Gene xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Research paper

Drug response prediction in high-risk multiple myeloma

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ARTICLE INFO

Keywords:
Drug response prediction
High-risk
Multiple myeloma

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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http://dx.doi.org/10.1016/j.gene.2017.10.071
Received 18 July 2017; Received in revised form 30 September 2017; Accepted 25 October 2017

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.