SUPPLEMENTARY MATERIALS AND METHODS

Quantification of the synergism of HIF-1α-siRNA or panobinostat with bortezomib and lenalidomide.

MMECs were treated with different concentrations of HIF-1α-siRNA and panobinostat, bortezomib and lenalidomide singularly and in combination: for HIF-1α-siRNA 10, 25, and 50 nM; panobinostat 10, 50, and 100 nM; bortezomib 5, 10, and 20 nM; lenalidomide 0.5, 0.25, and 1.75 µM. The potency of the combination was quantified with the Calcusyn software (Biosoft, Ferguson, MO, USA) and a combination index (CI) <1.0 indicated synergism, = 1.0 additive effect, and >1.0 antagonistic effect.

Matrigel assay with the conditioned media (CM) of BM cells

BM cells were treated with panobinostat 100 nM for 72 h. The treatment was performed for the first 48 h in DMEM with 20% FBS, and for the last 24 h in serum-free DMEM. The supernatants were centrifuged (380×g for 10 min), and stored at -80°C as CM. MMECs were isolated from panobinostat pretreated or not BM cells and used for matrigel assay in the presence of their respective BMCM.

Two-dimensional gel electrophoresis (2-DE) and protein identification.

Isoelectric focusing (IEF) as the first dimension on Protean cell apparatus with ready-made immobilized pH gradient (IPG, 11-cm, pH 3-10) strips and the Mini Protean system as second dimension (both from Bio-Rad, Hercules, CA, USA) were used. Each sample (300 μg) of untreated MMECs (control) or HIF-1α-siRNA- or panobinostat-treated MMECs was applied onto the IPG gel by in gel rehydration for 20 h. The IPG strips were then equilibrated in equilibration buffer (Bio-Rad) type I (added with dithiotreitol) and type II (added with iodoacetamide) both for 10 min. The second dimension was performed on a 4-12% gradient SDS-PAGE, and subjected to the Coomassie
staining method. Gels were digitized by the Gel-Logic1500 System (Eastman Kodak Co.), and
analyzed on PD-Quest software (Bio-Rad). After subtraction of the background values, each spot
was normalized according to the total quantity of the valid spots. Protein expression was quantified
by the spot volume; a $P \leq 0.05$ by the two-tailed Student’s $t$-test for equal or unequal variance
defined the differential expression. Spots excised from the SDS gels, destained, dried in acetonitrile
(ACN) and digested with trypsin were analysed by mass spectrometry (MS) with the MALDI
microMX (Micromass, Manchester, UK) equipped with a delayed extraction unit. The sample was
loaded onto MALDI target using 2 μL of the tryptic digest mixed 1:1 with an α-cyano-4-
hydroxycinnamic acid solution (10 mg/mL in 40% ACN, 0.1 % v/v trifluoroacetic acid). Peak list
was generated with Proteinlynx data using external calibration with lock mass of
adrenocorticotrophic hormone (2465.1989 Da), background subtraction combining all scans, and
deisotoping with a 5% threshold. The peak list was analyzed by PROWL using the database of
National Center for Biotechnology Information (NCBI).

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure S1

Caption: Lack of HIF-1α expression by BM macrophages in normoxia condition. Western blot
for HIF-1α (β-actin as loading control) shows lack of HIF-1α expression by BM macrophages from
MGUS and MM patients at different disease phases. Cells treated with deferoxamine (DFO, 380
μM for 6 h), an hypoxia-mimicking agent, were positive for the HIF-1α and used as positive
control. Data are representative of macrophages from MGUS patients (n=3), from MM patients at
remission (n=3), at diagnosis (n=3), at relapsed (n=3) and on refractory phase (n=3).

Supplementary Figure S2

Caption: HIF-1α protein correlates with VEGFR-2, FGFR-2 and HGF. A) Real-time RT-PCR
(normalized to β-actin) for VEGFR-2, FGFR-2, and HGF; B) Western blot measured by optical
density (OD) for VEGFR-2, and FGFR-2; C) ELISA for HGF of representative MMECs at different disease phases and MGECs. Data are expressed as means ± SD of MMECs from patients at diagnosis ($n=12$), in remission ($n=14$), relapsed ($n=10$), and refractory ($n=11$), and of MGECs ($n=20$). The statistical analysis was performed by comparing the levels of VEGFR-2, FGFR-2 and HGF mRNA and proteins in the groups of relapsed and refractory MM patients with those of patients at diagnosis or in remission and of patients with MGUS. *$P<0.05$; and **$P<0.01$ by Wilcoxon signed-rank test.

**Supplementary Figure S3**

Caption: HIF-1α protein negative MMECs are sensitive to the antiangiogenic effect of bortezomib and lenalidomide. HIF-1α protein negative MMECs of relapsed/refractory patients treated with bortezomib or lenalidomide were tested for angiogenesis on Matrigel. Measurement of vessel length and empty areas by the EVOS image software. Magnification= ×200; Scale bar: 50 µm. The statistical analysis was performed by comparing MMECs treated with bortezomib or lenalidomide with control. **$P<0.01$ by Wilcoxon signed-rank test.

**Supplementary Figure S4**

Caption: Panobinostat effect on MMECs in the presence of BM cells and proteomic analysis of MMECs upon treatment with HIF-1α siRNA or panobinostat. A) Angiogenesis of MMECs +/- the CM of the total BM cells (BMCM). B) Direct (left and middle panel) effects of panobinostat (Pan) on angiogenesis of MMECs pretreated with the drug and plated on matrigel, respectively in DMEM or in the untreated BMCM. Indirect effect of panobinostat on angiogenesis of MMECs plated on matrigel in the BMCM from BM cells pretreated with the drug (right panel). Measurement of vessel length and empty areas by the EVOS image software. Magnification= ×200; Scale bar: 50 µm. The pictures are representative of experiments with MMECs expressing HIF-1α and exposed to the untreated or Pan pretreated BMCM obtained from five different patients in the
refractory phase. C) Coomassie-stained 2-DE gels of whole protein lysates of MMECs treated with panobinostat and HIF-1α-siRNA. Red and green squares indicate upregulated or downregulated proteins, respectively. D) Enlarged panels of 2-DE gel maps show differentially expressed proteins in HIF-1α-siRNA- and panobinostat-treated MMECs vs the control. Two-DE gel maps are representative of experiments conducted on 5 relapsed/refractory patients.

Supplementary Figure S5

Caption: **HIF-1α protein does not correlate with AKT, and VHL.** HIF-1α, AKT, phospho (p) AKT and VHL (β-actin as loading control) measured as optical density (OD) on Western blot in MMECs from patients at different disease phases and in MGECs. The statistical analysis was performed by comparing the AKT, pAKT and VHL levels of the groups of relapsed or refractory MM patients with those of patients at diagnosis or in remission and of patients with MGUS. *P<0.05 and **P<0.01 by Wilcoxon signed-rank test.