MET dysregulation is a hallmark of aggressive disease in multiple myeloma patients

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Summary

Abnormal activation of MET/HGF (Hepatocyte Growth Factor) pathway has been described in several tumours and increased HGF plasmatic levels have been detected in patients with aggressive multiple myeloma (MM). *MET* and *HGF* mRNA expression was investigated in 105 samples of purified plasma cells derived from newly diagnosed MM patients treated with bortezomib-based induction therapy. Gene expression was compared with response to therapy and clinical outcome. *MET* gene copy number was also evaluated. *MET* mRNA expression was higher in $CD138^+$ than in $CD138^-$ cells (median 76·90 vs. 11·24; $P = 0·0009$). Low *MET* mRNA expression characterized patients with better response (complete response or very good partial response) compared to other patients (median 56·10 vs. 134·83; $P = 0·0006$). After a median follow-up of 50 months, patients with high *MET* mRNA expression displayed a worse progression-free survival (PFS; $P = 0·0029$) and overall survival (OS; $P = 0·0023$) compared to those with low *MET* mRNA levels. Patients with both high *MET* mRNA expression and high β2-microglobulin level (>5·5 mg/l) had further worse median PFS ($P < 0·0001$) and OS ($P < 0·0001$). Patients carrying 4 *MET* gene copies (8 out of 82, 9·8%) also had a short PFS. High *MET* mRNA expression identifies patients with dismal PFS and OS and the combination with high β2-microglobulin further characterizes patients with worse outcome.

Multiple myeloma (MM) is a haematological malignancy characterized by abnormal interactions between plasma cells (PCs) and the microenvironment. This aberrant cross-talk increases during disease progression, sustaining relentless cell growth and encouraging drug resistance (Hideshima *et al*, 2007). Proliferative and anti-apoptotic stimuli can occur

through cell-to-cell contact via adhesion proteins or can be mediated by soluble molecules. Several growth factors have been demonstrated to play a key role in sustaining myeloma cells growth and have been found to be elevated in the serum of MM patients (Podar *et al*, 2002). Among all these cytokines, Hepatocyte Growth Factor (HGF) is considered of cardinal importance because it is secreted by both bone marrow (BM) stromal cells and PCs. MET, the HGF receptor, is present on the PC membrane and favours both paracrine and autocrine stimulation (Wader *et al*, 2012). The interaction of HGF with MET is important under several physiological conditions including embryogenesis and tissue regeneration. Aberrant activation of the MET/HGF pathway determines uncontrolled cell growth, invasion and metastatization in several tumours (Danilkovitch-Miagkova & Zbar,2002).

MET is a tyrosine kinase receptor formed by two subunits (α and β) linked by disulphide bridges. The extracellular portion of MET is composed of one Sema domain, one plexin-semaphorin-integrin (PSI) domain and four immunoglobulin-plexin-transcription (IPT) domains while the intracellular portion includes the tyrosine kinase catalytic sites. The binding of HGF to MET determines dimerization of the receptor causing trans-phosphorylation of tyrosine kinase intracellular domains. This phenomenon activates several pathways, such as cell proliferation, differentiation, scattering and escape from apoptosis (Comoglio *et al*, 2008; Mahtouk *et al*, 2010). The oncogenic role of MET has been reported in several haematological malignancies and correlated with aggressive disease and poor outcome (Teofili *et al*, 2001; Tjin *et al*, 2006).

Multiple myeloma patients have higher levels of serum HGF compared to healthy subjects and high serum levels predict poor outcome (Börset *et al*, 1996). *In vitro* assays showed that exogenous stimulation of MM cell lines with HGF increases cell growth and reduces drug response (Holt *et al*, 2008). Moreover, MET can modulate the expression/activity of many growth factors (e.g. interleukin-6) and can be modulated by several cytokines and by trans-membrane molecules, such as the heparin sulfate proteoglycan CD138 (Derksen *et al*, 2002). The MET/HGF pathway also plays a fundamental role in modifying the bone metabolism, causing imbalance between bone formation and bone resorption and high levels of HGF and MET expression correlate with the presence of lytic bone lesions (Kristensen *et al*, 2013). Indeed, HGF stimulates osteoclast activity and inhibits bone morphogenetic protein (BMP)-induced osteoblastogenesis (Standal *et al*,2007).

All these data point to the importance of the MET/HGF pathway in sustaining myeloma growth, osteoblastogenesis deregulation and drug resistance. A quantitative determination of MET expression in MM PCs and a correlation with clinical outcome has never been deeply investigated. We evaluated whether *MET* mRNA expression correlates with other biological parameters, whether it can predict response and whether it can stratify patients with different clinical outcome. Finally, we investigated the occurrence of high *MET* gene copy number in MM PCs and its potential correlation with clinical outcome.

Methods

Patients

Biological samples were obtained from 105 patients with previously untreated MM enrolled in two clinical trials: the phase II PAD-MEL100-LP-L study and the phase III VMP *versus* VMPT-VT study. The PAD-MEL100-LP-L treatment schedule included: four 21-day PAD cycles as induction [bortezomib 1·3 mg/m^2 on days 1, 4, 8, and 11, pegylated liposomal doxorubicin 30 mg/m^2 on day 4 and dexamethasone (40 mg/d; cycle 1: days 1–4, 8–11, and 15–18; cycles 2–4: days 1–4)], and tandem melphalan at the dose of 100 mg/m^2 (MEL100) followed by stem-cell reinfusion. Four cycles of lenalidomide plus prednisone (LP) as consolidation therapy and lenalidomide alone (L) as maintenance were administered until relapse (Palumbo *et al*, 2010a). The VMP *versus* VMPT-VT phase III clinical trial compared nine cycles of bortezomib-melphalan-prednisone *versus* nine cycles of bortezomib-melphalan-prednisone-thalidomide and maintenance with bortezomib-thalidomide in elderly MM patients (Palumbo *et al*, 2010b). In the present study we analysed samples derived from patients treated with nine cycles of VMP (oral melphalan 9 mg/m^2 on days 1–4; oral

prednisone 60 mg/m^2 on days 1–4; intravenous bortezomib 1·3 mg/m^2 on days 1, 4, 8, 11, 22, 25, 29, and 32 during cycles 1–4 and on days 1, 8, 22, and 29 during cycles 5–9).

The following clinical and biological variables were recorded for enrolled patients: date of diagnosis, age at diagnosis, sex, haemoglobin level (g/l), serum creatinine level (µmol/l), serum albumin level (g/l), serum β2-microglobulin level (mg/l), immunoglobulin isotype, and PC infiltration detected on BM biopsy. On purified PCs obtained from BM aspiration, fluorescence *in situ* hybridization (FISH) analysis was performed to investigate the following chromosomal abnormalities: deletion of chromosome 13 (del13q14), deletion of chromosome 17 (del17p13), t(4;14), t(11;14) and t(14;16). After the beginning of the treatment, the following information was recorded: response to therapy, date of relapse, date of last follow-up, date of death, cause of death. All patients provided written informed consent in accordance with local institutional review board requirements and with the declaration of Helsinki.

Biological samples

At the time of diagnosis, BM was collected in our laboratory and PCs were purified using anti-CD138$^-$ coated magnetic MicroBeads and AutoMACS Pro Separator (Miltenyi Biotech GmbH, Bergisch Gladbach, Germany) following the manufacturer's specifications. Briefly, red cells were lysed using ammonium chloride buffer for 15 min at room temperature. Once filtered with 50 µm filter cups and centrifuged at 300 *g* for 10 min, the pellets were resuspended in 80 µl of AutoMACS Running Buffer per 2 × 10^7 total cells. Then 20 µl of CD138 MicroBeads per 2 × 10^7 cells were added. After incubation for 15 min at 4°C, cells were finally resuspended in 1 ml of Running Buffer. Magnetic separation was performed using the AutoMACS Separator (Miltenyi Biotech GmbH) with the appropriate program to collect the positive cell fraction. CD138 purity was evaluated by flow cytometry using anti-CD138 antibody (Becton Dickinson, Franklin Lakes, NJ, USA). Only samples with at least 90% of purified PCs were stored at −80°C together with the CD138$^-$ fraction of BM aspirate.

Real time polymerase chain reaction (PCR)

RNA was extracted from frozen pellet using the DNA/RNA Purification Kit (Norgen, Thorold, Canada) following the manufacturer's instructions. Complementary DNA was produced using High capacity cDNA RT Kit (Applied Biosytems, Foster Ciy, CA). Quantitative PCR (qPCR) was performed using an ABI Prism 7900 HT (Applied Biosystems) with a relative quantification based on the ΔΔCt approach. The JUM2 cell line was used as mathematical calibrator and *GUSB* as housekeeping gene. For optimal qPCR performances, the amplicon size of genes was reduced to <80 bp and MET and HGF expression was analysed by the Hs00179845 and Hs00900070 assays, respectively. All mRNA determinations were done in duplicate.

FISH analyses

FISH analyses were performed on purified CD138$^+$ cells obtained from BM at diagnosis following standard procedure as described elsewhere (Ross *et al*, 2012). At least 100 cells were scored for each abnormality. If 10% or more cells presented a deletion, or 15% or more cells carried a translocation, the sample was considered positive for that chromosomal abnormality. Based on published data (Munshi *et al*, 2011), patients were divided in two groups according to their FISH profile. Patients were included in high risk FISH group if they carried at least one of the following chromosomal alterations: del17p13, t(4;14), t(14;16). The standard risk group included patients for whom no chromosomal abnormality or t(11;14) or del13q14 were found (Munshi *et al*, 2011).

In addition to the common chromosomal alterations involved in MM prognosis, a specific investigation of *MET* gene copy number was performed. Slides for interphase FISH were prepared by employing fixed PC according to standard methods. A few drops of fixed cell suspension were applied onto the slides, which, after being air-dried, were washed with 2 × Standard Saline Citrate (SSC), pH 7·0, for 2 min. Slides were then dehydrated in an ethanol series (70, 85 and 100%), each for 2 min at room temperature. A commercially available*MET* amplification probe (Cytocell Ltd, Cambridge, UK) was employed for determining *MET* gain. The dual-color Aquarius probe consists of a 299 kb red probe spanning the *MET* gene (7q31) and a green centromeric probe for chromosome 7 provided as a control. Briefly, after pre-warming the probe and sample slides on a 37°C hotplate, 10 µl of probe were spotted on slides and coverslip were applied and

sealed with rubber cement. Sample and probe were simultaneously denatured on a 75°C hotplate for 5 min, then the hybridization was performed in a humid lightproof container at 37°C for 4–5 h. Post-hybridization washes were executed in 0·4 × SSC, pH 7·0, at 72°C for 2 min and then in 2 × SSC 0·05% Tween-20, pH 7·0, at room temperature for 30 s. Nuclei were identified by 4',6-diamidino-2-phenylindole (DAPI) staining and the hybridization signals of 100 cells for each patient were evaluated under a fluorescence microscope (Olympus BXM41; Olympus, Shinjuku, Tokyo, Japan). The ratio between *MET* gene and the chromosome 7 centromere (*MET*/CEP7 ratio) was used to define*MET* status: the *MET* gene copy number was considered abnormal if the *MET*/CEP7 ratio was ≥1·5 in at least 20% of cells. Samples with gain of *MET* signal were subsequently divided in those with a ratio of 1·5 and those with ratio >1·5 in order to differentiate samples according to their grade of amplification.

Statistical analysis

Differences in *MET* mRNA expression between groups were calculated using Wilcoxon rank-sum test and Kruskal-Wallis test. Correlations were assessed through Spearman coefficient. The relationships between β2-microglobulin - *MET* mRNA and *HGF* mRNA - *MET* mRNA were further investigated via linear regression models treating *MET* as a restricted cubic spline to allow for a nonlinear effect on the dependent variable (Hastie & Tibshirani, 1990). Treatment response was assessed using the standard International Myeloma Working Group Uniform Response Criteria (Durie *et al*, 2006). Progression-free survival (PFS) was defined as time from enrollment until the date of progression, relapse or death from any cause. Overall survival (OS) was defined as time from enrollment until the date of death or the date the patient was last known to be alive. PFS and OS were calculated using the Kaplan-Meier methods and comparison between groups were analysed using the log-rank test. Multivariate Cox models for OS and PFS were fitted and the decision regarding which covariate to include was made to achieve a reasonable trade-off between sample size/number of events and clinical relevance. To assess the discriminatory ability of each Cox model, we calculated the c-index: a value of 0·5 refers to no ability while a value of one means that the model is able to perfectly separate events from non-events.

Results

Patients characteristics

The main clinical and biological characteristics of the newly diagnosed MM patients are reported in Table 1. Median age was 70 years with 15 patients (14%) older than 75 years. According to FISH analysis, del13q14 was reported in 60/103 (58%) patients, del17p13 in 18/103 (17%) patients, t(11;14) in 15/98 (15%) patients, t(4;14) in 20/103 (19%) patients and t(14;16) in 3/98 (3%) patients. MET was not associated with any baseline characteristics.

Table 1. Baseline patient characteristics.

	All patients (*n* = 105)
VMP	52
PAD-MEL100-LP-L	53
Sex (female/male)	48/57
Median age, years, median (range)	70 (46–86)
Myeloma protein class	
IgG	51/105
IgA	34/105
Bence-Jones	20/105
Albumin, g/l, median (range)	3·8 (1·6–5·7)
β2-microglobulin, mg/l, median (range)	3·8 (0·7–11·0)
Haemoglobin, g/l, median (range)	105 (73–164)
Creatinine, μmol/l, median (range)	88 (52–220)
ISS stage	
I	33/94
II	43/94
III	18/94
FISH profile	
High risk	35/103
Standard risk	68/103
MET/CEP ratio = 1·5	22/82
MET/CEP ratio = 2	8/82

VMP, bortezomib, melphalan, prednisone; PAD, bortezomib, doxorubicin, dexamethasone; MEL100, Melphalan 100 mg/m^2; L, lenalidomide; P, prednisone; ISS, International Staging System; FISH, fluorescence *in situ* hybridization.

MET and HGF mRNA expression in MM patients

MET mRNA expression was found to be higher in CD138$^+$ cells than in CD138$^-$ fractions (median 76·90, range 0·81–586·10 vs. 11·24, range 0·1–86·22, respectively; *P* = 0·0009) (Fig 1A). Similarly, *HGF* mRNA expression was higher in CD138$^+$ cells than in the CD138$^-$population (median 2·07, range 0·1–65·34 vs. 0·49, range 0·11–3·22, respectively; *P* = 0·03).

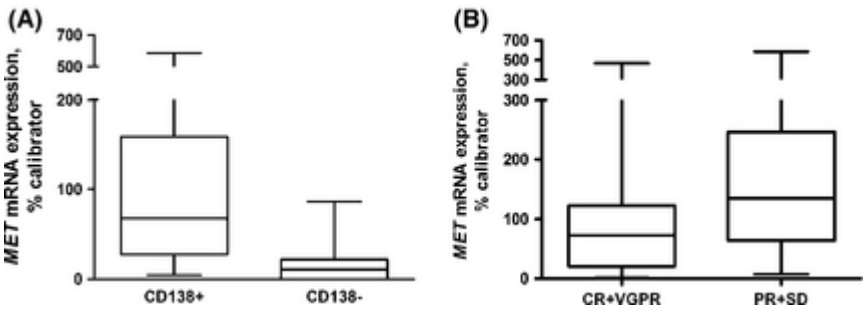

Figure 1. *MET* mRNA expression in different cell groups. (A) Tumour cells compared to surrounding cells: *MET* mRNA expression was evaluated in 20 matched CD138$^+$ purified cells and CD138$^-$ fraction obtained from bone marrow of multiple myeloma patients. *MET* expression levels were significantly higher in CD138$^+$ plasma cells compared to CD138$^-$ cells fraction. (B) *MET* mRNA expression according to response to therapy. *MET*mRNA levels were lower in patients with better response. Median mRNA expression was 56·10 in patients achieving at least a VGPR (*n* = 70) and 134·83 in those achieving PR or less (*n* = 35) (*P* = 0·0006).CR, complete response; VGPR, very good partial response; PR, partial response; SD, stable disease.

MET mRNA expression and response to therapy

Low *MET* mRNA expression in PCs characterized patients achieving a better response. At baseline, patients achieving a complete response (CR) had a median *MET* mRNA expression of 43·24 (range 4·03–466·26), those achieving a very good partial response (VGPR) a median value of 67·54 (0·81–416·51), those achieving partial response (PR) a median of 123·11 (7·44–517·35) and patients with stable disease (SD) a median of 170·66 (13·44–586·10). When patients were merged in two groups with distinct clinical outcome, those obtaining at least a VGPR (n = 70) had a median *MET* mRNA expression of 56·10 (range 0·81–466·26) whereas those achieving PR or SD (n = 35) exhibited a median value of 134·83 (range 7·44–586·10; P = 0·0006) (Fig 1B). No correlation was found between *HGF* mRNA levels and response to therapy (P = 0·64).

MET and HGF mRNA expression and clinical outcome

Multiple myeloma patients were divided in two groups according only to *MET* mRNA levels in purified PCs. Patients with low *MET* mRNA expression had a four-year PFS of 39% compared with the 20% of patients with a high *MET* value (Fig 2A; P = 0·0029). Four-year OS was significantly reduced in the high *MET* expression group (51%) compared with low *MET* level patients (77%) (Fig 2B; P = 0·0023). High *MET*mRNA expression identified patients with worse outcome enrolled in both trials. No differences were observed in PFS (P = 0·25) and OS (P = 0·36) with regard to *HGF* mRNA expression.

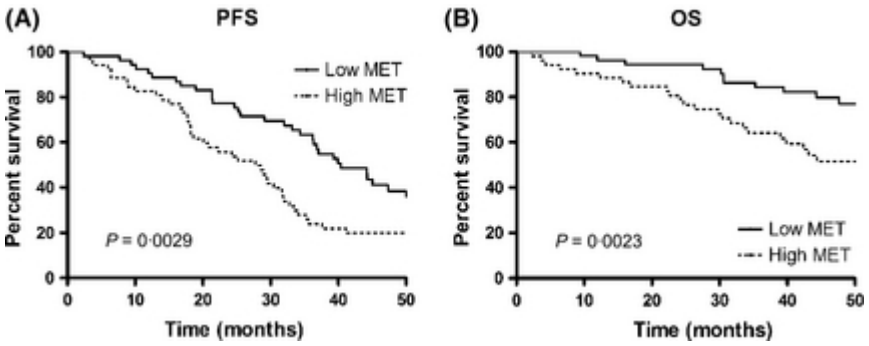

Figure 2. Clinical outcome according to *MET* mRNA value. (A) Progression-free survival (PFS) and (B) overall survival (OS) in 105 multiple myeloma patients according to *MET* mRNA expression alone. Low MET indicates patients with *MET* mRNA expression below the cut-off value, high MET those with *MET* expression above the cut-off value. Median *MET* mRNA value was used as cut-off. Patients with high *MET* mRNA had a four-year PFS of 20% compared to 39% in patients with low *MET* expression (P = 0·0029). Similarly, patients with high *MET* mRNA levels displayed a four-year OS of 51% compared to 77% for patients with low *MET* (P = 0·0023). Survival curves are reported until median follow-up time.

MET gene copy number and clinical outcome

Increased *MET* gene copy number was detected in 30/82 (37%) MM patients. Twenty-two patients carried three copies of *MET* (*MET*/CEP ratio 1·5) and eight patients harboured four copies (*MET*/CEP ratio 2) in more than 20% of analysed cells (Fig 3A). No deletions of the *MET*gene were identified in the analysed samples. No differences were detected in median *MET* mRNA values according to *MET* gene copy number: patients with normal *MET* gene copy number had an average *MET* mRNA value of 122·85 (range 1·08–516·51), patients carrying three *MET* copies had an average value of 109·52 (range 7·16–517·35) and those with four *MET* copies had a value of 116·38 (range 12·51–205·79) (P = 0·32). When *MET* gene copy number was considered alone, a lower PFS was observed in patients carrying four copies of the *MET* gene (n = 8; *MET*/CEP ratio >1·5) in comparison to those carrying two or three copies (n = 74; *MET*/CEP ratio ≤1·5). Median PFS of patients with *MET*/CEP ratio ≤1·5 was 32 months while that of patients with *MET*/CEP ratio >1·5 was 21 months (P = 0·03, Fig 3B). No statistical differences were observed in the OS curve (P = 0·63).

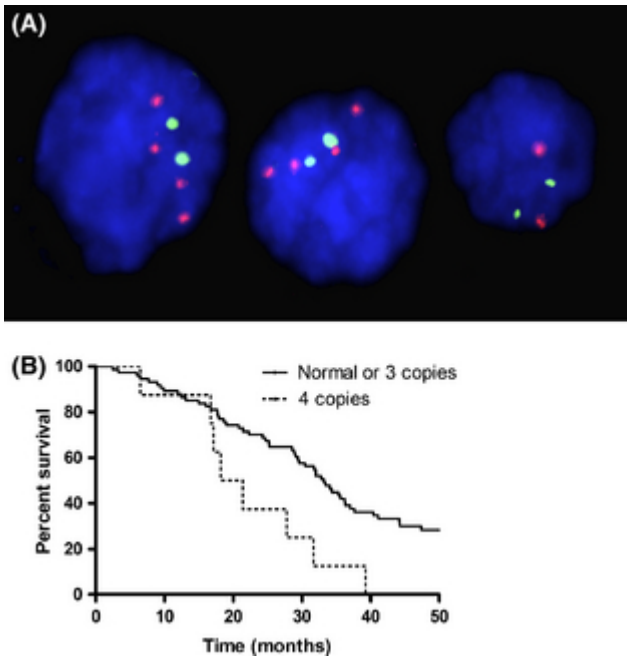

Figure 3. *MET* gene amplification using FISH probe. (A) Representative fluorescence *in situ* hybridization (FISH) analysis evaluating *MET* copy number: *MET* gene amplification (darker dots) is observed in two cells in the left and middle fields of the image while a normal ratio is observed in cell in the right field. Chromosome 7 centromeres (CEPs) are detected as green dots. (B) Progression-free survival (PFS) was calculated according to *MET*copy number alone: 74 patients with *MET*/CEP ratio of ≤1·5 displayed a median PFS of 32 months; eight patients with *MET*/CEP ratio of 2 had a median PFS of 21 months (*P* = 0·03). Survival curves are reported until median follow-up time.

Correlation of MET mRNA expression with other biological parameters

No relevant correlations were found between *MET* mRNA expression levels and other biological parameters, such as β2-microglubulin (Spearman's rho = −0·03, *P* = 0·77) and *HGF* mRNA (Spearman's rho = 0·21, *P* = 0·03). Linear regression model results did not show any effect, either linear or nonlinear, of *MET* mRNA on β2-microglobulin values (Fig 4). Differences in *MET* mRNA median values by *MET* copy number (Kruskal Wallis *P* = 0·60) and FISH risk (Wilcoxon *P* = 0·10) were not significant.

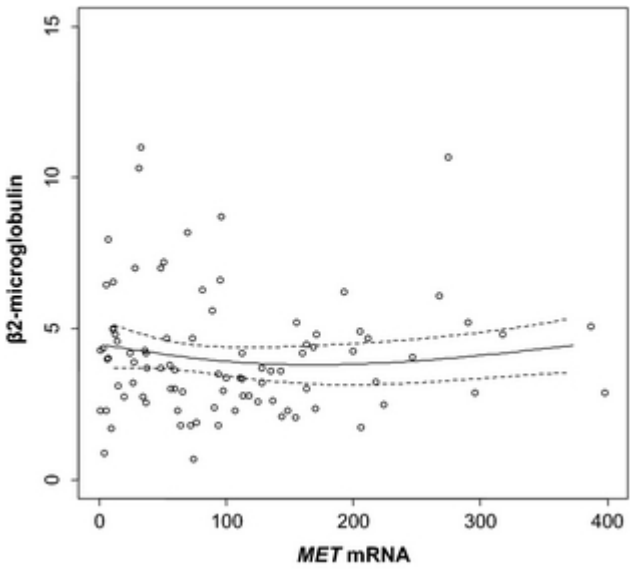

Figure 4. *MET* mRNA and β2-microglobulin: Crude effect of *MET* mRNA value, treated as a restricted cubic spline, on β2-microglobulin. Dotted lines indicate the 95% confidence bands.

In multivariate analysis including treatment protocol, FISH risk and β2-microglubulin, *MET* mRNA expression resulted as an independent prognostic factor of worse PFS and OS (Table 2). For the PFS models, with and without *MET* mRNA as predictor, the log-likelihood was −136·5 and −140·8, respectively, leading to a Likelihood Ratio Test (LRT) statistic of 8·6 (*P*-value = 0·003). For the OS models, with and without *MET* mRNA as predictor, the log-likelihood was −246·6 and −249·6, respectively, leading to a LRT statistic of 6·0 (*P*-value = 0·01). In both cases, models with *MET* mRNA as predictor performed better than models without it. After a median follow up of 50 months, a 20-unit increase in *MET* mRNA levels was associated with worse PFS [adjusted Hazard Ratio (HR) = 1·05; *P* = 0·001] and OS (adjusted HR = 1·09; *P* = 0·001). Nevertheless, the discriminatory ability of these survival models was poor, with a c-index equal to 0·69 and 0·64, for OS and PFS, respectively. Furthermore, the analysis could not disentangle the effect of treatment protocol and β2-microglubulin because the median β2-microglubulin level (4·7 mg/l) in the VMP study was significantly higher than in the PAD-MEL100-LP-L study (3·0 mg/l).

Table 2. Multivariate proportional hazard analysis on PFS and OS.

	PFS			OS		
	HR	95% CI	*P*	HR	95% CI	*P*
VMP *versus* PAD MEL100	1·97	1·08–3·66	0·028	1·07	0·49–2·31	0·864
FISH high *versus* standard risk	0·72	0·41–1·28	0·264	0·86	0·41–1·81	0·699
β2-microglobulin (3·5–5·5 mg/l) vs. ≤3·5 mg/l	1·42	0·76–2·63	0·268	2·00	0·84–4·71	0·115
β2-microglobulin >5·5 mg/l vs. ≤3·5 mg/l	1·45	0·66–3·19	0·354	4·17	1·60–10·81	0·003
MET mRNA (per 20-unit increase)	1·05	1·01–1·05	0·009	1·09	1·03–1·15	0·001

PFS, progression-free survival; OS, overall survival; HR, Hazard Ratio; CI, Confidence Interval; VMP, bortezomib, melphalan, prednisone; PAD, bortezomib, doxorubicin, dexamethasone; MEL100, Melphalan 100 mg/m^2; FISH, fluorescence *in situ* hybridization.

Patients with both high *MET* mRNA and high *MET* gene copy number (*n* = 15) were compared with the remaining (*n* = 67). The four-year PFS was 6% for patients with both high *MET* mRNA and *MET*/CEP ratio ≥1·5, compared to 31% in the remaining patients (*P* = 0·003; Fig 5A). Similarly, the four-year OS was 44% in patients with both abnormalities compared with 66% for all the others (*P* = 0·0023; Fig 5B).

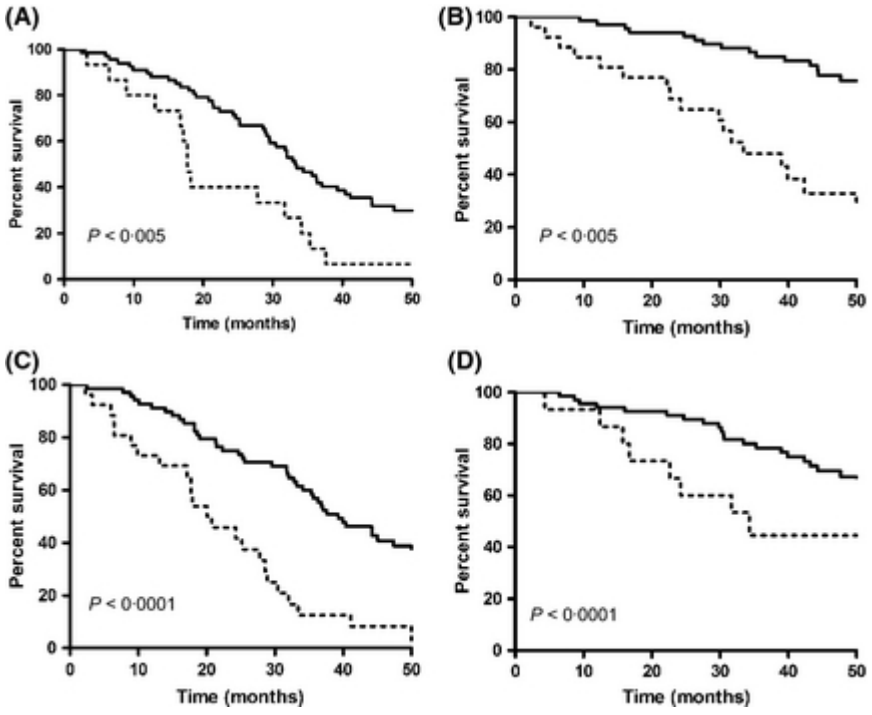

Figure 5. PFS and OS according to MET mRNA value and MET copy number or β2-microglobulin. Progression-free survival (PFS) and overall survival (OS) were calculated according to two biological parameters. Survival curves are reported until median follow-up time. (A, B) Patients with *MET* high risk status (concomitant high *MET* mRNA expression and *MET*/CEP ratio >1·5; *n* = 15; dotted line) were compared with the remaining patients (*n* = 67) continuous line). The four-year PFS was 6% for high risk *MET* patients and 31% for the remaining patients (*P* = 0·003). Four-year OS was 44% and 66%, respectively (*P* = 0·0023). (C, D) Patients with high *MET* mRNA expression and β2-microglobulin >5·5 mg/l (*n* = 26; dotted line) had a four-year PFS of 8% compared with 38% for the remaining patients (*n* = 68; continuous line) (*P* < 0·0001; Fig 5C). Similarly, the four-year OS was 32% and 76%, respectively (*P* < 0·0001; Fig 5D).

The combination of high *MET* mRNA expression and high β2-microglobulin level (>5·5 mg/l) identified patients with worse outcome (*n* = 26): the four-year PFS was 8% compared to 38% in all the others (*n* = 68) (*P* < 0·0001; Fig 5C) and the four-year OS was 32% and 76% respectively (*P* < 0·0001; Fig 5D).

Discussion

We report that *MET* mRNA expression identifies patient subgroups with different response to therapy and clinical outcome, independently of the already known risk factors. High *MET* mRNA expression characterizes patients with suboptimal response, short PFS and reduced OS. The combination of high *MET* mRNA expression and high β2-microglobulin levels has a further negative impact on outcome. *MET*gene copy number also characterized patients with dismal PFS. Several reports have demonstrated that the MET/HGF pathway is responsible for tumour growth and dissemination of malignant PCs (Trusolino *et al*, 2010). However, this is the first study providing quantitative analysis of *MET* and *HGF* mRNA expression and *MET* copy number in a large panel of samples derived from newly diagnosed MM patients for whom molecular, clinical and prognostic information is also available.

We first evaluated *MET* mRNA expression in BM PCs of MM patients and compared the results with the non-tumour fraction of BM aspiration. *MET* mRNA expression was superior in tumour cells, with a 7-fold higher median value compared to non-tumour cells. This is in line with the results obtained by Wader *et al* (2012) who used RT-PCR and confirms that MET expression is upregulated in MM PCs (Danilkovitch-Miagkova & Zbar, 2002).

We then investigated *MET* mRNA expression according to response to bortezomib-based induction therapy, based on the hypothesis that different levels of *MET* expression could predict drug sensitivity. We found that low *MET* mRNA values correlated with profound tumour reduction, whereas high MET expression predicted SD or poor response. This observation confirms that an altered MET/HGF pathway can be a hallmark of less responsive disease in patients receiving novel drug combinations with or without autologous stem cell transplantation.

The MET/HGF pathway is aberrantly activated in a huge number of epithelial cancers (Ke *et al*, 2007; Sawada *et al*, 2007) By stimulating multiple intracellular effectors, including the phosphatidylinositol-3-kinase/Akt axis, the focal adhesion kinase and the Ras/Raf/MEK/ERK cascade, the HGF/MET regulates cell proliferation, apoptosis, migration and differentiation of normal and neoplastic cells. *MET* mRNA expression has been reported to have a prognostic impact in solid tumours. Here we show that MM patients with high *MET* mRNA expression have shorter PFS and OS compared to those with low *MET* values. The prognostic role of *MET* mRNA is independent of other biological parameters that are commonly used to stratify MM patients (β2-microglobulin and FISH abnormalities). Despite the small number of patients included in this study, this finding seems to indicate that *MET* overexpression characterize those with dismal outcome. However, given that these estimates refer to crude comparisons, they should be carefuly interpreted.

Median *MET* mRNA values are similar regardless of the *MET* gene copy number. This unexpected finding may be due to the few patients carrying four *MET* copy number (*n* = 8). However, gain in *MET* copy number and high *MET* mRNA are individually associated with worst PFS, suggesting that these two mechanisms could independently exist in bad-prognosis MM patients. Further analyses on additional patients are required to clarify this issue.

MET can be activated by its ligand HGF; however, we did not find a strong correlation between *MET* and *HGF* mRNA expression. mRNA expression of *HGF* did not differentiate patients according to their response to therapy and their

clinical outcome. Although plasmatic HGF levels have been reported to have a predictive role in MM, the impact of HGF expression in MM seems to be similar to that observed in colon cancer, where only the expression of MET and its regulatory gene *MACC1* can identify groups of patients with different outcome (Stein *et al*, 2009; Galimi *et al*, 2011). Importantly, HGF is usually sequestered in its inactive form by stromal proteoglycans; therefore, the extent of HGF expression is not a direct function of its activity (Trusolino *et al*, 2010).

In addition to HGF stimulation, different mechanisms can sustain uncontrolled MET activation, including amplification of the *MET* gene, which causes protein overexpression and constitutive kinase activation (Cappuzzo *et al*, 2009). In several cancers, including non-small-cell lung carcinoma (NSCLC) and gastroesophageal adenocarcinoma, *MET* gene amplification is associated with aggressive disease (Graziano *et al*, 2011; Lennerz *et al*, 2011). We scrutinized *MET* gene copy number in the PCs of 82 MM patients and identified an abnormal *MET* copy number in 30 samples. Despite the small number of patients included in our series, we found that patients with four*MET* copies (*MET*/CEP ratio >1·5) had shorter PFS, while those carrying three *MET* copies (*MET*/CEP ratio 1·5) had a clinical outcome similar to those with a normal karyotype. Similar results were reported in a study evaluating the amplification of 1q21 (Hanamura *et al*,2006). In both situations, patients carrying four copies of the gene had poor prognosis (Hanamura *et al*, 2006). No data are available on the presence of *MET* mutations in MM patients. Notwithstanding the fact that *MET* mutations very rarely occur in solid cancers, analysing such molecular alterations in MM may deserve further investigation.

Different expression levels of *MET* mRNA should be taken into account to define the efficacy of current treatments for MM, and as an independent biomarker to predict prognosis. The identification of *MET* mRNA overexpression and increased *MET* copy number as a new prognostic marker in MM patients could be useful for stratification of patients who may benefit from the administration of MET inhibitors. Several compounds targeting MET have been recently developed, and many of them are now being tested in early clinical trials. Recently the results of a phase I trial using a selective oral inhibitor of MET (ARQ 197) have been reported (Yap *et al*, 2011). In patients with NSCLC receiving the epidermal growth factor receptor (EGFR) inhibitor, erlotinib, addition of the anti-MET antiboby METMab significantly increased PFS in patients with high MET expression (Spigel *et al*, 2011). Because MM is characterized by great heterogeneity, a personalized analysis of altered molecular pathway could be fundamental for successful future therapeutic intervention (Que *et al*, 2012). Targeting the MET/HGF pathway could also reduce the interaction of tumour PCs with the surrounding microenvironment, which may significantly increase treatment efficacy in resistant tumours often observed in the advanced stages of disease.

In conclusion, we demonstrate that MET overexpression in PCs of MM defines a sub-group of patients with low response to therapy and poor clinical outcome. The negative influence of MET overexpression is independent of other well-known prognostic factors such as β2-microglobulin level or FISH status. Alterations in *MET* gene copy number with a *MET*/CEP7 ratio of 2 also characterize patients with unfavourable outcome. Our findings confirm *in vitro* data on the role of MET/HGF in MM and strengthen the prospect of testing MET inhibitors in MM patients.

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Authors' contributions

Alberto Rocci, Manuela Gambella, Simona Aschero, Paola Omedé performed the research. Alberto Rocci, Ileana Baldi, Marco Ladetto, Mario Boccadoro, Antonio Palumbo designed the research study. Livio Trusolino, Federica Cavallo, Francesca Gay, Alessandra Larocca, Valeria Magarotto, Gianluca Isaia, Andrea Bertotti, Anna Marina Liberati, Lucio Catalano, Luca De Rosa, Pellegrino Musto, Roberto Vallone, Antonietta Falcone, Daniela Drandi, Paolo M. Camoglio provided essential data and materials for the study. Alberto Rocci, Manuela Gambella, Ileana Baldi analysed the data;

Alberto Rocci, Manuela Gambella wrote the manuscritp. All the authors had access to and approved the final version of the manuscript.

Conflict of interest

Alberto Rocci has received honoraria from Onyx; Federica Cavallo has received honoraria from Celgene, Janssen-Cilag, Onyx and served on the advisory committee for Celgene; Francesca Gay has received honoraria from Celgene and Janssen-Cilag, and served on the advisory committee for Celgene; Alessandra Larocca has received honoraria from Celgene and Janssen-Cilag; Pellegrino Musto has received honoraria from Celgene; Marco Ladetto has served on the speaker bureau for Celgene, Janssen-Cilag, Roche, Bayer, Amgen, Mundipharma and research support from Celgene, Pfizer, Mundipharma, Roche, and financial support from Amgen, Roche and Italfarmaco; Mario Boccadoro has received research funding from and served on the advisory board for Celgene and Janssen-Cilag; Antonio Palumbo has received honoraria from Celgene, Janssen-Cilag, Merck and Amgen, and served on the advisory board for Celgene and Janssen-Cilag. All the other authors declare no competing conflicts of interest.

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