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To B(ortezomib) or Not to B: The Stroma's the Thing*

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Abstract

The proteasome inhibitor bortezomib has been widely used to treat patients with multiple myeloma (MM). However, some patients show primary or secondary resistance. In recent work published in *The Journal of Pathology*, Beyar-Katz *et al.* demonstrate that bortezomib treatment stimulates a host inflammatory response, which in turn promotes MM cell migration, viability and proliferation. These effects appear to be mediated by pro-inflammatory “M1”-like stromal macrophages partly via secretion of cytokine IL-16. These unexpected findings imply that the binary M1/M2 definition of macrophages may not accurately describe the complexity and heterogeneity of macrophages associated with MM tumor growth and progression and further suggest that bortezomib treatment stimulates host-driven tumor-promoting activity in addition to its cytotoxic activity, thus leading to potential bortezomib resistance in MM patients. Understanding the underlying mechanisms may identify novel targets to overcome or prevent bortezomib resistance.

Keywords

Multiple myeloma; bortezomib; proinflammatory macrophages; IL-16; host response

The proteasome inhibitor bortezomib is FDA-approved for the treatment of both multiple myeloma (MM) and mantle cell lymphoma in the first-line and relapsed/refractory settings. Bortezomib may be used as a single agent, but it is almost always used in combination with other chemotherapeutic agents such as lenalidomide or dexamethasone. Bortezomib has demonstrated improved outcomes across the spectrum of MM therapy, perhaps most notably in patients with high-risk cytogenetic features [1]. Nevertheless, decreased response rates with drug re-challenge reveal the phenomenon of treatment resistance. Primary and secondary resistance to bortezomib therapy is an emerging clinical problem. Elucidation of

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the underlying resistance mechanisms is critical to developing novel approaches to subvert or prevent refractory disease. A recent study showed that high expression of tight junction protein 1 (TJP1) in MM cells may serve as a biomarker for such resistance [2]. Beyond defects intrinsic to malignant plasma cells, Beyar-Katz and colleagues identified an unexpected mechanism by which bortezomib induces accumulation of pro-inflammatory macrophages that promote MM disease progression, reported in *The Journal of Pathology* [3].

As a first step, Beyar-Katz *et al.* examined host responses to bortezomib and found that plasma from bortezomib-treated non-tumor-bearing Balb/c mice induced migration and invasion by both mouse and human MM cell lines *in vitro*. In addition, bortezomib-treated plasmas exerted protective effects on MM cells when they were treated directly with bortezomib, but not when they were co-cultured with the anti-mitotic chemotherapeutic paclitaxel. Furthermore, they found that plasma from bortezomib-treated mice induced systemic angiogenesis, which is critical for plasma cell expansion. Taken together, the results demonstrate that bortezomib treatment in tumor-free mice induces pro-tumorigenic effects. To further support this notion, they performed *in vivo* experiments using SCID mice preconditioned with bortezomib; the pretreated mice had significantly higher MM tumor growth and mortality rates as compared with mice treated with vehicle. Similarly, mice challenged with MM cells pretreated with plasma from bortezomib-treated mice exhibited plasma cell tumor growth and metastasis in the lungs and bones.

Having demonstrated that the host response after bortezomib treatment contributes to MM cell aggressiveness, the authors explored the underlying mechanisms. Cytokine array analysis revealed that IL-6, IL-10 and amphiregulin levels were significantly increased in the plasma of bortezomib-treated mice. However, none of these seemed to contribute directly to plasma cell aggressiveness. Surprisingly, pro-inflammatory macrophages were significantly increased in Matrigel plugs containing plasma from bortezomib-treated mice. Depletion of macrophages with liposomal clodronate completely abrogated the enhanced mortality from bortezomib preconditioning. These macrophages express some conventional M1-like markers such as CCL4, IL-6 and IL-1 β but reduced or absent levels of IL-10 and IL-4. This result is notable, since M1-like macrophages have been considered to be tumoricidal, while M2-like macrophages promote tumor progression and metastasis [4]. The presented data suggests that the binary M1/M2 definition of macrophages does not accurately describe the complexity and heterogeneity of macrophages associated with MM tumor growth and progression [5]. More important and accurate descriptions of macrophages may rely on their functional activity. Indeed, those seemingly “M1-like” pro-inflammatory macrophages from bortezomib-treated mice promote mouse mortality and bone metastasis of plasma cells.

Why does bortezomib instigate the accumulation of proinflammatory M1-like macrophages and the subsequent promotion of MM cell aggressiveness? One potential link could be the cytokine IL-16, since these macrophages secrete high levels of IL-16; IL-16 plasma levels were likewise elevated in bortezomib-treated mice. The addition of IL-16-neutralizing mAb to the conditioned medium of macrophages from bortezomib-treated mice significantly reduced MM cell viability. However, exogenous addition of IL-16 did not promote plasma cell proliferation. The authors argued that this may be due to IL-16 secretion from MM cells,

which could be saturated in the co-culture system. If this is the case, specific knockdown of IL-16 from MM cells should directly address this issue. The relative contribution of IL-16 from macrophages of bortezomib-treated mice in mediating enhanced plasma cell aggressiveness and metastasis is still unclear. Taken together, the results show that bortezomib treatment induces the mobilization of pro-inflammatory macrophages in addition to its cytotoxic effect directly on MM cells. These macrophages may promote MM cell aggressiveness and survival via the secretion of IL-16 and promotion of angiogenesis.

In the course of their work, the authors pose a fascinating and troubling paradox: why does bortezomib, a proteasome inhibitor with a solid reputation as a lymphotoxic and anti-inflammatory molecule, drive MM cell aggressiveness and inflammatory macrophage activity? Bortezomib negatively impacts nuclear factor kappa B (NF κ B) signaling, a master regulator of anti-apoptotic and pro-inflammatory cellular behavior. The answer may reside in the malignant plasma cell's microenvironment, or stroma.

The malignant plasma cell cannot prosper without a "nurturing" stroma, and stromal macrophages and mesenchymal stem cells are required ingredients for MM cells to prosper in their niche [6]. While bortezomib is directly toxic to MM cells, it has a paradoxical effect on stromal macrophages, causing increased IL-6 and IFN- γ levels, which accounts for the clinical phenomenon of febrile response after drug administration [7]. Bortezomib may also upregulate IL-8 in monocyte/macrophage lineage-derived cells. Thus, bortezomib has the undesirable potential to exacerbate stromal inflammation through its effects on macrophages, which may provide a more nurturing microenvironment for MM cells. That paradigm explains why pretreatment of *healthy* mice with bortezomib actually harmed them when they were injected with MM cells. Parallel outcomes have resulted from other tumor models. In a murine lung cancer model, bortezomib stimulated macrophage activity and production of IL-1 β and chemokines CXCL1 and 2, leading to tumor amplification [8].

Bortezomib "gums up" the proteasome, resulting in accumulation of improperly folded proteins and proteotoxic cellular stress; the unfolded protein response (UPR) is triggered, leading to apoptosis. The UPR may play an unwitting role in bortezomib's resistance-promoting activity. The UPR drives macrophages to produce IL-1 β [9] and the ER stress response and UPR drive production of proinflammatory cytokines [10]. Therefore, intrinsic to bortezomib's mechanism of action are off-target effects that promote an inflammatory stroma.

The authors' findings regarding IL-16 are aligned with other studies. IL-16 promotes a variety of malignancies, including MM [11]. Furthermore, IL-16 expression is increased in MM bone marrow stroma from patients. The bone marrow environment in MM patients showed elevated concentrations of several cytokines and tumor growth factors, including IL-16 [12]. Those results support the authors' findings and a model of resistance in which bortezomib turns on the UPR and antagonizes NF κ B signaling, stimulating proinflammatory stromal macrophages to nurture malignant plasma cells through elaboration of IL-16 into the microenvironment.

Therefore, it is likely that direct tumor killing dominates at the beginning of bortezomib treatment in MM patients, leading to high overall response rates. On the other hand, bortezomib treatment also induces host responses that promote angiogenesis and MM proliferation and survival, resulting in a final outcome of bortezomib resistance (Figure 1). However, it would be premature to make recommendations regarding bortezomib treatment in MM patients. Clinical studies are needed to further confirm the authors' hypothesis using human MM samples. This is particularly important given the fact that recent studies of bortezomib resistance have yielded inconclusive outcomes or non-statistically significant outcomes. Plasma from newly-diagnosed MM patients treated with bortezomib, dexamethasone, and cyclophosphamide showed a non-significant trend towards enhanced human MM cell viability [3]. In addition, myeloid cell accumulation in MM patients is independent of bortezomib treatment.

A particularly useful study design would be the comparison of host responses in MM patients in the initial treatment versus the relapsed/refractory setting. That study would examine whether plasma samples from newly-diagnosed MM patients treated with bortezomib have tumor-promoting capabilities and concomitant increased IL-16 levels. More interestingly, cytokine/chemokine profiles from same patients who are treated with bortezomib in the different settings may help determine whether bortezomib treatment indeed stimulates human stromal host responses, in turn promoting MM progression and invasiveness. These studies will provide insight into strategies for overcoming bortezomib resistance and whether proteasome inhibitors should be used in consecutive lines of therapy. There is no debate on whether to use bortezomib—the issue is how to overcome resistance. To paraphrase Shakespeare, the stroma's the thing wherein we'll improve treatment engineering.

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Figure 1. Host stromal response mediated by bortezomib treatment results in MM aggressiveness and potential bortezomib resistance

Bortezomib treatment induces proteotoxic cellular stress and the unfolded protein response (UPR), leading to MM cell death/apoptosis. Stromal proinflammatory macrophages are also activated to secrete chemokines/cytokines such as IL-16 to promote angiogenesis and increase MM cell invasiveness, viability and proliferation, leading to potential bortezomib resistance.