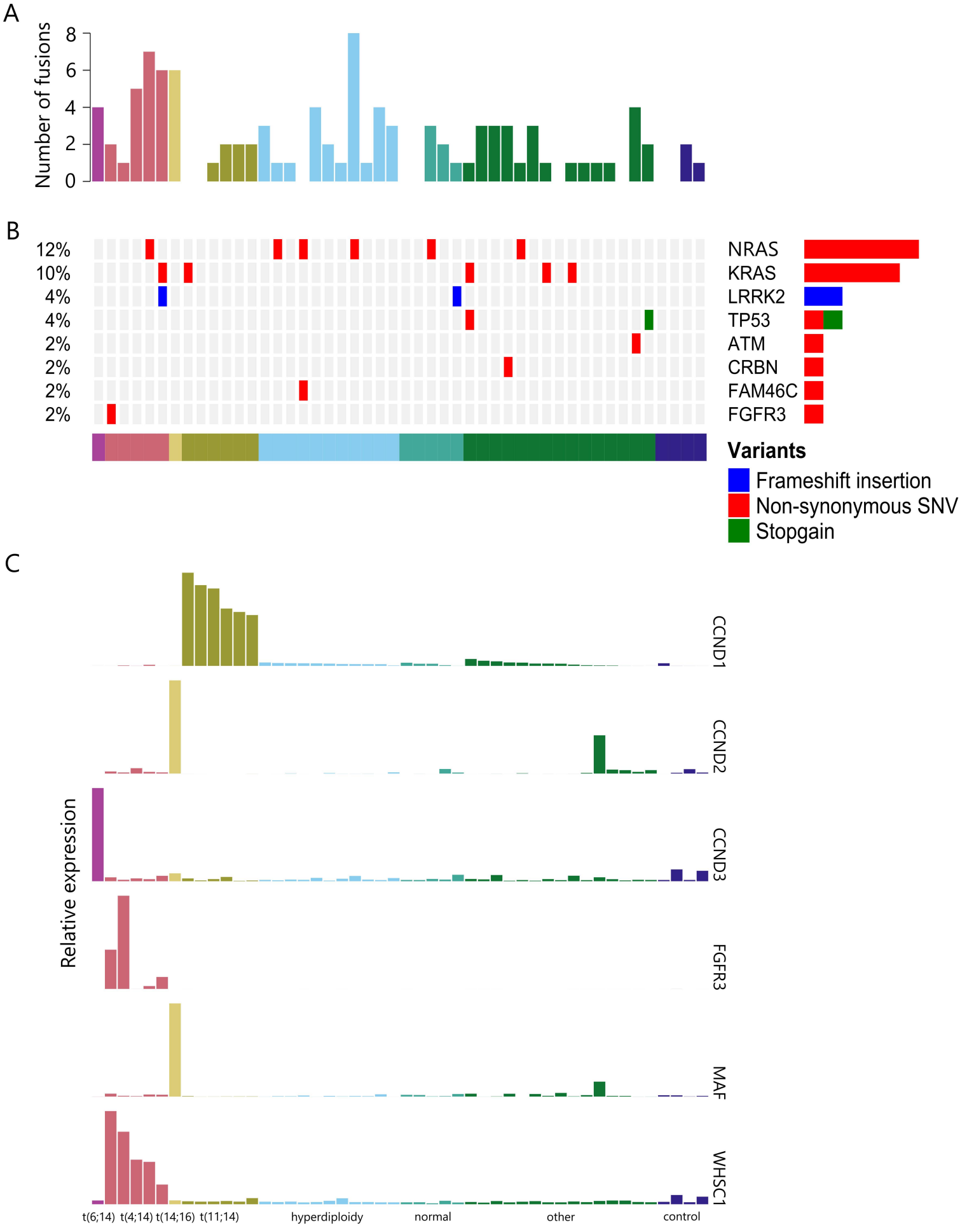
Figure 3 – The seven-gene signature can be used as a classifier to select between bortezomib-based therapy and lenalidomide/dexamethasone (RD) in the independent CoMMpass dataset. (A) Kaplan-Meier plot showing the progression-free survival (PFS) of patients who received RD therapy (n=40) within CoMMpass and who were predicted to benefit (n=11; broken line) or not (n=29; solid line) from bortezomib-based therapy by the seven-gene signature following training in PADIMAC. The p-value and hazard ratios (HRs) are those obtained from Cox regression analysis. (B) HRs for disease progression of bortezomib-good versus bortezomib-standard patients who received RD in CoMMpass. Predictions were made by the seven-gene signature, trained in PADIMAC, and followed repeated (n=100) training/validation splits (Signature). The HRs are compared with a null dataset of HRs obtained following permutations of the assignments (Null). The p-value is that of the Wilcoxon-Mann-Whitney test, under the null hypothesis that the distributions of observed and null performances are the same. (C) Kaplan-Meier plot showing the PFS of patients who received bortezomib-based therapy or RD within CoMMpass (n=187) and who received the correct (n=68; broken line) or incorrect (n=119; solid line) therapy predicted by the seven-gene signature following training in PADIMAC. The p-value and HR are those obtained from Cox regression analysis. (D) Kaplan-Meier plot showing the overall survival (OS) of patients who received bortezomib-based therapy or RD within CoMMpass (n=187) and who received the correct (n=68; broken line) or incorrect (n=119; solid line) therapy predicted by the seven-gene signature following training in PADIMAC. The p-value and HR are those obtained from Cox regression analysis. (E) Kaplan-Meier plot showing the OS of patients (n=276) who received bortezomib/lenalidomide/dexamethasone (VRD; n=208; solid line) or who received bortezomib-based therapy or RD within CoMMpass and who received

the correct therapy predicted by the seven-gene signature (n=68; broken line) following training in PADIMAC. The p-value and HR are those obtained from Cox regression analysis. (F) Kaplan-Meier plot showing the OS of patients who received VRD in CoMMpass (n=208; solid line) and who were predicted to benefit from RD (n=164; solid line) or from bortezomib-based therapy (n=44; broken line) by the seven-gene signature following training in PADIMAC. The p-value and HR are those obtained from Cox regression analysis.

Figure 4 – The seven-gene signature accurately predicts bortezomib- or lenalidomide-responsiveness in further independent datasets. (A) Kaplan-Meier plot showing the progression-free survival (PFS) of patients who received single-agent bortezomib within the Millennium studies (n=173) and who were predicted to benefit from bortezomib-based therapy (n=38; broken line) or from RD therapy (n=135; solid line) by the seven-gene signature following training in PADIMAC. The p-value and hazard ratios (HRs) are those obtained from Cox regression analysis. (B) Kaplan-Meier plot showing the overall survival (OS) of patients who received single-agent bortezomib within the Millennium studies (n=188) and who were predicted to benefit from bortezomib-based therapy (n=40; broken line) or RD therapy (n=148; solid line) by the seven-gene signature following training in PADIMAC. The p-value and HRs are those obtained from Cox regression analysis. (C) HRs for PFS and OS of patients predicted to benefit from bortezomib-based therapy who received bortezomib in the Millennium studies. Predictions were made by the seven-gene signature, trained in PADIMAC with repeated (n=100) training/validation splits (Signature). The HRs are compared with a null dataset of HRs obtained following permutations of the assignments (Null). The p-values are those of the Wilcoxon-Mann-Whitney test, under the null hypothesis that the distributions of observed and null performances are the same. (D) Kaplan-Meier plot showing the PFS of patients who received RD within the plasma cell leukemia study (n=18) and who were predicted to benefit (n=8; solid line) or not (n=10; broken line) from RD

therapy by the seven-gene signature following training in PADIMAC. The p-value is that obtained from Cox regression analysis. (E) Kaplan-Meier plot showing the OS of patients who received RD within the plasma cell leukemia study (n=18) and who were predicted to benefit (n=8; solid line) or not (n=10; broken line) from RD therapy by the seven-gene signature following training in PADIMAC. The p-value is that obtained from Cox regression analysis. (F) HRs for PFS and OS of patients predicted to benefit from lenalidomide-based therapy and who received RD in the plasma cell leukemia study. Predictions were made by the seven-gene signature, trained in PADIMAC with repeated (n=100) training/validation splits (Signature). The HRs are compared with a null dataset of HRs obtained following permutations of the assignments (Null). The p-value is that of the Wilcoxon-Mann-Whitney test, under the null hypothesis that the distributions of observed and null performances are the same.

Figure 5 – The signature loses predictive ability in the transplant setting. (A) Kaplan-Meier plot showing the progression-free survival (PFS) of patients who received bortezomib, adriamycin, and dexamethasone (PAD) within the HOVON-65/GMMG-HD4 study (n=143) prior to autologous stem cell transplant (ASCT) and who were predicted to benefit from bortezomib-based therapy (n=58; broken line) or from RD therapy (n=85; solid line) by the seven-gene signature following training in PADIMAC. The p-value and hazard ratios (HRs) are those obtained from Cox regression analysis. (B) Kaplan-Meier plot showing the overall survival (OS) of patients who received PAD within the HOVON-65/GMMG-HD4 study (n=143) prior to ASCT and who were predicted to benefit from bortezomib-based therapy (n=58; broken line) or RD therapy (n=85; solid line) by the seven-gene signature following training in PADIMAC. The p-value and HRs are those obtained from Cox regression analysis.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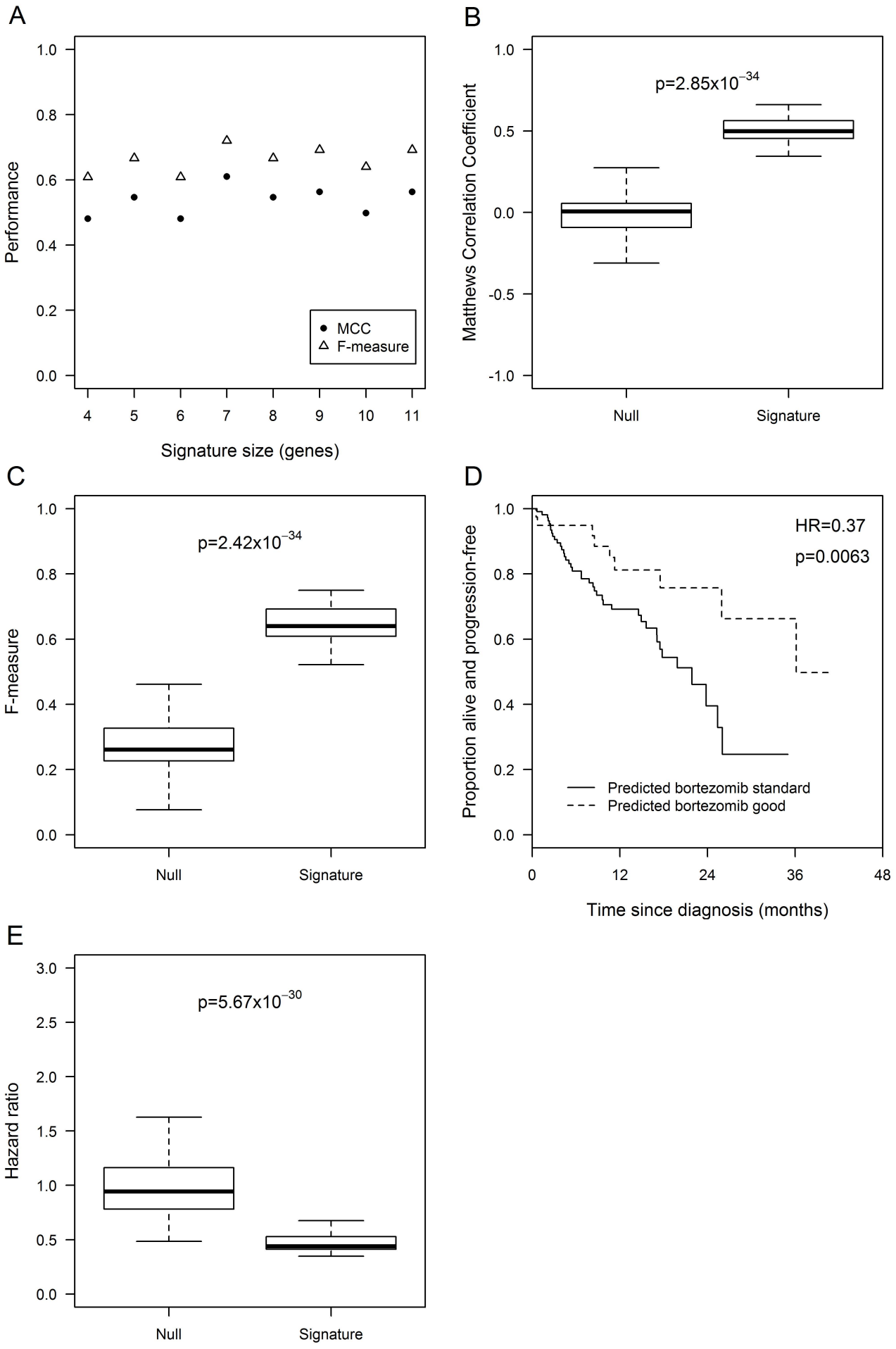
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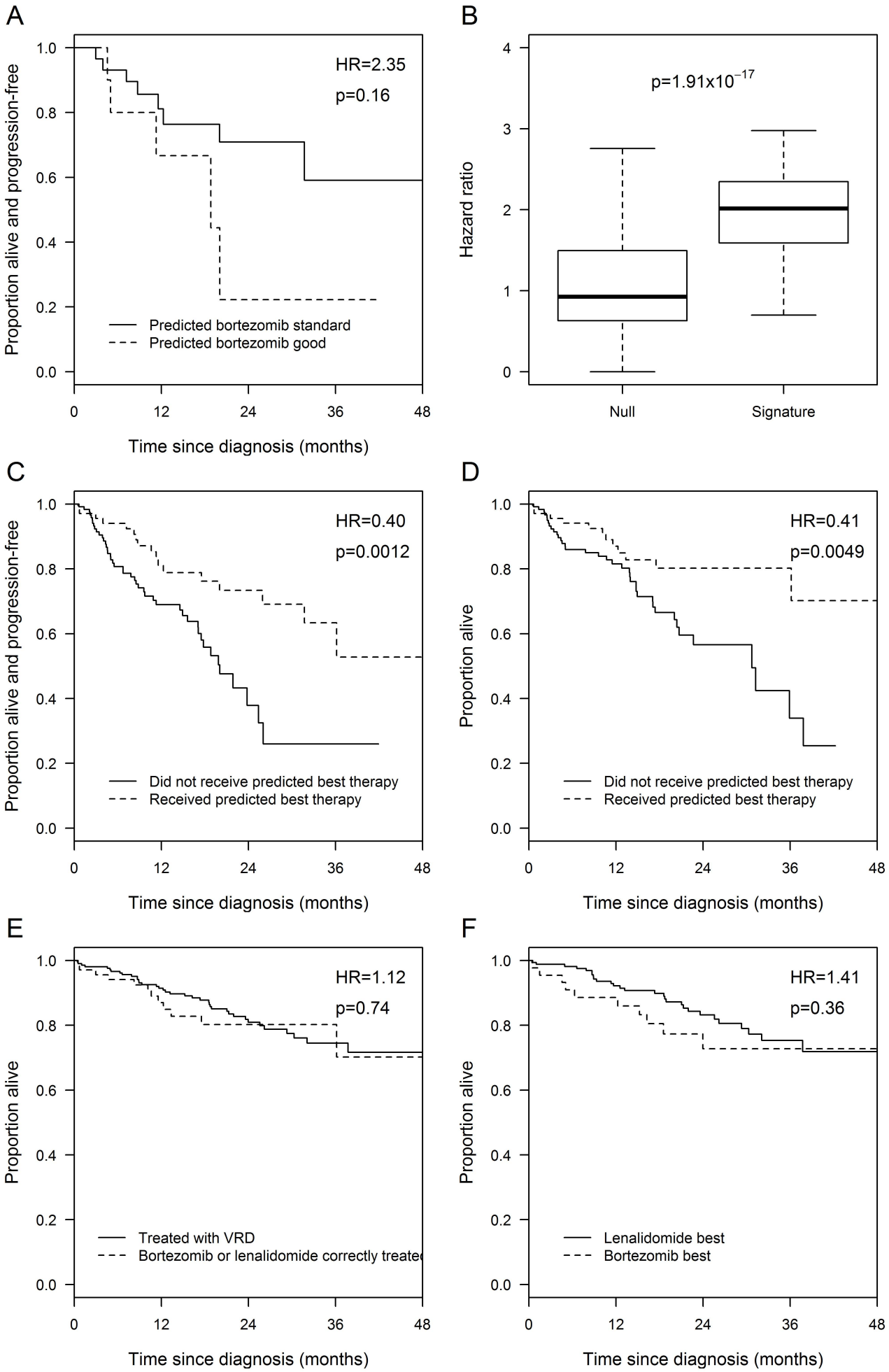
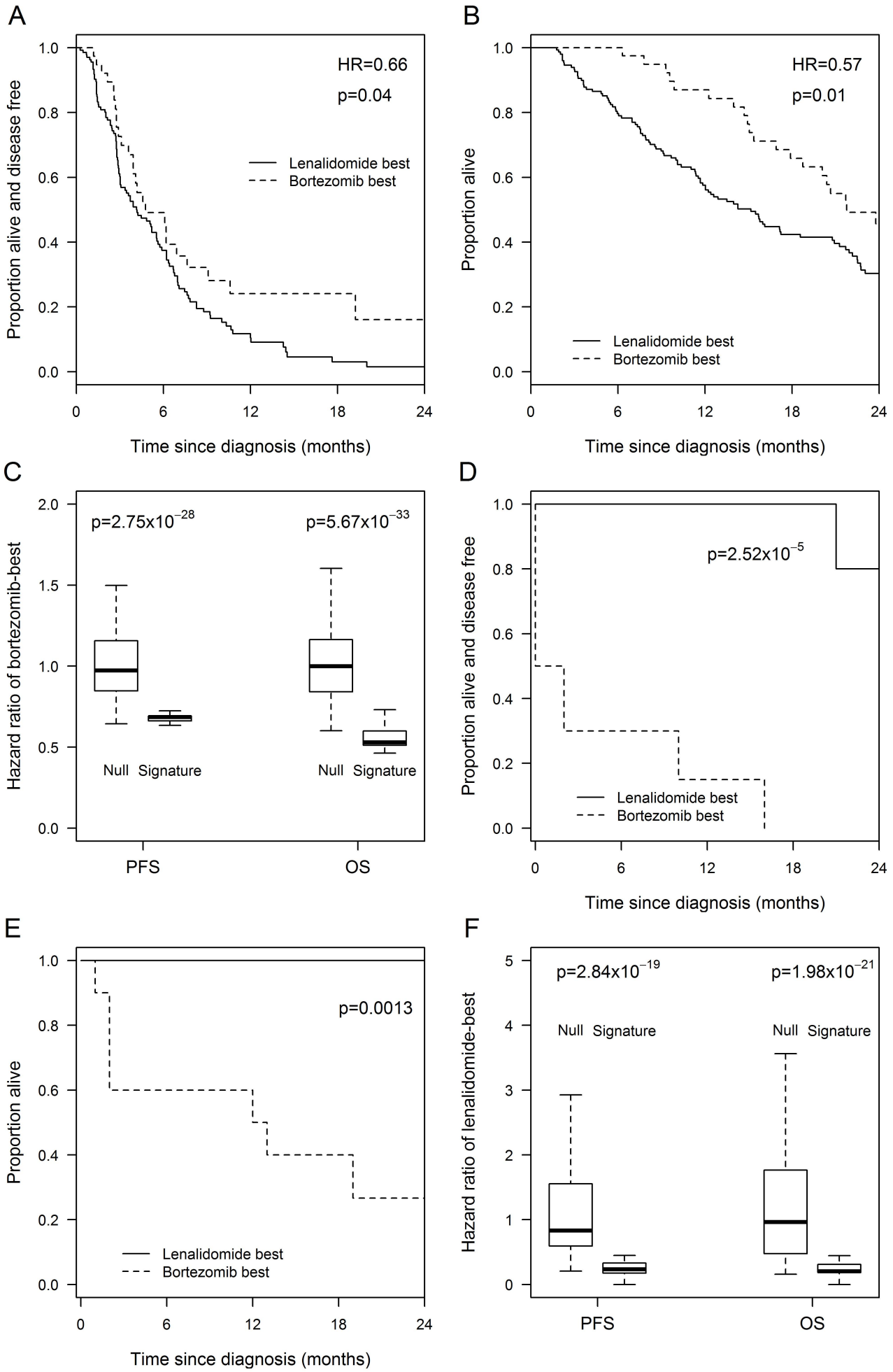
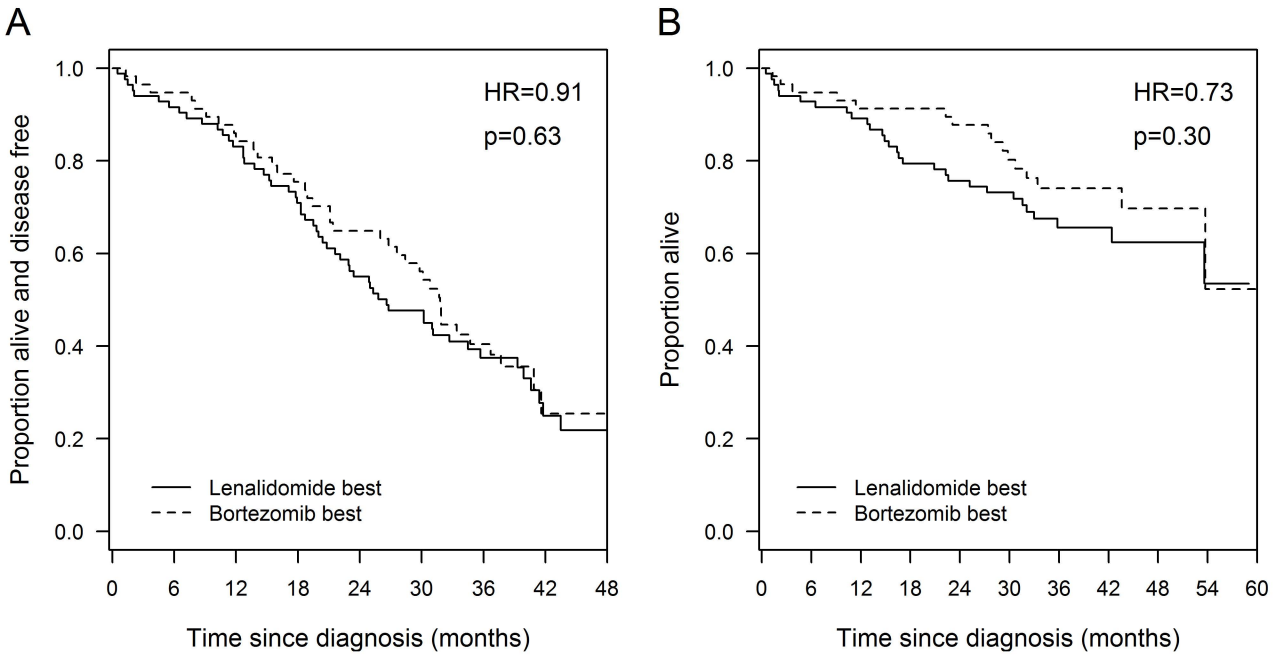
Null
Signature
C
F-measure
p=2.42x10^-34
Null
Signature
D
Proportion alive and progression-free
HR=0.37
p=0.0063
Predicted bortezomib standard
Predicted bortezomib good
Time since diagnosis (months)
E
Hazard ratio
p=5.67x10^-30
Null
Signature


A
HR=2.35
p=0.16
Proportion alive and progression-free
Predicted bortezomib standard
Predicted bortezomib good
Time since diagnosis (months)
B
p=1.91x10^-17
Hazard ratio
Null
Signature
C
HR=0.40
p=0.0012
Proportion alive and progression-free
Did not receive predicted best therapy
Received predicted best therapy
Time since diagnosis (months)
D
HR=0.41
p=0.0049
Proportion alive
Did not receive predicted best therapy
Received predicted best therapy
Time since diagnosis (months)
E
HR=1.12
p=0.74
Proportion alive
Treated with VRD
Bortezomib or lenalidomide correctly treate
Time since diagnosis (months)
F
HR=1.41
p=0.36
Proportion alive
Lenalidomide best
Bortezomib best
Time since diagnosis (months)


A
Proportion alive and disease free
HR=0.66
p=0.04
Lenalidomide best
Bortezomib best
Time since diagnosis (months)
B
Proportion alive
HR=0.57
p=0.01
Lenalidomide best
Bortezomib best
Time since diagnosis (months)
C
Hazard ratio of bortezomib-best
p=2.75x10−28
p=5.67x10−33
Null
Signature
Null
Signature
PFS
OS
D
Proportion alive and disease free
p=2.52x10−5
Lenalidomide best
Bortezomib best
Time since diagnosis (months)
E
Proportion alive
p=0.0013
Lenalidomide best
Bortezomib best
Time since diagnosis (months)
F
Hazard ratio of lenalidomide-best
p=2.84x10−19
p=1.98x10−21
Null
Signature
Null
Signature
PFS
OS


A
Proportion alive and disease free
1.0
0.8
0.6
0.4
0.2
0.0
HR=0.91
p=0.63
Lenalidomide best
Bortezomib best
0
6
12
18
24
30
36
42
48
Time since diagnosis (months)
B
Proportion alive
1.0
0.8
0.6
0.4
0.2
0.0
HR=0.73
p=0.30
Lenalidomide best
Bortezomib best
0
6
12
18
24
30
36
42
48
54
60
Time since diagnosis (months)