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Research paper

Drug response prediction in high-risk multiple myeloma

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ABSTRACT

A Drug Response Prediction (DRP) score was developed based on gene expression profiling (GEP) from cell lines and tumor samples. Twenty percent of high-risk patients by GEP70 treated in Total Therapy 2 and 3A have a progression-free survival (PFS) of more than 10 years. We used available GEP data from high-risk patients by GEP70 at diagnosis from Total Therapy 2 and 3A to predict the response by the DRP score of drugs used in the treatment of myeloma patients. The DRP score stratified patients further. High-risk myeloma with a predicted sensitivity to melphalan by the DRP score had a prolonged PFS, HR = 2.4 (1.2–4.9, $P = 0.014$) and those with predicted sensitivity to bortezomib had a HR 5.7 (1.2–27, $P = 0.027$). In case of predicted sensitivity to bortezomib, a better response to treatment was found ($P = 0.022$). This method may provide us with a tool for identifying candidates for effective personalized medicine and spare potential non-responders from suffering toxicity.

1. Introduction

A new promising therapy approach for multiple myeloma is to use gene expression profiling (GEP) as basis for precision medicine. This method was developed from GEP available from the NCI-60 panel of 60 human cancer cell lines that was established as an *in vitro* system for discovery of cytotoxic drugs for treatment of cancer (Shoemaker, 2006). GEP data from all cell lines are available and a characteristic gene expression pattern from cancer cells responsive to drugs can be identified. Based on these GEP patterns, a unique predictor for the cytotoxic effect of individual drugs on cancer cells has been established. This method may provide us with a tool for effective personalized medicine for myeloma and in particular patients with high-risk myeloma who have an unmet need for new treatment strategies. The prognosis of myeloma has improved considerably and the focus is now on a subgroup of myeloma patients with poor outcome (*i.e.* high-risk myeloma). The International Myeloma Work Group (IMWG) defines high-risk myeloma patients as patients who die within 2 years from diagnosis despite the use of novel agents. High-risk myeloma is a heterogeneous population of myeloma, recently defined by IMWG (Sonneveld et al., 2016). Several scoring systems have been described for high-risk myeloma (Supplementary Table S1). These scoring systems are mainly established on younger patients included in treatment protocols with high dose melphalan and hematopoietic stem cell support (Supplementary Tables S1 and S2). The majority of the scorings systems are based on GEP *e.g.* the TC classification, GEP70, GEP80, GEP5, Erasmus MC-92, IMF-15, MRC-IX-6 (Bergsagel and Kuehl, 2005; Kaiser et al., 2013; Mikhael et al., 2013; Shaughnessy et al., 2007; Heuck et al., 2014; Shaughnessy et al., 2011; Kuiper et al., 2012; Decaux et al., n.d.; Dickens et al., 2010) and others are based on cytogenetic abnormalities, ISS stage and LDH (Chng et al., 2014; Moreau et al., 2014; Palumbo et al., 2015). The mSMART includes both cytogenetic abnormalities and high-risk by GEP (Mikhael et al., 2013). The chromosomal translocations in myeloma such as t(4;14), t(6;14), t(11;14), t(14,16) and t(14,20) result in high expression levels of *MMSET/FGFR3*, *CCND3*, *CCND1*, *MAF*, and *MAFB*. In 2014 Tian et al. demonstrated that high expression levels of *MMSET/FGFR3*, *CCND1* and *MAF* by GEP can capture information on these translocations determined by FISH (virtual karyotyping) (Tian et al., 2014). Recently, Shaughnessy et al. showed that the mutational burden in myeloma is reflected in the GEP

Abbreviations: GEP, gene expression profiling; PFS, progression free survival; DRP, drug response prediction; TT, Total therapy; IMWG, The International Myeloma Work Group; ASCT, autologous stem cell transplantation; *MMSET*-high, high expression levels of *MMSET*; *MAF*-high, high expression levels of *MAF*; MDS, multidimensional scaling; VTD, velcade-thalidomide-dexamethasone

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data (ASH 2016; abstract 4450). This is of importance as targeted treatment is being explored in myeloma and comprises treatment with MEK inhibitors in patients with oncogenic mutations in *KRAS*, *NRAS* or *BRAF* genes (Heuck et al., 2016). The Bcl2 pathway is also targeted in myeloma and preliminary data suggest that venetoclax preferably works for patient with t(11;14) (Moreau et al. IMW 2017 abstract 026). A new strategy is to identify high-risk patients by GEP and use GEP for drug response prediction and treat patients according to these results with personalized medicine. A specific Drug Response Prediction (DRP) score has been developed by the Medical Prognosis Institute (Knudsen et al., 2014). The method has proven reliable in 29 out of 37 trials (Supplementary Fig. S1).

The aim of this study is to test whether the DRP score could predict drug sensitivity, progression free survival (PFS) and drug response in high-risk myeloma by GEP70 and virtual karyotyping in patients treated primarily with Total Therapy (TT).

2. Materials and method

2.1. Clinical trials

The treatment details of the clinical studies are presented in Supplementary Table S2. TT2 was a phase 3 trial randomizing patients between a control arm and added thalidomide. Following combination chemotherapy for induction, tandem autologous stem cell transplantation (ASCT) with high-dose melphalan were applied, consolidated with combination therapy and maintained on interferon and dexamethasone (Barlogie et al., 2006). TT3 also applied melphalan-based tandem transplants and incorporated bortezomib in induction and consolidation treatments and maintenance employed bortezomib, thalidomide and dexamethasone (Barlogie et al., 2007). Patients treated in the HOVON65/GMMG-HD4study received randomized induction treatment with vincristine plus adriamycin and dexamethasone or bortezomib plus adriamycin and dexamethasone, followed by ASCT and randomized maintenance treatment with thalidomide or bortezomib depending on induction treatment (Scheid et al., 2014). Patients treated in the GIMEMA MMY-3006 study were randomized to bortezomib plus thalidomide and dexamethasone (VTD) or thalidomide and dexamethasone (TD), harvest of stem cells followed by high-dose melphalan and ASCT and consolidation VDT or TD (Tacchetti et al., 2014). In the CREST, the SUMMIT and APEX study myeloma patients with relapsed disease were treated with bortezomib with and without dexamethasone (Richardson et al., 2005; Richardson et al., 2003; Jagannath et al., 2004).

2.2. Prediction of outcome by drug response prediction

The NCI-60 panel was established as an *in vitro* system for discovery of cytotoxic drugs in the treatment of cancer (Shoemaker, 2006) The 50% growth inhibition and 50% lethal concentration show a characteristic molecular pattern for each drug. This method can be used to identify new drugs of potential tumor-specific interest and for testing response to drugs for individual patients. Transcriptional data, protein levels, and data on function are available for thousands of drugs. The Drug Response Prediction (DRP™) was created from the pattern of mRNA expression from these60 cell lines by correlating growth inhibition to mRNA expression as described previously (Knudsen et al., 2014). Cell killing and growth inhibition to different cytotoxic drugs has been established from downloaded data from the DTP web site (http://dtp.nci.nih.gov) (Shoemaker, 2006). The individual drug response profile established from the different cell lines was filtered through mRNA expression from more than 3500 fresh frozen tumor samples to exclude outliers in the analysis. The DRP score is a scale from 0 to 100 and the population median is in our experience a good cut-off between responders and non-responders. The higher the score, the more likely is a response (Supplementary Fig. S1).

2.3. GEP data from clinical studies

We used 4 data sets of available GEP data from myeloma cells taken from patients enrolled in the TT, HOVON65/GMMG, the GIMEMA MMY-3006 study, the CREST, SUMMIT, and APEX study. We used pretherapeutic GEP data from myeloma cells from patients enrolled in the TT2 (N = 351) andTT3A (N = 181) protocols, (GEO:National Center for Biotechnology Information [NCBI], http://www.ncbi.nlm.nih.gov/geo/; accession number GSE2658) (Shaughnessy et al., 2007), the HOVON65/GMMG-HD4study; N = 328, of which 38 poor quality samples were excluded (NCBI-GEO repository) (http://www.ncbi.nlm.nih.gov/geo; accession number: GSE19784) (Kuiper et al., 2012), the-GIMEMAMMY-3006 study, N = 118 (NCBI-GEO repository) (http://www.ncbi.nlm.nih.gov/geo; accession number:GSE68871) (Terragna et al., 2016). Furthermore, we used GEP data from post-therapeutic myeloma cells from patients with relapsed disease and enrolled in the CREST, SUMMIT and APEX study (N = 264) (NCBI-GEO repository) (http://www.ncbi.nlm.nih.gov/geo accession number GSE9782) (Mulligan et al., 2007).

2.4. Stratification of high-risk myeloma

High-risk myeloma can be identified by GEP and IgH translocations. The GEP70 scoring system was developed on 351 patients in a training set from patients treated with TT2 and validated in a test set of 181 patients treated with TT3 (Shaughnessy et al., 2007). GEP from the training (TT2) set was used to identify patients with high-risk by GEP70. To analyze whether stratification by GEP70 into low and high-risk myeloma separates patients into two entities, GEP data from patients included in TT2 and TT3A, were analyzed by multidimensional scaling. The most frequent IgH translocations that confer a poor outcome are translocation t(4;14) and t(14;16) found in 12–15% and 3–5% of the cases, respectively. Translocation t(4;14) and t(14;16) alter the expression levels of the genes *MMSET* and *MAF* and can be predicted with high accuracy by spiked gene expression levels with a technique known as *virtual karyotyping* (Decaux et al., n.d.). GEP data from TT2 and TT3 were used to identify patients with t(4;14) and t(14;16) by virtual karyotyping. The heterogeneity of myeloma was analyzed by dividing patients into entities stratified by GEP70, high expression levels of the genes *MMSET* (*MMSET*-high) and *MAF* (*MAF*-high).

2.5. Prediction of sensitivity to drugs, prediction of progression estimates, and drug response for high-risk myeloma by GEP70 by the DRP score

Myeloma from patients treated in TT3A was used for analysis for predicted sensitivity by the DRP score to drugs included in the TT strategies. The GEP data from the HOVON65/GMMG-HD4 were used to validate the results. GEP data from TT2 and TT3A were used for evaluation of progression free survival (PFS) based on predicted sensitivity to melphalan by the DRP score. GEP data from TT3A were used to predict PFS based on predicted sensitivity to bortezomib and thalidomide by their DRP scores. GEP data from the GIMEMA MMY-3006 were used to predict response to treatment by VTD based on predicted sensitivity to bortezomib by the DRP score. GEP data from the CREST, SUMMIT and APEX study were used to predict response based on predicted sensitivity to bortezomib for patients with relapsed myeloma (Mulligan et al., 2007).

2.6. Statistical analysis

Statistical analysis of PFS was performed by Cox proportional hazards with 95% confidence intervals as well as a Wald test. The Wald test gave similar results as the log rank test and the likelihood ratio test on the Cox proportional hazards models. Comparison of response with predicted sensitivity was performed with a one-sided Pearson product moment correlation test.

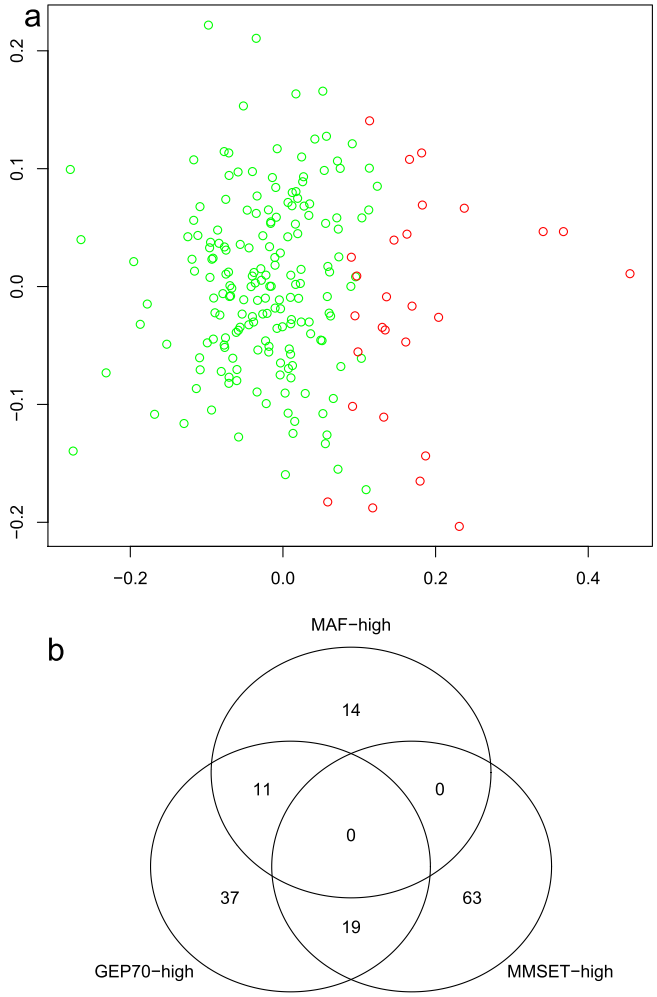


Fig. 1. A and B: Heterogeneity of high-risk myeloma by GEP70, high expression levels of *MAF* or *MMSET*.
The Fig. 1A and B illustrate the heterogeneity of high-risk myeloma patients included in TT2 and TT3A when stratified by GEP70, and *MAF*-high or *MMSET*-high. Figure A: multidimensional scaling showing GEP70 high and low-risk in TT3A as a continuum instead of two separate populations. Green dots are patients with low-risk by GEP70 and red dots patients with high-risk by GEP70. Fig. 1B: Venn diagram showing overlaps in patients with high-risk identified by GEP70, *MAF*-high and *MMSET*-high (virtual karyotyping). Both the TT2 and TT3A cohorts were used in this plot. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3. Results

3.1. High-risk myeloma as a disease entity

Multidimensional scaling (MDS) was performed with GEP data from patients stratified by GEP70 into high and low-risk myeloma. As shown in Fig. 1A MDS presented high- and low-risk myeloma by GEP70 as a continuum rather than two separate populations. We identified patients with t(4;14) and t(14;16) by virtual karyotyping. A Venn diagram from patients with high-risk by GEP70, *MMSET*-high and *MAF*-high is presented in Fig. 1B. None of the patients had high-risk features by all 3 criteria and to be expected none of the patients with *MAF*-high were included in the group of patients with *MMSET*-high and *vice versa*. Eleven of 28 patients (44%) with *MAF*-high were high-risk by GEP70. Nineteen of 82 patients (23%) with *MMSET*-high were classified as having high-risk by GEP70. Thirty-seven of 144 patients (26%) were only high-risk by GEP70, 14 of 144 patients (9.7%) were only had high-risk by *MAF*-high and 63 of 144 patients (44%) had only high-risk by *MMSET*-high. Fifty-three percent of patients with high-expression levels of *MMSET* or *MAF* did not exhibit high-risk features by GEP70.

3.2. Heterogeneity of myeloma in predicted sensitivity to drugs when stratified by GEP70, high expression levels of MAF or MMSET

A predicted drug response by the DRP score to melphalan, bortezomib, etoposide, doxorubicin, cisplatin, vincristine, cyclophosphamide, lenalidomide and thalidomide was found for myeloma patients treated in TT3A stratified by GEP70, *MMSET*-high, or *MAF*-high and is presented in Fig. 2A–C. The mean DRP score was higher for all drugs among high-risk patients by GEP70 and varied from 58 to 78. All differences between mean DRP score for high- and low-risk myeloma in Fig. 2A were significant in a two-sided *t*-test with the exception of cyclophosphamide. The highest mean DRP was found for IMiDS, vincristine, doxorubicin and bortezomib. The HOVON65/GMMG-HD4 GEP dataset confirmed the results (Supplementary Fig. S2). Myeloma from patients included in TT3A with *MMSET*-high had lower mean predicted sensitivity to all drugs by the DRP score than myeloma with low levels of *MMSET* and the mean score from myeloma with high expression levels of *MMSET* ranged from 35 to 52. A higher mean DRP score was found for thalidomide, lenalidomide, cisplatin and bortezomib (Fig. 2B). Among patients treated in TT3A with *MAF*-high the predicted sensitivity by the DRP score was lower for all drugs and varied from 35 to 50. The predicted sensitivity by the DRP score was higher for IMiDS, bortezomib, cisplatin and vincristine (Fig. 2C). Among high-risk patients identified by either GEP70, *MAF*-high, or *MMSET*-high a predicted sensitivity by the DRP score of more than 70 was found for few patients, indicating that responders to drugs can be found among high-risk patients irrespectively of risk stratification.

3.3. Prediction of progression estimates for high-risk myeloma by GEP70 by the DRP score of melphalan, bortezomib and thalidomide in newly diagnosed myeloma

Twenty percent of high-risk patients by GEP70 treated in TT2 and TT3A have a PFS of more than 10 years (Johnson et al., 2011). Tandem high dose melphalan is included in TT2 and TT3A and is the most intensive alkylating drugs used in the regimes. TT2 is a randomized study with added thalidomide and TT3 added further treatment Bortezomib (Supplementary Table 2). The DRP scores stratified progression estimates for high-risk patients further. Patients with myeloma with a predicted sensitivity to melphalan and a DRP score of more than 50 had a prolonged PFS, HR = 2.4 (1.2–4.9; $P = 0.014$) (Fig. 3A). The DRP score for bortezomib was applied to myeloma from patients treated in TT3A (Fig. 3B). Patients with myeloma with a predicted sensitivity to bortezomib by the DRP score of more than 66 had a prolonged PFS, HR 5.7 (1.2–27; $P = 0.027$). No significantly better PFS was found for patients with myeloma and predicted sensitivity by the DRP score to thalidomide HR 2.3 (0.6–8.8, $P = 0.21$).

3.4. Response estimates to bortezomib by the DRP score for newly diagnosed high-risk patients by GEP70 and in patients with relapsed disease

GEP data from newly diagnosed patients treated with VTD in the GIMEMA MMY-3006 study were used for analysis. Predicted drug response to bortezomib by the DRP score correlated to response to treatment with VTD for high-risk patients by GEP70 ($P = 0.022$) (Supplementary Fig. S3). GEP data from patients treated at relapse with bortezomib with and without dexamethasone in the CREST, SUMMIT and APEX were used and a predicted drug response to bortezomib by the DRP score correlated to response to treatment ($P = 0.001$) (Supplementary Fig. S4).

3.5. Predicted sensitivity to B-cell drugs for high-risk myeloma by GEP70

Fig. 4 presents predicted sensitivity by the DRP score to B-cell drugs

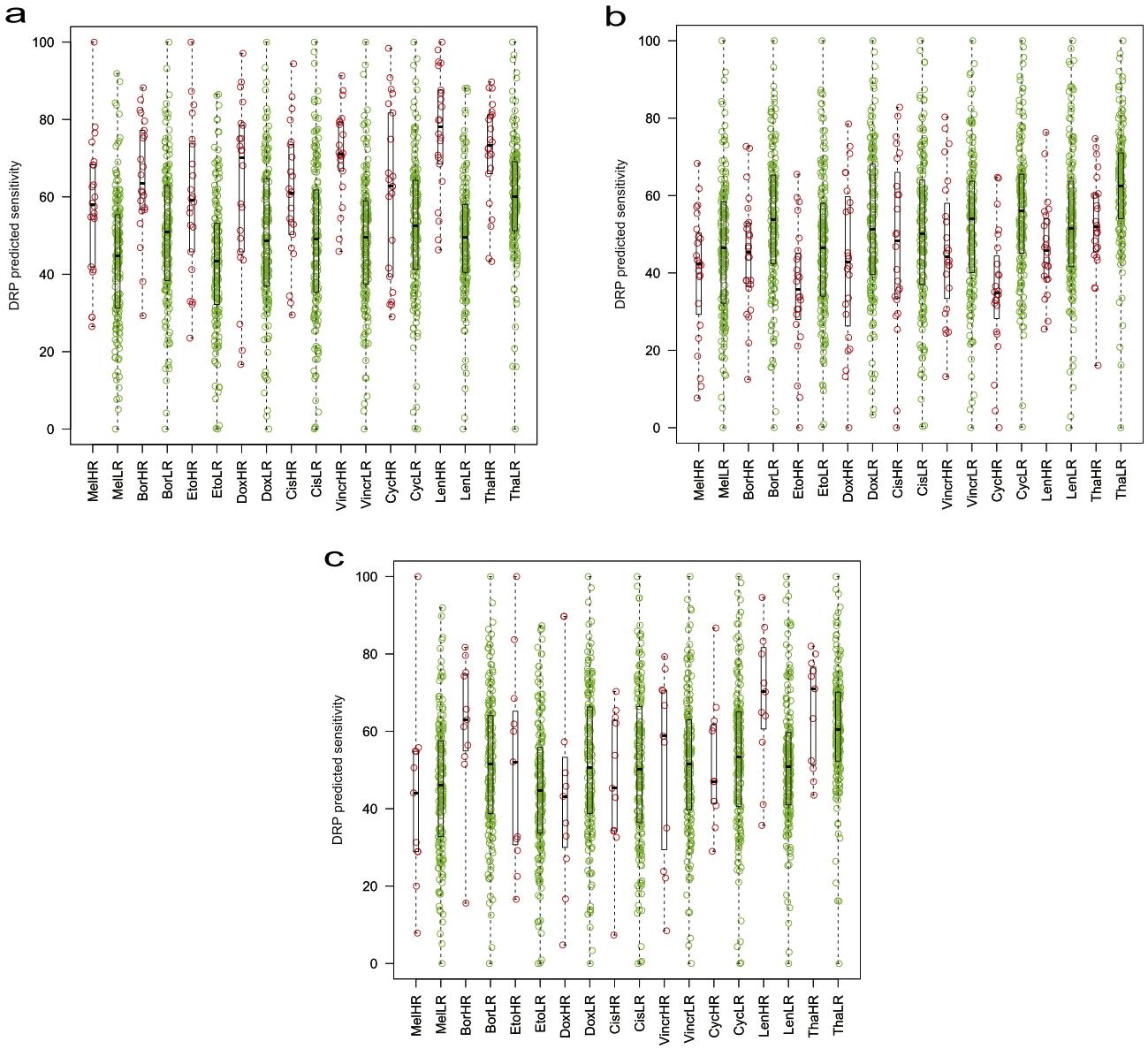


Fig. 2. The heterogeneity of predicted drug response by DRP for myeloma stratified by GEP70, high expression levels of MAF and MMSET.
The Fig. 2A, B and C illustrate the DRP score for the drugs melphalan (Mel), bortezomib (Bor), etoposide (Eto), doxorubicin (Dox), cisplatin (Cis), vincristine (Vin), cyclophosphamide (Cyc), lenalidomide (Len) and thalidomide (Thal) in myeloma stratified by GEP70 and by *MMSET*-high or *MAF*-high. The DRP scores were established on GEP data from patients treated in TT3A. (A) The DRP scores for patients with high-risk (HR) and low-risk by GEP70. (B) The DRP scores for high-risk by *MMSET*-high. (C) The DRP scores for high-risk by *MAF*-high.

used in myeloma, and other B-cell diseases portrayed in a heat map. The figure shows the number of patients with a GEP profile that may predict sensitivity to a drug. Green and red signals represent myeloma samples that are predicted sensitive or insensitive, respectively. GEP data from 67 newly diagnosed high-risk patients by GEP70 included in TT2 and TT3 were tested for predicted drug response by the DRP score to lenalidomide, ibrutinib, trametinib, venetoclax, panobinostat, carfilzomib, veliparib, vincristine, cisplatin, doxorubicin, etoposide, cyclophosphamide, melphalan and bortezomib. Green indicates patients with a DRP score above the population median for this drug. A predicted sensitivity by the DRP score for at least one drug was found for all high-risk patients. Myeloma samples from 7 patients were only predicted sensitive by the DRP score for one of the drugs tested. Of these, myeloma from five patients were predicted sensitive by the DRP score to ibrutinib, one to panobinostat, and one to vincristine. Thirty-four patients (51%) had myeloma that predicted sensitivity by the DRP score to venetoclax, a score that was independent of translocation t (11;14) by *virtual karyotyping* (Supplementary Fig. S5). Twenty-eight patients (42%) had myeloma with predicted sensitivity by the DRP score to carfilzomib and bortezomib, 23 (34%) had myeloma that predicted insensitive by the DRP score to carfilzomib and bortezomib, and myeloma in 12 patients (18%) were predicted sensitive by the DRP score to carfilzomib but not bortezomib. Furthermore, myeloma from 8 patients (12%) was predicted sensitive to bortezomib but not carfilzomib. Finally, eleven patients had myeloma with predicted insensitivity to all drugs used in TT3A (vincristine, cisplatin, doxorubicin, etoposide, cyclophosphamide, melphalan and bortezomib). Myeloma from all these patients was also predicted insensitive by the DRP score to lenalidomide, carfilzomib and venetoclax. Ten of these 11 patients had myeloma with predicted sensitivity to ibrutinib, two with predicted sensitivity to panobinostat, and three with predicted sensitivity to veliparib.

4. Discussion

Identification of high-risk myeloma patients by the GEP70 scoring

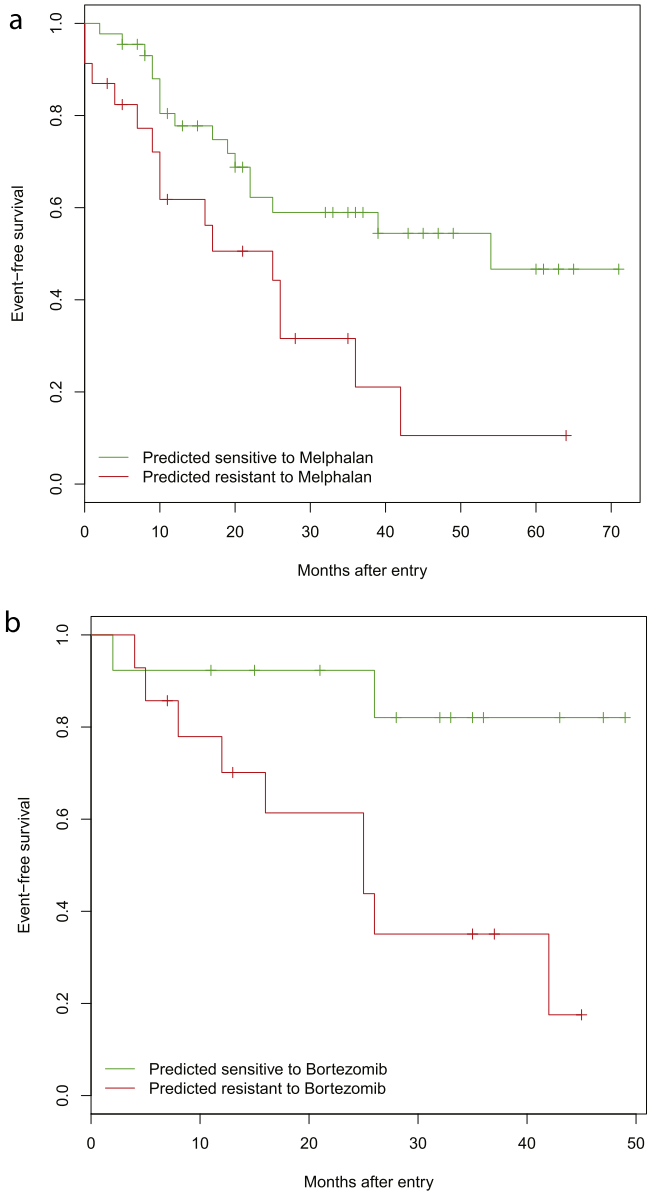


Fig. 3. Progression free survival for high-risk patients by GEP70 treated in Total Therapy stratified by DRP score to melphalan and bortezomib.
The Fig. 3A and B illustrate the progression free survival for high-risk patients by GEP70 when stratified by the DRP score for predicted sensitivity to melphalan and bortezomib. (A) Patients treated in TT2 and TT3 with a predicted sensitivity by DRP to melphalan of more than 50. Green line: patients with predicted sensitivity to melphalan. Red line: patients with predicted insensitivity to melphalan (B) Patients treated in TT3 with a predicted sensitivity of more than 66 to bortezomib. Green line: patients with predicted sensitivity to bortezomib. Red line: patients with predicted insensitivity to bortezomib. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

system has been validated in the transplant, non-transplant, and relapse settings (Shaughnessy et al., 2007; Barlogie et al., 2014). In 2014 Barlogie et al. published outcome of patients treated in the TT2 and TT3A protocols that include active drugs used in other B-cell diseases as well as IMiDs and proteasome inhibitors (Johnson et al., 2011). Although complete response duration (CRD) for low-risk patients by GEP70 in TT3 was more than 60% at 10 years the CRD for high-risk patients in TT2 and TT3A had not improved since the incorporation of thalidomide and bortezomib in the TT strategies. However, about 20% of patients with high-risk myeloma by GEP70 had a CRD of 8 years (Johnson et al., 2011). Shaughnessy et al. and Terragna et al. have established methods based on GEP data from myeloma to predict outcome of patients treated with bortezomib (Shaughnessy et al., 2011; Terragna et al., 2016). The GEP80 scoring system was established on myeloma taken from patients 48 h after a test dose of bortezomib (Shaughnessy et al., 2011). Here, gene expression alterations in myeloma were attributed to the protein ubiquitination pathway and myeloma with high proteasome mRNA activity had worse outcome. A gene expression signature of 42 genes has recently been published that distinguish good and poor responders to proteasome inhibitors based on *in vitro* chemo sensitivity of human myeloma cell lines (Paiva et al., 2017). In the present study, we describe another method for prediction of drug response based on GEP data from NCI60 cell lines killed by drugs. The DRP score uses differential baseline GEP data from cells surviving treatment as compared to cells killed by the tested drug and thereby accounts for drug induced deregulation of genes. The hypothesis is that tumor cells killed by drugs share biological response pathways that can be addressed independently of origin of the cancer. The method is therefore based on the cytotoxic effect of drugs and do not accounts for drug effects that require interaction with the surrounding microenvironment including the immune system. We applied the DRP score of melphalan, bortezomib and thalidomide to available GEP data from high-risk patients by GEP70 treated in TT2 and TT3, GIMEMA MMY-3006, APEX, SUMMIT and CREST to test if the DRP score could be used for prediction of PFS and response to treatment. The method predicted response and outcome to bortezomib and outcome of melphalan. The HR for better PFS in patients with predicted sensitivity to bortezomib by the DRP score was 5.7 and for melphalan it was 2.4. Biologically well-defined molecular subgroups, of which four are strongly influenced by specific translocations involving the immunoglobulin heavy chain locus, have been described with different outcome (Richardson et al., 2003). Patients in the MF subgroup (high expression levels of *MAF* and *MAFB*), patients in the MS subgroup (high expression levels of *FGFR3* and *MMSET*) and patients in the PR subgroup (subgroup with high proliferation index) have the worst outcome. In this study, we focus on predicted drug response in high-risk patients identified by the GEP70 scoring system and by *virtual karyotyping* of the high-risk cytogenetic t(4;14) and t(14,16). None of the high-risk patients had all high-risk markers, and patients with *MAF*-high and *MMSET*-high were mutually exclusive. Forty-three percent of patients with either high *MAF* or *MMSET* levels were also high-risk byGEP70. The predicted sensitivity to drugs used in TT differed in relation to risk stratification byGEP70 and a high expression level of *MAF* or *MMSET* and thereby support the heterogeneity in high-risk myeloma. Predicted mean sensitivity to all drugs was higher for patients when stratified by GEP70 but the mean predicted sensitivity to all drugs was lower for patients with high-risk stratified by either high expression levels of *MAF* or *MMSET* and may indicate different biology of these subgroups. Paiva et al. have recently shown that the myeloma cell population expands from fully differentiated plasma cells to less differentiated clones with poor outcome and that the latter cell clones become enriched after chemotherapy (Berglof et al., 2015). Their study indicates that the less differentiated plasma cells have plasticity resembling preceding B-cell stages. We therefore used the DRP method for predicted response to other B-cell drugs such as venetoclax, idelalisib, trametinib, veliparib and ibrutinib as well as established drugs in treatment of myeloma. In this panel of drugs a predicted effective treatment was found for all high-risk patients by GEP70 and therefore suggest that the predicted response by DRP should be tested on up-coming GEP results for the individual drugs and in clinical trials. In patients with myeloma that were predicted insensitive to treatment used in the TT strategy (including treatment with bortezomib) 10 out of 11 patients had myeloma with predicted sensitivity to ibrutinib by the DRP score. The effect of ibrutinib is not only confined to inhibition of the Bruton's tyrosine kinase (BKT) but can also inhibit other kinases (Murray et al., 2015). However, the exact off-target effect of ibrutinib in other diseases, including myeloma, is still under investigation (Murray et al., 2015). One study on bortezomib

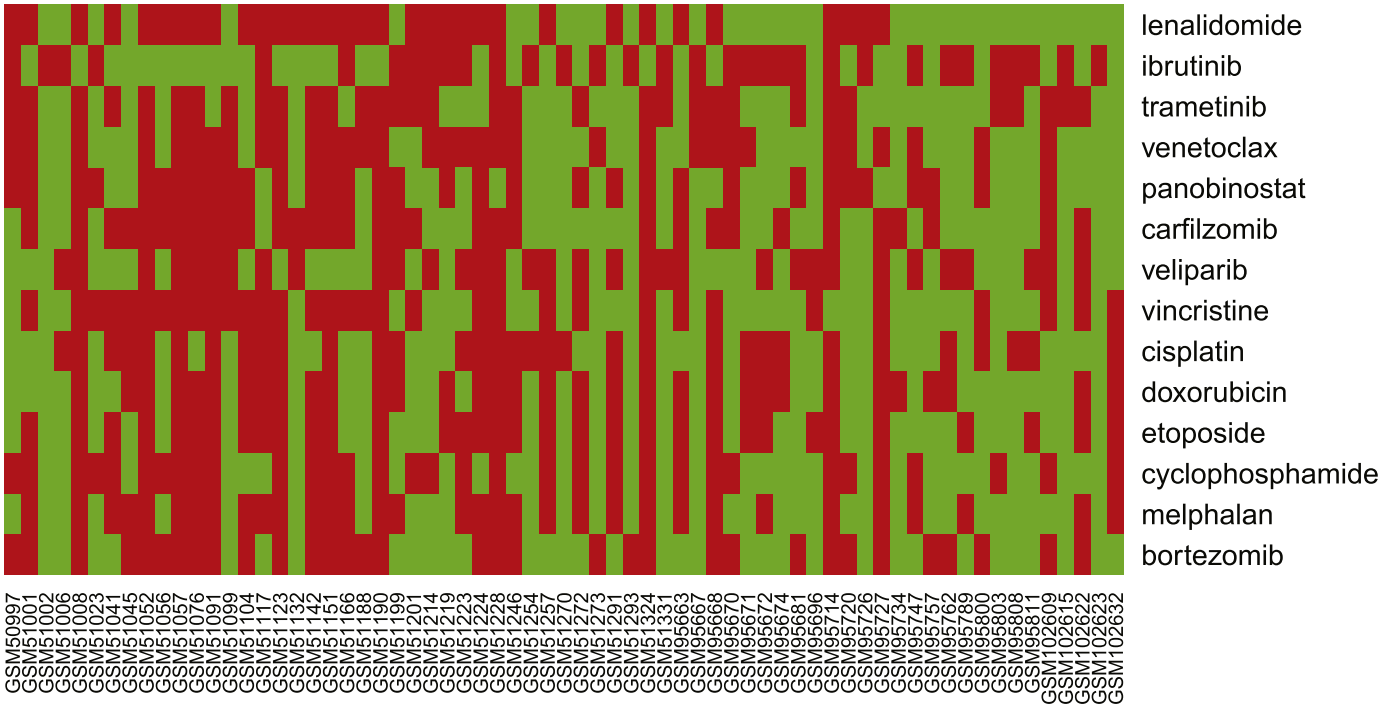


Fig. 4. Heat map of drug sensitivity by the DRP score for high-risk patients by GEP70.
The figure portrays drug sensitivity by the DRP score among 67 high-risk patients treated in TT2and TT3A. The individual high-risk patients are presented on the X-axis. The Y-axis shows drug tested by the DRP method used in B-cell diseases including myeloma. Green signals are myeloma with predicted sensitivity to drugs by the DRP score. Red signals are myeloma with predicted insensitivity to drugs by the DRP score. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

resistant cell lines and primary malignant plasma cells has shown increased tyrosine kinase activity in patients with relapsed disease and preliminary data from this study indicates that ibrutinib may re-sensitize the myeloma cells to bortezomib by interference of the NF-κB p65 pathway. We still need to study the difference of the DRP score in myeloma from patients at diagnosis as compared to myeloma from patients with relapsed disease.

5. Conclusion

In conclusion, our study confirms the results from others of the biological heterogeneity of myeloma. The DRP score predicted outcome of bortezomib and melphalan among high-risk patients by GEP70. We present a new DRP score that identifies myeloma patients with a high predicted sensitivity to drugs irrespective of classification of high-risk. To improve outcome for high-risk patients we suggest application of the DRP method in clinical trials on drugs used in treatment of other B-cell diseases. The perspective is to use the DRP score for all myeloma patients to find effective drugs and spare non-responders from toxicity.

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Authorship

AJV and SK conceived and designed the work, interpreted the results, drafted the manuscript and approved the final version. SHP, JBC, PBJ, PG interpreted the results, revised the manuscript and approved the final version. BB acquired data, interpreted the results, revised the manuscript and approved the final version.

Conflict of interests

A.J. Vangsted, S. Helm-Petersen, J. B. Cowland, and B. Barlogie have no conflict of interests. P. Gimsing receives compensation as a consultant for Oncology Venture and Medical Prognosis Institute, Hørsholm, Denmark, P.B. Jensen and S. Knudsen are employees if of Medical Prognosis Institute, Hørsholm, Denmark, which focuses on developing and commercializing microarray-based technology for use in clinical care for cancer. However, this does not alter their adherence to all the Gene policies on sharing data and materials.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gene.2017.10.071.

References

Barlogie, B., Tricot, G., Anaissie, E., Shaughnessy, J., Rasmussen, E., van Rhee, F., et al., 2006. Thalidomide and hematopoietic-cell transplantation for multiple myeloma. N. Engl. J. Med. 354 (10), 1021–1030.
Barlogie, B., Anaissie, E., van Rhee, F., Haessler, J., Hollmig, K., Pineda-Roman, M., et al., 2007. Incorporating bortezomib into upfront treatment for multiple myeloma: early results of total therapy 3. Br. J. Haematol. 138 (2), 176–185.
Barlogie, B., Mitchell, A., van Rhee, F., Epstein, J., Morgan, G.J., Crowley, J., 2014. Curing myeloma at last: defining criteria and providing the evidence. Blood 124 (20), 3043–3051.
Berglof, A., Hamasy, A., Meinke, S., Palma, M., Krstic, A., Mansson, R., et al., 2015. Targets for ibrutinib beyond B cell malignancies. Scand. J. Immunol. 82 (3), 208–217.
Bergsagel, P.L., Kuehl, W.M., 2005. Molecular pathogenesis and a consequent classification of multiple myeloma. J. Clin. Oncol.: Off. J. Am. Soc. Clin. Oncol. 23 (26), 6333–6338.
Chng, W.J., Dispenzieri, A., Chim, C.S., Fonseca, R., Goldschmidt, H., Lentzsch, S., et al.,

2014. IMWG consensus on risk stratification in multiple myeloma. Leukemia 28 (2), 269–277.
Decaux, O., Lode, L., Magrangeas, F., Charbonnel, C., Gouraud, W., Jezequel, P., 2008. Prediction of survival in multiple myeloma based on gene expression profiles reveals cell cycle and chromosomal instability signatures in high-risk patients and hyperdiploid signatures in low-risk patients: a study of the Intergroupe Francophone du Myelome. J. Clin. Oncol.: Off. J. Am. Soc. Clin. Oncol. 26 (29), 4798–4805.
Dickens, N.J., Walker, B.A., Leone, P.E., Johnson, D.C., Brito, J.L., Zeisig, A., et al., 2010. Homozygous deletion mapping in myeloma samples identifies genes and an expression signature relevant to pathogenesis and outcome. Clin. Cancer Res.: Off. J. Am. Assoc. Cancer Res. 16 (6), 1856–1864.
Heuck, C.J., Qu, P., van Rhee, F., Waheed, S., Usmani, S.Z., Epstein, J., et al., 2014. Five gene probes carry most of the discriminatory power of the 70-gene risk model in multiple myeloma. Leukemia 28 (12), 2410–2413.
Heuck, C.J., Jethava, Y., Khan, R., van Rhee, F., Zangari, M., Chavan, S., et al., 2016. Inhibiting MEK in MAPK pathway-activated myeloma. Leukemia 30 (4), 976–980.
Jagannath, S., Barlogie, B., Berenson, J., Siegel, D., Irwin, D., Richardson, P.G., et al., 2004. A phase 2 study of two doses of bortezomib in relapsed or refractory myeloma. Br. J. Haematol. 127 (2), 165–172.
Johnson, S.K., Heuck, C.J., Albino, A.P., Qu, P., Zhang, Q., Barlogie, B., et al., 2011. The use of molecular-based risk stratification and pharmacogenomics for outcome prediction and personalized therapeutic management of multiple myeloma. Int. J. Hematol. 94 (4), 321–333.
Kaiser, M.F., Walker, B.A., Hockley, S.L., Begum, D.B., Wardell, C.P., Gonzalez, D., et al., 2013. A TC classification-based predictor for multiple myeloma using multiplexed real-time quantitative PCR. Leukemia 27 (8), 1754–1757.
Knudsen, S., Jensen, T., Hansen, A., Mazin, W., Lindemann, J., Kuter, I., et al., 2014. Development and validation of a gene expression score that predicts response to fulvestrant in breast cancer patients. PLoS One 9 (2), e87415.
Kuiper, R., Broyl, A., de Knegt, Y., van Vliet, M.H., van Beers, E.H., van der Holt, B., et al., 2012. A gene expression signature for high-risk multiple myeloma. Leukemia 26 (11), 2406–2413.
Mikhael, J.R., Dingli, D., Roy, V., Reeder, C.B., Buadi, F.K., Hayman, S.R., et al., 2013. Management of newly diagnosed symptomatic multiple myeloma: updated Mayo Stratification of Myeloma and Risk-Adapted Therapy (mSMART) consensus guidelines 2013. Mayo Clin. Proc. 88 (4), 360–376.
Moreau, P., Cavo, M., Sonneveld, P., Rosinol, L., Attal, M., Pezzi, A., et al., 2014. Combination of international scoring system 3, high lactate dehydrogenase, and t (4;14) and/or del(17p) identifies patients with multiple myeloma (MM) treated with front-line autologous stem-cell transplantation at high risk of early MM progression-related death. J. Clin. Oncol.: Off. J. Am. Soc. Clin. Oncol. 32 (20), 2173–2180.
Mulligan, G., Mitsiades, C., Bryant, B., Zhan, F., Chng, W.J., Roels, S., et al., 2007. Gene expression profiling and correlation with outcome in clinical trials of the proteasome inhibitor bortezomib. Blood 109 (8), 3177–3188.
Murray, M.Y., Zaitseva, L., Auger, M.J., Craig, J.I., MacEwan, D.J., Rushworth, S.A., et al., 2015. Ibrutinib inhibits BTK-driven NF-kappaB p65 activity to overcome bortezomib-resistance in multiple myeloma. Cell Cycle (Georgetown, Tex). 14 (14), 2367–2375.
Paiva, B., Puig, N., Cedena, M.T., de Jong, B.G., Ruiz, Y., Rapado, I., et al., 2017. Differentiation stage of myeloma plasma cells: biological and clinical significance. Leukemia 31 (2), 382–392.
Palumbo, A., Avet-Loiseau, H., Oliva, S., Lokhorst, H.M., Goldschmidt, H., Rosinol, L., et al., 2015. Revised international staging system for multiple myeloma: a report from international myeloma working group. J. Clin. Oncol.: Off. J. Am. Soc. Clin. Oncol. 33 (26), 2863–2869.
Richardson, P.G., Barlogie, B., Berenson, J., Singhal, S., Jagannath, S., Irwin, D., et al., 2003. A phase 2 study of bortezomib in relapsed, refractory myeloma. N. Engl. J. Med. 348 (26), 2609–2617.
Richardson, P.G., Sonneveld, P., Schuster, M.W., Irwin, D., Stadtmauer, E.A., Facon, T., et al., 2005. Bortezomib or high-dose dexamethasone for relapsed multiple myeloma. N. Engl. J. Med. 352 (24), 2487–2498.
Scheid, C., Sonneveld, P., Schmidt-Wolf, I.G., van der Holt, B., el Jarari, L., Bertsch, U., et al., 2014. Bortezomib before and after autologous stem cell transplantation overcomes the negative prognostic impact of renal impairment in newly diagnosed multiple myeloma: a subgroup analysis from the HOVON-65/GMMG-HD4 trial. Haematologica 99 (1), 148–154.
Shaughnessy Jr., J.D., Zhan, F., Burington, B.E., Huang, Y., Colla, S., Hanamura, I., et al., 2007. A validated gene expression model of high-risk multiple myeloma is defined by deregulated expression of genes mapping to chromosome 1. Blood 109 (6), 2276–2284.
Shaughnessy Jr., J.D., Qu, P., Usmani, S., Heuck, C.J., Zhang, Q., Zhou, Y., et al., 2011. Pharmacogenomics of bortezomib test-dosing identifies hyperexpression of proteasome genes, especially PSMD4, as novel high-risk feature in myeloma treated with total therapy 3. Blood 118 (13), 3512–3524.
Shoemaker, R.H., 2006. The NCI60 human tumour cell line anticancer drug screen. Nat. Rev. Cancer 6 (10), 813–823.
Sonneveld, P., Avet-Loiseau, H., Lonial, S., Usmani, S., Siegel, D., Anderson, K.C., et al., 2016. Treatment of multiple myeloma with high-risk cytogenetics: a consensus of the international myeloma working group. Blood 127 (24), 2955–2962.
Tacchetti, P., Terragna, C., Galli, M., Zamagni, E., Petrucci, M.T., Pezzi, A., et al., 2014. Bortezomib- and thalidomide-induced peripheral neuropathy in multiple myeloma: clinical and molecular analyses of a phase 3 study. Am. J. Hematol. 89 (12), 1085–1091.
Terragna, C., Remondini, D., Martello, M., Zamagni, E., Pantani, L., Patriarca, F., et al., 2016. The genetic and genomic background of multiple myeloma patients achieving complete response after induction therapy with bortezomib, thalidomide and dexamethasone (VTD). Oncotarget 7 (9), 9666–9679.
Tian, E., Sawyer, J.R., Heuck, C.J., Zhang, Q., van Rhee, F., Barlogie, B., et al., 2014. In multiple myeloma, 14q32 translocations are nonrandom chromosomal fusions driving high expression levels of the respective partner genes. Genes Chromosom. Cancer 53 (7), 549–557.