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Novel Strategies in the Treatment of Multiple Myeloma: From Proteasome Inhibitors to Immunotherapy

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Abstract

The proteasome serves as the catalytic core of the Ubiquitin (Ub) protein degradation pathway and has become an intriguing target in drug development and cancer therapy. Successful pharmacologic inhibition of the proteasome with the small molecule bortezomib led to US Food and Drug Administration (FDA) regulatory approval for the treatment of mantle cell lymphoma and multiple myeloma (MM) and has been extended to a steadily increasing number of clinical trials to assess efficacy and safety in other hematologic malignances and solid tumors. Proteasome inhibition results in the accumulation of multi-ubiquitinated proteins, which are normally degraded through the tightly regulated Ub pathway. The Ub-Proteasome pathway is responsible for the selective degradation of many proteins that regulate the cell cycle and growth. Inhibition of the proteasome generates the accumulation of multi-ubiquitinated proteins that eventually leads to apoptosis although the exact mechanism of cell death is not completely understood. A specialized form of the proteasome, known to as the immunoproteasome, processes intracellular and viral proteins to generate peptides that are then presented at the cell surface bound as antigens (Ags) bound to the Major Histocompatibility Complex (MHC) class I molecule receptor. Importantly, inhibitors of the immunoproteasome decrease the processing and generation of MHC class I Ags and alter tumor cell recognition by the principal cellular effectors of the immune system. Hence, proteasome inhibitors may be employed as therapeutics to regulate the production of tumor specific Ags and for the selective removal of tumor cells through recognition by cytotoxic T lymphocytes (CTLs), natural killer (NK) cells and dendritic cells (DC). Proteasome inhibitors have been validated as effective cytotoxic agents and may have further potential as novel immunotherapeutic strategies.

Keywords: Proteasome; Bortezomib; Multiple Myeloma; Cytotoxic; Immunotherapy

Introductory Remarks on the Ubiquitin-Proteasome Proteolytic Pathway

In eukaryotic cells, the Ub-Proteasome proteolytic pathway is the major intracellular system for the selective degradation of nuclear and cytosolic proteins (1,2). Proteins are targeted for degradation through the covalent ligation to Ub, a highly conserved 76 amino acid polypeptide (3,4). Multi-ubiquitinated proteins are then degraded in an ATP-dependent manner by a high molecular mass structure known as the 26S proteasome (5,6). The Ub-Proteasome pathway maintains cellular homeostasis through dynamic switches in protein functional states to control essential cellular processes such as cell-cycle progression and programmed cell death. Deregulation of ubiquitination in tumor models results in malignant transformation and tumor progression likely due to the altered degradation of oncoproteins and tumor suppressor proteins (7-11).

Three enzymatic components are required to covalently link Ub chains onto protein substrates that are destined for degradation. E1 (Ub-activating enzyme) and E2's (Ub-conjugating proteins) prepare Ub for conjugation, but the key enzyme in the process is the E3 (Ub-protein ligase), because it recognizes a specific target protein and catalyzes the transfer of activated Ub (3,4). The specificity of target selection in the Ub-Proteasome pathway is through E3 Ub ligases that bind substrates for degradation and catalyze the transfer of activated Ub from the E2 to a lysine residue on the target. Subsequently additional Ub moieties are then attached to lysines that are present in Ub, yielding a substrate-anchored chain of Ub molecules.

The 26S proteasome is a ~2.5-MDa highly organized structure that recognizes and degrades ubiquitinylated substrates targeted for destruction. The 26S proteasome contains a barrel-shaped proteolytic core complex (the 20S proteasome), capped at one or both ends by 19S regulatory complexes. The 20S proteasome is a multicatalytic protease that exhibits various peptidase activities to function as the catalytic core the 26S proteasome and more broadly the functional core of the Ub-Proteasome pathway. All peptidase activities for proteolytic cleavage of the protein substrate reside within the 20S structure. In mammalian tissues, the 20S proteasome is comprised of up to 14 different proteins, with each subunit represented twice. These are classified as either α subunits or β subunits based on their similarities to the two subunits found in the 20S proteasome from the archaebacterium *Thermoplasma acidophilum.* The α and β subunits form four seven-membered rings that stack on top of each other to form a barrel-shaped structure.

Targeting the Ub-Proteasome Pathway in Multiple Myeloma

Multiple myeloma (MM) is a neoplasia hallmarked by the clonal expansion of malignant plasma cells (PCs) and the accumulation of a monoclonal immunoglobulin (Ig) (12,13). MM is the second most commonly diagnosed hematologic malignancy in the Western world

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and despite conventional treatment that includes high-dose therapy with autologous hematopoietic stem cell transplantation (auto-HSCT) is generally considered incurable (14,15). Proteasomal-dependent turnover of multi-ubiquitinated substrates has been targeted therapeutically with the small molecule inhibitor bortezomib and has demonstrated significant clinical benefit in MM patients (16-19). In the CREST study, relapsed MM patients received bortezomib and overall response rates (ORR) reached 50% and, with the addition of dexamethasone (DEX) RRs rose to 62% (20). The Assessment of Proteasome inhibition for Extending remissions (APEX) trial then compared bortezomib with high dose DEX in MM patients that had relapsed after one or more prior therapies and demonstrated an ORR of 38% in the bortezomib arm vs. 18% in the arm that received DEX alone (21). At one year, overall survival (OS) was 80% in those that had received bortezomib compared to 67% in the DEX arm. Advances in mechanistic understanding and treatment modalities have extended median survival to greater than six years and 10% of MM patients survive beyond 10 years (16,17). Novel immunomodulatory agents (IMIDs) such as thalidomide and the analogs lenalidomide and pomalidomide as well as proteasome inhibitors have significantly improved prognosis but patient survival remains highly variable and patient response to therapy cannot be accurately predicted. Furthermore, nearly one-half of the MM patients that receive bortezomib do not respond to treatment and therapeutic efficacy is compromised by the emergence of drug-resistance- the molecular basis of which remains elusive. Thus, novel therapeutic approaches are urgently needed for the treatment of MM.

Immunotherapy and the Immunoproteasome as a Target in Multiple Myeloma

Immunotherapy for hematologic malignancies such as MM offers therapeutic interventions that may utilize the host immune system to target and eradicate malignant cells. An advance in understanding how tumor cells evade immune surveillance mechanisms has assisted the development of immune-based therapies. One potential benefit of immunotherapy is the ability to eradicate tumor cells that are not eliminated by cytotoxic or targeted methods. Furthermore, an immune response may be generated through a mechanism completely independent of proteasome inhibition and therefore avoid the generation of resistance to proteasome inhibitors. Finally since the effectors of an immune response are long-lived (relative to cytotoxic chemotherapy) they offer the opportunity for a durable anti-tumor effect through sustained immune vigilance. Whether promising preclinical and phase I clinical trials will ultimately translate into improved, long-term OS remains to be determined.

Early reports established a role for the proteasome in the processing and generation of class I MHC Ag's (22-24), and proteasome inhibitors have been used to study class I Ag processing and presentation *in vitro* (25-30). The proteasome, or a specialized form known as the immunoproteasome, cleaves intracellular proteins within tumor cells to generate peptide fragments that then are transported to the cell surface (Figure 1). The peptides are inserted into the binding pocket of class I MHC molecules to facilitate tumor cell recognition by the CTL. Importantly, a single peptide~MHC class I complex may trigger cytolysis of a tumor cell. It is also noteworthy that the proteasome is not a static structure. Exposure of cells to cytokines, such as g-interferon, induces partial replacement of the three catalytic subunits with new subunits referred to as LMP-2, -7, and -10. Proteasome inhibitors were shown to reduce the generation of endoplasmic reticulum (ER) leader-derived T cell epitope and may either up- or down-regulate Ag presentation at non-toxic doses (31,32). Furthermore, bortezomib was shown to alter viral Ag processing with increased susceptibility to lymphocytic choriomeningitis virus (LCMV) infection *in vivo* (33). Therefore, the reduction of class I Ag presentation of virus-derived peptides may suppress the CTL response and allow virus replication (Figure 2). However, while immunoproteasome inhibition may overcome resistance to conventional drugs and nonspecific proteasome inhibitors, e.g., bortezomib, it also may generate unwanted effects. Therefore, the immunoproteasome may be selectively targeted with greater specificity and less toxicity (34). Finally, alterations in the Ag processing machinery have been detected in transformed PCs and are associated with reduced recognition by $CD8^+$ T cells (35). The changes in the Ag processing machinery may allow PCs to elude

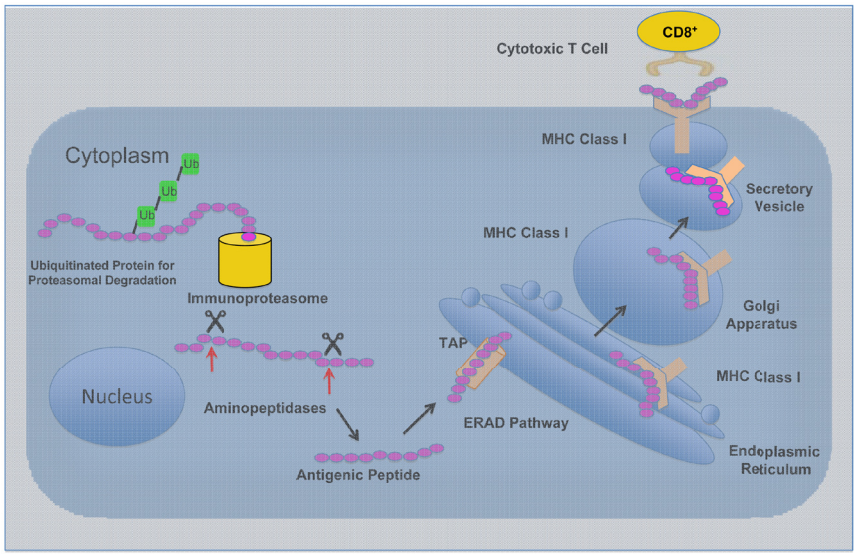

Figure 1: Processing of Intracellular Proteins by the Immunoproteasome Generates Peptides that Serve as Class I MHC Antigens at the Tumor Cell Surface.

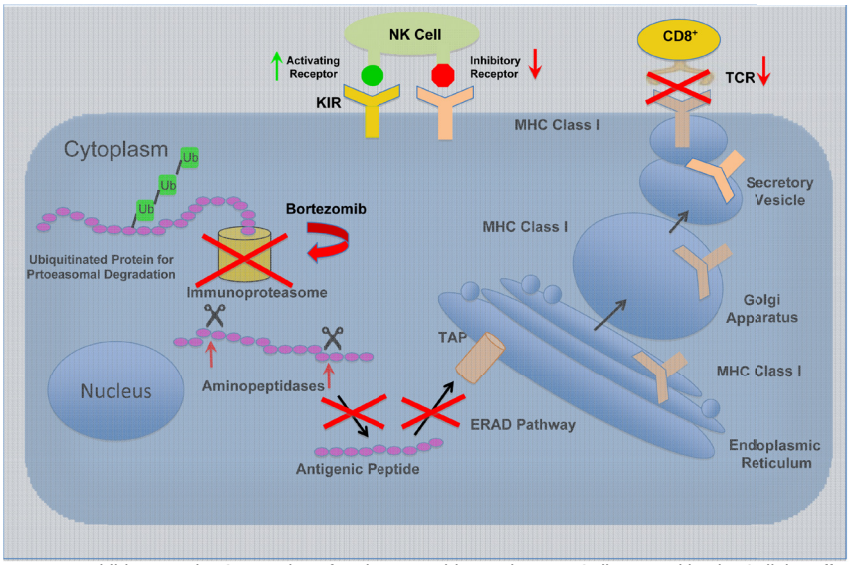

Figure 2: The Effect of Proteasome Inhibitors on the Generation of Antigen Peptides and Tumor Cell Recognition by Cellular Effectors of the Immune System.

immune surveillance and be a part of the MGUS to MM sequence in myelomagenesis.

Tumor Specific Antigens as Therapeutic Targets in Multiple Myeloma

Since the immunoproteasome is responsible for the generation of antigenic peptides it is noteworthy to report the identity those that have been reported or detected. A number of recent studies have identified tumor specific Ags that have been either detected on MM cells or have been associated with MM. These Ags include survivin (36), Muc-1 (37), telomerase (38), Sp-17 (39), PRAME, MAGE-A family members (40), Wilms tumor gene (WT1), (41) and gp96 (heat-shock proteins) (42). These Ags may be used as targets for cytotoxic and immunologic modalities to elicit selective tumor cell removal. To generate a robust, sustained immune response, the host immune system must recognize Ags as being foreign, over expressed or inappropriate. An ideal Ag would be expressed exclusively on the tumor cell and in high numbers; the selective interaction between the Ag and immune system effector, e.g. CTL or NK cell, should produce a rapid, sustained cytotoxic effect. The immune response should then be upregulated to elicit a response directed against as many myeloma cells as possible.

Idiotype (Id) proteins have been targeted in multiple lymphoproliferative disorders as immunotherapy against malignant B-cells (43). In myeloma, studies have shown that host response is inadequate to control the tumor cell proliferation as seen with the higher probability of a T_H1 lymphocytic response with increased IFN-γ levels and IL-2 in indolent and early-stage myeloma versus T_H2 response with increased IL-4 in patients with late-stage myeloma. This is mainly an MHC class II restricted effect with little role for CTL activity and suggested that MM cells do not produce an immunogenic Id protein. This differs from other B-cell malignancies. However, Wen et al. demonstrated that exposure of myeloma patient specific Id protein on the cell surface to cultured, leukapheresed peripheral blood mononuclear cells (PBMCs) produced DCs as Ag-presenting cells (APCs) to suggest that under these circumstances, MM cells may process and present Id proteins as MHC antigens (44).

Another antigenic target is the Dickkopf-1 (DKK1) protein, which normally is only found in placenta and mesenchymal stem cells (MSCs) but is aberrantly found in elevated levels in bone marrow and myeloma PCs by immunohistochemical (IHC) staining (45). DKK-1 is a secreted protein that inhibits the Wnt/[b]-catenin signaling pathway by interacting with the co-receptor protein Lrp-6 (46,47) and expression is associated with lytic bone lesions (48). DKK1 peptides attached to HLA-A*0201 cells produced a cytotoxic response from CTLs primed against this peptide using DCs as APCs. DKK-1 also produced a killing response in the MM cell lines U266 and IM-9, as well as HLA-A*0201-positive myeloma cells. However, this method did not kill DKK1+/HLA-A*0201-negative cells nor lyse HLA-A*0201 B lymphocytes (49). DKK1 may be a universal tumor-associated Ag to produce myeloma cell-directed vaccine.

Immune surveillance may play a role in preventing the MGUS to MM progression and the identification of immune responsive Ags in MGUS may provide insight into myelomagenesis and immune-based therapeutic applications. A serologic analysis of recombinant cDNA expression library (SEREX) approach screened an MM cDNA library with sera from 3 MGUS patients (50). Ten Ags were identified with specific antibody responses in MGUS patients. A response against the Oral-facial-digital type I syndrome (OFD1) was seen in 6/29 (20.6%) MGUS patients but 0/11 newly diagnosed MM patients. Interestingly, 3/11 (27.2%) MM patients following autologous SCT showed responses to OFD1. OFD1 functions in the Hedgehog (Hh) and Wingless (Wnt) pathways and may represent a critical step in the transformation of the pre-malignant MGUS condition to the malignant state. The cancer testis Ags MAGE-3 and NY-ESO-1 are normally present in male germ cells, ovarian cells and gestational trophoblasts but atypically occur in MM and may also induce a selective CTL-mediated response (43).

Antibody-based Immunotherapy in Multiple Myeloma

Monoclonal antibodies (mAb) recognize Ags on the tumor cell to induce complement-mediated lysis as well as ADCC and have been successful in solid tumors such as melanoma and renal cell carcinoma (51,52). CS1 is a transmembrane glycoprotein that shares a structural similarity with surface Ig. Myeloma cells commonly express CS1 and increased CS1 blood levels seen in MM patients are indicative of active disease. A fully humanized mAb (HuLuc63, Elotuzamab)

was developed to exploit these properties (53) and demonstrated inhibition of MM cell adhesion to stromal cells, induced ADCC *in vitro* and injections into xenograft myeloma mice caused tumor regression. Elotuzumab has been combined with bortezomib in a phase I, dose escalation trial as well as with lenalidomide (LEN) and dexamethasone in a phase I/II trial. The bortezomib combination showed a best response of rate 60% with 40% achieving a >/= partial response (PR) and median time to progression (TTP) of 9.6 months (54). Meanwhile, the LEN/DEX combination showed an ORR of 82% (64% PR, 18% VGPR) and an adjusted ORR of 95% (73% PR, 23% VGPR) in patients that had not been exposed to lenalidomide. At present, the median TTP had not been reached in the phase II portion and further studies will address CS-1 mAb efficacy in MM (55).

β_2-microglobulin (β_2M) is a component of the MHC class I molecule but is non-covalently bound to the α-chain and freely exchanges with serum β_2M. The serum level of β_2M is greatly increased in active MM and increased levels are associated with poor prognosis. A mAb directed against β_2M specifically induced apoptosis in 90% of myeloma cells without harming normal lymphocytes, plasma cells, stem cells or osteoclasts (56-58). Moreover, injection into mice produced a therapeutic response without damage to the hematopoietic system or murine organs (58). The novel mechanism thought to be at work here is the transfer of lipid rafts to the MHC class I molecule and removal from the IL-6/IGF-I receptor with consequent down regulation of the proliferation signaling and up regulation of apoptosis.

Interleukin-6 (IL-6) is a major cytokine that promotes MM cell growth and CNTO328 is a chimeric mAb directed against IL-6 that has shown modest clinical benefit. CNT0328 produced stable disease in 5/14 MM patients but no significant response as a single agent. Ten patients went on to have DEX added to their regimen and 5/10 achieved a PR (59). CD56 is a membrane glycoprotein with structural similarity to Ig and is seen in 70-90% of MM cells. CD56 prevents apoptosis and up regulates and promotes cellular proliferation (60). IMGN901 is a humanized anti-CD56 mAb conjugated to a potent chemotherapeutic maytansinoid (DM-1). In a phase I study in MM patients with relapsed/refractory disease, 3/18 patients showed a minor response (MR) and at least 8/18 patients had stable disease (SD). Treatment lasted at least 24 weeks in 5 patients and at least 42 weeks in 2 (61). Other targets under evaluation include CD74 (), IGF-IR (62), AVE1642 (53), HM1.24/BST-2/CD317 (anti-HM) (63), and TNF-related apoptosis-inducing ligand (TRAIL) (53). In addition, a humanized anti-DKK1 mAb, BHQ880, has been used in human myeloma mouse models (64).

Multiple Myeloma-Specific Cytotoxic T Cells

Agents targeted against key immunosuppressive, oncogenic or anti-apoptotic factors may sensitize tumor cells to CTL-mediated death. Progress in understanding the mechanisms of Ag processing and cancer cell escape from the normal processes that govern the removal of unwanted cells further support a CTL-based approach to cancer therapy. CTLs recognize target cells using clonally unique T cell receptors (TCRs) that confer specificity for Ags expressed on the surface of targets. To investigate this approach, a HSCT donor was immunized with MM Ig prior to transplantation it was shown that tumor Ag-specific immunity could be transferred to the recipient (65). Detection of a lymphoproliferative response, a parallel response in the carrier protein, recovery of a recipient CD4+ T-cell line with unique specificity for myeloma idiotype, and demonstration by in-situ hybridization that the cell line was of donor origin, proved that a myeloma idiotype-specific T-cell response was successfully transferred to the recipient. The idiotypic structure of clonal Ig expressed on the B-cell surface can be regarded as a tumor-specific Ag and a potential target for anti-idiotypic T and B-cells in an immune response (66). Active immunization using the autologous monoclonal Ig as a vaccine induces tumor-specific immunity in murine B-cell tumors and in patients with B-cell lymphoma. An anti-idiotypic T-cell response was amplified 1.9 to 5-fold in 3/5 patients during immunization and in two of the patients induction of idiotype-specific immunity was associated with a gradual decrease of $CD19^+$ B cells. This warrants further study to optimize the immunization schedule to achieve long-lasting T-cell immunity.

Another potential target is HM1.24 Ag, which is preferentially over expressed in MM but not in normal cells. T cells specific for the HM1.24 antigen were generated from MM patients using stimulation with protein-pulsed dendritic cells (67). HM1.24-primed T cells responded selectively to HM1.24-loaded autologous peripheral blood mononuclear cells (PBMCs) in an IFN-γ ELISPOT assay to indicate the promise of HM1.24 as a target Ag. To further explore the potential of HM1.24 as a target, Jalili et al. selected 4 HM1.24-derived peptides that possessed binding motifs for HLA-A2 or HLA-A24 by using 2 computer-based algorithms (68). The ability of these peptides to generate CTLs was then examined in 20 healthy donors and 6 patients with MM by a reverse immunologic approach. DCs were induced from PBMCs harvested from patients with MM, and autologous $CD8^+$ T cells were stimulated with HM1.24 peptide–pulsed DCs. Interferon-g–production and cytotoxic responses from $CD8^+$ T cells were both observed after stimulation with either HM1.24-126 or HM1.24-165 peptides. Importantly, HM1.24-specific CTLs were also induced from peripheral blood stem cell (PBSC) harvests of MM patients and these CTLs were able to kill MM cells in an HLA-restricted manner. This finding demonstrates the existence of functional DCs and HM1.24-specific CTL precursors within PBSC harvests and provides the rationale for cellular immunotherapy in combination with autologous PBSC transplantation in MM.

Prior studies of Id-protein specific CTLs for myeloma-lysed tumor cells indicated that the mechanism of cytotoxicity was mediated primarily through the perforin pathway because concanamycin A, but not brefeldin A, down-regulated CTL activity (69). Since CTLs continuously re-circulate through the body as a surveillance mechanism, antigenic stimulation of CTLs may serve as a potentially useful means to fight systemic disease. Evidence of T cell reactivity against survivin Ag in patients with MM suggests that this Ag might be effective (70). Both cytolytic and IFN-γ-producing responses to autologous myeloma cells were generated in 6/7 MM patients after stimulation *ex vivo* with DCs that had processed autologous tumor cells. The antitumor effectors recognized fresh autologous tumor but not non-tumor cells in the bone marrow, myeloma cell lines, DCs loaded with tumor-derived Ig or allogeneic tumor. Importantly, these $CD8^+$ effectors developed with similar efficiency by using T cells. Therefore, even in the setting of clinical tumor progression, the tumor bed of myeloma patients contains T cells that can be activated readily by DCs to kill primary autologous tumor.

IMIDs are standard of care treatment for MM and one of mechanism of action proposed includes T cell clonal expansion. A retrospective trial examined MM patients that received prednisone +/- thalidomide maintenance following auto-HSCT (71). T cell expansions were seen in 48% of patients pre-transplant and 68% after 8-month maintenance therapy and the T cell expansions, previously shown to be clonal, were predominantly $CD8^+$ (93%). Thalidomide therapy was associated with a significant increase in the percentage of patients with multiple

expansions of CTL clones. The presence of expansion was associated with a significantly longer median in progression-free survival (PFS) (32.1 vs. 17.6 months) and OS. Novel methods to expand anti-myeloma derived CTL clones in the pre-clinical setting involve fusing myeloma cells to DCs to bring tumor Ags in proximity to the APCs and hence, stimulate more CTLs and form a more intense, longer-lasting immunity. These methods were used in mouse plasmacytoma models and anti-myeloma humoral and CTL responses were seen, leading to longer lives in the mice; however, no reduction in tumor size was seen. Further study led to the finding that more mature DCs rather than immature DCs used in the fusion cells led to a stronger immune response with higher levels of cytokines and CTL activity (72). Another technique used involves alternative APCs, namely CD40 activated B-cells loaded with myeloma Ags. These methods may induce intense, myeloma-specific CTL responses similar to that seen in the *in vitro* killing of cultured myeloma cell lines (73).

Modulation of Natural Killer Cell Activity through Proteasome Inhibitors

NK cells are of lymphoid origin, constitute up to 20% of PBMCs and function to lyse tumor and virus-infected cells that lack MHC class I molecules (74,75). NK cells express surface receptors that either inhibit or activate cell lysis. Importantly, inhibitory receptors with different specificities for class I molecules have been identified. The two main receptor groups are the killer Ig-like receptors (KIR) that bind HLA-class I molecules and the heterodimeric receptors CD94-NKG2A/B that recognize HLA-E. The absence of even a single MHC-I allele sensitizes tumor cells to NK-mediated cytotoxicity. NK target recognition is achieved by the absence of syngeneic MHC molecules through dominant NK-inhibitory receptors. Alternatively, NK cells may utilize the presence of allogeneic MHC molecules or MHC-like molecules by NK activating receptors to recognize targets. Should a cell down regulate MHC expression, such as that seen in malignancy, that cell is less susceptible to CTL-induced lysis but may actually become more susceptible to NK recognition. Bortezomib was shown to sensitize tumors to death receptor signaling pathways used by both NK and T cells. Bortezomib simultaneously results in divergent effects on NK and T cell function since bortezomib sensitized cells to NK cell-induced apoptosis but also altered tumor Ag presentation and paradoxically reduced tumor-specific T cell effector response (76).

Additional cell surface receptors affected by bortezomib include the tumor necrosis factor–related apoptosis-inducing ligand (TRAIL) receptors DR4 and DR5. Compared with cycling populations, quiescent $CD34^+$ Chronic Myelogenous Leukemia (CML) cells have higher surface expression of DR4 and DR5 (77). Cells treated with bortezomib were shown to up regulate TRAIL receptor expression on quiescent $CD34^+$ CML cells, and furthermore enhanced their susceptibility to cytotoxicity by *in vitro* expanded allogeneic donor NK cells. The results suggest that donor-derived, NK cell–mediated Graft-versus-Leukemia (GVL) effects may be improved by sensitizing residual quiescent CML cells to NK-cell cytotoxicity.

Human leukocyte antigen (HLA) class I molecules expressed by tumor cells play a central role in the regulation of NK cell-mediated immune responses (78,79). When one such molecule, KIR-ligand (KIR-L), is mismatched, NK cells have potent antileukemic effects in the setting of heavily T cell–depleted haplo-identical allogeneic transplantation. This is problematic for the majority of MM patients, as most are unlikely to be able to tolerate the associated toxicity of such a regimen. It is postulated that mature NK cells express at least one inhibitory receptor for autologous HLA class I to preserve self-tolerance. In contrast, NK cells avidly lyse tumor cells that do not display such inhibitory KIR-L. The humanized IgG_4 anti-KIR blocking antibody IPH2101 enhances NK cell IFN-γ and granzyme B release against MM cells (80) and proceeded to phase I trials without dose-limiting toxicity reported (81).

Dendritic Cell-based Therapy and Proteasome Inhibitors

Dendritic cells (DCs) are potent APCs that efficiently survey for incoming pathogens. Encounter of DCs with pathogen leads to DC activation, migration to secondary lymphoid organs and maturation (82, 83). Mature DCs stimulate not only quiescent, naive $CD4^+$ and $CD8^+$ T cells but also B cells to initiate a primary immune response through the optimal use of co-stimulatory, adhesion, and MHC molecules. A strong secondary immune response is mounted, which requires a relatively small number of DCs and low level of Ag. Given their central role in controlling immunity, DCs are logical targets for treatment of MM but preliminary reports of DC-based immunotherapy have demonstrated low clinical responses. Vaccination with tumor Ag-pulsed DCs are protective in animal models and have induced potent tumor-specific immunity and durable regression of human solid tumors and B-cell lymphoma in these models (84). The results indicate that DCs pulsed with Id protein could be used to induce the type 1 anti-Id response in patients with MM.

Heterokaryons generated by the fusion of DC's with tumor cells combine the machinery needed for immune stimulation with presentation of a large repertoire of tumor cell-specific Ags. Subsequent fusions of MM cells with DC as a vaccination strategy were shown to be potent stimulators of autologous patient T cells and more importantly, fusion cell-primed autologous PBMCs demonstrated MHC-restricted cytolysis (85). In a murine model, vaccination of DCs fused with mouse 4TOO plasmacytoma cells was associated with induction of antitumor humoral and CTL responses (86). Immunization with the fusion cells protected mice against tumor challenge and extended the survival of tumor-established mice without eradication of the tumor cells while addition of IL-12 further eradicated disease.

A novel extension of the heterokaryon approach fused Id-protein pulsed DCs that were then induced to undergo maturation with exposure to CD40 ligand (CD40L) (87). The experimental premise is that mature DC's are better at stimulating $CD4^+$ and $CD8^+$ cell activation than immature DC's, but myeloma, as with other cancers, can impair the T-helper cells. Thus, CD40 activation of DC's is similarly impaired. Vaccination with CD40L-stimulated DC's may reduce the effect on host immune system. In a murine model, bortezomib sensitized B16 melanoma tumor cells to the lytic effects of immune effector cells (88) but also impaired the immune stimulatory capacity of myeloid DC's (89).

While bortezomib has the potential to enhance DC-mediated anti-tumor immunity, it has also been reported to impair several stimulatory properties of monocyte-derived DC's. The precise role of proteasome inhibitors and the effect on the innate and adaptive immunity as combined therapy is still unclear. It has been shown that the uptake of human MM cells by DC's after bortezomib-induced death leads to antitumor immunity and depends upon cell surface exposure of hsp90 on dying cells (90). Bortezomib impaired several properties of DC's such as phagocytic capacity, maturation in response to LPS and TNF-a and CD40L and reduced cytokine production (91). Bortezomib was found to down-regulate MyD88, an essential adaptor for TLR signaling as well.

Conclusions

The clinical success of proteasome inhibitors in the treatment of MM and an increasing number of hematological malignancies validates the proteasome as viable therapeutic target. Bortezomib combined with IMIDs and a number of chemotherapeutic agents in MM has demonstrated improved RR, CR and OS comparable or superior to those achieved in the auto-HSCT. Furthermore, bortezomib-based therapy may be more applicable particularly in the elderly or frail patient populations. Future efforts are directed the identification of synergistic drug combinations that produce more durable responses, less toxicity and prolonged survival for patients to make certain plasma cell dyscrasias increasingly chronic and treatable diseases. Clinical response to agents or cells that enhance immunity alone is rare; however, it is more likely to show benefit when combined with other agents such as proteasome inhibitors. Durable tumor regression and potential cures of metastatic solid cancers can be achieved by a variety of cellular immunotherapy strategies, including cytokine therapy, dendritic cell–based vaccines, and immune-activating antibodies, when used in so-called immune-sensitive cancers such as melanoma and renal cell carcinoma. However, these immunotherapy-based strategies have very low tumor response rates, usually in the order of 5% to 10% of treated patients. The antitumor activity of adequately stimulated tumor antigen–specific T cells is limited by local factors within the tumor milieu and that pharmacologic modulation of this milieu may overcome tumor resistance to immunotherapy. By understanding the mechanisms of tumorigenesis and of cancer cell immune escape, it may be possible to design rational combinatorial approaches of novel therapies such as proteasome inhibitors able to target immunosuppressive or antiapoptotic molecules in an attempt to reverse resistance to immune system control. The Ub-Proteasome pathway is an ideal target for immunopotentiating drugs that block key oncogenic mechanisms in cancer cells resulting in a proapoptotic cancer cell milieu while at the same time do not negatively interfere with critical CTL functions and may actually augment NK-cell based tumor lysis. Further studies are warranted and in progress to incorporate these immunotherapeutic approaches with existing and emerging novel, biologic approaches aimed at the improved treatment of MM.

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