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A GRP78-Directed Monoclonal Antibody Recaptures Response in Refractory Multiple Myeloma with Extramedullary Involvement

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Introduction

The development of drug resistance still represents the main obstacle of current multiple myeloma therapy. Whereas survival has continuously improved for newly diagnosed patients, the prognosis in the relapsed-refractory (RR) setting is still adverse. In a multicenter analysis of 286 patients, the mean overall survival (OS) of bortezomib (btz) and lenalidomide (len) dual-refractory patients was 9 months (1), and even in the era of next-generation proteasome and cereblon-blocking therapies such as carfilzomib and pomalidomide, OS has only marginally improved varying from 12 to 14 months in recent studies (2–4). Even worse is the situation for extramedullary relapsed patients who mostly die from refractory disease within the first year (5, 6). Of note, the majority of multiple myeloma patients will finally become RR, illustrating the urgent medical need for new options for patients in this situation.

Glucose regulated protein (GRP) 78 is a heat shock protein (HSP) 70 family member with chaperone activity. It serves as main sensor for misfolded proteins in the endoplasmatic reticulum (ER) and triggers the unfolded protein response. Furthermore, surface-expressed and secreted/soluble variants have been described for various cancer entities. In multiple myeloma, recent articles have highlighted GRP78's role in the mediation of resistance toward proteasome inhibitors (PI) mainly by promoting autophagosome formation—a compensatory mechanism that restores protein degradation in the presence of a blocked proteasome (7, 8), and a similar mechanism was previously described for the BRAF600E inhibitor vemurafenib in melanoma (9). On the other hand, removal of GRP78 by drugs or shRNA increased susceptibility to PI *in vitro* (7, 8). Furthermore, multiple myeloma cells surviving PI treatment showed increased GRP78 protein expression, and in multiple myeloma patients, GRP78 expression was associated with progressive disease (8). These observations are in line with previous findings from solid cancer showing an association between GRP78 expression, stage of disease, invasiveness, and drug resistance (10). It has also been reported that

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Translational Relevance

Monoclonal antibodies emerge as highly active compounds in the treatment of multiple myeloma, leading to FDA approval of two molecules just in 2015. In a translational approach, we evaluated the fully human anti-GRP78 antibody PAT-SM6 *in vitro* and report on a first patient who experienced partial remission after treatment with PAT-SM6 in combination with novel agents. We show that surface-expressed GRP78 suits as target for immunotherapy of multiple myeloma, especially when considering late-stage or relapse-refractory patients. Furthermore, we identified already approved anti–multiple myeloma drugs to positively modulate GRP78 surface expression, and, as a consequence, to act synergistically with PAT-SM6. These results form the basis for upcoming clinical trials evaluating anti-GRP78 immunotherapy particularly in combination with novel agents in larger cohort of patients with multiple myeloma.

stressed cells from solid cancer frequently translocate GRP78 from the cytosol to the plasma membrane for reasons that remain elusive (11). We have previously shown that GRP78 is stably and consistently expressed on the cell surface of multiple myeloma where it can serve as target for immunotherapy (12). We and others have developed therapeutic antibodies against cell surface GRP78 with promising preclinical and clinical results as single agents (13, 14). PAT-SM6 is an IgM-type human antibody targeting GRP78 with broad reactivity to cancer including multiple myeloma (13). When evaluated with primary multiple myeloma cells *in vitro*, PAT-SM6 induced apoptosis in a dose dependent manner and complement was fixed and activated as a second mode of action (12). Single-agent PAT-SM6 was investigated in a dose-escalating phase I study in relapsed and refractory multiple myeloma (RRMM) patients. Twelve heavily pretreated patients received four applications of PAT-SM6 with doses ranging from 0.3 to 6 mg/kg. Antibody treatment was well tolerated, and MTD was not reached. A disease stabilization rate of 33% was observed; however, objective responses according to the International Myeloma Working Group (IMWG) criteria were not seen (15).

In this article, we show for the first time that treatment of drug-resistant multiple myeloma with the anti-GRP78 antibody PAT-SM6 in combination with novel agents can lead to synergistic anti–multiple myeloma activity *in vitro*, and induces a clinical objective response *in vivo* as demonstrated in an index patient with RRMM and extramedullary involvement.

Materials and Methods

Cell lines

Human myeloma cell lines (HMCL) are derived from primary myeloma cells cultured in RPMI 1640 medium supplemented with 5% fetal calf serum (OPM2, MM.1S, MM.1S-DR, and LP1-LR) and 3 ng/mL recombinant IL6 for IL6-dependent cell lines (XG5-BR) as previously described (16). LP1, OPM-2, and MM.1S were purchased from DSMZ and have been authenticated by DNA profiling as described in detail on the cell bank's website.

MM.1S-DR, LP1-LR, and XG5-BR were obtained after long-time exposure of parental cell lines, MM.1S, LP1, and XG5, to dexamethasone (dex), len, and btz, respectively, resulting in the following resistance status: LP1-LR: len resistant; MM1.S-DR: dex resistant; and XG5-BR: len, dex, and btz resistant as previously described (17, 18). Resistance to respective drugs was routinely reconfirmed. All HMCL used in this article have been previously extensively characterized, authenticated by phenotype analysis, and resistant cell lines were identified with human leukocyte antigen typing (16).

Antibodies

Anti-GRP78 antibody PAT-SM6 (fully human IgM) was produced as outlined elsewhere (12) and provided by Patrys Ltd. Anti-GRP78 control mAb (rabbit IgG, ET-21) was obtained from Sigma-Aldrich. ChromPure IgM was used as isotype control (Dianova) and anti-CD138 (Dako) as positive control.

PAT-SM6 immunostaining on bone marrow paraffin sections

Immunohistochemistry with PAT-SM6 antibody, GRP78 antibody, or control antibodies of intra- and extramedullary multiple myeloma infiltrates on paraffin sections was performed by trained pathologists with blinded sample groups as previously described (12).

FACS

Surface GRP78 expression was evaluated on HMCL MM.1S, OPM2, MM.1S-DR, XG5-BR, and LP1-LR (2×10^6 cells per conditions). For analysis, cells were washed in PBS and stained directly by isotype control Dylight 488 (rabbit IgG Dylight 488; Abcam; 1:25) or rabbit polyclonal anti-GRP78 Dylight 488 (Novus Biologicals; 1:25).

To evaluate the expression of GRP78/binding of PAT-SM6 antibody on plasma membranes in response to treatment with multiple myeloma drugs, HMCL MM.1S, OPM2, and LP1-LR were preincubated with dex (500 nmol/L), len (500 nmol/L), or btz (2 nmol/L) for 48 hours. Cells were washed in PBS and stained with PAT-SM6 and isotype control (ChromPure IgM; Dianova; 5 μg/mL) followed by anti-human IgM PE (Dako; 1:100).

Direct and indirect flow cytometric analysis was performed using a FACS LSRII with Diva Software (Beckman Coulter). Three independent experiments were done for each cell line.

MTT

Cells ($1–1.5 \times 10^4$) were plated into 96-well plates followed by drug treatment for 72 hours. Cell viability was measured using MTT (3-(4,5-dimethylthiazol-2- yl)-2,5-diphenyltetrazolium bromide) colorimetric assay. Three different dose levels were tested: level 1: PAT-SM6 111 nmol/L, len 250 nmol/L, btz 1 nmol/L, Dex 250 nmol/L; level 2: PAT-SM6 222 nmol/L, len 500 nmol/L, dex 500 nmol/L, btz 2 nmol/L; and level 4: PAT-SM6 444 nmol/L, len 1,000 nmol/L, dex 1,000 nmol/L, btz 4 nmol/L. At the end of each treatment, cells were incubated with 1 mg/mL MTT for 3.5 hours at 37°C; lysis buffer was added and dye absorbance was measured at 570 nm after 18 hours of incubation. All experiments were repeated 3 times, and each experimental condition was repeated at least in duplicate wells in each experiment.

Compassionate use patient's characteristics

A 62-year-old male, with newly diagnosed multiple myeloma (IgG kappa, hyperdiploid karyotype) and osteolytic lesions

underwent induction therapy with PAD (btz, doxorubicin, and dex) followed by stem cell collection and single autologous stem cell transplantation (auto SCT) resulting in a biopsy-proven complete remission. Six months later, a serological relapse occurred that was salvaged with 3 cycles of PAD/len. Initially, a serological response could be documented, but in the third cycle, still being on len therapy, the patient noticed subcutaneous nodules and swelling of the right testis, and extramedullary spread was diagnosed by PET-CT. A single-patient treatment use of PAT-SM6 in combination with len and btz was initiated after informed consent.

Statistical analysis

Data were expressed as mean ±SEM. Statistical analyses were conducted using Mann–Whitney or unpaired Student t tests. Descriptive statistics were used for analyzing immunohistochemical stainings.

For the synergism study between PAT-SM6 and myeloma drugs on cell growth inhibition, a combination index (CI) was performed using the data obtained from MTT assay. Drug combination studies were based on concentration effect curves generated as a plot of the fraction of unaffected cells versus drug concentration, in accordance to the Chou and Talalay method (19) using CalcuSyn software (Biosoft). The resulting CI values indicate a synergistic effect in drug combinations when < 1, an antagonistic effect when > 1, and an additive effect when equal to 1.

Study approval

The patient was treated on the basis of a single-patient treatment use after written informed consent. This retrospective review was approved by the local ethics committee of the University of Würzburg.

Results

GRP78 expression in early and late stages of multiple myeloma

Differences in GRP78 surface expression from monoclonal gammopathy of undetermined significance (MGUS) to late-stage multiple myeloma relapses were studied in paraffin-embedded bone marrow trephine biopsies of cases with MGUS ($n = 10$); primary diagnosis of multiple myeloma ($n = 11$) and relapsed multiple myeloma ($n = 29$) including 15 patients with len/btz-refractory disease and 5 patients with extramedullary disease. As expected, GRP78 expression defined by PAT-SM6 was present in all samples of multiple myeloma, whereas in 3 of 10 samples of MGUS, no expression was found (Fig. 1A). Comparing mean number of PAT-SM6–positive cells, patients with primary diagnoses of multiple myeloma had 89% positive cells (range, 80 to 100) and 98% (80 to 100) in the relapsed setting, respectively. Semiquantitative analysis of staining intensity using a (+) to (+++) scale showed highest expression in multiple relapses (11/15) and in extramedullary lesions (5/5), although high expression was also present in some cases at primary diagnosis (5/9) and MGUS (3/10; Fig. 1B; Supplementary Table S1).

GRP78 surface expression in sensitive and resistant cell lines

GRP78 surface expression of sensitive (MM1.S, OPM-2) and resistant cell lines (MM1.S-DR, LP-1-LR, XG5-BR) was determined by flow cytometry using a rabbit anti-GRP78 IgG antibody (direct staining) or PAT-SM6 IgM antibody followed by secondary antibodies (indirect staining). FACS analysis showed that GRP78 is

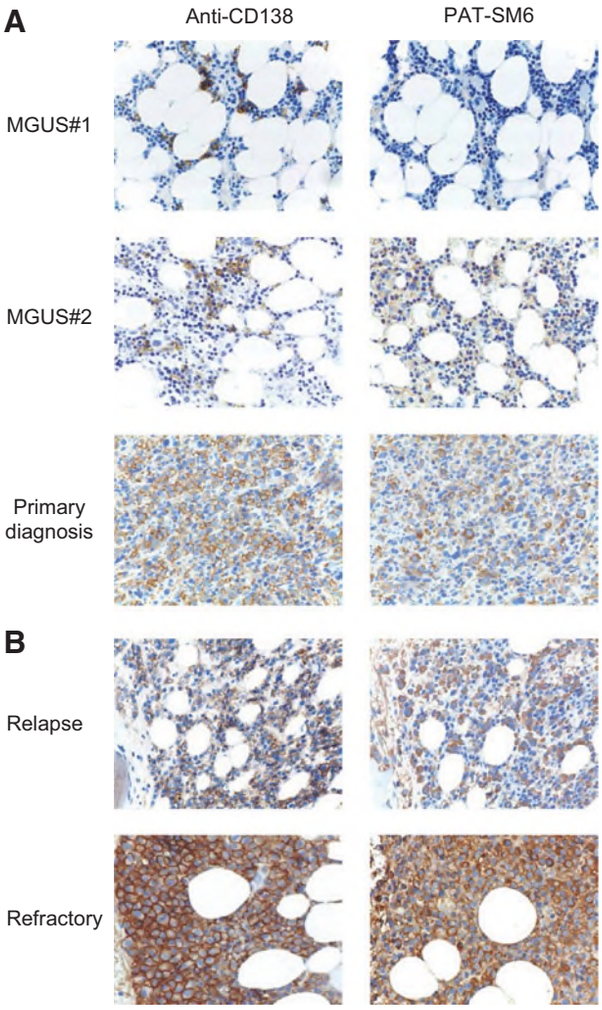
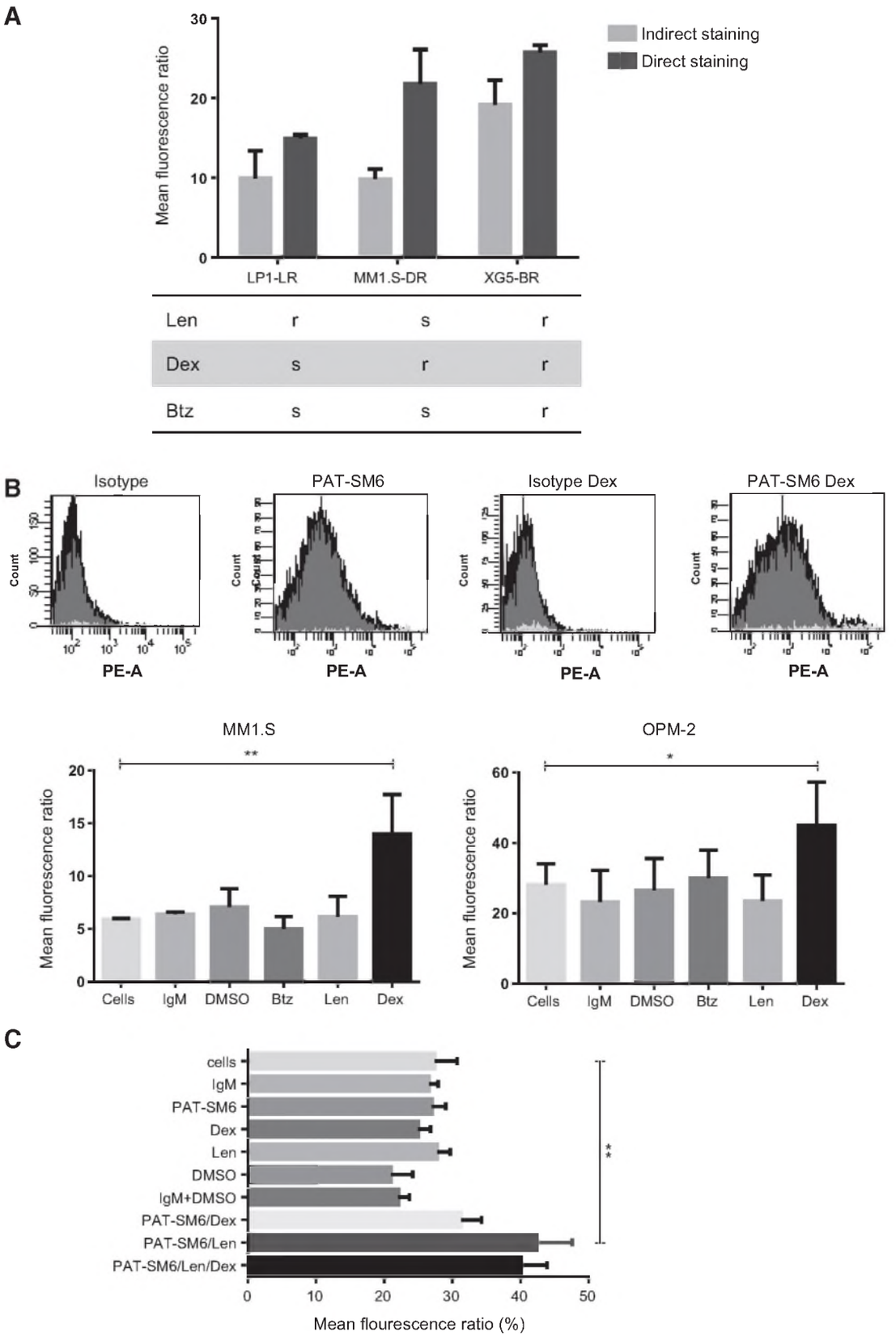


Figure 1.
Surface GRP78 expression from MGUS to RRMM. **A,** two cases of MGUS and a case of primary diagnosis stained immunohistochemically with positive control CD138 and anti-GRP78 antibody PAT-SM6 are shown. In MGUS#1, GRP78 expression was missing in total, whereas in MGUS#2, GRP78 expression was present clearly but moderate in almost all CD138-positive cells. Expression further increases in primary diagnosed multiple myeloma case. **B,** refractory relapse shows highest GRP78 expression when compared with sensitive relapse. Images were captured using a Leica DM BL microscope, the Leica ICC HD digital camera, and the Leica LAS EZ V2.1.0 software. Representative images were from patient specimens (magnification, x200).

expressed on the plasma membrane of all evaluated multiple myeloma cell lines. Among those, triple resistant cell line XG5-BR expressed highest level of sGRP78 in both direct and indirect stainings. Furthermore, MM1.S-DR expressed higher level of GRP78 than its parental cell line MM1.S sensitive to dex (Fig. 2A).

GRP78 cell surface translocation upon treatment with antimyeloma drugs

We then investigated changes in GRP78 surface expression in response to treatment with anti–multiple myeloma drugs. After a 48-hour preincubation with len, btz, dex, or PAT-SM6, cells were washed and sGRP78 expression was analyzed by FACS. Mean


A
Indirect staining
Direct staining
Mean fluorescence ratio
30
20
10
0
LP1-LR
MM1.S-DR
XG5-BR
Len r s r
Dex s r r
Btz s s r
B
Isotype
PAT-SM6
Isotype Dex
PAT-SM6 Dex
Count
PE-A
MM1.S
OPM-2
**
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Mean fluorescence ratio
Cells
IgM
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cells
IgM
PAT-SM6
Dex
Len
DMSO
IgM+DMSO
PAT-SM6/Dex
PAT-SM6/Len
PAT-SM6/Len/Dex
**
Mean flourescence ratio (%)

fluorescence ratio (MFR) was calculated by dividing specific fluorescence through isotype control fluorescence. When only viable cells were gated, MFR was slightly decreased in comparison to the analysis of all cells. However, no specific difference in sGRP78 expression between viable and all cells was found.

Whereas, btz and len treatment had no impact on GRP78 surface expression, preincubation of dex (500 nmol/L) significantly increased binding of PAT-SM6 to MM1.S and OPM-2 cells ($P < 0.05$; Fig. 2B). Considering double combinations, PAT-SM6 pretreatment in combination with len and/or dex increased GRP78 expression in LP1-LR (Fig. 2C) and OPM-2 (data not shown).

Activity of anti–multiple myeloma agents in combination with GRP78 antibody PAT-SM6 *in vitro*

Combination effects were studied in sensitive myeloma cell lines MM1.S and OPM-2 and resistant cell lines LP1-LR (len resistant), MM1.S-DR (dex resistant), and XG5-BR (len, dex, and btz resistant). Single-, dual-, and triple-agent combinations were evaluated in varying doses using a simple proliferation assay (MTT) allowing a high throughput analysis. Pretesting experiments were done to determine dose effect curves and the optimal dose ranges for each of the respective drugs as described previously (20). Within the evaluated doses, single-agent dex showed strongest growth inhibition (max. 54%) in sensitive cell lines, whereas *in vitro* activity of len and PAT-SM6 and btz was moderate (max. 36% and 20%, and 25%, respectively). Of note, in triple resistant XG5-BR, only PAT-SM6 led to a significant growth inhibition (Supplementary Fig. S1A). In sensitive cell lines, double combinations of PAT-SM6 with len or dex were synergistic across all evaluated doses and also synergistic with btz at higher doses in OPM-2 cells (Fig. 3). In resistant cell lines, again the combination of anti GRP78 antibody with len or dex resulted in synergistic growth inhibition at higher concentrations in LP1-LR and MM1.S-DR cell lines. In contrast, antagonistic effects were observed in combination with btz in XG5-BR cells.

When triple combinations were studied, PAT-SM6/len/dex led to strong synergistic inhibition of LP1-LR cells and to additive effects in triple resistant cell lines XG-5-BR. Btz in combination with PAT-SM6/dex showed synergy at low doses only in MM1.S-DR cells (Fig. 3). In summary, based on these *in vitro* experiments, len and dex were the best combination partners for PAT-SM6 showing activity in both, sensitive and resistant cell lines.

PAT-SM6, bortezomib, and lenalidomide treatment in a patient with drug-refractory multiple myeloma

The patient presented with relapsed IgG kappa multiple myeloma at the end of cycle three of PAD-Rev salvage therapy (containing btz, doxorubicin, len, and dex) with a new subcutaneous swelling at the right shoulder and the left testicle. Prior lines of therapy before PAT-SM6/len/btz was initiated are presented in Fig. 4A. Serologically tumor burden was low as expressed by an M protein in serum of 3.9g/L, balanced-free light chain ratio, and beta-2 microglobulin of 1.5 mg/L. However, a PET-CT revealed multiple (>10) intraosseous focal lesions at the skull, spine, pelvis, ribs, and the long bones. Furthermore, extramedullary involvement of right sphenoid sinus, the left testicle as well as a subcutaneous nodule of $1.7 \times 1.4 \times 0.4$ cm adjacent to the left scapula was diagnosed. Taking into account that the manifestations occurred under quadruple therapy including both novel agent classes, resensitization was considered a rational approach. On the basis of an individual patient treatment use, PAT-SM6 was administered at a dose of 10 mg/kg on days 1, 3, and 8 combined with len 10 mg on days 1 to 10 and btz 1.3 mg/m^2 on days 1 and 8 after informed consent. Treatment was well tolerated, and the patient noticed a rapid decrease of the manifestation at the left shoulder. An ultrasound examination at day 8 confirmed response showing an almost complete resolution of the soft tissue involvement at the scapula site. A PET-CT was initiated at day 14 showing a metabolic response of all lesions including nearly complete resolution of the extramedullary manifestations at the testicle and scapula (Fig. 4A and B). Of note, all lesions responded to therapy, but displayed varying SUV reduction ranging from 28% to 80%. A second cycle was given, and therapy was well tolerated. However, PET-CT scan at week 8 showed progressive disease of intra- and extramedullary sites. A biopsy from relapsed soft-tissue involvement at the scapula was taken, and sGRP78 expression was assessed. Histologically, an infiltration of highly anaplastic CD138$^+$ plasma cells was found showing a preserved high sGRP78 expression in all multiple myeloma cells (Fig. 4C). The study was stopped and the patient continued with several lines of polychemo- and experimental radiotherapy followed by salvage auto- and allogeneic stem cell transplantation, but unfortunately died 9 months after extramedullary progression from refractory disease.

Discussion

Availability of tumor- or lineage-specific antigens with robust expression throughout various stages of hematologic malignancies is crucial for the design of successful antibody-based therapies. In target expression analysis, we observed that in multiple myeloma cell lines as well as in patients' specimens, myeloma evolution and drug resistance go along with increased surface

Figure 2.

sGRP78 expression in sensitive and resistant human multiple myeloma cell lines at baseline and in response to anti–multiple myeloma drugs. Surface expression was determined using a fluorochrome-conjugated rabbit anti-GRP78 IgG antibody (direct staining) as well as therapeutic antibody PAT-SM6 (human anti-GRP78 IgM) followed by conjugated secondary antibody (indirect staining) and analyzed by FACS. MFR was calculated by dividing specific fluorescence through isotype control fluorescence. **A,** sGRP78 baseline expression of drug-resistant cell lines LP1-LR (len resistant), MM1.S-DR (dex resistant), and XG5-BR (len, dex, and btz triple resistant). sGRP78 was found across all cell lines, and triple resistant XG5-BR showed highest expression in both, direct and indirect staining. Error bars correspond to mean with SEM ($n = 3$). **B,** changes in sGRP78 expression in response to treatment with anti–multiple myeloma agents dex (500 nmol/L), len (500 nmol/L), and btz (2 nmol/L). MM1.S and OPM-2 cells were incubated with respective drugs and appropriate controls for 48 hours, washed, and stained with PAT-SM6 or anti-GRP78 IgG antibody followed by FACS. Representative histograms of indirect staining of dex-treated MM1.S cells are shown in FACS histograms, illustrating an increase of sGRP78 expression upon dex treatment. Diagrams summarize results showing dex to increase sGRP78 expression in MM1.S and OPM-2 cell lines. Unpaired *t* test; *, $P < 0.05$; **, $P < 0.01$. Error bars correspond to mean ± SD ($n = 3$). **C,** changes in sGRP78 expression in response to single-, dual-, and triple-drug combinations in resistant LP1-LR cells. Pretreatment with dual combinations PAT-SM6/len or PAT-SM6/dex as well as triple combination PAT-SM6/len/dex showed an increase in sGRP78 expression. Unpaired *t* test; **, $P < 0.01$. Error bars correspond to mean ± SD ($n = 3$).

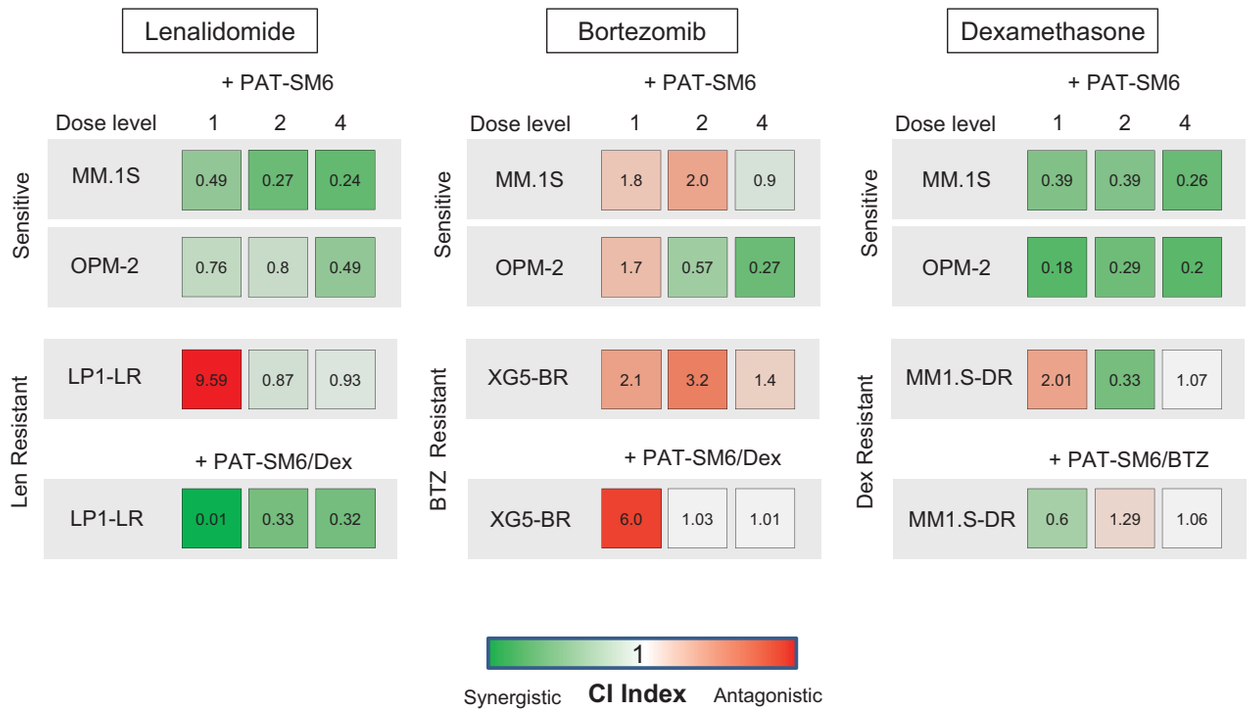

Figure 3.
Activity of anti–multiple myeloma agents in combination with GRP78 antibody PAT-SM6 *in vitro*. Anti–multiple myeloma effect of dual and triple combinations was determined by MTT proliferation assay (in triplicates, experiments repeated three times) followed by synergy quantification using the median effect principle of Chou and Talalay. Three equimolar dose levels were analyzed: Dose level 1: PAT-SM6 111 nmol/L, len 250 nmol/L, btz 1 nmol/L, dex 250 nmol/L; level 2: PAT-SM6 222 nmol/L, len 500 nmol/L, dex 500 nmol/L, btz 2 nmol/L; and level 4: PAT-SM6 444 nmol/L, len 1,000 nmol/L, dex 1,000 nmol/L, btz 4 nmol/L. Values above 1 (red) are consistent with an antagonistic effect, values equal to 1 (white) are consistent with additive effect, and values less than 1 (green) are consistent with a synergistic effect.

GRP78 expression which is in line with previous data from solid cancers, demonstrating an increase in more advanced and refractory stages (21–23). This may reflect a functional role of sGRP78 in the mediation of drug resistance, but could also occur as an epiphenomenon without biologic significance. In respect of immunotherapy, this difference is of less importance because robust target expression is the main requirement. Thus, the preserved target expression at relapse in the PAT-SM6 treated patient is an important observation. Of note, other multiple myeloma targets such as CD38 show a decrease of expression from normal plasma cells to advanced stages or plasma cell leukemia, which may alter binding features and distribution of addressing immunotherapies in relapsed patients (24).

To build rationales for reasonable combinations with other anti–multiple myeloma drugs, we tested whether sGRP78 expression changed upon treatment with len, btz, or dex and found an increase in dex and PAT-SM6/len–pretreated cells. Consequently, PAT-SM6 dual and triple combinations with len and/or dex showed synergistic cytotoxicity across sensitive and resistant cell lines. Unexpectedly, btz did not impact sGRP78 expression, and synergistic effects with PAT-SM6 could only be observed in sensitive OPM-2 cells. Of note, results achieved from cell lines, especially in multiple myeloma, need to be interpreted with caution, because they hardly reflect the enormous heterogeneity of this disease.

The presented case report has clearly shown efficacy of a GRP78-targeting antibody in drug-refractory and extramedullary myeloma. The patient experienced progressive disease while being treated with the four-drug combination btz, len, doxorubicin, and dex (PAD Rev regimen) demonstrating resistance against all of the used compounds.

Further de-escalated therapy using len and btz with PAT-SM6 led to a rapid response in both intra- and extramedullary lesions, and although duration of response was limited, it proves the principal utility of PAT-SM6 as a GRP78-addressing antibody therapy. However, this single patient was treated on compassionate grounds, and further controlled studies are warranted to investigate response rates and more effective dose schedules of this drug combination.

If we speculate on the exact mechanism by which PAT-SM6 recaptured response in the patient, we need to take the following into consideration: *In vitro* PAT-SM6 showed a direct cytotoxic effect on multiple myeloma cells by the induction of apoptosis and the amount of cytotoxicity was positively correlated with the degree of antigen expression. This was previously shown for several multiple myeloma cell lines (12), and the results of the current study recapitulate this finding by the observation that PAT-SM6 showed single-agent activity in XG5-BR cells that exhibit highest sGRP78 expression. Furthermore, we confirmed PAT-SM6 activity *in vivo* in the murine 5T33 multiple myeloma model where PAT-SM6 reduced multiple myeloma outgrowth in a dose-dependent manner (data not shown). However, in the previous phase I study, single-agent PAT-SM6 led to disease stabilization in some patients with RRMM but not to objective

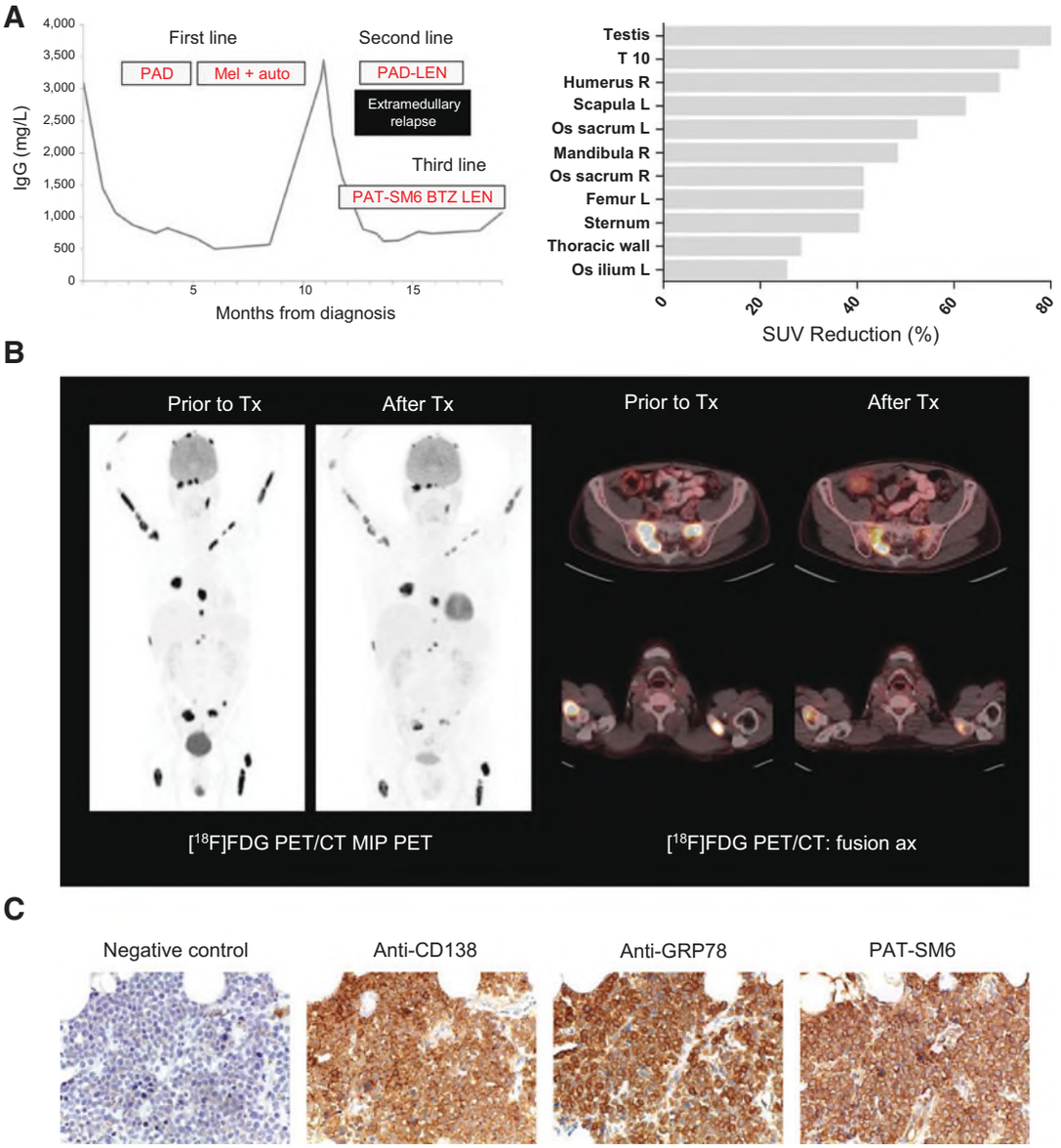

Figure 4.
PAT-SM6, btz, and len treatment in a patient with drug-refractory multiple myeloma. **A,** left, prior lines of therapy before PAT-SM6/len/btz was initiated. Right, metabolic response (SUV reduction) in PET after first cycle of PAT-SM6/len/btz related to different focal lesions. **B,** maximum intensity projections (MIP) and transaxial PET-CT fusions before and after PAT-SM6 therapy. **C,** histologic re-examination at relapse after PAT-SM6/len/btz showed preserved GRP78 expression and PAT-SM6 binding (magnification, x200). Images were captured using a Leica DM BL microscope, the Leica ICC HD digital camera, and the Leica LAS EZ V2.1.0 software.

responses according to the IMWG criteria (15). We speculate that both late-stage multidrug-resistant disease and concomitant len treatment led to high sGRP78 expression and consequently to a significant cytotoxicity of PAT-SM6 via induction of apoptosis. However, in the end, the ultimate evidence remains elusive, and ongoing studies will investigate the role of soluble GRP78 in the mediation of drug resistance as well as the impact of PAT-SM6 on the unfolded protein response. Speculating on the mechanism of resistance to PAT-SM6 combination therapy, upregulation of protective molecules such as CD55 and CD59 could preserve cells from complement-mediated lysis as it was recently observed for daratumumab (25, 26). Furthermore, newly acquired mutations may alter intracellular signaling and finally prevent the induction of apoptosis by PAT-SM6. Ongoing research currently investigates these hypotheses.

Typical paths of drug development suggest that successful new drugs display single-agent activity, and indeed a recent analysis revealed that also in multiple myeloma, single-agent activity is the best predictor for FDA approval (27). In regard to the upcoming monoclonal antibodies in multiple myeloma, to date robust single-agent activity can only be observed in the anti–CD38-addressing antibodies daratumumab and SAR650984 (28, 29).

However, this paradigm is changing, especially since the approval of panobinostat—a pan-deacetylase inhibitor with only modest activity as single-agent but with high response rates in the combination with btz. In the Panorama 2 trial, around 40% of btz-refractory patients responded to a combination with panobinostat with a progression-free survival of 5.4 months, which clearly indicates that the concept of resensitization is feasible (30). Of note, this recapture of response was achieved by an increase of side effects, including grade 3 diarrhea and fatigue in >20% of the patients. In addition, elotuzumab, a humanized IgG 1 antibody targeting CS-1, showed a disease stabilization rate of only 26.5% in the relapsed setting (31), but when combined with len and dex (Rd), overall response rate (ORR) and progression-free survival were significantly superior in a randomized trial (79% vs.66% ORR and 19.4 vs. 14.9 months, respectively; refs. 32, 33). In contrast with panobinostat, this combination therapy was well tolerated, and only infusion-related reactions, which had been manageable, added to the toxicity profile of Rd.

The situation is comparable for the anti-GRP78 antibody PAT-SM6. Within the investigated doses, no single-agent activity was seen in phase I, but in the combination with novel agents, efficacy was achieved. It may be argued that a clinical benefit of 2-month progression-free survival as it was observed in the reported patient is not significant. But it needs to be taken into consideration that we have treated a highly aggressive multiple myeloma refractory to available standard therapeutics with an investigational agent in an ambiguous dose schedule. Further studies are planned to identify effective doses and synergistic combination partners in clinical trials to increase depth and duration of response in future patients suffering from resistant disease.

We have shown that surface GRP78 can be targeted effectively by a monoclonal antibody, but also intracellular GRP78 increasingly emerges as molecular target for multiple myeloma therapy. Previous preclinical studies have shown that suppression of intracellular GRP78 (e.g., by the antidiabetic drug metformin) particularly enhance the cytotoxicity of proteasome inhibitors such as btz (7, 34). Thus, a cross-talk between surface and intracellular GRP78 is likely (35). In solid cancer, sGRP78 is known to regulate the PI3K/AKT pathway, which itself interacts with the unfolded protein response (36, 37). We have previously reported that PAT-SM6 is rapidly internalized upon binding to malignant cells which would also allow a direct interaction with intracellular GRP78 (38). Further studies are warranted to elucidate the biologic function of GRP78 and to find additional strategies to overcome drug resistance in multiple myeloma.

Conclusions

In this article, we demonstrate that sGRP78 can serve as robust target for immunotherapy of multiple myeloma and particularly show that anti-GRP78 antibody PAT-SM6 is active in combination with novel agents in late-stage multiple myeloma with extramedullary involvement. Further studies are warranted to elucidate whether this strategy is limited to late-stage multiple myeloma and how therapies need to be designed to translate this concept to a successful clinical myeloma treatment.

Disclosure of Potential Conflicts of Interest

S. Brändlein reports receiving a commercial research grant from Patrys Ltd. Melbourne, Australia. No potential conflicts of interest were disclosed by the other authors.

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