Atacicept (TACI-Ig) inhibits growth of *TACI^high^* primary myeloma cells in SCID-hu mice and in coculture with osteoclasts

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Abstract

APRIL (a proliferation-inducing Ligand) and BLyS/BAFF (B-lymphocyte stimulator/B-cell-activating factor of the TNF (tumor necrosis factor) family have been shown to be the survival factors for certain myeloma cells *in vitro*. BAFF binds to the TNF-related receptors such as B-cell maturation antigen (BCMA), transmembrane activator and CAML interactor (TACI) and BAFFR, whereas APRIL binds to TACI and BCMA and to heparan sulfate proteoglycans (HSPG) such as syndecan-1. *TACI* gene expression in myeloma reportedly can distinguish tumors with a signature of microenvironment dependence (*TACI^high^*) versus a plasmablastic signature (*TACI^low^*). We tested the effect of atacicept (formerly TACI-Ig, which blocks APRIL and BAFF) and BAFFR-Ig (which blocks BAFF only) on primary myeloma growth in the SCID-hu model and in coculture with osteoclasts. With only few exceptions, atacicept and to a lesser extent BAFFR-Ig, inhibited growth of *TACI^high^* but not *TACI*^low^ myeloma samples *in vivo* and *ex vivo*, and the response rate was inversely correlated with *TACI* expression. Most *TACI^high^* myeloma cells were molecularly classified as being low risk with our recently described 70-gene model. APRIL and BAFF were highly expressed by osteoclasts and were upregulated in myeloma cells after coculture with osteoclasts. Our findings suggest that APRIL plays an essential role in the survival of *TACI^high^* bone marrow-dependent myeloma cells and *TACI* gene expression may be a useful predictive marker for patients who could benefit from atacicept treatment.

Keywords

myeloma; APRIL; BAFF; TACI; gene expression; microenvironment

Introduction

In multiple myeloma, a plasma cell malignancy, a majority of the tumor cells reside in the hematopoietic bone marrow (BM). As a consequence of interaction with certain microenvironmental components, myeloma cells are protected from spontaneous and drug-induced apoptosis.[1,2] In late-stage disease, myeloma cells become independent of BM microenvironmental signals and extramedullary disease is not uncommon. Recent clinical studies indicate that using drugs that simultaneously target myeloma cells and their supportive BM microenvironment (for example, thalidomide, bortezomib) provides a promising approach to control disease progression and overcome drug resistance.[3,4] Interaction of myeloma cells with cellular and extracellular components results in upregulation of growth factors such as

interleukin (IL)-6[5−7] and vascular endothelial growth factor (VEGF).[8] However, inhibiting their activity in patients with myeloma results in partial or no response,[9,10] suggesting that additional growth factors and/or cell-to-cell interactions may be involved in the growth and survival of myeloma cells within the BM.

Two TNF (tumor necrosis factor) family members known to play key roles in normal B-cell biology, BLyS/BAFF (B-lymphocyte stimulator/B cell activating factor of the TNF family) and APRIL (A PRoliferation-Inducing Ligand), also promote the survival of various malignant B-cell types, including myeloma.[11−13] BAFF binds to three TNF-R-related receptors, BCMA (B-cell maturation antigen), TACI (transmembrane activator and CAML interactor), and BAFFR (BAFF-receptor), whereas APRIL binds to TACI and BCMA and to heparan sulfate proteoglycans (HSPG) such as syndecan-1 (CD138).[11−13] All myeloma cells express high levels of syndecan-1 and one or more of these three TNF-R-related receptors. Culture of myeloma cells with BAFF and/or APRIL leads to enhanced survival of these malignant cells *in vitro*[14,15] and promotes their adhesion to stromal cells.[16] APRIL and BAFF induce myeloma cell adhesion and survival through activation of nuclear factor-κB (NF-κB), phosphatidylinositol-3 (PI-3) kinase/Akt and mitogen-activated protein kinase (MAPK) pathways and upregulation of the antiapoptotic factors MCL-1 and BCL-2 in myeloma cells. [14,16] Although myeloma cells can express very low levels of BAFF and APRIL, circulating levels of these cytokines in myeloma patients is higher than that in normal individuals, suggesting that BM microenvironmental cells are a major source of these factors in myeloma patients.[14,16] It has been shown that differences in *TACI* gene expression can distinguish tumors with a BM microenvironment-dependent signature (*TACI^{high}*) from those with a plasmablastic signature (*TACI^{low}*),[17] suggesting that *TACI^{high}* myeloma cells may be more sensitive to growth factor withdrawal.

Inhibition of BAFF and APRIL using a soluble receptor, TACI-Ig, in culture of L363 and RPMI8226 cell lines causes cultured myeloma cells to die rapidly.[14] In coculture of certain myeloma cell lines with the supporting osteoclasts or dendritic cells, TACI-Ig addition resulted in reduced myeloma cell growth and diminished clonogenic potential.[18,19] In the current study, we further tested the involvement of APRIL and BAFF in myeloma pathogenesis using two inhibitors—atacicept (TACI-Ig) and BAFFR-Ig—in our *in vivo*[20,21] and *ex vivo*[22,23] experimental systems for primary myeloma. Whereas atacicept binds and neutralizes both BAFF and APRIL, BAFFR-Ig blocks only BAFF y. For our *in vivo* model, SCID- (severe combined immunodeficiency) hu mice are constructed by implanting a human fetal bone into which primary human myeloma cells are directly injected. Myeloma cells from the majority of patients interact with the human BM, grow exclusively in or on the implanted bone and produce myeloma manifestations, including stimulation of osteoclastogenesis and angiogenesis of human origin, suppression of osteoblastogenesis and induction of severe osteolytic bone disease. This SCID-hu system has been successfully used to study the role of BM microenvironment in myeloma.[24−26] We also recently established a coculture system for the growth of primary myeloma cells *ex vivo* using authentic bone-resorbing osteoclasts, which are typically activated in myelomatous BM. Cultured osteoclasts consistently support long-term survival and proliferation and protect myeloma cells from drug-induced apoptosis.[22,23] Osteoclasts have been shown to express high levels of APRIL and BAFF and thus may represent an important paracrine source of these survival factors.[17] Here, for the first time we demonstrate the association between *TACI* gene expression by myeloma cells and their response to inhibitors of APRIL and BAFF *in vivo* and *ex vivo*.

Materials and methods

Myeloma cells

Myeloma cells were obtained from heparinized BM aspirates from 17 patients with active myeloma during scheduled clinic visits. Signed Institutional Review Board-approved informed consent forms are kept on record. Pertinent patient information used in animal experiments is provided in Table 1. The BM samples were separated by density centrifugation using Ficoll-Paque (specific gravity, 1.077 g ml^{-1}; Amersham Biosciences Corp., Piscataway, NJ, USA), and the proportion of myeloma plasma cells in the light-density cell fractions was determined by CD38/CD45 flow cytometry. Aliquots of BM cells were taken for *in vivo* studies. The rest of the BM cells were used for isolation of plasma cells by CD138 immunomagnetic bead selection (Miltenyi-Biotec, Auburn, CA, USA).[27] The isolated plasma cells were subjected to global gene expression profiling as described previously.[27–29]

Construction of primary myelomatous SCID-hu mice

SCID-hu mice were constructed as described previously.[20,21,24] Briefly, 6- to 8-week-old CB. 17/Icr-SCID mice were obtained from Harlan Sprague Dawley (Indianapolis, IN, USA) and were housed and monitored in our animal facility. The University of Arkansas for Medical Sciences (UAMS) Institutional Animal Care and Use Committee approved all experimental procedures and protocols. The human fetal bones were obtained from Advanced Bioscience Resources (ABR, Alameda, CA, USA). The bone (femur or tibia) was inserted subcutaneously through a small (5 mm) incision. The incision was then closed with sterile surgical staples, and engraftment of the bones was allowed to take place for 6–8 weeks. For each experiment, 5–10 $\times 10^6$ mononuclear cells containing >15% plasma cells in 100 μl of phosphate-buffered saline (PBS) were injected directly into the implanted bone. Mice were periodically bled from the tail vein and changes in the levels of circulating human Ig (hIg) of the tumor M-protein isotype were used as an indicator of myeloma growth. When hIg levels reached 15 μg ml^{-1} or higher, two to three mice injected with cells from the same patient were used for each experiment. Usually, the hosts with the higher hIg levels, indicative of higher tumor burden, were selected for treatment, while the others served as controls. At the end of each experiment, the myelomatous human bone was histologically processed and bone sections were stained with hematoxylin and eosin (H&E).

In vivo treatment with soluble receptors

In 9 of 11 experiments, myeloma-bearing SCID-hu mice were injected three times a week intraperitoneally with approximately 10 mg kg^{-1} atacicept (200 μg per mouse; *n* = 11), 10 mg kg^{-1} BAFFR-Ig (*n* = 9) or with vehicle (PBS; *n* = 11) for 4–6 weeks. Hosts engrafted with cells from patients 10 and 11 were similarly treated with 5 mg kg^{-1} atacicept. TACI–Fc5 (atacicept) and BAFFR–Fc5 Fc fusion proteins were provided by ZymoGenetics Inc., Seattle, WA, USA. These soluble receptors were constructed using the extracellular domain of TACI or BAFFR fused to a form of the Fc portion of human IgG$_1$ that contains mutations preventing complement binding and FcγR engagement.

Determination of human Ig levels

Levels of human κ and λ light chains were determined by ELISA as described previously.[20,21] All samples from each experiment were analyzed in the same assay at the end of each experiment to avoid potential complications from interassay variability.

Myeloma plasma cells and osteoclasts cocultures

Highly purified bone-resorbing osteoclasts were prepared as described previously.[22,23] Briefly, human peripheral blood mononuclear cells were cultured at 2.5×10^6 cells per ml in α-minimum

essential medium (MEM) supplemented with 10% fetal bovine serum (FBS), antibiotics, RANKL (50 ng ml^{-1}) and macrophage colony-stimulating factor (25 ng ml^{-1}) (osteoclast medium) for 10–14 days, at which time the cultures contained large number of multinucleate osteoclasts exhibiting bone-resorbing activity.[22,23]

For coculture experiments, osteoclasts were washed three times with phosphate-buffered saline (PBS) to detach and remove nonadherent cells. To test the effects of atacicept and BAFFR-Ig inhibition on osteoclast-induced tumor growth/survival, myeloma plasma cells (0.5–1 × 10^6 cells per ml in osteoclast medium) were cultured with osteoclasts in 24-well plates (1 ml per well) in the presence or absence of 1–10 μg ml^{-1} atacicept or BAFFR-Ig for 5–7 days. At the end of each experiment, myeloma plasma cells were collected and subjected to an MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay.[22]

Immunohistochemistry

Cytospin slides of myeloma cells (40 000 per slide) were fixed with HistoChoice (Amersco, Solon, Ohio) for 20 min, thoroughly washed and incubated in citrate buffer in a water bath (80 °C, 30 min) for antigen retrieval. Following peroxidase quenching with 3% hydrogen peroxide for 10 min, the slides were incubated with monoclonal antibodies against human APRIL (Axxora, San Diego, CA, USA; 10 μg ml^{-1}) or BAFF (ZymoGenetics Inc. 15 μg ml^{-1}) for 60 min. The assays were completed using immunoperoxidase kit from DAKO (Carpinteria, CA, USA) and counterstaining with hematoxylin.

Statistical analysis

All values are expressed as mean ± s.e.m. Student's paired *t*-test was used to test the effect of treatment on myeloma burden. The Spearman's rank correlation coefficient analysis was used to determine the association between levels of *TACI* gene expression and changes in myeloma burden *in vivo*. The Fisher's exact test was used to determine the between levels of *TACI* gene expression and global gene expression-based prognosis systems.

Results

Antimyeloma response of atacicept and BAFFR-Ig in SCID-hu mice

Myeloma cells from 11 patients were successfully engrafted in SCID-hu mice and used for this study. The growth pattern of myeloma cells in this model has been well characterized.[20,21,24] In nine experiments, myeloma growth was restricted to the implanted BM (medullary myeloma), while myeloma cells from patients 8 and 10 grew also on the outer surface of the implanted bone (extramedullary myeloma). When SCID-hu mice had established myeloma as indicated by increased hIg,[24] hosts engrafted with myeloma cells from different patients were treated with atacicept (*n* = 11), BAFFR-Ig (*n = 9*) or PBS (*n = 11*) for 4–6 weeks. Atacicept and BAFFR-Ig did not cause apparent toxicities in any of the experiments.

For assessing antitumor response, reduced tumor burden defined as reduction of hIg levels from pretreatment levels and growth rate delay defined as reduced myeloma burden by more than 25% compared with PBS-treated hosts.[30] Compared with PBS-treated hosts, in which tumor burden (hIg) increased from pretreatment levels in all experiments, atacicept treatment markedly reduced tumor burden from pretreatment level in six experiments (patients 1, 2, 3, 5, 6 and 7), inhibited myeloma growth rate in three experiments (patients 4, 9 and 11) and had no antitumor effect in two experiments (patients 8 and 10) (Table 1). In contrast, BAFFR-Ig treatment resulted in tumor reduction in one experiment (patient 2), growth rate inhibition in five experiments (patients 1, 3, 4, 6 and 9) and no effect in three experiments (patients 5, 7 and 8) (Table 1). Overall changes in hIg levels relative to pretreatment values in atacicept-treated hosts were significantly lower than that in PBS-treated hosts (*P*<0.05, Figure 1). BAFFR-Ig

treatment also resulted in reduced hIg from pretreatment levels but the change was insignificantly different than in control PBS-treated hosts ($P<0.08$, Figure 1).

The *in vivo* effect of atacicept and BAFFR-Ig on hIg levels and tumor burden in three representative experiments is shown in Figure 2. Histological examinations of myelomatous bone sections from these experiments further confirmed changes in myeloma burden as assessed by measurement of hIg levels and demonstrated parallel changes in myeloma cell infiltration following treatment (Figure 2). Since atacicept significantly inhibited myeloma growth in primary myelomatous SCID-hu mice more consistently than BAFFR-Ig, we focused our further analyses and *in vitro* work on atacicept.

Association between response to atacicept in SCID-hu mice and myeloma cell *TACI* expression and molecular classification

Next, we looked for an association between *TACI* gene expression in myeloma plasma cells and changes in myeloma burden in PBS- and atacicept-treated hosts (Figure 3a). It has been shown previously that global gene expression profiling can be successfully used to assess *TACI* expression accurately by myeloma plasma cells and the differences in *TACI* gene expression can distinguish tumors with a BM microenvironment-dependent signature (*TACI^high*) from those with a plasmablastic signature (*TACI^low*).[17] Using global gene expression profiling on CD138-selected myeloma plasma cells from newly diagnosed myeloma patients enrolled in total therapy 2 clinical trial in our institute,[28] we measured a *TACI* signal level of <800 in 25% of all subjects and designated those samples as *TACI^low*[17]. Myeloma cells expressing *TACI* mRNA at a signal level >800 were designated *TACI^high*. In our *in vivo* experiments, myeloma cells from 4 of the 11 patients were *TACI^low* (patients 8–11; Table 1). Atacicept treatment in six of seven *TACI^high* cases led to reduced serum hIg compared with pretreatment levels, and two of the four *TACI^low* cases exhibited delayed growth (patients 9 and 11), whereas none of the *TACI^low* myeloma had reduced hIg level from pretreatment following treatment with atacicept ($P<0.01$, Fisher's exact test, Table 1).

In the control SCID-hu mice treated with PBS, we observed an inverse correlation between the levels of *TACI* gene expression and increased hIg compared with pretreatment levels ($r= -0.7$, $P<0.05$, Spearman's rank correlation coefficient), suggesting that *TACI^low* myeloma cells, which are typically associated with a proliferative phenotype,[17] also have higher growth rate in our *in vivo* model system (Figure 3a). An even higher inverse correlation rate was observed between *TACI* gene expression level and changes in tumor burden following treatment with atacicept ($r_{Spearman's} = -0.8$, $P<0.01$), reflecting the ability of atacicept treatment to reduce tumor burden efficiently in the *TACI^high* myeloma-bearing mice. In contrast, most myeloma cases expressed very low levels of *BAFFR* (as expected, since BAFFR is downregulated as B cells differentiate into plasma cells) and a high levels of *BCMA* (Table 1). Levels of *BAFFR* or *BCMA* expression were not associated with response to atacicept or BAFFR-Ig (data not shown).

To further analyze the association within *TACI* expression level, response to atacicept in SCID-hu mice and patients' outcome, we utilized two of our myeloma plasma cell molecular classification models: the 70-gene model, which accurately defines low- and high-risk patients[29] and the 7-group model, which segregates all cases into seven disease subtypes based on known genetic lesions.[28] Based on the 70-gene model, five cases from our *in vivo* study (patients 1, 4, 5, 7 and 9) were classified as the low-risk group and six were classified as the high-risk group (patients; 2, 3, 6, 8, 10 and 11). While four of the five low-risk cases (80%) were *TACI^high*, three of six high-risk cases (50%) were *TACI^low* (Table 1). We then sought to analyze the response to atacicept of low- and high-risk myeloma cases. The analysis revealed that atacicept reduced tumor burden from pretreatment level in low-risk myeloma, and that atacicept-treated hosts had lower hIg levels than that in matching control hosts by the end of

the experiment (P<0.03). However in atacicept-treated hosts harboring high-risk myeloma, hIg levels were increased from pretreatment and were not statistically significantly different from hIg levels in matching PBS-treated control hosts (Figure 3b).

We also looked for the association between *TACI* expression level and the gene expression profile-based prognostic system using the 7-group model.[28] To increase statistical validity, we tested patients whose samples used in the *in vivo* and *in vitro* study (n = 17, Table 1). Based on the 7-group model, 7 of 10 (70%) *TACI*[high] cases were categorized in the subgroups associated with superior clinical outcome (low bone, hyperdiploid, CCND1-2) groups, while 6 of 7 *TACI*[low] cases (85%) belonged to the poor outcome (proliferation, MAF, MMSET) groups (P<0.036, Fisher's exact test, Table 1).[28]

Role of APRIL and BAFF in the osteoclast-induced myeloma growth

To further shed light on the role of APRIL and BAFF in myeloma and validate our *in vivo* findings of an antimyeloma effect of atacicept, we employed our primary myeloma–osteoclast coculture system. We previously demonstrated the stimulatory effect of highly purified bone-resorbing osteoclasts on long-term *in vitro* survival of primary myeloma plasma cells.[22] To shed light on the molecular consequences of this interaction, myeloma cells from eight patients were cocultured with osteoclasts, generated from mobilized blood from eight different patients for 4 days. We then performed global gene expression profiling and looked for changes in expression levels of APRIL and BAFF in pre and cocultured myeloma plasma cells, and in osteoclasts cultured alone or cocultured with myeloma cells.[31,32] As reported previously,[17] osteoclasts express high levels of both APRIL and BAFF compared with myeloma cells, which had low but detectable levels of these cytokines. After coculture, expression level of APRIL and BAFF remained high in the osteoclasts and was also significantly upregulated in the myeloma plasma cells themselves (Figure 4a). Using immunohistochemistry for APRIL and BAFF, we further demonstrated increased production of these cytokines in cocultured myeloma cells, thus excluding the possibility of fault microarray results due to contamination with osteoclasts (Figures 4b and c). These data support previous reports on autocrine production of these APRIL and BAFF by myeloma cells.[14,15]

To test the effect of atacicept on myeloma cell growth *ex vivo*, myeloma plasma cells from seven patients were cocultured with osteoclasts in the absence or presence of atacicept for 5–7 days. Atacicept-inhibited survival of myeloma cells by >35% in three of six experiments (Table 1; P<0.03).[18] As in the SCID-hu mice, the antimyeloma effect of atacicept was evident on *TACI*[high] but not *TACI*[low] cases and, as in the *in vivo* experiments, was inversely correlated with *TACI* expression level (r= −0.9, P<0.01; Table 1; Figure 5a). For the three *TACI*[high] myeloma samples, atacicept-inhibited myeloma cell survival in a dose-related manner (Figure 5b). In three experiments (patients 13 (*TACI*[high]), 15 and 16 (*TACI*[low]); Table 1), we also tested the *ex vivo* effect of BAFFR-Ig on myeloma growth. BAFFR-Ig had no antitumor effect in coculture of myeloma cells with osteoclasts, regardless of the level of *TACI* expression by the tumor cells, suggesting that APRIL plays a key role in myeloma survival in this coculture system.

Discussion

APRIL and BAFF have been shown to be upregulated in the myeloma microenvironment, stimulate survival and proliferation of myeloma cells *in vitro* through autocrine and paracrine mechanisms and protect tumor cells from drug-induced apoptosis.[15–18,33] In this study, we demonstrated that osteoclasts express high levels of APRIL and BAFF and that these cytokines are upregulated by myeloma cells after coculture with osteoclasts. Atacicept effectively reduced the growth of *TACI*[high] primary myeloma cells in SCID-hu mice and in long-term coculture with the supportive osteoclasts. For the first time, we also demonstrated using global

gene expression profiling that antimyeloma efficacy of atacicept *in vivo* and *ex vivo* was significantly correlated with expression of *TACI* by myeloma cells. These results are in accordance with the previous reports demonstrating the ability of TACI-Ig to induce apoptosis of *TACI*-expressing myeloma cell lines *in vitro*[14,18] and to inhibit the stimulatory effects of osteoclasts[18] and dendritic cells[19] on myeloma cells. Our findings that atacicept more consistently demonstrated antimyeloma effects in our experimental systems than BAFFR-Ig suggest that APRIL, either alone or together with BAFF, plays a role in the growth and/or survival of myeloma cells from patients of specific subtypes.

Our data strongly support a recent report associating *TACI^high* myeloma with a BM microenvironment-dependence signature, suggesting that these myeloma cells are likely to be more sensitive to growth factor withdrawal than *TACI^low* myeloma cases harboring plasmablastic and proliferative gene signatures.[17] The significant inverse correlation that we found between *TACI* expression and the growth rate of myeloma cells in SCID-hu mice (Figure 3) emphasizes the clinical relevance of this experimental system, as *TACI^low* patients were reportedly characterized by an increased percentage of stage III myeloma, reduced hemoglobin level and increased bone lesions.[17] Studies by Moreaux *et al.*,[17] and our current data indicate that global gene expression profiling for assessing *TACI* expression and molecular classifications may prove to be a useful approach for tailoring therapeutic intervention with APRIL and BAFF inhibitors in myeloma. These findings are supported by the data demonstrating high correlation in expression of *TACI* at the RNA and protein levels[14,17] as well as with other genes critically involved in myeloma pathogenesis.[27,28,32]

The majority of *TACI^high* cases were associated with low-risk myeloma according to our recently reported 70-gene model[29] or molecularly classified subgroups with superior survival based on the 7-group model (Table 1).[28] Furthermore, the majority of *TACI^high* samples tested *in vivo* and all three *TACI^high* cases tested *ex vivo* responded to atacicept. Our *in vivo* study also identified exceptional *TACI^high* cases that responded to atacicept but were classified as high-risk myeloma (*in vivo* studies patients 2, 3 and to a lesser extent patient 6; *ex vivo* studies patient 13), while two *TACI^low* cases (*in vivo* studies patient 9; *ex vivo* studies patient 15) were molecularly classified in the low-risk group (Table 1). This suggests that some myeloma cells from cases with high-risk classifications may also still maintain a certain degree of dependency on the BM microenvironment compared with myeloma cells from most high-risk cases. If true, this refinement suggests that even some high-risk subtype patients may also benefit from microenvironment-targeted therapy. Intriguingly, two *in vivo* experiments with *TACI^low* myeloma (patients 9 and 11) showed a partial response to atacicept. The heterogeneous effect of atacicept may be related to yet unknown functions of APRIL and/or BAFF in myelomatous BM or confounding factors associated with the model systems.

In contrast to atacicept, BAFFR-Ig (which binds to BAFF but not APRIL) partially inhibited growth of myeloma cells *in vivo* and had no antitumor effect in cocultures. It has been demonstrated previously that *TACI*-expressing myeloma cells had a higher response to APRIL than to BAFF, and that certain myeloma cells responded to APRIL but not to BAFF.[14] Our study suggests that blocking BAFF by itself may not be sufficient to impact myeloma progression. However, since BAFF has been demonstrated to protect myeloma cells from drug-induced apoptosis, combination therapy of anti-BAFF compounds with antimyeloma agents such as dexamethasone and lenalidomide may help overcome *de novo* drug resistance.[16] Previous reports indicated that heparan sulfate proteoglycans (HSPGs) are important for APRIL-induced tumor growth by mediating the binding of APRIL to tumor cells and activating TACI signaling.[34,35] Syndecan-1 is produced and shed from myeloma plasma cells and is involved in myeloma cell metastasis and growth.[36–38] A recent study also demonstrated that syndecans, including syndecan-1, directly bind to tumor cells and activate TACI signaling.[39]

These studies may suggest that in contrast to BAFF, APRIL plays a nonredundant role in the survival of myeloma due to its interaction with syndecan-1 and possibly other HSPGs.

Myeloma is typically associated with increased osteoclast activity and severe osteolytic lesions in more than 80% of patients.[40] Recent experimental studies suggest that myeloma bone disease drives tumor progression.[24,30,41] Cultured osteoclasts alone support long-term survival of myeloma cells *in vitro*[22,42] and protect them from drug-induced apoptosis.[23,32] The molecular mechanisms by which osteoclasts affect myeloma cells are progressively being revealed. IL-6 and osteopontin, two myeloma growth factors produced by osteoclasts, play certain roles in osteoclast-induced myeloma growth.[22,42] We have reported that fibroblast activation protein (FAP), a cell-surface serine protease with dual dipeptidyl peptidase (DPP) and collagenase proteolytic activity, was consistently upregulated in cocultured osteoclasts and supported myeloma cell survival.[31] In the current study, we further confirmed that osteoclasts are a major source of APRIL and BAFF in myelomatous bone[17] and that the direct interaction between osteoclasts and myeloma cells results in upregulation of these survival factors by myeloma cells. The coculture experiments revealed that, as in our animal studies, growth of *TACI^{high}* myeloma cells was severely attenuated in the majority of cases by atacicept but not by BAFFR-Ig, suggesting that APRIL is a critical survival component in myeloma-osteoclast cocultures.

In summary, our studies in SCID-hu mice and myeloma-osteoclast cocultures have revealed that atacicept, and to a lesser extent BAFFR-Ig, effectively attenuates growth of *TACI^{high}* myelomas, the majority of which were molecularly classified into the low-risk, superior survival subgroups and are associated with a BM-dependence gene signature. Thus, myeloma patients whose tumors can be selectively classified according to high *TACI* gene expression may benefit from atacicept treatment.

Acknowledgments

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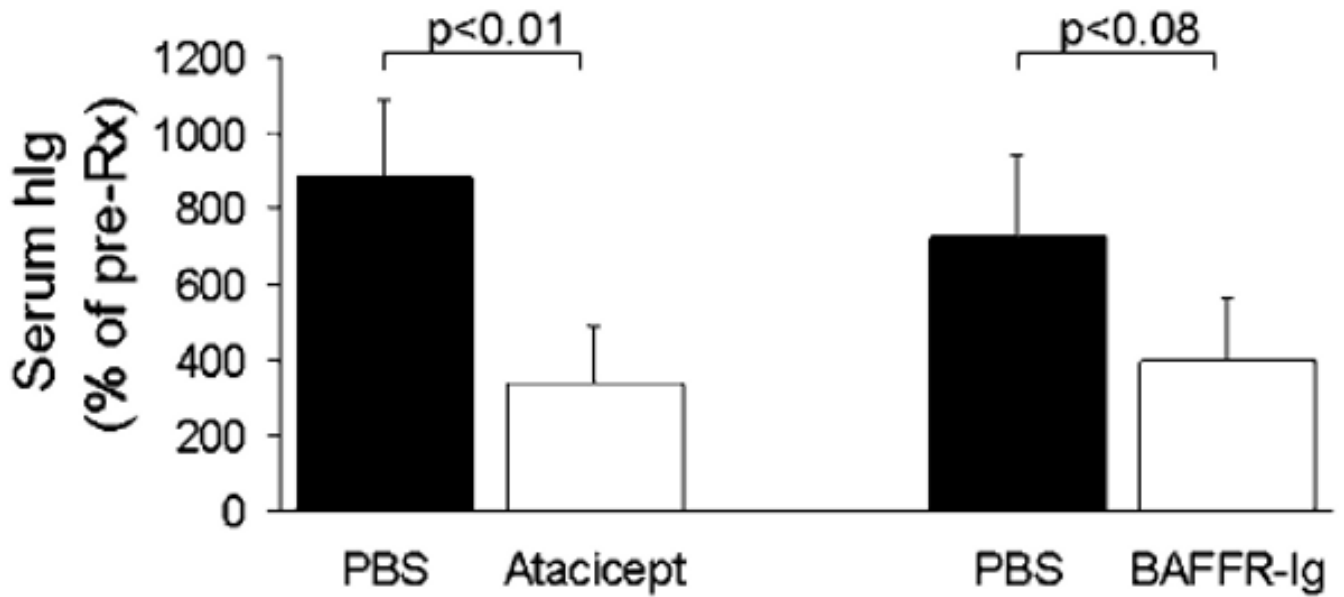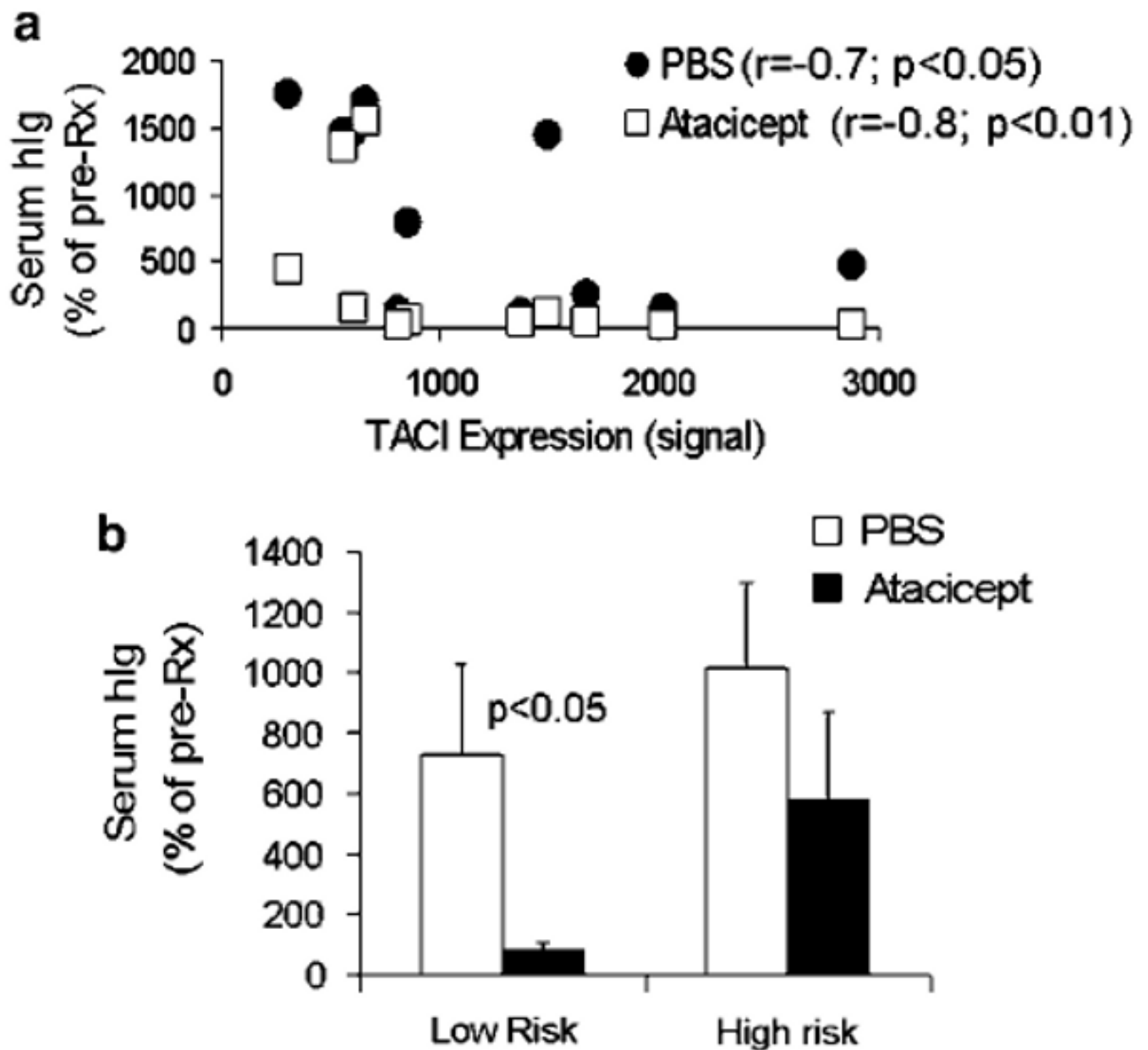
Figure 1.
Significant antimyeloma efficacy of atacicept, but not BAFFR-Ig, in SCID-hu mice. Myelomatous SCID-hu mice engrafted with myeloma cells from different patients were treated with atacicept ($n = 11$), BAFFR-Ig ($n = 9$) or PBS ($n = 11$) for 4–6 weeks. Serum hIg levels, reflecting tumor burden, were assessed by ELISA and expressed as percentage of pre-Rx level. BAFFR, B-cell activating factor of the TNF family receptor; PBS, phosphate-buffered saline.
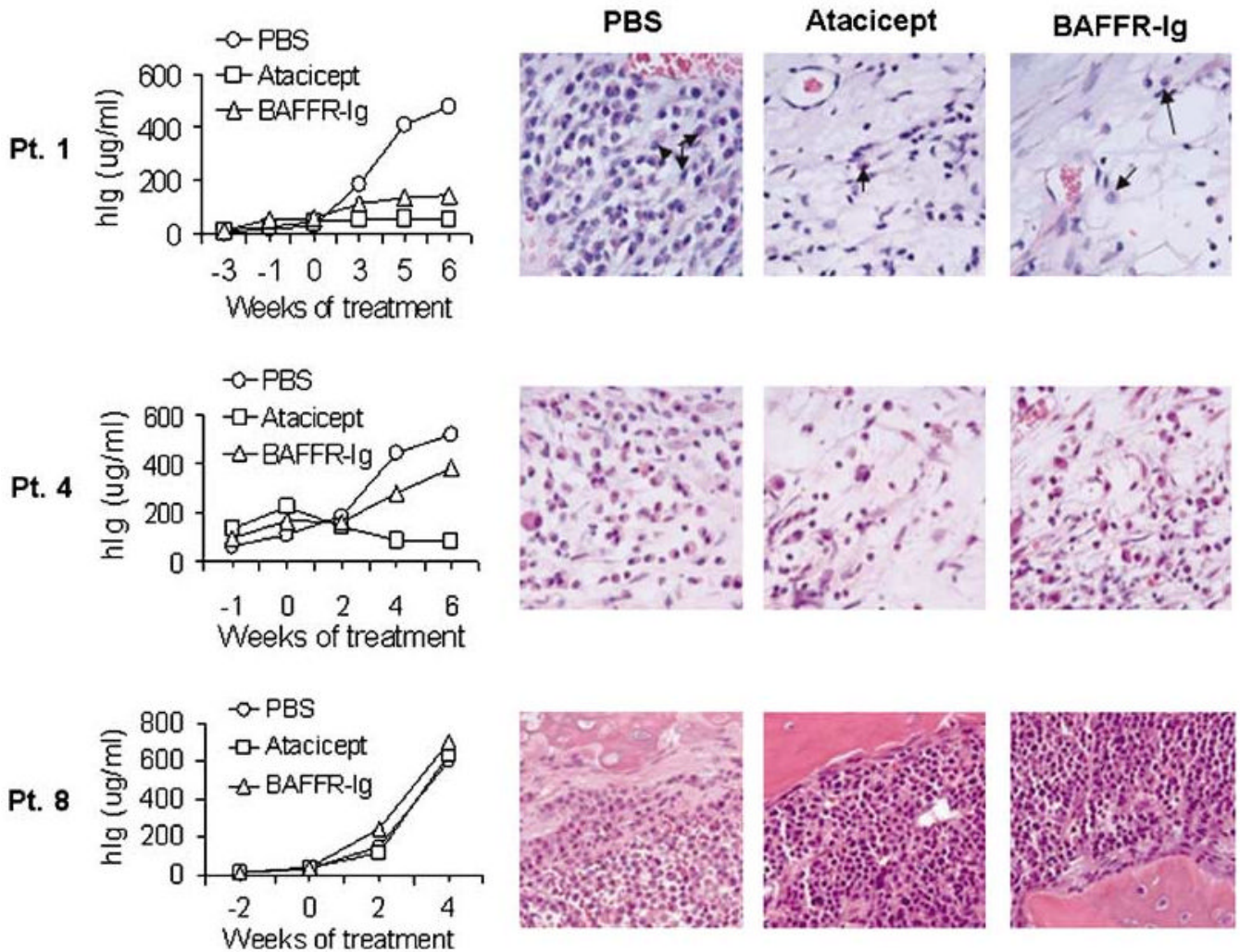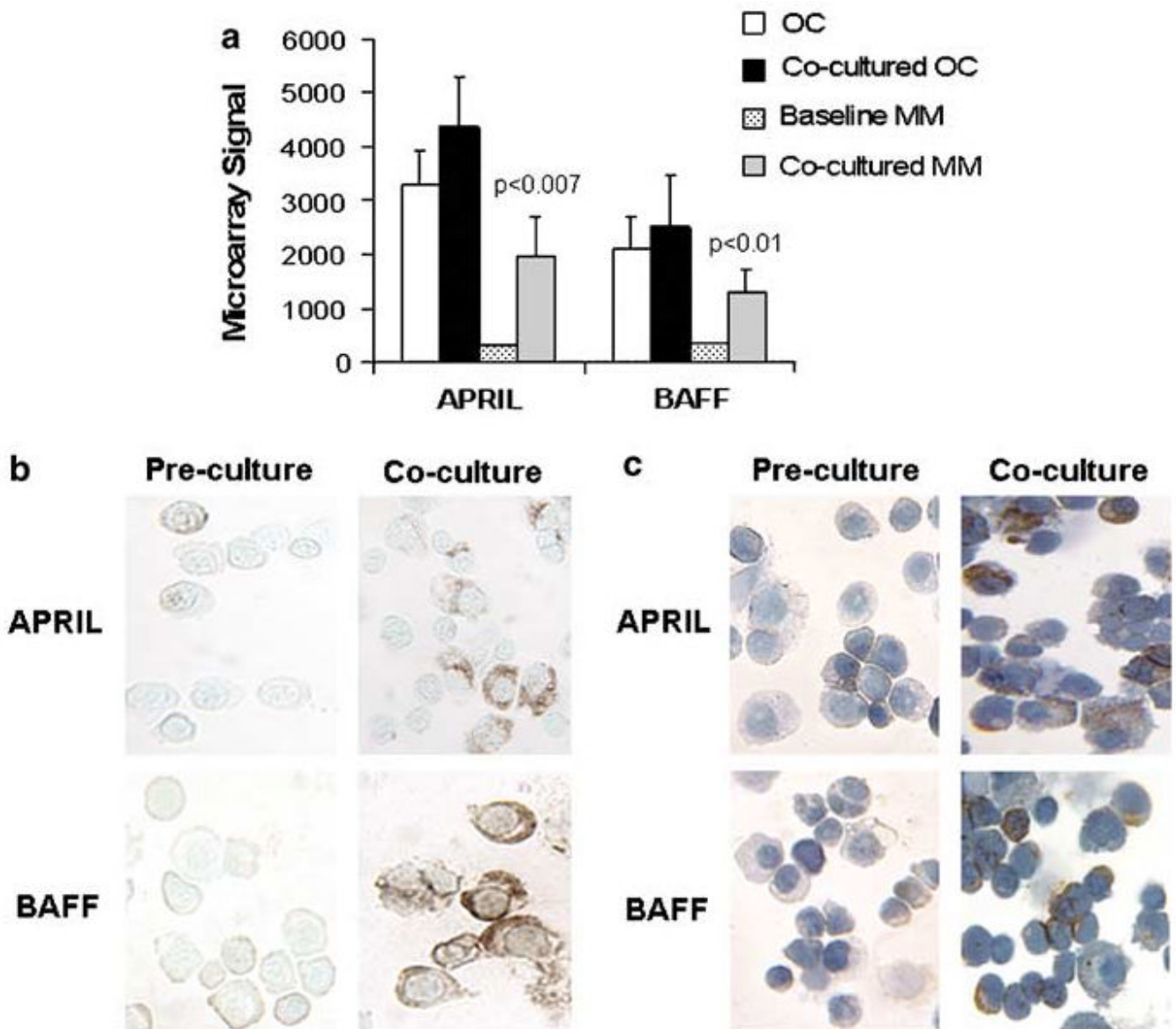
Figure 2.
Heterogeneous *in vivo* antimyeloma effect of atacicept and BAFFR-Ig. Three representative experiments (patients 1, 4 and 8) are shown, demonstrating time course effect on serum hIg (left panel) and histological examinations of myelomatous bone sections from the engrafted mice (right panel). Note parallel changes in hIg levels and myeloma cell infiltration of the implanted human bone in SCID-hu mice following treatment. Both atacicept and BAFFR-Ig inhibited growth of myeloma cells in patient 1 and had no effect on the growth of myeloma cells in patient 8. The myeloma cells in patient 4 responded to atacicept but not to BAFFR-Ig. Arrows indicate myeloma plasma cells. BAFFR, B-cell activating factor of the TNF family receptor.
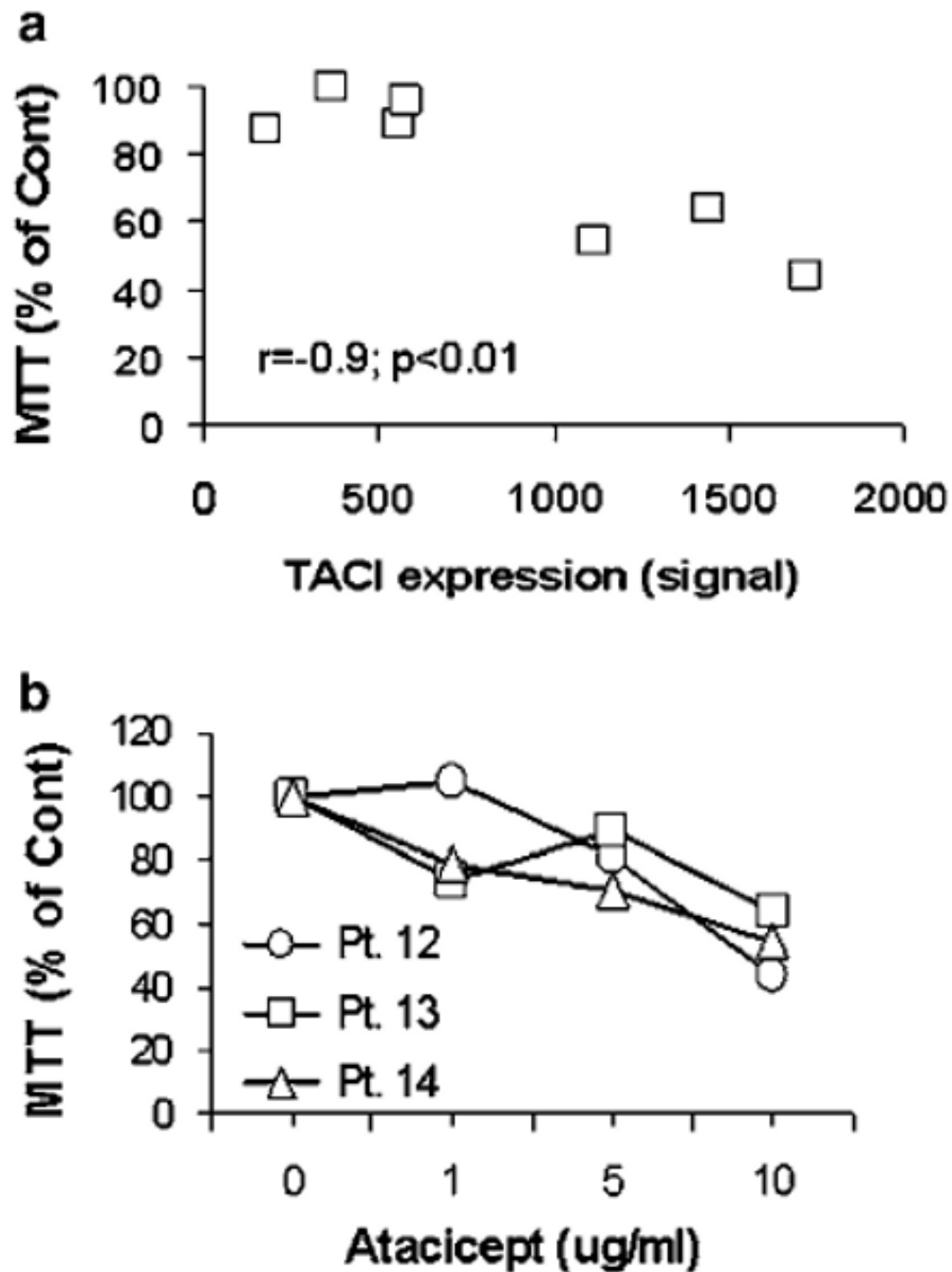
Figure 3.
Response to atacicept is associated with *TACI* gene expression in myeloma cells and is higher in low-risk myeloma. (**a**) Correlation coefficients (Spearman's rank test) between myeloma growth and *TACI* expression by myeloma cells in PBS- and atacicept-treated SCID-hu mice were −0.7 (*P*<0.05) and −0.8 (*P*<0.01), respectively. (**b**) Response to atacicept was determined in myeloma samples that were classified into low-risk (*n* = 5) and high-risk (*n* = 6) groups according to the 70-gene model.[29] Note that the low-risk myeloma were highly sensitive to the antimyeloma effect of atacicept.

Figure 4.
APRIL and BAFF expression and production in myeloma-osteoclast cocultures. (**a**) APRIL and BAFF gene expression in MM and osteoclasts (OC, $n = 8$) at baseline and after coculture as determined by microarray. Note the high expression of these cytokines by osteoclasts and their upregulation in myeloma cells after coculture. (**b, c**) Immunohistochemical staining for APRIL and BAFF in precultured and cocultured myeloma cells isolated from two patients. Note the increased production of these cytokines in cocultured myeloma cells. APRIL, a proliferation-inducing Ligand; BAFF, B-cell activating factor of the TNF family; MM, myeloma cells.

Figure 5.
Antimyeloma response of atacicept in myeloma-osteoclast cocultures. (**a**) Primary myeloma cells ($n = 7$) were cocultured with osteoclasts in the absence and presence of atacicept (10 μg ml^{-1}) for 5–7 days and then subjected to MTT assay. Percentage inhibition of myeloma cell growth is plotted against *TACI* gene expression determined by microarray analysis. Response to atacicept was highly correlated with *TACI* expression in myeloma cells ($r= -0.9$, *P*<0.01). (**b**) Atacicept-inhibited growth of *TACI*high myelomas (patients 12, 13 and 14; see Table 1) in a dose-related manner. MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

Table 1

Patients' characteristics, changes in hIg levels in control-, ataccept- and BAFFR-Ig-treated SCID-hu mice, and myeloma cell response to ataccept in coculture with osteoclasts[a]

In vivo studies

Patient/Stage[b]	Prior therapy	Isotype	70-gene model[c]	7-group model[d]	TACI signal[b]	BAFFR signal[e]	BCMA signal[f]	hIg (% of pretreatment)[g]		
								PBS	Ataccept	BAFFR-Ig
1 IIIa	NO	IgGκ	Low risk	Low bone	2882	254	17 034	471	36	240
2 IIIa	NO	IgAκ	High risk	MAF	2015	36	10 773	150	34	85
3 IIIa	NO	IgGλ	High risk	Proliferation	1669	22	15 759	244	54	114
4 IIIa	NO	IgGκ	Low risk	Myeloidy	1491	101	10 635	1448	120	244
5 IIa	NO	IgGκ	Low risk	Hyperdiploid	1370	37	17 630	120	51	120
6 IIIa	NO	IgGκ	High risk	CCND1-2	855	71	20 346	798	68	150
7 IIIa	NO	IqAλ	Low risk	CCND1-2	805	819	13 643	137	47	346
8 IIIa	NO	IgGκ	High risk	Proliferation	655	42	14 247	1699	1541	1710
9 IIIb	NO	IgGλ	Low risk	Low bone	599	104	11 589	1465	148	315
10 IIIa	NO	IgGλ	High risk	Proliferation	559	55	3985	1466	1354	ND
11 IIIa	YES	IgAλ	High risk	Proliferation	303	30	22 122	1747	439	ND

Ex vivo studies

Patient	Stage[d]	Prior therapy	Isotype	70-gene model[c]	7-group model[d]	TACI signal[h]	BAFFR signal[e]	BCMA signal[f]	MTT (% of control)[i]	
									Ataccept	BAFFR-Ig
12 IIIa	NO	IgGκ	Low risk	CCND1-2	1720	485	14 658	44	ND
13 IIIb	NO	IgGλ	High risk	Proliferation	1444	59	17 575	64	105
14 IIIa	NO	IgGλ	Low risk	Hyperdiploid	1113	56	16 244	54	ND
15 IIa	NO	IgGκ	Low risk	MMSET	574	820	15 418	96	93
10 IIIa	NO	IgGλ	High risk	Proliferation	559	55	3985	89	ND
16 IIIb	YES	IgGκ	High risk	Proliferation	362	108	13 740	100	102
17 IIIb	YES	Freeλ	High risk	Proliferation	175	28	3388	88	ND

Abbreviations: BAFFR, B-cell activating factor of the TNF family receptor; BCMA, B-cell maturation antigen; hIg, human Ig; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; SCID, severe combined immunodeficiency.

[a]Patients were numbered in each study according to *TACI* signal expression level.

[b]Stage at diagnosis, according to the Durie-Salmon staging system.

[c]Patients were segregated into low- and high-risk groups according to Shaughnessy et al.[29]

[d]Patients were segregated into low- and high-risk groups according to Zhan et al.[28]

[e]BAFFR expression was analyzed by microarray. Signal level >107 is considered high.

[f]BCMA expression was analyzed by microarray. Signal level > 15 000 is considered high.

[g]Circulating hIg was determined by ELISA and calculated as percent of pretreatment level (pre-Rx).

[h]TACI expression was analyzed by microarray. Signal level > 800 is considered *TACI*[high].

[i]Response to ataccept and BAFFR-Ig (10 μg ml^{-1}) was determined using MTT assay following 5–7 days of treatment. Results are expressed as percentage of control.