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Prospects of combining adoptive cell immunotherapy with bortezomib

“...bortezomib could serve as an effective combinatorial treatment along with other approaches of cancer immunotherapy to break tumor-induced tolerance in CD8⁺ T cells and elicit a robust antitumor immune response in cancer patients.”

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Bortezomib, also known as PS-341 and Velcade™, is a front-line drug approved in the USA and EU for the treatment of multiple myeloma [1] and mantle cell lymphoma [2]. Its use has been extended lately to advanced stage non-small-cell lung cancer [3]. Bortezomib inhibits the ubiquitin-proteasome proteolytic pathway, which cancer cells rely heavily upon due to their high rate of aberrant protein generation and need of rapid protein turnover. Therefore, interference with the proteasome system became an attractive therapeutic strategy against cancer. Intracellular pathways requiring protein degradation for activation, such as the NFκB pathway, which maintain cancer cell resistance to apoptosis, are also one of the targets of this drug. The reversible nature of proteasome inhibition by bortezomib sets it apart from other commercially available proteasome inhibitor drugs. While bortezomib's boronic acid moiety prevents it from being transported out of the cell by the multidrug resistance system, its transient half-life (9–15 h) makes it tolerable to patients when given at the doses of 1 mg/kg body weight, approximately 15–20 nM by blood volume. Bortezomib can sensitize solid tumor cells to apoptosis by amplifying tumor cell caspase-8 activation in the death-inducing signaling complex following death receptor ligation by death ligands such as TRAIL or its receptor agonist monoclonal antibodies [4,5]. Unfortunately, clinical trials in patients with solid tumors showed that bortezomib lacks therapeutic efficacy as a

single agent. Moreover, there were concerns over the negative side effects reported on immune cells, particularly on CD4⁺ T cells and dendritic cells, when using high doses of bortezomib [6–8].

New data show that administration of the proteasome inhibitor bortezomib (1 mg/kg body weight; ≤20 nM) has complementary immunostimulatory effects following adoptive transfer of CD8⁺ T cells in mice bearing kidney or mammary adenocarcinomas. It was observed that lymphocyte effector functions were significantly improved following bortezomib treatment by mechanisms involving the upregulation of multiple Notch components and lymphocyte activation signaling molecules [9,10]. These results are in contrast with the effects of bortezomib observed *in vitro* where the doses used were high (>100 nM) – doses that cannot be extrapolated to the therapeutic situation *in vivo*. Only 15–20 nM concentrations of bortezomib are found to be tolerable *in vivo* in both mice and humans [5,11]. Growing evidence suggests that bortezomib given at 15–20 nM doses exhibits an intrinsic ability to increase the levels of immunostimulatory cytokines, and components of Notch and NFκB signaling pathways in lymphocytes, thereby amplifying their effector functions either directly or indirectly. Thus, bortezomib could be an attractive candidate for exploration in combination with antitumor T-cell and/or natural killer cell adoptive therapy [12,13].

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Augmentation of lymphocyte-stimulatory cytokine signaling in the tumor environment by bortezomib opens the possibility of overcoming tumor-induced suppression of numerous immune regulatory networks, which renders immunotherapy inefficient. Recently published studies evaluated therapeutic efficacy of the combination of bortezomib and adoptive T-cell transfer in mice with established renal or mammary adenocarcinoma tumors, which expressed defined low-avidity epitopes of model antigen hemagglutinin (HA) [14]. Notably, adoptive transfer of CD4⁺ T or CD8⁺ T cells from MHC class I and II-restricted HA-specific T-cell receptor-transgenic Cln4 and 6.5^{+/+} mice, respectively, followed by bortezomib administration resulted in reduced numbers of metastatic tumor pulmonary nodules and enhanced survival of tumor-bearing mice. No negative effect on the number and viability of CD4⁺ or CD8⁺ T lymphocytes, or tissue-resident CD11c⁺CD8⁺ dendritic cells was observed in tumor-bearing mice. Nor did bortezomib affect the expression of costimulatory molecules on dendritic cells or MHC class I or II-associated antigen presentation to HA-specific T cells. In addition to direct antitumor effects, bortezomib had stimulatory effects on the host immune system. Data also suggest that bortezomib stabilized expression of IL2 receptor- α and intracellular T-cell receptor CD3 ζ chains, enhanced IFN- γ production and sustained FasL-mediated CD8⁺ T-cell cytotoxicity in tumor-bearing mice. These effects correlated with increased phosphorylation of nuclear and cytoplasmic NF κ B p65 levels in CD8⁺ T cells, likely due to the inhibition of their proteasomal degradation by bortezomib. Moreover, a high surface expression of Fas was observed on tumor cells in bortezomib-administered mice, and in human melanoma tissue from a responsive patient who received combination therapy that included bortezomib. These findings underscore the high clinical potential of an optimized regime of bortezomib administration for enhancing CD8⁺ T-cell function, thereby improving the outcome of adoptive T-cell therapy of cancer.

“...bortezomib can act as a safe and potent immunotherapeutic drug in sustaining antitumor immune effector functions and in addressing tumor-associated immunosuppression...”

Another important lymphocyte-stimulatory feature of bortezomib administration that was observed in these studies was the upregulation of the components of Notch signaling that plays a crucial role in functional differentiation and maturation of T cells among several other key biological processes [10]. Tumors downregulate Notch signaling in lymphocytes to

suppress their effector function [15]. In various mouse tumor models, analysis of endogenous antitumor CD8⁺ T-cell response after bortezomib treatment showed that bortezomib can rescue tumor-induced downregulation of Notch in lymphocytes while enhancing their effector function. CD8⁺ T cells in tumor-bearing mice following bortezomib administration not only showed increased Notch1/2 activation but also exhibited increased expression of T-cell activation molecules CD25 and CD44, with significantly improved production of IFN- γ and expression of eomesodermin, perforin and granzyme-B. T-box transcription factor eomesodermin is not only important for the differentiation of cytolytic CD8⁺ T cells, but also maintains the memory CD8⁺ T-cell repertoire [16]. These bortezomib-mediated enhancements of CD8⁺ T-cell effects associate with increased crosstalk between Notch and NF κ B signaling pathways important for T-cell cytolytic function [17,18]. The findings predict that bortezomib can act as a safe and potent immunotherapeutic drug in sustaining antitumor immune effector functions and in addressing tumor-associated immunosuppression if well optimized in conjunction with other immune therapies.

Immunosuppressive chronic inflammation in the tumor microenvironment dampens immune effector response by interfering with immunostimulatory Notch and cytokine signaling. This diminishes the outcome of anticancer therapeutic protocols. It was observed that bortezomib affected cytokine production in the tumor microenvironment, thereby polarizing the cytokine milieu in favor of antitumor T-cell immunity to reduce tumor burden. Using a therapeutic regimen that mimics the clinic [5], bortezomib administration in tumor-bearing mice at a dose of 1 mg/kg body weight increased the levels of immunostimulatory cytokines IL-2, IL-12 and IL-15 and enhanced their downstream signaling by increasing the expression of their receptors on tumor-infiltrating CD8⁺ T cells in both endogenous or adoptive transfer of CD8⁺ T cells specific to a low-avidity HA_{518–526} epitope, resulting in reduced pulmonary metastatic nodules [19]. Specifically, bortezomib treatment increased IL-2 production by CD4⁺ T cells, IL-12 and IL-15 production from CD11c⁺ dendritic cells, and enhanced the expression of IL-2 receptor- α chain, IFN- γ , granzyme-B, as well as the transcription factor eomesodermin on endogenous or adoptively transferred HA-specific CD8⁺ T cells from bortezomib-treated tumor-bearing mice. These effects of bortezomib treatment were supported by the activation of downstream PI3K/Akt/STAT5 pathways observed in CD8⁺ T cells following bortezomib treatment. Interestingly, bortezomib treatment restored IFN- γ synthesis and inhibited metastasis in tumor-bearing mice

even in the presence of neutralizing antibodies to IL-12 and IL-15. This indicates that bortezomib can activate alternative effector pathways that could strengthen tumor immune surveillance. These observations highlight the immunostimulatory potential of bortezomib with a capacity to sustain CD8⁺ T-cell effector function in the tumor microenvironment of solid tumors independent of the type of tumor.

Therefore, besides the established role of bortezomib in sensitizing tumors to cell death, it may act as a multifaceted immunomodulatory drug and can provide immune stimulatory effects along with immunotherapeutic strategies such as adoptive T-cell therapy. Moreover, by suppressing constitutive activation of NFκB in tumor cells, it also abrogates their resistance to cell death [20]. By its direct antitumor activity and intrinsic nature to modulate immune cells, bortezomib acts to release brakes on the immune system from the tumor-associated suppressive microenvironment, thereby making the combination therapy including bortezomib potentially more efficient than either therapy alone. Therapeutic restoration of lymphocytic Notch signaling and effector function could enhance antitumor

immune surveillance following a carefully optimized bortezomib regimen, which could abrogate tumor resistance to cytolysis and overcome tumor-associated immunosuppression. Thus, by restoring the Notch–NFκB mediated effector signaling and increasing the immunostimulatory signaling via cytokines such as IL-2, IL-12 and IL-15 in antitumor CD8⁺ T cells, bortezomib could serve as an effective combinatorial treatment along with other approaches of cancer immunotherapy to break tumor-induced tolerance in CD8⁺ T cells and elicit a robust antitumor immune response in cancer patients.

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