SUPPLEMENTARY APPENDIX

GRP78-directed immunotherapy in relapsed or refractory multiple myeloma - results from a phase 1 trial with the monoclonal immunoglobulin M antibody PAT-SM6

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Supplementary

Supplementary Table S1: Summary of clinical assessment findings

Patient Number	Dose Group (mg/kg)	Nr of prior therapy lines	Refractory to novel agents used in prior therapy	Response to PAT-SM6 (IMWG Criteria)	Time to next therapy (since last dosing with PAT-SM6)	Salvage therapy regimen	Response to salvage regimen
#1	0.3	4	BZT	PD	28 days	BZT, Len, Cyclo, Dex	PR
#2	0.3	5	no	PD	9 days	Benda, Pred, Thal	PR
#3	0.3	3	Len	PD	75 days	HD-Treo with ASCT	SD
#4	1	5	Poma, BZT	SD	8 days	Benda, BZT	PR
#5	1	4	BZT, Thal	PD	41 days	Benda, BZT, Dex	SD
#6	1	4	no	PD	50 days	BZT, Mel	SD
#7	3	4	no	SD	138 days	Carf, Cyclo, Dex,	PR
#8	3	6	Carf, Len	PD	43 days	Poma, Dex	PD
#9	3	7	Len	PD	12 days	Carf, Cyclo, Dex	PR
#10	6	6	no	SD	30 days	Carf, Len, Dex	VGPR
#11	6	4	Len, Thal	SD	157 days	Poma, Dex	PR
#12	6	2	no	PD	30 days	BZT, Len, Doxo, Dex	PR

Abbreviations: BZT, Bortezomib; Len, Lenalidomide; Poma, Pomalidomide; Thal, Thalidomide; Carf, Carfilzomib; Benda, Bendamustine; Mel, Melphalan; Dex, Dexamethasone; HD-Treo with ASCT, High dose Treosulfan with autologous stem cell transplantation; Cyclo, Cyclophosphamide; Pred, Prednisone; Doxo, Doxorubicin;
VGPR, very good partial response; PR, partial response; SD, stable disease; PD, progressive disease

Supplementary Methods: Immunmonitoring

20 ml of blood was collected from each patient: before the first administration of PAT-SM6 and at each post—administration time point (Days 0, 1, 2, 3, 7, 8, 9, 10). An additional sample was collected at the end of trial (approximately 36 days after the first administration of PAT-SM6). Peripheral blood mononucleated cells (PBMCs) were isolated using Biocoll separating solution (Biochrome) according to manufacturer's manual. The surface of PBMCs was stained in multiple colors for several immune system activation markers using fluorescent antibodies (BD Bioscience, Heidelberg, Germany) and analyzed by flow cytometry. For intracellular Foxp3 and Helios staining, PBMCs were previously permeabilized using the Permeabilization/Fixation kit (ebiosciences, Frankfurt, Germany). All data were analyzed using FlowJo analysis software (Tree Star, Ashland, Oregon). T cells were tested using following multi-color immunophenotyping reagent panel (Supplemental Table S2).

Supplementary Table S2: Immunmonitoring FACS panel

Subset								
Lymphocyte Panel	CD3 PerCP	CD4 FITC	CD8 V450	CD56 APC	CD20 PE	CD14 APCCy7		
T cell Subset	CD3 PerCP	CD4 APC	CD8 V450	CD45RA FITC	CD45R0 PE			
Memory Subset	CD4 PerCP	CD8 V450	CD62L APC	CD45R0 FITC	CD27 PE	CD28 PECy7		
Activity Subset	CD3 PerCP	CD4 V500	CD8 V450	CD25 FITC	CD69 APC	CD134 PE		
Treg Panel	CD4 PerCP	CD25 FITC	CD39 APC	CD127 PECy7	Foxp3 PE	Helios PacBlue		
NK/NKT-cell Panel	CD3 PerCP	CD56 V450	CD16 PECy7	CD6 FITC	NKGD2D APC	CD319 PE		
γ/δ T cell Panel	CD3 APC-H7	CD4 V500	CD8 PerCP	TCR FITC	CD45RA APC	CD45R0 PE	HLA DR V450	CD25 PECy7

Abbreviations: CD, cluster of differentiation; PerCP, Peridinin chlorophyll; APC, Allophycocyanin; FITC, Fluorescein isothiocyanate; PE, Phycoerythrin; PECy7, Phycoerythrin cyanine; Foxp3, forkhead box P3; PacBlue, Pacific Blue; APC-H7, Allophycocyanin – cyanine; HLA, Human leukocyte antigen; APCCy7, Allophycocyanin – cyanine; TCR, T cell receptor; Treg, regulatory T cells; NK, natural killer cell; NKT, natural killer T cell

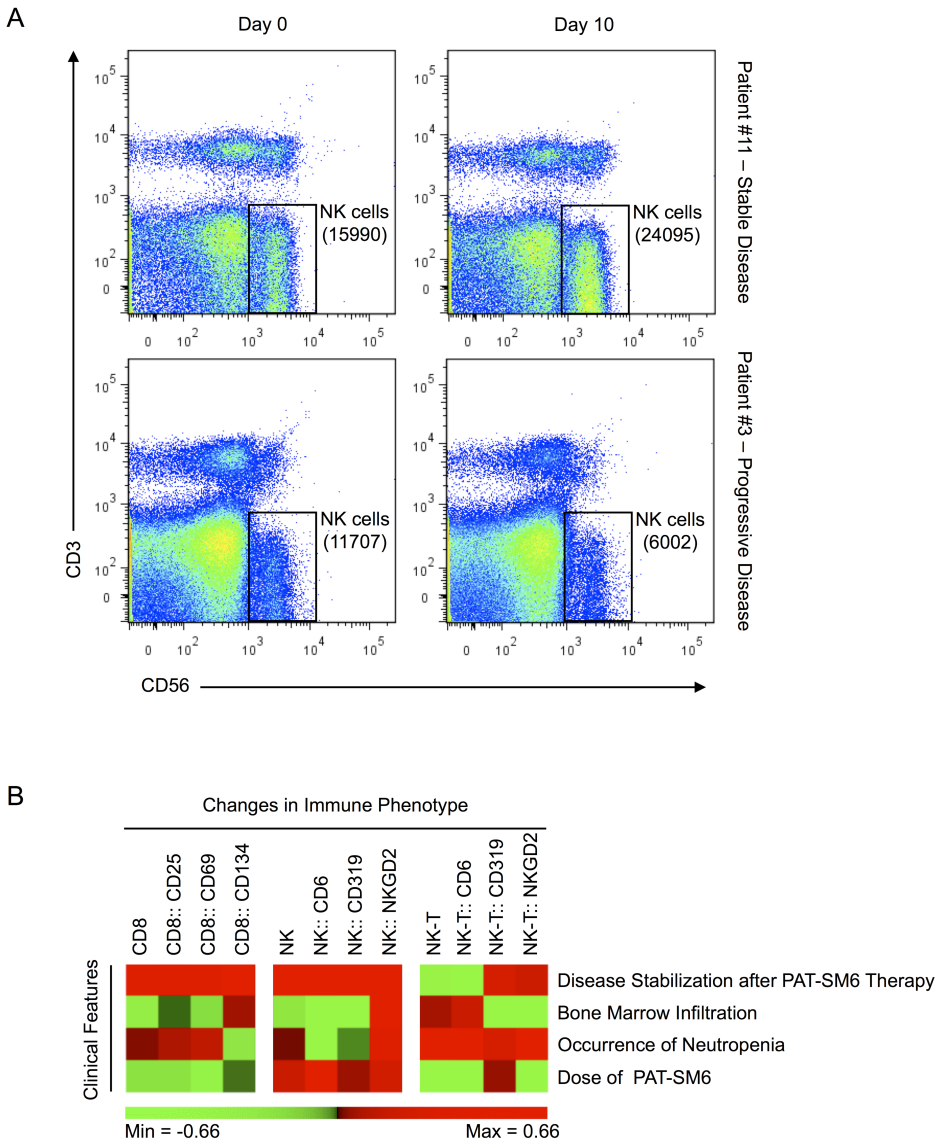
Supplementary Data: Immunmonitoring

Immune monitoring of all patients was conducted by measuring levels of various immune cell populations including the T cell subsets like memory and activated CD4 and CD8 cells, γ/δ T cells, NK/NKT and T regulatory (Treg) cells (see table S2). The levels of these cells in patient's blood at day 0 of the trial (before PAT-SM6 treatment) were compared with levels measured at day 10. Nearly all tested subsets consistently showed decreased levels in majority of patients, except for Treg cells.

The absolute values of levels of immune cells in PAT-SM6 treated patients were correlated with patients' response to PAT-SM6 as defined by IMWG (stable disease) and a representative sample is shown in Figure S1A. Patients showing stable disease after PAT-SM6 treatment showed an increase in CD8+ T cells and NK cells during the time of dosing. In contrast, the majority of patients who were progressing after PAT-SM6 treatment showed a decrease in CD8 and NK cells. In Figure S1A, FACS analysis (lymphocyte gated) of PBMC cells from a patient where PAT-SM6 treatment induced stabilization of MM disease, showed increased numbers of NK cells (Patient #11 upper panels). On the other hand, in patient #3 where MM disease progressed irrespective of PAT-SM6 treatment, the levels of NK cells decreased (lower panels).

Figure S1B shows the heat map with the correlation coefficients relating the changes in immune phenotype (levels of CD8, NK and NK-T cell markers with subsequent activation markers) to the clinical features. Positive correlation was observed between activity of CD8 and NK cells and subsequent activation markers and stabilization of MM disease. In contrast, levels of NK-T cells, the degree of MM bone marrow infiltration, the occurrence of neutropenia and the dosing of PAT-SM6 seems not to correlate with disease stabilization.

Supplementary Figure S1: Immunmonitoring



A, Flow cytometric analysis of PBMCs from MM patients before (day 0) and after (day 10) PAT-SM6 administration

Comparison between changes in the number of NK cells (CD56+/CD3-) in a patient with stable disease (upper panels) and a patient with progressive disease (lower panels) after PAT-SM6 treatment. In case of patient #11 (stable disease), the number of NK cells increased at the end of the treatment, in contrast with the case of patient #3 (progressive disease), where a decrease in the number of NK cells was observed. (Cells in the lymphocyte gate were analyzed)

B, Heat map visualization of correlation patterns between the changes in patients` immune phenotype and clinical behavior after PAT-SM6 administration

The change in activity, NK and NK-T markers was calculated subtracting the absolute values of each marker before PAT-SM6 treatment to the absolute values of the corresponding marker on day 10 post PAT-SM6 treatment. The correlation coefficient between the changes in the immune phenotype and various clinical features was calculated using the Pearson Correlation test, where 1 is total positive correlation, 0 is no correlation, and −1 is total negative correlation. Heat maps were generated using the PermutMatrix software.