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Productively combining proteasome inhibition with the immunotherapy of cancer

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For a number of years, there has been much enthusiasm for harnessing the immune system in order to improve cancer therapy. Immunotherapy of cancer patients has demonstrated that T cells (particularly CD8⁺ T cells) play a critical role in mediating antitumor responses. However, to date, these immunotherapeutic strategies have only had a modest level of success in cancer patients [1]. Advances in the understanding of antigen recognition by T cells, plus improved technology, has resulted in the detection of specific T cells in cancer patients that recognize tumor antigens. Consequently, it has been assumed that if these cells could be adequately expanded and triggered for effector function by appropriate vaccination, a therapeutic benefit to cancer patients would ensue. Unfortunately, although the presence of large numbers of these antitumor T cells could be unequivocally demonstrated following vaccination, this has not translated into a significant therapeutic benefit [2]. An alternative approach has been the isolation of T cells from patients, followed by their expansion and activation *ex vivo* and subsequent adoptive transfer back into tumor-bearing patients. Once again, although significant responses were observed in a small number of patients with renal cancer or melanoma, most patients did not show durable responses to this adoptive transfer regimen. This led to elegant studies in mice to determine how the antitumor efficacy of adoptive T cell transfer could be optimized, particularly in the context of a large tumor burden. These findings were then applied in the design of clinical trials for treatment of cancer patients with adoptive immunotherapy. Recently, it has been reported that response rates of up to 50% can be obtained in melanoma patients, with adoptive cell transfer of T cells and interleukin-2 (IL-2) following non-myeloablative chemotherapy. This suggests that if the immune response to cancer cells is triggered in an appropriate manner, significant antitumor effects are possible [1,3].

One of the major problems in generating effective immune responses to tumors is the immunosuppression that is associated with tumor development or the presence of a large tumor burden. These immunosuppressive effects prevent the appropriate activation of antitumor T cells, resulting in a state of tolerance. Thus, tumor-specific T cells can be present, yet only possess a very limited functional capacity. These immunosuppressive effects can be derived from the cancer cells themselves, or the tumor microenvironment that develops during tumor growth and progression. A number of immunosuppressive factors have been described in the literature and these include tumor gangliosides, immunosuppressive members of the B7-H family, immunosuppressive T regulatory cells, myeloid-derived suppressive cells (MDSC), arginase-producing cells and various

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immunosuppressive cytokines such as IL-10 or TGF-beta. Thus, the tumor and its microenvironment can generate a suppression of both local and systemic immune responses [4]. It therefore seems logical that these immunosuppressive effects must be targeted and overcome in order for immunotherapy to succeed. This, however, remains a daunting task, particularly if these immunosuppressive effects are multiple and their mechanisms of action are somewhat redundant. One direct way to circumvent these immunosuppressive effects of the tumor could be to dramatically reduce the tumor burden. As such, the immunosuppressive effects emanating from the tumor might also be reduced. However, at present the best ways to reduce tumor burden are surgery, radiation and chemotherapy. These treatments are inherently immunosuppressive in their own right, so it is difficult to envisage how they can be optimally combined with immunotherapy in most instances.

Encouragingly, in the last few years, significant advances have been made, identifying novel agents that more specifically target cancer cells, and thus exhibit less undesirable side-effects. One such agent, that has unexpectedly provided some direct therapeutic benefit in cancer patients, has been the proteasome inhibitor bortezomib [5]. Bortezomib (Velcade) is a dipeptide boronic acid analog that is a potent specific and reversible proteasome inhibitor. Surprisingly, levels of proteasome inhibition of 70–80%, which are tolerated by normal non-transformed cells, prove to be fatal to some tumor cells. Thus, a narrow therapeutic window exists in which the anticancer effects of bortezomib administration can occur in the absence of unwanted toxicities. A substantial therapeutic benefit was demonstrated for bortezomib administration in patients with multiple myeloma, and it gained FDA approval for the treatment of multiple myeloma in 2003. Bortezomib's activity in a wide range of other cancers is currently being assessed. Thus, bortezomib could potentially be combined with immunotherapeutic regimens, particularly if its immunosuppressive effects were limited. Ideally, such a combination of bortezomib and immunotherapy would result in the generation of T cell memory, thus, also limiting reoccurrence of the tumor.

The study by Tseng et al. [7] has provided encouraging preliminary data that this type of approach may have some merit. Previous studies by Kim et al. [6] had described elegant antitumor vaccination strategies directed against the E7 antigen of the human papilloma virus (HPV). These strategies involved the administration of DNA vaccines using a gene gun, to deliver the vaccine to the dendritic cells of the skin. The optimal DNA constructs contained a modified non-transforming version of the E7 protein, combined with immune-enhancing CpG sequences and a gene for the calcium-binding protein calreticulin. It was thought that, following gene gun delivery of the DNA vaccine, calreticulin functioned to improve the uptake and trafficking of the E7 protein by the dendritic cells. Immunization with this DNA vaccine resulted in enhanced immune responses directed against E7 and some therapeutic effects in vivo against a tumor TC-1 that expressed the E7 antigen. Tseng et al. [7] coupled bortezomib treatment of the mice to this vaccination strategy (Fig. 1), to show that the combination provided a far superior antitumor effect than either agent alone in the TC-1 tumor model. Although there is the caveat that this is a relatively small tumor burden and the E7 protein is inherently immunogenic, the therapeutic benefit of the combination was clearly superior to either agent alone. More importantly, these regimens seemed to cooperate in vivo, since bortezomib treatment of tumor-bearing mice prior to DNA vaccination resulted in an increased number of E7-specific CD8⁺ T cells when compared to DNA vaccination alone. Interestingly, in the absence of tumor this did not occur, suggesting that it was the bortezomib effects on the tumor that improved the immune response to DNA vaccination. It seems likely that the initial bortezomib treatment resulted in apoptotic destruction of the tumor cells, causing an increased release of tumor antigens. These antigens (e.g., E7) were then subsequently taken up by antigen-presenting dendritic cells and presented to CD8⁺ T cells initiating an immune response (Fig. 2). This response was then further amplified following DNA vaccination with E7. The increased numbers of

effector CD8⁺ T cells were then capable of destroying the remaining tumor cells. However, previous studies injecting tumor lysates have not always generated significant immune responses; so, is there anything special about the way bortezomib kills tumor cells in vivo?

During the last few years there have been a number of provocative studies suggesting that the manner of tumor cell death can determine how the immune system responds. Obeid et al. [8] reported that if tumor cells in mice were killed by treatment with anthracyclines, then an antitumor immune response developed. However, in the same mouse model, tumor destruction with other DNA-damaging agents did not result in the development of antitumor immunity. Intriguingly, the molecular basis of this immunogenic cell death appeared to be the presence of calreticulin on the surface of the dying tumor cells. Thus, it was suggested that only when calreticulin was expressed on the surface of the dying tumor cells was the death perceived as immunogenic. Other studies have shown that heat-shock proteins such as HSP70 or HSP90 as well as calreticulin can also promote an immune response by targeting tumor antigens to dendritic cells [9]. In this context, it was recently reported that bortezomib treatment of multiple myeloma cells increased cell surface levels of HSP90 on dying tumor cells, promoted the uptake of tumor antigens by DC and improved generation of antitumor immunity [10]. Thus, the possibility that bortezomib can preferentially promote an immunogenic form of death in cancer cells is certainly worthy of further investigation.

Nonetheless, proteasome inhibition results in many diverse biological effects [5]. Indeed, there are reports that bortezomib can also have inhibitory effects on the functions of dendritic cells [11]. In addition, administration of bortezomib has been shown to be immunosuppressive enough to block graft-versus-host disease in vivo [12]. Therefore, how can these immunosuppressive effects of bortezomib be reconciled with enhancing immune responses to cancer vaccines? One possible answer to this may lay in the timing of bortezomib administration. Khan et al. [13] showed that bortezomib could be successfully combined with cytokine-based immunotherapy in a mouse model of neuroblastoma, but appropriate scheduling of bortezomib administration was crucial. In the study of Tseng et al. [7], bortezomib was administered prior to the DNA vaccine. It is likely that bortezomib administration promoted both tumor destruction and antigen release. However, since the effects of bortezomib are transient, any deleterious effects on the immune response to subsequent DNA vaccination may have been minimized.

Interestingly, bortezomib has also been shown to sensitize tumor cells to the cytotoxic effects of TNF-related apoptosis-inducing ligand (TRAIL) and natural killer (NK) cells in vitro [14–16]. Furthermore, bortezomib, in combination with agonist antibodies to the TRAIL receptor DR5, has been shown to provide therapeutic benefit in mouse tumor models [17]. The cell death induced by the combination of bortezomib and TRAIL was clearly apoptotic; however, whether it was also immunogenic was not investigated in these studies. It is encouraging that bortezomib may potentially be combined with immunotherapy in a number of different ways, either potentiating the generation of an immune response or sensitizing tumor cells to immune effector cells. However, the relative importance of these multiple effects of bortezomib for therapeutic benefit, as well as the optimal timing of administration of this proteasome inhibitor during immunotherapy still remains to be determined.

References

1. Gattinoni L, Powell DJ Jr, Rosenberg SA, Restifo NP. Adoptive immunotherapy for cancer: building on success. *Nat Rev Immunol.* 2006; 6:383–393. [PubMed: 16622476]
2. Rosenberg SA, Yang JC, Restifo NP. Cancer immunotherapy: moving beyond current vaccines. *Nat Med.* 2004; 10:909–915. [PubMed: 15340416]

3. Dudley ME, Wunderlich JR, Yang JC, Sherry RM, Topalian SL, Restifo NP, Royal RE, Kammula U, White DE, Mavroukakis SA, Rogers LJ, Gracia GJ, Jones SA, Mangiameli DP, Pelletier MM, Gea-Banacloche J, Robinson MR, Berman DM, Filie AC, Abati A, Rosenberg SA. Adoptive cell transfer therapy following non-myeloablative but lymphodepleting chemotherapy for the treatment of patients with refractory metastatic melanoma. *J Clin Oncol.* 2005; 23:2346–2357. [PubMed: 15800326]
4. Zou W. Immunosuppressive networks in the tumour environment and their therapeutic relevance. *Nat Rev Cancer.* 2005; 5:263–274. [PubMed: 15776005]
5. Adams J, Palombella VJ, Sausville EA, Johnson J, Destree A, Lazarus DD, Maas J, Pien CS, Prakash S, Elliott PJ. Proteasome inhibitors: a novel class of potent and effective antitumor agents. *Cancer Res.* 1999; 59:2615–2622. [PubMed: 10363983]
6. Kim JW, Hung CF, Juang J, He L, Kim TW, Armstrong DK, Pai SI, Chen PJ, Lin CT, Boyd DA, Wu TC. Comparison of HPV DNA vaccines employing intracellular targeting strategies. *Gene Ther.* 2004; 11:1011–1018. [PubMed: 14985791]
7. Tseng CW, Monie A, Wu CY, Huang B, Wang MC, Hung CF, Wu TC. Treatment with proteasome inhibitor bortezomib enhances antigen-specific CD8+ T-cell-mediated antitumor immunity induced by DNA vaccination. *J Mol Med.* 2008 Jun 10. [Epub ahead of print] doi:[10.1007/00109-008-0370-y](https://doi.org/10.1007/00109-008-0370-y).
8. Obeid M, Tesniere A, Ghiringhelli F, Fimia GM, Apetoh L, Perfettini JL, Castedo M, Mignot G, Panaretakis T, Casares N, Metivier D, Larochette N, van EP, Ciccocanti F, Piacentini M, Zitvogel L, Kroemer G. Calreticulin exposure dictates the immunogenicity of cancer cell death. *Nat Med.* 2007; 13:54–61. [PubMed: 17187072]
9. Tesniere A, Panaretakis T, Kepp O, Apetoh L, Ghiringhelli F, Zitvogel L, Kroemer G. Molecular characteristics of immunogenic cancer cell death. *Cell Death Differ.* 2008; 15:3–12. [PubMed: 18007663]
10. Spisek R, Charalambous A, Mazumder A, Vesole DH, Jagannath S, Dhodapkar MV. Bortezomib enhances dendritic cell (DC)-mediated induction of immunity to human myeloma via exposure of cell surface heat shock protein 90 on dying tumor cells: therapeutic implications. *Blood.* 2007; 109:4839–4845. [PubMed: 17299090]
11. Nencioni A, Garuti A, Schwarzenberg K, Cirmena G, Dal BG, Rocco I, Barbieri E, Brossart P, Patrone F, Ballestrero A. Proteasome inhibitor-induced apoptosis in human monocyte-derived dendritic cells. *Eur J Immunol.* 2006; 36:681–689. [PubMed: 16479541]
12. Sun K, Welniak LA, Panoskaltsis-Mortari A, O'Shaughnessy MJ, Liu H, Barao I, Riordan W, Sitcheran R, Wysocki C, Serody JS, Blazar BR, Sayers TJ, Murphy WJ. Inhibition of acute graft-versus-host disease with retention of graft-versus-tumor effects by the proteasome inhibitor bortezomib. *Proc Natl Acad Sci USA.* 2004; 101:8120–8125. [PubMed: 15148407]
13. Khan T, Stauffer JK, Williams R, Hixon JA, Salcedo R, Lincoln E, Back TC, Powell D, Lockett S, Arnold AC, Sayers TJ, Wigginton JM. Proteasome inhibition to maximize the apoptotic potential of cytokine therapy for murine neuroblastoma tumors. *J Immunol.* 2006; 176:6302–6312. [PubMed: 16670342]
14. Sayers TJ, Brooks AD, Koh CY, Ma W, Seki N, Raziuddin A, Blazar BR, Zhang X, Elliott PJ, Murphy WJ. The proteasome inhibitor PS-341 sensitizes neoplastic cells to TRAIL-mediated apoptosis by reducing levels of c-FLIP. *Blood.* 2003; 102:303–310. [PubMed: 12637321]
15. Hallett WH, Ames E, Motarjemi M, Barao I, Shanker A, Tamang DL, Sayers TJ, Hudig D, Murphy WJ. Sensitization of tumor cells to NK cell-mediated killing by proteasome inhibition. *J Immunol.* 2008; 180:163–170. [PubMed: 18097016]
16. Lundqvist A, Abrams SI, Schrupp DS, Alvarez G, Suffredini D, Berg M, Childs R. Bortezomib and depsipeptide sensitize tumors to tumor necrosis factor-related apoptosis-inducing ligand: a novel method to potentiate natural killer cell tumor cytotoxicity. *Cancer Res.* 2006; 66:7317–7325. [PubMed: 16849582]
17. Shanker A, Brooks AD, Tristan CA, Wine JW, Elliott PJ, Yagita H, Takeda K, Smyth MJ, Murphy WJ, Sayers TJ. Treating metastatic solid tumors with bortezomib and a tumor necrosis factor-related apoptosis-inducing ligand receptor agonist antibody. *J Natl Cancer Inst.* 2008; 100:649–662. [PubMed: 18445820]

Fig. 1.
Combination of bortezomib treatment and DNA vaccination increases T cell responses and results in therapeutic benefit

Fig. 2.
Tumor apoptosis in response to bortezomib results in tumor destruction. Dendritic cells take up tumor antigens and present them to T cells. T cells proliferate and differentiate