Immunoproteasome and Immune Checkpoints Modulation for Cancer Therapy

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Immunoproteasome is a noncanonical form of proteasome with enzymological properties optimized for the generation of antigenic peptides presented in complex with class I MHC molecules. This enzymatic property makes the modulation of its activity a promising area of research. Nevertheless, immunotherapy has emerged as a front-line treatment of advanced/metastatic tumors providing outstanding improvement of life expectancy, even though not all patients achieve a long-lasting clinical benefit. To enhance the efficacy of the currently available immunotherapies and enable the development of new strategies, a broader knowledge of the dynamics of antigen repertoire processing by cancer cells is needed. Therefore, a better understanding of the role of immunoproteasome in antigen processing and of the therapeutic implication of its modulation is mandatory.

Keywords: immunoproteasome ; ubiquitin–proteasome system ; immune checkpoints ; proteasome inhibitors ; immunotherapy

1. Introduction

Cancer immunotherapy is conceptually based on therapeutic stimulation of cancer immunosurveillance, a theory that states that tumor cells, mostly through the phenotypic alteration and the repertoire of tumor-associated neoantigens they often present, can be recognized and targeted by the immune system in the attempt to prevent disease progression. Nowadays, the dynamics of cancer and immune system crosstalk have been unequivocally unveiled to be extremely complex and has been renamed as cancer immunoediting. According to the three Es theory, this process comprises three defined phases: elimination of cancer cells by the immune system; equilibrium between tumor growth and control by the immune system; escape of neoplastic cells from immunosurveillance. The first two phases in some way coincide with the concepts described by the former theory, but the second phase can last long, running asymptomatically, until it is overrun by the third one, which results in tumor growth and dissemination. In an immunocompetent host, this breakthrough results from a positive selection of tumor clones that are able to evade the inhibitory control of the immune system by downregulating/masking antigen epitopes or by increasing the immunosuppressive properties of the tumor microenvironment [1][2].

Modern approaches of cancer immunotherapy, designed to restore a robust degree of immune activity against tumor cells, encompass immune checkpoint blockade, adoptive cellular therapies, and cancer vaccines [1][2][3][4][5]. Among these therapeutic interventions, immune checkpoint inhibitors (ICKi) have substantially revolutionized the oncology field by prolonging the survival of patients affected by highly aggressive/advanced stage cancers, such as metastatic melanoma and non-small-cell lung cancer (NSCLC). This approach is currently based on the use of monoclonal antibodies targeting inhibitory ICKs, such as CTLA4 (cytotoxic T lymphocyte antigen 4) and PD-1 (programmed cell death 1) or PD-L1 (programmed cell death 1 ligand) that regulate activated T lymphocytes function, switching off the immune response. A number of preclinical and clinical studies have revealed that antibodies raised against the immune checkpoint molecules enhance the antitumor immunity through not completely identified mechanisms of action, which differ depending on the target and specificity of the monoclonal antibody used [1][3][4][5]. Despite the undoubtful clinical success of ICK blockade, the on-field experimentation has opened several tasks that are worth being addressed: organ involvement and cohorts of signs and symptoms; second, the clinical efficacy is limited to subgroups of responder patients or, in many other subjects, after an initial response, drug resistance takes over. The latter event is generally due to the genetic and phenotypic heterogeneity of cancer cells and/or to tumor microenvironment remodeling during disease progression and dissemination [5][6][7]. Nonetheless, the overall efficacy of ICKi is affected by multiple factors, spanning from the expression and distribution of the target within the tumor microenvironment, the tumor mutational rate, and alterations of antigen presentation [8][9][10]. In this context, a crucial role is played by components of the host microenvironment that infiltrate the

tumor and exert immunosuppressive effects, thus counteracting ICKi activity (i.e., infiltration by T regulatory cells (Tregs), dendritic cells, immunosuppressive myeloid cells, cancer-associated fibroblasts) [11].

Cancer cell immunogenicity is a key determinant for ICKi efficacy: malignancies which do not express tumor-specific antigens are not potentially susceptible to this approach. This is somewhat strengthened by the evidence that an immunotherapy-non-responsive cancer can turn into an immunotherapy-responsive one upon enhancement of tumor immunogenicity [8][9][10][12].

Hence, a better knowledge of the "immunopeptidome" (the repertoire of peptides bound to and presented by MHC molecules) and how tumor-specific antigen repertoire change during tumor progression is expected to improve the current therapeutic strategies; thus, a challenging issue is the identification and characterization of the MHC-I-presented peptides that modulate T cell-based tumor response [12][13][14].

At the molecular level, the generation of effective T cells that fight cancer requires a functional and efficient machinery for the multistep and multicellular mediated process, including antigen processing and presentation. The intracellular antigen processing pathway almost exclusively deals with the ubiquitin proteasome system (UPS) activity by which proteins are first ubiquitinated and then degraded. Indeed, the UPS carries out multiple functions in cells and in the onset and progression of different human pathologies spanning from neurodegeneration to cancer [15]. A major role in antigen processing is covered by a specialized and inducible form of proteasome (i.e., the multi-catalytic machine which degrades ubiquitinated proteins) named "immunoproteasome" [16]. This review focuses on immunoproteasome, its involvement in antigen generation, and on the therapeutic implications of its modulation to halt cancer progression. Finally, the potential crosstalk between proteasome modulators and immune checkpoint inhibitors is discussed.

2. Ubiquitin–Proteasome System: Cellular Biocomputing Machinery

In living cells, the proteome is constantly tuned through a complex, entwined, and multi-subcellular compartments network which coordinates the synthesis, folding, conformational upkeep and degradation of individual proteins [1]. The removal of undesired proteins is carried out by two main intracellular proteolytic systems, namely the ubiquitin–proteasome system (UPS) and autophagy [17][18][19][20]. The UPS is the major actor in the turnover of more than half of intracellular proteins that play fundamental roles in several facets of cell life, such as cell cycle, apoptosis, DNA repair, antigen presentation, inflammation, cellular response to environmental stress, and morphogenesis of neuronal networks [21][22][23]. The UPS's hierarchical organization includes two intertwined and consecutive steps, i.e., the covalent ATP-dependent attachment of ubiquitin (Ub) polymers to a given substrate (target protein conjugation cascade), which is catalyzed by three classes of ubiquitin ligases, E1 (Ub-activating enzyme), E2 (Ub-conjugating enzyme), and E3 ligase, and its degradation by the 26S proteasome, followed by recycling of ubiquitin moieties along with the release of cleared protein oligopeptides [15][24][25][26][27][28]. In the final step of ubiquitination, the E3 ligase, which is committed with substrate specificity [25][26][27][29][30], mediates the formation of an isopeptide bond between the carboxyl C-terminal group of Ub and the ε-amino group of the lysine residue of the target protein. Thereafter, the reaction can be repeated multiple times, allowing the polyubiquitin chain to increase by 6 Ub moieties in average, in which each subsequent Ub monomer is connected to the previous one through an isopeptide covalent bond similar to that of the first Ub–substrate bond [30][31][32][33][34][35]. The process of ubiquitylation is a highly dynamic and reversible equilibrium; in fact, deubiquitinases or deubiquitinating enzymes (DUBs) can reverse the effect of E3 ligases by removing ubiquitin from target proteins. Furthermore, they mediate the polyubiquitin chain release during the hydrolysis of substrates by the proteasome [36][37].

3.Immunoproteasome as a Specialized Apparatus of Self-Target Designation

In the early nineties, proteasome was discovered as the crucial player for the class I MHC-restricted antigen processing pathway and two proteasome genes, namely *PSMB9* (*LMP2*) and *PSMB8* (*LMP7*), which encode two alternative subunits of the 20S, β1 and β5, respectively, were identified in close proximity of the transporter associated with the antigen-processing (*TAP*) gene in the MHC class II genomic region [38][39]. Concurrently, it was shown that synthesis and incorporation of these subunits into the 20S was driven by interferon γ (IFNγ) [40][41][42][43]. Thus, immunoproteasome, also known as inducible proteasome, is a specialized form of the 20S with a prominent role in immunity. Immunoproteasome preferentially and cooperatively incorporates three immune subunits, β1i, β2i (MECL-1), and β5i, to replace the constitutive catalytic subunits into the β ring of the 20S within its biogenesis pathway. The preferential assembly of inducible subunits is likely due to the higher affinity of β5i than of β5c for the proteasome maturation protein (POMP) which mediates the β ring formation [15][44][45]. The i20S assembly is four times faster than c20S, clearly reflecting the need for a rapid and transient response upon exposure to a proinflammatory stimulus. In fact, IFNγ induces, via the

JAK/STAT signaling, the transcription of immune catalytic subunits, the *MHC-I* and *TAP* genes, thus enhancing the entire class I antigen presentation machinery [46][38][40][47]. Immunoproteasome is constitutively expressed at the basal level in hematopoietic cells and has a shorter half-life than c20S (average 27 h for immunoproteasome and 133 h for constitutive proteasome). Such a rapid turnover has the purpose of efficiently adapting to the environmental changes [48][49]. It has been shown that during the course of viral, bacterial, and fungal infections, immunoproteasome replaces up to 90% of the c20S pool [50][51]. As a matter of fact, besides the pioneering contribution of IFNγ, immunoproteasome was shown to be further transcriptionally induced by a plethora of inflammatory stimuli, such as IFNα and IFNβ, tumor necrosis factor α (TNF-α), lipopolysaccharides (LPS), as well as by redox unbalance [52][53][54][55]. It is important to recall that the different forms of proteasome particles can coexist inside the cells as well as the different peptide antigens generated. Anyway, in dependence to the different stimuli the cells are exposed to, the relative abundance of antigens generated by each specific subpopulation can be adapted [56].

A side-by-side comparison of the three different substrate-binding pockets of the c20S and i20S points out the enzymological differences of the two complexes [57]. In general, the i20S is characterized by increased chymotrypsin-like and trypsin-like activities, but a lower caspase activity [58]. In detail, the caspase-like subunit β1 accommodates peptides with an acidic residue in the P1 position, whereas β1i binds to peptides with a hydrophobic residue in the same position, exerting a branched-chain amino acid-preferring activity.

The folded trimeric complex formed by a given peptide and the MHC-I ligand cleft is exposed to the cell surface for presentation to the immune cells. The requisites for tight peptide MHC class I binding are essentially two: (1) the length of 8–9 amino acids and (2) an anchor of basic or hydrophobic residues located at the C-terminus or within the peptide sequence [59]. MHC-I does not accept C-termini with acidic anchor residues; thus, the substitution of β1c with β1i produces antigenic peptides with hydrophobic C-termini that can efficiently bind to MHC-1 molecules. Additionally, the structural properties of β5i also contribute to generating peptides with preferred C-terminal anchor amino acids for MHC-I molecules. In fact, the β5i S1 pocket accommodates larger hydrophobic amino acids chains than β5c (which presents, instead, a “small neutral amino acids-preferring activity”) and is characterized by a more hydrophilic environment around the catalytic threonine favoring the chymotryptic-like catalytic properties of the inducible subunit. Despite the β5 and β1 subunits, the active sites of β2c and β2i seem to be structurally identical, rendering this substitution more enigmatic, even though several studies reported an increase in trypsin-like activity of i20S with respect to c20S [59][57].

Additional forms of proteasome bearing a mix of standard and inducible subunits were identified. These intermediate proteasomes, which represent from one third to one half of the overall proteasome content in different tissues, such as liver, colon, and kidney, contain these triads of subunits, β1/β2/β5i or β1i/β2/β5i. Remarkably, a recently discovered mechanism of antigen generation through which proteasome increases the repertoire of antigens for presentation to the immune system is the “proteasome-catalyzed peptide splicing”: spliced peptides, which are made by two not contiguous fragments of parental proteins, are produced efficiently both by immunoproteasome and constitutive particles [60][61][62][63]. The existence of these mechanisms along with the copresence of different proteasome populations beyond the constitutive proteasome involved in antigen–peptide generation broadens the repertoire of antigens produced by a cell [49][64][65]. However, whether the incorporation of immune subunits triggers qualitative or quantitative effects on peptide repertoire generation is still not resolved since different studies report somewhat controversial results. In fact, a number of studies highlighted the positive role of immunoproteasome mainly against viral and bacterial antigens, whereas some studies reported that immunoproteasome expression can abrogate the presentation of some tumor epitopes [64][65][66][67][68][69][70][71].

Anyway, a defect in antigen presentation was found in the triple-inducible-subunit KO mice, and this alteration is now reported to be much broader qualitatively and quantitatively than that previously described in any of the β1i, β2i, or β5i single-subunit KO mice and still far greater than the sum of the defects these single-subunit KO animals were reported to bear [49][72][73]. Moreover, analysis of MHC class I-bound peptides shows that the antigen repertoire of KO mice differs from that of WT mice, reinforcing the hypothesis that immunoproteasomes generate peptides that, apparently, cannot be produced by constitutive proteasomes [66][74][75]. On the other hand, other studies suggest that immunoproteasomes affect the quantity rather than the quality of the given generated peptides, influencing also in this case the immune response [70][76][77]. Thus, some antigens are exclusively produced by the immunoproteasome or the constitutive proteasome, while others can be processed by both, and some others can be preferentially processed by intermediate-type proteasomes [78][79]. Of note, this distinction between the quantitative and qualitative effects of the antigen repertoire depending on the expression rate of immune–constitutive or mixed proteasome is not simply semantic. In fact, it is of basic significance not only to better understand the enzymatic properties of the different proteasome populations, but also to better define the MHC class I-dependent CD8+ T cell response in the context of specific physiopathological conditions [80].

4.Immunoproteasome: An Emerging Target in Cancer

Alterations of different genes belonging to the UPS are a hallmark of cancer. UPS dysregulation may occur at multiple levels, spanning from genetic modifications (i.e., mutations, amplifications, deletions), transcriptional network alterations (i.e., p53; NRF-1 and NRF-2) to epigenetic and post-translational modifications [15][81].

A number of studies underline the role of the i20S in cancer progression, strengthening its therapeutic potential. The tumor microenvironment is profoundly different from that of healthy tissues; this is also due to the presence of tumor-infiltrating lymphocytes (TILs) that release IFNγ and other inflammatory cytokines. Interestingly, immunoproteasome seems to possess both pro- and antitumorigenic properties, which are associated with the modulation of cytokine expression and tumor-associated peptide presentation, respectively [79].

Tumor cells can evade recognition by cytotoxic T lymphocytes (CTLs or CD8+ T cells) through downregulation of MHC-I at the cell surface or, additionally, by reducing immunoproteasome subunits expression [79][82]. In fact, non-small-cell lung cancer that undergoes the epithelial–mesenchymal transition shows a reduced immunoproteasome subunits expression: this leads to a dramatic drop of heterogeneity of the antigen/peptide repertoire produced by tumor cells and to poor clinical outcomes [83]. Thus, it has been proposed that a decrease in immunoproteasome expression might represent a mechanism of immune escape in tumor cells which present with a mesenchymal phenotype since this downregulation is associated with a decline in the amount and diversity of MHC-I-presented peptides [83]. In accordance with these data, transforming growth factor β (TGF-β)-induced epithelial–mesenchymal transition leads to a decrease in the immunoproteasome content [83]. Moreover, in the early stage of NSCLC, low expression of the i20S is linked to an increased risk of recurrence and metastasis onset [83]. At the molecular level, one proposed mechanism which links carcinogenesis with immunoproteasome deficiency is the differential expression of the β5i subunit. Two main β5i variants have been described, which are both induced by IFNγ: LMP7E2 and LMP7E1. LMP7E2, usually expressed in normal cells and in certain cancer types, is regularly incorporated into the mature i20S. However, many cancer cell lines express only the LMP7E1 isoform that does not interact with the 20S assembly chaperone POMP and thus cannot be integrated into the mature i20S, leading to a deficiency of functional immunoproteasome [84]. Moreover, a polymorphism at amino acid 49 of LMP7 (K49 instead of Q49), localized at the pre-sequence of β5i, reduces the rate of proteasome assembly and is associated with a higher risk of developing colon carcinoma [85]. On the other hand, overexpression of immunoproteasome subunits due to an increase in IFNγ production by TILs correlates with a better prognosis in different tumors, such as melanoma and breast cancer [79][81]. Immunoproteasome expression is not only triggered by paracrine production of proinflammatory cytokines (such as IFNγ) by immune cells, but it is constitutively elevated in hematological malignancies [86][87]. In myeloid leukemia cells, the i20S increase was associated with a higher survival rate [88]. Interestingly, the upregulation of immunoproteasome by IFNγ overcomes resistance to the proteasome inhibitor bortezomib and sensitizes hematological malignant cells (such as multiple myeloma and leukemia) to a selective immunoproteasome inhibitor ONX0914 [89]. This opens up the perspective of developing therapeutic approaches based on selective inhibition of immunoproteasome subunits different from those targeting the constitutive ones. Moreover, some evidence indicates that immunoproteasome alterations can have an impact on the onset of inflammation-driven carcinogenesis: indeed, β5i inhibition prevents colitis associated with colon carcinoma [90]. Thus, the emerging complex picture indicates that the altered expression of immunoproteasome subunits (mainly LMP2 and LMP7) is common in various tumors, but the extent of the expression and its biological significance vary depending on cancer type and grading [51][90]. As a matter of fact, the immunopeptidome changes in the context of tumor microenvironment and depending on the relative abundance of constitutive or inducible proteasome. For example, a number of cancer antigens derived from members of the melanoma antigen gene protein family (MAGE), whose expression is restricted to reproductive tissues but which are also aberrantly expressed in a wide variety of cancer types, such as $MAGA3_{(114–122)}$, $MAGEC_{(42–50)}$, and $MAGEA2_{(338–344)}$, are produced by immunoproteasome but not by the constitutive proteasome [61][91]. Since the identification and characterization of neoantigens is of clinical relevance, modern strategies which combine genomic, proteomic, and immunopeptidomic approaches are a powerful way of discovering novel presented antigens and tumor-associated antigens, paving the way to the novel therapeutic potential [92][93]. As mentioned in the previous section, intermediate proteasomes broaden the repertoire of MHC-I antigenic peptides and, intriguingly, are involved in the production of unique tumor antigens. In fact, it has been reported that some peptides derived from proteins belonging to the melanoma antigen gene (MAGE) family are generated by intermediate forms. Specifically, the β1i–β2–β5i intermediate produces the $MAGE\text{-}A10_{(254–262)}$ peptide, whereas the β1–β2–β5i intermediates generate the $MAGE\text{-}C2_{(191–200)}$ and $MAGE\text{-}A3_{(271–279)}$ peptides. On the other hand, other antigenic peptides, such as $MAGE\text{-}A3_{(114–122)}$ and $MAGE\text{-}C2_{(42–50)}$, are produced with equal efficiency by the i20S and intermediate proteasomes [49][94][95]. Moreover, intermediate proteasomes were detected in a number of tumor cells, including lung carcinoma, myeloma, osteosarcoma, and melanoma [96][97][49][94]. Despite this evidence, the role of these forms of proteasome in cancer onset and development is poorly known.

5.Immunoproteasome and Immune Checkpoint Inhibitors: A Glance to the Future?

As mentioned in the previous sections, the production of tumor-associated antigenic peptides recognized by CTLs is a process that starts in the cytoplasm with the degradation of cellular proteins mainly by immunoproteasome [98][99]. Peptide antigens produced by cancer cells are commonly classified into two main groups, namely with high and low specificity. Antigens with high tumor specificity are encoded by viral genes (expressed only in infected cells), mutated genes (generated by the intrinsic instability of cancer cells and hereafter referred to as neoantigens), and cancer germinal genes (expressed as a result of genome-wide demethylation occurring in germinal cells) [56][100]. Moreover, it is known that tumorigenesis is strictly related to genetic diversity and high mutational burden of cancer cells, which increase the possibility of production of neoantigens [81][93][101][102]. This high mutational heterogeneity and neoantigens frequency positively correlate with the response to ICKi therapy. In fact, ICKi are particularly effective against cancers that present with a high burden of mutations and are characterized by DNA mismatch repair deficiency, such as colorectal cancer and NSCLC [103][104][105]. Thus, neoantigens have been proposed to be a prognostic marker for positive clinical outcomes [81][93][101][102]. As a matter of fact, one of the main reasons of acquired resistance to the ICKi therapy seems to be the loss of neoantigens recognized by circulating T cells, suggesting that tumors are “able” to eliminate some mutations during the acquisition of a resistant phenotype [105]. Remarkably, despite the approval of the ICK therapy for cancers characterized by a high mutational burden, a very recent study failed to support the concept that a high mutational burden is a positive biomarker for the ICKi treatment in all solid tumors. In fact, a high mutational burden seems to behave as a predictive marker of response to ICK-based therapies only when the $CD8^+$ infiltration level correlates with the neoantigen load (such as melanoma, lung, bladder cancers, and colon cancer). On the other hand, for tumors in which no relationship between $CD8^+$ levels and the neoantigens load is reported (such as glioma), the high mutational burden failed to predict a positive response to therapy [106]. Thus, it clearly emerges that additional tumor type-specific studies should be performed to unveil the role of this biomarker in the ICKi response. Anyway, the identification of an additional biomarker as well as of non-invasive techniques that monitor the microenvironment before and during the course of the treatment (e.g., imaging-based radiogenomics) are urgently needed for selecting patients who will benefit from immunotherapy [107][108].

In light of the plethora of antigenic peptides produced by the proteasome pathway, it is not surprising that alterations of the proteasome activity and composition could be linked to antigen processing and the ICKi response. Of note, the local production of IFNγ within the tumor microenvironment by infiltrating T lymphocytes positively correlates with clinical outcomes to the ICKi therapy and cancer vaccination in tumors like metastatic melanoma [109][110]. In fact, the upregulation and secretion of chemotactic cytokines (such as IFNγ and TNF-α) increase the recruitment of additional immune cells and alter the tumor microenvironment, stimulating the inhibition of immune exclusion of cancer signatures [111]. Interestingly, some studies show that the primary response to anti-CTLA4 antibodies required a high-level exposure of MHC-1 on the surface of cancer cells at baseline; on the other hand, the response to anti-PD-1 antibodies is linked to a pre-existing IFNγ transcriptome signature [112]. In a recent study, the transcriptome of baseline and on-therapy tumor biopsies from a cohort of 101 patients with advanced melanoma included within the CheckMate 038 study (https://clinicaltrials.gov/ct2/show/NCT01621490, accessed date: 25 July 2021) and treated with nivolumab alone or in combination with ipilimumab has been analyzed [113]. These data, together with in vitro studies, suggest that the immune activation, which follows the administration of ICKi, is associated with the expression of IFNγ response genes, mediated by the increase of T cell infiltration. Among the different sets of genes induced by IFNγ, the most relevant in mediating the response to the therapy are those involved in antigen-presenting machinery [113]. Thus, a combination therapy of ICKi with agents that independently trigger the intratumoral production of IFNγ could become meaningful [113][114][115][116]. Consistently with the role of IFNγ signature in driving ICKi response, it has been proposed that upregulation of immunoproteasome subunits in tumor cells might be also involved in this process [109][113]. In fact, the local production of IFNγ induced by ICKi, which are routinely used in the treatment of advanced/metastatic melanoma, leads to the modulation of proteasome composition, thus inducing the generation of antigenic peptides [109][110]. Consistent with this observation, the expression of immunoproteasome subunits β1i and β5i was associated with a better prognosis in the case of tumors with a high mutational burden (i.e., melanoma and NSCLC) and positively correlated with the response to ICKi and the survival rate of patients [83][109][117]. Thus, it has been proposed that at least in some tumors the expression level of the β1i and β5i subunits might represent a predictive marker of response to ICKi [109][118]. As a matter of fact, the overexpression of these subunits is linked to longer survival and improved response to the ICKi therapy in melanoma patients and the proposed mechanism underlying this connection consists in enhanced reactivity of TILs toward melanoma cells as a consequence of an altered repertoire of the presented antigens [109]. Importantly, it has also been reported that immunoproteasome subunit overexpression sometimes occurs regardless of IFNγ and T cell infiltration, suggesting that these subunits should be independent prognostic biomarker with respect to the IFNγ level and the rate of T cell infiltration in the context of tumor microenvironment [109]. Thus, this last observation opens up a possible and yet

poorly investigated scenario concerning the role of immunoproteasome in mediating the ICKi response independently of the IFNγ pathway. Therefore, even though many crucial points deserve to be clarified, it clearly emerges that immunoproteasome expression seems to be an important predictive marker in colorectal cancer, melanoma, and NSCLC, for which the ICKi therapy has proven to be effective. Thus, an intriguing therapeutic strategy that should be explored in the near future is the combination of ICKi and drugs that directly modulate immunoproteasome activity and/or induce immunoproteasome expression in order to increase its pool inside the cells.

6. Conclusion

Cancer cells are often more dependent on a proper integrity and functionality of UPS than nonmalignant cells due to the rapid proliferation rate, increased metabolic activity, and continuous exposure to a variety of extrinsic stress perturbations (such as nutrient deprivation, hypoxia, and acidosis) under which cancer cells live. All these conditions lead to a decrease in protein quality control and make UPS a suitable target for cancer therapy [111][112]. Accordingly, a number of proteasome based-strategies have been proposed, spanning from (i) inhibition of proteasome proteolytic activity, (ii) modulation of the abundance of proteasome regulatory particles (i.e., 19S or PA28) and of their interaction with the 20S to (iii) modulation of the activity of enzymes involved in proteasome subunit post-translational modifications and (iv) interference with transport of natural low-molecular-weight proteasome activators (e.g., spermine) [46][119][120][121][122][123][124][125]. Additionally, a series of strategies targeting the ubiquitination cascade have been studied. One of the most intriguing and novel therapeutic approaches involves the use of PROTAC (proteolysis-targeting chimeric molecules), which are hetero-bifunctional molecules that recruit specific target proteins to the E3 ligase, thus inducing the increase of target ubiquitination and degradation. This strategy has already been applied to the degradation of a number of selected targets [126][127][128]. However, even though promising, it is still in its infancy for application to immunotherapy. On the other hand, the most deeply investigated strategy consists in the use of broad-specificity inhibitors of the 20S activity, like bortezomib, carfilzomib, and ixazomib that inhibit proteolysis of all the proteasome forms present in different cells [15][46]. The second approach encompasses the identification of specific inhibitors targeting inducible tissue-specific forms of proteasome, mainly the i20S, which is involved in the production of antigenic peptides. Though this mechanism is well-known, how such inhibitors might either decrease or stimulate cancer cell recognition by T cells is debated [46]. As a matter of fact, a challenging but poorly investigated issue is the precise in vivo impact of broad-specificity 20S proteasome inhibitors commonly used in clinical practice on antigen presentation by cancer cells [56], and it should deserve more attention. A key question for improving the efficacy and safety profile of immunotherapy includes the identification of the most appropriate strategy to optimize the antigenic peptide repertoire of the tumor required for an efficient immune response [56]. Notably, some recent data suggest that enhanced immunoproteasome activity might play an important role in the response of melanoma to ICKi [109]. It seems to indicate that, at least in some tumors, the more efficient strategy could be to “enhance” immunoproteasome expression and activity. Thus, the choice of the best strategy whether to inhibit or activate immunoproteasome should take into consideration the biological features of the specific tumor that has to be treated. Even though additional in vitro and in vivo investigation needs to be performed, the current evidence indicates that selective modulation of proteasome activity might have a role in improving the outcome of the ICKi or other immunotherapeutic approaches.

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