Until recently, malignant disease has been viewed as a purely cell-autonomous disease mediated by the progressive accumulation of genetic and epigenetic alterations in prema-
lignant and malignant cells that override cell-intrinsic tumor-
suppressive mechanisms, including senescence and apoptosis. 
Nowadays, this view has been challenged, and it has become
increasingly clear that malignant disease is also the result of
inefficient immunosurveillance, meaning that tumors can
only develop after their recognition and after elimination
by the immune system has failed. Beyond academic considera-
tions on the etiology of neoplastic disease, this concept has
major practical implications. Indeed, the recent approvals
of immune-checkpoint blockers (mostly targeting PD-1 and
PD-L1) across a wide spectrum of previously intractable
malignant diseases illustrates the possibility to transiently
reactivate failed immunosurveillance to obtain tangible ben-
efits in a substantial (though admittedly insufficient) fraction
of patients with cancer.

The cell-autonomous vision of cancer has profoundly influ-
enced the way in which cancer drugs have been conceived and
developed. After the widespread implementation of chemo-
therapeutic cytotoxicants that indiscriminately kill proliferat-
ing cells of any kind, explaining their major side effects, the
idea arose to develop “targeted” or “personalized” anticancer
agents as part of a novel “precision medicine.” Thus, drugs
should target the oncogene to which a cancer cell is “addicted,”
for example, an activating mutation in an oncogenic kinase,
thereby acting on malignant but not normal cells. Alternatively,
drugs should target “non-oncogene addiction” (NOA), the
phenomenon where tumor cells present stress phenotypes that
render them vulnerable to specific agents. One such drug that
targets NOA is bortezomib, an inhibitor of the 26S proteasome
used for the treatment of multiple myeloma (MM). Now, in the
current issue of *Blood Cancer Discovery*, Gulla and colleagues
report that bortezomib has the capacity to induce potent anti-
cancer immune responses.

Although anticancer drugs, be they nonspecific chemother-
peutics or targeted agents, were supposed to mediate their
effects by direct cytostatic or cytotoxic effects on malignant
cells, it turned out that their long-term effects in patients
relied on the induction of antitumor immune responses. Thus,
several major chemotherapeutic agents, including anthracy-
clines and oxaliplatin, induced “immunogenic cell death”
(ICD), meaning that they stress and kill cancer cells in such a
way that they become recognizable to the immune system (2).
Accordingly, accumulating evidence supports the idea that
anticancer immune response elicited by chemotherapy deter-
mines patient prognosis (2). Ironically, imatinib, the first drug
developed to target an oncogenic tyrosine kinase, BCR–ABL
[the ABL kinase constitutively activated by a chromosomal
translocation, in Philadelphia chromosome–positive chronic
B-cell leukemias (CLL, chronic lymphocytic leukemia)] turned
out to owe its long-term success against CLL [and later against
gastrointestinal stroma tumors (GIST), which depend on
another oncogenic kinase, KIT, also inhibited by imatinib] to
immune responses as well (3). In this case, cell-autonomous
effects on CLL or GIST cells increase their susceptibility
to immune attack by cytotoxic T cells (CTL) and natural
killer cells coupled to a reduction of immunosuppressive cell
types (such as regulatory T cells and myeloid-derived suppres-
sor cells). Moreover, imatinib inhibits nonmutated tyrosine
kinases in immune cells, in particular in dendritic cells (DC),
increasing their immunostimulatory action (3). Thus, targeted
therapies acting on oncogenic kinases may have immunologic
off-target effects that explain their therapeutic success.

Bortezomib has been suspected of having immunostimulat-
ory effects since the discovery that it induces the expres-
sion of heat shock protein 90 on MM cells, favoring their
recognition by DCs (4). The translocation of intracellular

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**IN THE SPOTLIGHT**

**Bortezomib Induces Immunogenic Cell Death in Multiple Myeloma**

Laurence Zitvogel1,2,3,4,5,6 and Guido Kroemer1,6,7,8,9,10

**Summary:** As a general rule, successful antineoplastic treatments induce an antitumor immune response, even if
they were initially designed to target cancer cell–autonomous pathways. In this issue of *Blood Cancer Discovery*,
Gulla and colleagues reveal that the proteasome inhibitor bortezomib induces immunogenic stress and death in
multiple myeloma cells, thus explaining its therapeutic efficacy.

See related article by Gulla et al. (1).
proteins to the cell surface is indeed a hallmark of ICD. In particular, the endoplasmic reticulum (ER) chaperone calreticulin (CALR) translocates from the ER lumen to the plasma membrane surface, where it acts as an “eat-me” signal to favor the phagocytosis of dying cells by DCs. CALR exposure has been observed in response to a wide range of ICD-inducing chemotherapeutics (2) and some targeted agents such as crizotinib, a tyrosine kinase inhibitor (5). Gulla and colleagues now report that bortezomib induces classic, CALR-dependent ICD while providing molecular and translational insights into the therapeutic relevance of bortezomib-induced ICD (1).

Gulla and colleagues found that bortezomib induces CALR exposure to the surface of human or mouse MM cells and that CALR is required for the phagocytosis of bortezomib-treated MM cells by monocyte-derived DCs (1). Coculture of bortezomib-treated human MM cells, DCs, and T lymphocytes led to an increase in the frequency of CD4+ effector memory (EM) cells, CD8+ EM cells, and CD8+ T EM cells reexpressing CD45RA (TEMRA) that was not seen when bortezomib-treated MM cells were removed from the coculture or when bortezomib alone was added to cocultures of DCs and T cells. When bortezomib-killed murine MM cells were injected into syngeneic immunocompetent mice, they induced an immune response that prevented the growth of MM cells injected 1 week later. This vaccination effect was reduced when the Calr gene was inactivated in MM cells. Moreover, established MMs only responded to bortezomib treatment in vivo when established in immunocompetent rather than in immunodeficient mice. Although knockout of Calr in MM cells did not affect their apoptotic response to bortezomib in vitro, it did abolish the efficacy of bortezomib against MM tumors developing in immunocompetent mice. These results demonstrate that a CALR-dependent immune response is required for optimal therapeutic efficacy of bortezomib against MM. Driven by this consideration, Gulla and colleagues determined the effects of bortezomib on the transcriptome of MM tumors evolving in immunocompetent mice. In CALR-expressing (but not in CALR-deficient) MM, bortezomib induced 90 immune-related genes that composed the “ICD signature.” High expression of the human orthologs of the mouse genes found in this signature correlated with clinical outcome of MM patients treated with bortezomib-based regimens (1).

Of note, among these 90 genes, 57 could be classified as interferon-stimulated genes, and bortezomib was able to activate a type-1 interferon response in MM cells in vitro, meaning that genes encoding interferon alpha1 (IFNIA), interferon beta1 (IFNB1), and chemokine (C-X-C motif) ligand 9 (CXCL9, a ligand acting on the receptor CXCR3 on T cells to attract them into the tumor bed) were upregulated (1). This response apparently was decisive for the bortezomib effects against MM because a neutralizing antibody specific for STING, and activating phosphorylation of beta1 (IFNB1), and CXCL9 gene transcription by bortezomib; and abolished the capacity of bortezomib-treated human MM cells to stimulate CD4+ EM and CD8+ EM cells in cocultured immune cells (Fig. 1). Nonetheless, the STING pathway appeared to be suboptimally activated in response to bortezomib, meaning that a synthetic STING agonist, ADUS-100, could trigger a stronger TBK1 phosphorylation in vivo. When combined with bortezomib, ADUS-100 also improved therapeutic responses in a mouse MM model, as it increased the infiltration of the tumors by T lymphocytes. Such synergistic effects were lost upon knockout of STING in mouse MM cells or in immunodeficient mice (1). Of note, in MM patients, STING mRNA levels positively correlated with the expression of the 57 interferon-stimulated genes found in the ICD cluster, pleading for the clinical relevance of this pathway (1).

In response to cGAS activation, STING is known to translocate to the ER (8), suggesting a possible intersection with the CALR exposure pathway ignited at the ER. However, knockout of STING did not interfere with bortezomib-induced CALR exposure (1). Conversely, knockout of CALR prevented the bortezomib-mediated induction of interferon-stimulated genes in vivo, in the MM mouse model (1), suggesting that CALR is somehow required for the cytosolic DNA–cGAS–STING–TBK1→type-1 interferon pathway to be activated (Fig. 1). However, this conjecture requires further exploration by in vitro experimentation. In particular, the exact level at which CALR deficiency interrupts this pathway remains to be determined. Cancer cells can escape immune-surveillance by suppressing the CALR exposure pathway at multiple levels (9). Hence, it would not be surprising that MM cells developed such deficiencies as a result of immunoselection, in particular after relapse from bortezomib-based therapeutic regimens.
In summary, the work by Gulla and colleagues reveals that ICD accompanied by CALR exposure and viral mimicry is an important clinical feature of MM treatment by bortezomib (1), echoing recent results on another efficient anti-MM drug, belantamab mafodotin, which also acts as a potent ICD inducer (10). This anti-BCMA antibody conjugated to maleimidocaproyl monomethyl auristatin F was FDA approved in August 2020 for the treatment of relapsed or refractory MM. Logically, clinical studies evaluating the possibility of combining bortezomib or belantamab mafodotin with anti-PD-1 antibodies will be underway soon (ClinicalTrials.gov Identifiers NCT03848845 and NCT04258683). It will be interesting to see whether the combination of ICD inducers with other immuno-therapies, including STING agonists, will further improve the clinical management of MM.

**Authors’ Disclosures**

L. Zitvogel reports a patent for compounds and uses thereof to induce an immunogenic cancer cell death in a subject issued; a patent for compounds regulating calreticulin, KDEL receptor, and/or Erp-57 cell-surface exposure and uses thereof to evaluate the efficiency of a cancer treatment issued; a patent for inhibitors of protein phosphatase 1, GADD34, and protein phosphatase 1/GADD34 complex, preparation and uses thereof pending; a patent for kits and methods for detecting the ability to induce an immunogenic cancer cell death in a subject pending, and a patent for calreticulin for its use as a medication for the treatment of cancer in a mammal pending.

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