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Central Role of the Antigen-Presentation and Interferon-γ Pathways in Resistance to Immune Checkpoint Blockade

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Abstract

Resistance to immunotherapy is due in some instances to the acquired stealth mechanisms of tumor cells that lose expression of MHC class I antigen–presenting molecules or downregulate their class I antigen–presentation pathways. Most dramatically, biallelic β2-microglobulin (B2M) loss leads to complete loss of MHC class I expression and to invisibility to $CD8^+$ T cells. MHC class I expression and antigen presentation are potently upregulated by interferon-γ (IFNγ) in a manner that depends on IFNγ receptor (IFNGR) signaling via JAK1 and JAK2. Mutations in these molecules lead to IFNγ unresponsiveness and mediate loss of recognition and killing by cytotoxic T lymphocytes. Loss of MHC class I augments sensitivity of tumor cells to be killed by natural killer (NK) lymphocytes, and this mechanism could be exploited to revert resistance, for instance, with interleukin-2 (IL-2)-based agents. Moreover, in some experimental models,

potent local type I interferon responses, such as those following intratumoral injection of Toll-like receptor 9 (TLR9) or TLR3 agonists, revert resistance due to mutations of JAKs.

INTRODUCTION

Immune checkpoint blockade (ICB) therapy has improved the treatment landscape of cancer, but it is limited by primary resistance, as when ICB fails to provide any objective response upon first administration, and by acquired resistance, as when a cancer initially responds but regrows after a period of time while on therapy (Sharma et al. 2017). The main reason for primary resistance is the lack of a significant preexisting T cell–based immune response to the cancer that had been kept in check by one of the major immune checkpoints, such as the cytotoxic T lymphocyte antigen 4 (CTLA-4) or programmed death receptor 1 (PD-1). If the immune system was not trying to attack the cancer and was not limited by a particular immune checkpoint, then releasing such an immune checkpoint with a blocking antibody cannot be expected to successfully treat the cancer. Since carcinogen-induced cancers and cancers that accumulate DNA damage through alterations of the mismatch repair machinery are the most responsive to ICB (Ribas & Wolchok 2018), it is reasonable to assume that primary resistance is mainly due to the low antigenicity of the cancer, which limits the ability of T cells to differentially recognize cancer cells from normal cells (Schumacher & Schreiber 2015). However, some degree of antigenicity leading to weak antitumor immune responses is expected in most patients with cancer. The hope is that such preexisting weak immune responses could be invigorated by combination therapies at least in a subset of patients with primary resistance.

The study of ICB resistance is facilitated when patient-derived biopsies from when the cancer was sensitive are paired with those from when the cancer has already acquired resistance. Finding genetic or nongenetic changes that compare baseline, responding, and acquired resistance biopsies provides rich understanding of the mechanisms of response and resistance to ICB. It is easier to definitively demonstrate genetic alterations to be mechanistically related to resistance to any therapy, but even with targeted therapies genetic alterations only explain a subset of cases. If a recurrent genetic event in a relevant pathway is detected in the acquired resistance biopsy that was not present at baseline, then even if overall it is an infrequent finding, its biological significance is very high, as it highlights what cancer cells can do to escape from the therapy. In this review, we focus on cancer cell–intrinsic changes that lead to resistance to ICB and on the key signaling pathways that are involved in response and resistance to this mode of immunotherapy. It is important to consider that other postulated mechanisms of resistance are not tumor cell–intrinsic and are mediated by diverse mechanisms such as stromal inflammatory components (Pérez-Ruiz et al. 2020) or the composition of gut microbiota (Baruch et al. 2021).

PRIMARY AND ACQUIRED RESISTANCE TO ICB: A KEY ROLE FOR TUMOR CELL–INTRINSIC ANTIGEN PRESENTATION AND IFNγ SIGNALING

Clinical studies have provided ample evidence for the central role of $CD8^+$ T cells in ICB outcomes, showing that the likelihood of benefit from therapy is higher for patients with $CD8^+$ T cell–inflamed (hot) metastases than for patients with noninflamed (cold) lesions (Cristescu et al. 2018, Eroglu et al. 2018, Jerby-Arnon et al. 2018, Taube et al. 2014, Tumeh et al. 2014). Other studies have correlated clinical responses with elevated expression of cytolytic markers and interferon signatures as surrogates for T cell activation in tumor biopsies (Ayers et al. 2017, Grasso

et al. 2020, Liu et al. 2019, Van Allen et al. 2015). The presence in the tumor stroma of activated CD8$^+$ T cells recognizing tumor antigens seems to depend on mechanisms of presentation of tumor antigens taken up and processed by specialized antigen-presenting conventional type 1 dendritic cells (cDC1s) (Sánchez-Paulete et al. 2016, 2017).

How do CD8$^+$ T cells achieve cancer control? Primed tumor-infiltrating CD8$^+$ T cells become triggered to kill when their T cell receptors (TCRs) engage cognate HLA-I (human leukocyte antigen class I)-antigen complexes on tumor cells. T cell activation induces the release of perforin- and granzyme-containing granules and the secretion of the inflammatory cytokine interferon-γ (IFNγ). Granule-mediated cytolysis of the target cell has long been considered the major antitumor effector mechanism of CD8$^+$ T cells, in line with data associating the clinical benefit of immunotherapy, including ICB, with cytolytic maker expression in melanoma biopsies (Rooney et al. 2015, Van Allen et al. 2015). In contrast to cytolytic granules, operating only at the T cell–target cell interface (immune synapse), IFNγ distributes in the microenvironment, where it acts also on neighboring tumor cells (Neubert et al. 2017, Sanderson et al. 2012).

Binding of IFNγ to the ubiquitous surface IFNγ receptor 1/2 (IFNGR1/2) complex leads to the activation of the receptor-associated kinases JAK1 and JAK2, which phosphorylate STAT1, driving transcription of primary interferon-responsive genes including *IRF1* as inducers of secondary response genes (Alspach et al. 2019), ultimately turning on directly or indirectly more than 500 genes in cancer cells (Grasso et al. 2020). Therefore, via the JAK1/2-STAT1 signaling axis, IFNγ impacts on multiple cancer cell–intrinsic processes. This cytokine contributes to disease control by enhancement of antigen presentation, expression of IFNγ pathway signaling molecules, production of chemokines, inhibition of cell proliferation, and induction of cell death (**Figure 1**). The emergence of IFNγ-resistant tumor cells, as observed in primary and acquired

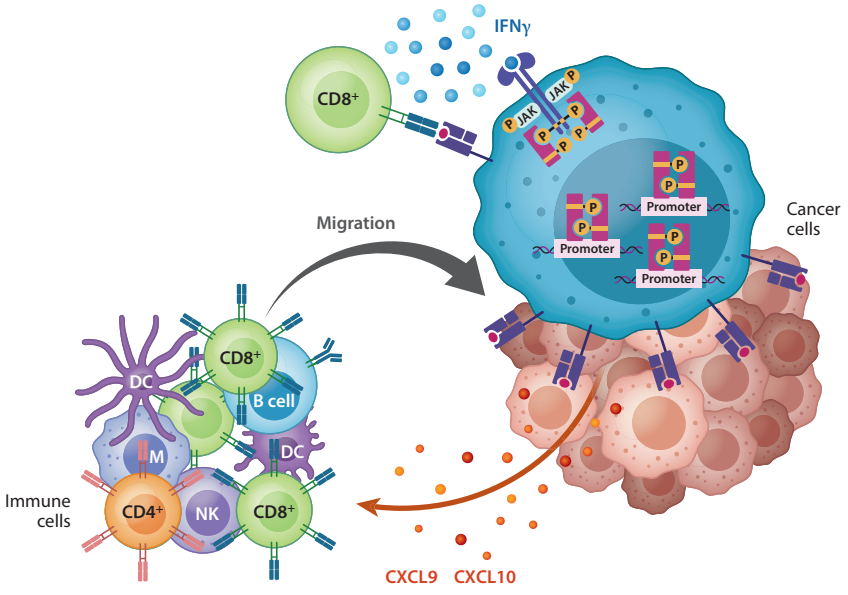

Figure 1

Cancer cells become enablers of an immune response through the expression of IFNγ response genes, which upregulate the antigen-presentation machinery, amplify the interferon response, and induce the chemokines CXCL9 and CXCL10 to attract more immune cells. Abbreviations: DC, dendritic cell; M, macrophage; NK, natural killer cell.

resistance to ICB, indicates a strong T cell–selective pressure that forces tumor cells to dampen or switch off the IFNGR1/R2-JAK1/2-STAT1 signaling cascade in order to survive (Gao et al. 2016, Shin et al. 2017, Sucker et al. 2017, Zaretsky et al. 2016). Alternatively, tumor cells evade selective T cell pressure in ICB by downregulation or loss of antigen presentation (Chowell et al. 2018, Gettinger et al. 2017, Sade-Feldman et al. 2017, Such et al. 2020, Zaretsky et al. 2016). Below we highlight both processes, tumor cell–intrinsic IFNγ signaling and antigen presentation, as the mechanistic bases of antitumor $CD8^+$ T cell immunity and the development of resistance to ICB.

LONG-DISTANCE CYTOSTATIC AND CYTOTOXIC EFFECTS OF IFNγ ON TUMOR CELLS

TCR-dependent $CD8^+$ T cell activation elicits the directed transport of cytolytic granules and IFNγ-containing vesicles toward the immunological synapse. While the release of perforin and granzymes is restricted to the T cell–tumor cell interface, the leakiness of the synapse for IFNγ enables it to spread into the tumor microenvironment (Sanderson et al. 2012) (**Figure 2**). Activation of the JAK1/2-STAT1 signaling pathway in bystander tumor cells can have cytostatic and cytotoxic effects. Cytokine spreading might explain how cancer control can be achieved despite low numbers of tumor-infiltrating $CD8^+$ T cells and low killing rates. In fact, studies in different murine tumor models (melanoma, lymphoma) have demonstrated that small numbers of adoptively transferred tumor antigen–specific $CD8^+$ T cells arrested the growth of tumor cells that were several times higher in number (Beck et al. 2019, Matsushita et al. 2015). Recent imaging studies that analyzed the spatiotemporal activity of IFNγ in the tumor microenvironment have demonstrated that the long-distance cytokine effects ranged between 100 and 800 μm, corresponding to 30–40 cell layers away from the T cell–tumor cell contact site (Hoekstra et al. 2020, Thibaut et al. 2020) (**Figure 2**). Moreover, analyses on mosaic tumor transplants consisting of antigen-positive and antigen-negative tumor cells have shown that low numbers of antigen-positive cells

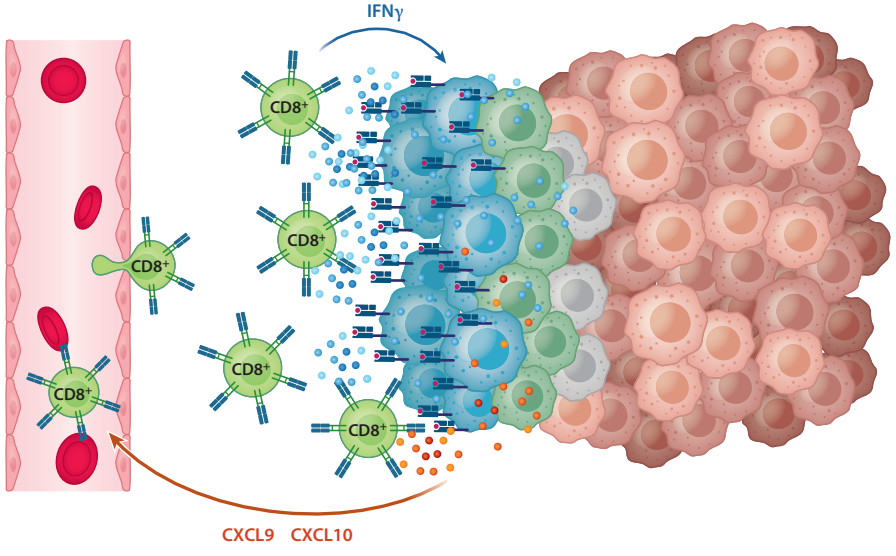

Figure 2

IFNγ gradient in a cancer being attacked by $CD8^+$ T cells and the attraction of more IFNγ-producing T cells through downstream chemokines CXCL9 and CXCL10.

were sufficient to block the proliferation of higher numbers of antigen-negative cells and that this control was abrogated when tumor cells lacked intact IFNγ signaling (Hoekstra et al. 2020). Overall, these studies demonstrated that contact-dependent cytolytic activity of T cells is not sufficient to achieve cancer control and that long-distance cytostatic and cytotoxic effects of IFNγ on bystander tumor cells critically contribute to antitumor effects. The importance of intact tumor cell–intrinsic IFNγ signaling in $CD8^+$ T cell–based cancer immunotherapy including ICB was recapitulated by genome-wide in vivo and in vitro CRISPR/Cas9-based knockout screens, revealing loss of tumor control upon genetic inactivation of IFNGR1/R2-JAK1/2-STAT1 pathway components (Kearney et al. 2018, Lawson et al. 2020, Manguso et al. 2017, Pan et al. 2018, Patel et al. 2017). Accordingly, elevated expression of interferon pathway components in tumor biopsies has been associated with clinical responses to ICB (Ayers et al. 2017, Grasso et al. 2020, Kim et al. 2021, Liu et al. 2019, Van Allen et al. 2015).

Mechanistically, IFNγ signaling in tumor cells induces a G0/G1 cell cycle arrest (Acquavella et al. 2015, Braumüller et al. 2013, Gollob et al. 2005, Matsushita et al. 2015). The cell cycle arrest can even be permanent when T cells secrete both IFNγ and tumor necrosis factor α (TNFα). As shown in different tumor models, combined IFNγ and TNFα signaling induces tumor cell senescence, involving the activities of the cell cycle inhibitors p16 (CDKN2A) and p21 (CDKN1A), thereby reducing disease progression (Ahmetlic et al. 2021, Braumüller et al. 2013, Brenner et al. 2020).

Prolonged IFNγ signaling can also trigger cell death (Hoekstra et al. 2020, Sucker et al. 2017, Thibaut et al. 2020). Different types of cytokine-induced programmed cell death have been reported, including apoptosis, necroptosis, and ferroptosis (Chin et al. 1997, Thapa et al. 2011, Wang et al. 2019). Necroptosis has been observed when tumor cells become deficient in caspase 8–dependent apoptosis as a consequence of T cell–selective pressure (Rooney et al. 2015, Thapa et al. 2011). Ferroptosis is induced upon lethal phospholipid peroxidation and has recently been suggested as a major mechanism by which $CD8^+$ T cells achieve tumor control in ICB (Wang et al. 2019). The type of tumor cell response to IFNγ signaling will most likely be determined by different factors, including local cytokine concentration, duration of JAK1/2-STAT1 signaling, and cancer cell transcriptional programs that influence intrinsic pathway activation. Interestingly, in melanoma, cytokine-induced dedifferentiation of tumor cells is an indicator of beneficial IFNγ signaling in response to ICB (Kim et al. 2021).

THE MULTIPLE EFFECTS OF IFNγ ON TUMOR ANTIGEN PRESENTATION

The spreading of IFNγ into the microenvironment affects not only proliferation and survival but also antigen presentation of bystander tumor cells. Already in the 1990s it was demonstrated that chemically induced tumors, lacking intact IFNγ signaling and displaying a stable MHC-I (major histocompatibility complex class I)-low phenotype, were less efficiently controlled by $CD8^+$ T cells than tumors with intact responsiveness to IFNγ (Kaplan et al. 1998). Immunohistochemistry analyses of human cancers have shown that tumor cells frequently display an HLA-I-low or even HLA-I-negative phenotype, which has been postulated to protect from $CD8^+$ T cell recognition (Aptsiauri et al. 2018, Rodig et al. 2018). JAK1/2-STAT1 pathway activation by IFNγ can counteract HLA-I downregulation and resensitize tumor cells to $CD8^+$ T cells (Sucker et al. 2017), demonstrating that IFNγ signaling, antigen presentation, and T cell sensitivity of tumor cells are closely linked. Accordingly, clinical benefit from ICB has been associated with enhanced expression of antigen-presentation markers in pretreatment biopsies (Such et al. 2020).

HLA-I antigen presentation is dependent on the interplay of a complex cellular machinery. Surface HLA-I antigen–presenting molecules are trimeric complexes consisting of the antigen peptide bound to a fitting binding groove in variable heavy chains, associated with the constant light-chain β2-microglobulin (B2M). In humans, classical HLA-I heavy chains are encoded by the three highly polymorphic loci *HLA-A*, *HLA-B*, and *HLA-C* in the *HLA* locus in the short arm of chromosome 6, with a maternal and paternal allele for each of them. The allelic polymorphisms of codominant expressed HLA-I molecules provide a diversity of peptide binding motifs to ensure the presentation of a large repertoire of 8–9-mer peptides from the cell proteome. Besides HLA-A, HLA-B, HLA-C, and B2M, IFNγ dramatically upregulates the expression of molecules involved in antigen processing, including proteasome subunits (LMP2, LMP7), peptidases (ERAP1, ERAP2), and antigen peptide transporters (TAP1, TAP2), and molecules involved in antigen peptide-loading onto HLA heavy chains (TAPBP) (Alspach et al. 2019).

The processed antigens in tumor cells can roughly be divided into four groups: neoantigens, germline antigens, differentiation antigens, and overexpressed antigens. Neoantigens are cancer specific, originating from expressed nonsynonymous somatic mutations, insertions and deletions, and chromosomal aberrations. The number of tumor mutations in exomes, defined as tumor mutational burden (TMB), is well accepted as a surrogate measure for neoantigenicity. Clinical responses in ICB have been associated with high TMB (Liu et al. 2019, Van Allen et al. 2015), and this association was even stronger when high TMB coincided with high $CD8^+$ T cell infiltration (hot tumors) (Cristescu et al. 2018). Neoantigen-specific T cells are considered very potent antitumor effectors, which have not been tolerized and express high-avidity TCRs (Oliveira et al. 2021). Additionally, T cells recognizing shared antigens (tissue differentiation antigens) can display potent antitumor activity and are present at high frequency in the peripheral blood and tumors of some cancer patients, especially in skin melanoma (Oliveira et al. 2021).

IFNγ determines the T cell sensitivity of tumor cells not only via the expression level of the HLA-I antigen-processing and -presentation machinery (APM), but also via its impact on the peptide epitope repertoire derived from a given tumor antigen. This repertoire is largely defined by the catalytic subunits of the proteasome, which degrade antigen proteins into peptides. IFNγ induces expression of the immunoproteasome subunits LMP2 and LMP7, which display different catalytic activities compared to the corresponding subunits of regular proteasomes and generate distinct although still overlapping peptide products. Clinical benefit to ICB in melanoma has been associated with LMP2/LMP7 subunit expression, in line with the observation that the peptide epitopes preferentially generated by the immunoproteasome lead to enhanced activation of tumor-infiltrating $CD8^+$ T cells (Kalaora et al. 2020). For loading into the HLA binding groove, some antigen peptides require further postproteasomal trimming by IFNγ-inducible peptidases, such as ERAP1 (Textor et al. 2016). Thus, IFNγ affects HLA-I antigen presentation at multiple levels that generally contribute to enhanced tumor cell immunogenicity.

In addition to HLA-I, IFNγ also induces the expression of HLA class II (HLA-II) molecules, which present antigens to $CD4^+$ T cells, a heterogeneous group of T lymphocytes primarily known for their role in promoting (type 1 helper T cells) or suppressing (regulatory T cells) antitumor $CD8^+$ T cell responses. Recent studies have demonstrated a role for HLA-II in response to ICB (Johnson et al. 2016, Oh et al. 2020, Rodig et al. 2018) and provided evidence for direct antitumor activity of $CD4^+$ T cells by either release of senescence- and cell death–inducing type I effector cytokines (IFNγ, TNFα) or HLA-II-dependent granzyme/perforin-dependent tumor cell killing (Braumüller et al. 2013, Cachot et al. 2021, Müller-Hermelink et al. 2008). Thus, besides $CD8^+$ T cells, $CD4^+$ T cells might also contribute to tumor immune surveillance by similar effector mechanisms. Importantly $CD4^+$ T lymphocytes are able to license dendritic cells to prime $CD8^+$ T cells in a CD40L-CD40-dependent fashion (Ferris et al. 2020).

DEFECTIVE TUMOR CELL–INTRINSIC ANTIGEN PRESENTATION AND IFNγ SIGNALING IN PRIMARY AND ACQUIRED RESISTANCE TO ICB

Primary and acquired resistance to ICB is established when tumor cells evade $CD8^+$ T cell recognition and effector mechanisms by altered antigen presentation (Chowell et al. 2018, Gettinger et al. 2017, Sade-Feldman et al. 2017, Such et al. 2020, Zaretsky et al. 2016) and IFNγ signaling (Gao et al. 2016, Shin et al. 2017, Sucker et al. 2017, Zaretsky et al. 2016), respectively. Genome-wide CRISPR/Cas9-based knockout screens have confirmed defective antigen presentation and IFNγ signaling as key resistance mechanisms in cancer immunotherapy (Kearney et al. 2018, Manguso et al. 2017, Pan et al. 2018, Patel et al. 2017). Tumor cell–intrinsic resistance can be achieved by either genetic alterations or nongenetic mechanisms that counteract IFNγ signaling and antigen presentation.

ESCAPE FROM T CELL RECOGNITION

$CD8^+$ T lymphocytes may turn into cells licensed to kill other cells upon recognition of their cognate antigen on their targets. To expand and acquire such effector functions, $CD8^+$ T lymphocytes first need to have met the antigen recognized by their TCRs on specialized professional antigen-presenting cells, which provide costimulatory receptors and cytokines in addition to antigen presentation (Sánchez-Paulete et al. 2017). A subset of dendritic cells termed cDC1s specialize in capturing antigens from third-party cells including tumor cells and cross-present them to naïve and memory $CD8^+$ T cells (Anderson et al. 2021). This cross-priming mechanism by dendritic cells is key for the efficacy checkpoint inhibitors, since in the absence of these phenomena there is no baseline immune response amenable to being depressed. Indeed, weakness or failure of this antigen cross-presentation mechanism is considered a reason for primary therapy resistance that has been associated with tumor-intrinsic gene alterations such mutations in the β-catenin pathway, which lead to reduced chemoattraction of cDC1 into the tumor as a consequence of reduced CCL4/CCL5 expression (Luke et al. 2019, Ruiz de Galarreta et al. 2019, Spranger et al. 2015).

Once an immune response mediated by cytotoxic T lymphocytes (CTLs) is ongoing in the tumor microenvironment, antigen recognition leads to the formation of cytolytic synapses of CTLs with tumor cells and the production of cytokines such as IFNγ and chemokines that attract other T cells into the malignant tissue (**Figure 2**). The killing process is contingent on cell-to-cell contact and degranulation of the CTLs. Effector killing mechanisms include the formation of pores in the plasma membrane of target cells by polymerized perforin, which permits the entrance of granzyme B, which triggers caspase activation and apoptosis. In addition, in lytic synapses the action of FASL and TRAIL on their counterreceptors FAS and DR4/5 triggers apoptosis of target cells. These latter mechanisms require that tumors express such proapoptotic cell counterreceptors that can be downregulated under immune selective pressure.

The most important event for killing is antigen recognition of a few cognate peptides presented by MHC-I molecules. If tumor-intrinsic antigen presentation is absent or weakened, then $CD8^+$ T cells, even if properly preactivated, cannot execute their function. Experimental evidence shows that complete loss of MHC-I expression (because of *B2M* biallelic loss) or loss of the alleles presenting the relevant neoantigens is conducive to tumor escape (McGranahan et al. 2017, Rosenthal et al. 2019, Sucker et al. 2014, Zaretsky et al. 2016).

Hence, tumor cells may evade T cell recognition by inactivating genetic alterations in distinct components of the HLA-I APM. Several studies have linked genetic alterations abrogating HLA-I antigen presentation in tumor cells to primary and acquired resistance to ICB (Chowell et al. 2018,

Gettinger et al. 2017, Sade-Feldman et al. 2017, Zaretsky et al. 2016). Most frequently described have been inactivation of single or multiple HLA genes and loss of *B2M*. *B2M* loss establishes an HLA-I-negative $CD8^+$ T cell–resistant tumor cell phenotype. To gain *B2M* deficiency, there must be a complex genetic evolution consisting in a loss-of-function mutation plus alterations in chromosome 15q (Bernal et al. 2012, Grasso et al. 2018, Gurjao et al. 2019, Sucker et al. 2014, Zhao et al. 2016). Longitudinal sample analyses in melanoma have revealed the early acquisition of chromosomal losses, followed by inactivating mutations in tumor subclones (Sucker et al. 2014, Zhao et al. 2016). Strong $CD8^+$ T cell–mediated selective pressure can lead to multiple resistant tumor clones, as in one patient showing distinct inactivating mutations in *B2M* (Zhao et al. 2016). There are mechanisms by which $CD8^+$ T cells kill neighboring bystander tumor cells, with a prominent role for FAS-FASL interactions, in order to prevent the escape of antigen- or HLA-loss variants (Upadhyay et al. 2021). Moreover, in murine mosaic tumor transplants consisting of B2m-positive and B2m-negative tumor cells, T cell–derived IFNγ can suppress the outgrowth of bystander MHC-I-loss variants (Hoekstra et al. 2020). Similar mechanisms might be active in human tumors, as suggested by occasional reports of patients with baseline *B2M*-knockout tumors that responded to ICB (Benci et al. 2019, Grasso et al. 2020, Liu et al. 2019, Rizvi et al. 2018, Sade-Feldman et al. 2017). However, *B2M*-knockout tumors could be targets of tumor-reactive $CD4^+$ T cells (Germano et al. 2021, Middha et al. 2019, Nagasaki et al. 2020), as shown in Hodgkin's disease, where Reed–Sternberg cells frequently show B2M loss and are probably controlled upon successful PD-1 blockade immunotherapy by $CD4^+$ T cells (Nagasaki et al. 2020).

Each HLA-I allele is able to present peptides with different binding motifs; therefore, the broader the repertoire of HLA-I molecules (HLA divergence), the better the response to ICB (Chowell et al. 2019). In this regard, *HLA* loss of heterozygosity (LOH) is associated with resistance to ICB (Montesion et al. 2021). As a result of the coevolution of cancer cells and ongoing $CD8^+$ T cell–mediated immune responses, neoantigen-driven *HLA* LOH and *B2M* loss have been observed in lung cancer and melanoma (Rosenthal et al. 2019, Schrors et al. 2017). This is reminiscent of the elimination, equilibrium, and escape phase paradigms discovered by Robert Schreiber's group in the interaction of immunity and cancer in mouse fibrosarcoma models (Mittal et al. 2014).

In addition to genetic mutations and alterations, transcriptional and posttranscriptional downregulation of HLA-I antigen presentation has been associated with primary resistance to ICB in melanoma (Lee et al. 2020, Rodig et al. 2018, Such et al. 2020). Transcriptional suppression has been proposed to be associated with low IRF2 levels (Kriegsman et al. 2019), TGFβ signaling (Lee et al. 2020), and epigenetic silencing (Burr et al. 2019).

Importantly, upon antigen recognition, CTLs release IFNγ, which diffuses over the surrounding tissue (Hoekstra et al. 2020, Thibaut et al. 2020), demanding neighboring cells to upregulate their APM. If all goes well, this leads to alertness for subsequent antigen recognition events (**Figure 1**). Accordingly, T cell–derived IFNγ could counteract HLA-I downregulation and restore T cell recognition of tumor cells. However, HLA-I-low or HLA-I-negative tumor cell phenotypes frequently lack T cell infiltrates (Al-Batran et al. 2005, Perea et al. 2017). As recently demonstrated, tumors might even develop complex resistant phenotypes showing HLA-I APM transcriptional silencing on a *JAK*-deficient genetic background (Sucker et al. 2017). Tumor cells with defective JAK1/2-STAT1 signaling maintain their $CD8^+$ T cell–resistant HLA-I-negative/-low phenotypes even in an IFNγ-rich microenvironment.

The importance of IFNγ for proper levels of tumor-intrinsic antigen presentation is paramount. However, it has been shown that uncoupling antigen presentation from IFN signaling loss is possible. Recent studies in murine and human melanoma models have demonstrated that

therapies targeting tumor cell–intrinsic innate immune receptors uncouple antigen presentation from IFNGR signaling and restore antigen presentation in *JAK1*-mutant cells based on the activity of transcription factors NF-κB and IRF (IFN regulatory factor) (Kalbasi et al. 2020, Such et al. 2020, Torrejon et al. 2020).

Invisibility to $CD8^+$ T cell recognition might increase susceptibility to lysis by natural killer (NK) cells because of downregulation of HLA-I surface molecules, which downregulate NK cytolysis directly via KIRs (killer cell immunoglobulin-like receptors) or indirectly via CD94/NKG2A interactions with HLA-E (Muntasell et al. 2017). Moreover, $CD4^+$ T cells could also at least partially tackle alterations in HLA-I, since to operate they mainly need to recognize antigens presented by HLA-II molecules on stromal cells. Agents that invigorate NK and $CD4^+$ T cells might prevent or even revert resistance due to HLA-I loss or downregulation (Germano et al. 2021, Torrejon et al. 2020). T cell- and NK cell–stimulating cytokines such as constructs based on interleukin-2 (IL-2) or IL-15 (Rouanne et al. 2020, Wrangle et al. 2018), or anti-NKG2A antibodies such as monalizumab (André et al. 2018) might be useful to restore at least partial sensitivity to ICB.

ATTEMPTS TO DIRECTLY USE IFNγ TO TREAT HUMAN CANCER

Given the mechanisms discussed thus far, it has made sense to test recombinant IFNγ (rIFNγ) either systemically or delivered locally to treat cancer. Such clinical trials have been performed in the past with very limited clinical activity as monotherapy (Gleave et al. 1998, Schiller et al. 1996, Von Hoff et al. 1990). When delivered intraperitoneally for advanced cases of ovarian cancer, rIFNγ showed some activity to deal with residual disease upon second-look laparotomies (Pujade-Lauraine et al. 1996), but it was not sufficiently effective or was even detrimental if given subcutaneously in combination with chemotherapy in first-line treatment of patients with ovarian cancer (Alberts et al. 2008, Windbichler et al. 2000). Of note, administration of therapeutic doses of rIFNγ was limited by leukopenia and fever. All of those trials were performed before the ICB era; therefore, the potential combinability of IFNγ and PD-(L)1 blockade remains to be explored, and this could be interesting particularly in ovarian cancer. This combination possibility is important since IFNγ is the most potent known inducer of surface PD-L1 expression on tumor cells (Garcia-Diaz et al. 2017).

RESISTANCE TO IFNγ

Similar to antigen presentation, cancer cells switch off or downregulate IFNγ signaling by genetic and nongenetic mechanisms in order to evade tumor-suppressive cytokine activity and establish primary and acquired resistance to ICB (Gao et al. 2016, Shin et al. 2017, Sucker et al. 2017, Zaretsky et al. 2016). Defective IFNγ signaling protects cancer cells from cytotoxic and cytostatic cytokine effects and counteracts HLA-I APM upregulation, thereby preserving poor tumor cell immunogenicity (Gao et al. 2016, Hoekstra et al. 2020, Sucker et al. 2017). Moreover, defective IFNγ signaling blocks PD-L1 upregulation on tumor cells. Overall, this leads to a PD-L1-low/HLA-low tumor cell phenotype that resists anti-PD-1 ICB (Garcia-Diaz et al. 2017, Shin et al. 2017).

Even though deep sequencing of human tumor biopsies has found inactivating mutations present in all genes of the IFNGR1/R2-JAK1/2-STAT1-IRF1 pathway (Gao et al. 2016, Sucker et al. 2017), functional relevance in resistance to ICB has mainly been attributed to JAK1 and JAK2 (Gulhan et al. 2020, Shin et al. 2017, Zaretsky et al. 2016). In melanoma, the development of JAK1/JAK2 deficiency follows patterns similar to the evolution of B2M loss, involving allele

losses due to early chromosomal aberrations and subsequent inactivating mutations in tumor subclones (Shin et al. 2017, Sucker et al. 2017). The *JAK2* gene is located on chromosome 9p, to which also map the *CD274* gene, encoding PD-L1, and the tumor-suppressor gene *CDKN2A* (Horn et al. 2018, William et al. 2021). Deletion of *CDKN2A* by chromosome 9p loss is a frequent and early genetic alteration in melanoma and other cancers that generally leads to the codeletion of *JAK2* and *CD274* (Horn et al. 2018). Loss of *CDKN2A* has been identified as a mechanism by which tumors may escape IFNγ-induced senescence (Braumüller et al. 2013, Brenner et al. 2020). Thus, aneuploidy in chromosome 9p affects antitumor immune responses at the level of JAK2, PD-L1, and CDKN2A, explaining its association with nonresponsiveness to ICB (Davoli et al. 2017, Roh et al. 2017).

However, tumor cells gain resistance to IFNγ by not only genetic but also nongenetic mechanisms. Genome-wide CRISPR/Cas9-based knockout screens have identified different regulators of IFNγ responses in tumor cells. As such, APLNR, a G protein–coupled receptor, interacts with JAK1 to enhance JAK-STAT signaling. Inactivating mutations in *APLNR* have been detected in tumors from patients refractory to immunotherapy (Patel et al. 2017). The phosphatase PTPN2 functions as a negative regulator of JAK-STAT signaling. Accordingly, its inactivation enhanced antitumor $CD8^+$ T cell responses in vitro and in vivo (Lawson et al. 2020, Manguso et al. 2017). Additionally, epigenetic regulators have been identified that counteract IFNγ responses in tumor cells. PBAF, a form of the SWI/SNF chromatin remodeling complex, dampens the expression of IFNγ target genes (Pan et al. 2018), and similar observations have been described for histone methyltransferase EZH2, the enzymatic component of PRC2 (polycomb repressive complex 2) (Wee et al. 2014).

Nongenetic resistance to IFNγ enables tumors to still exploit protumorigenic cytokine activities. As such, chronic IFNγ signaling in tumor cells has been demonstrated to upregulate not only PD-L1 but also ligands of other inhibitory checkpoint receptors (Benci et al. 2016, Garcia-Diaz et al. 2017), thereby counteracting effective $CD8^+$ T cell activation. Moreover, studies in murine tumor models have demonstrated that T cell–derived IFNγ enhances the genome instability of tumor cells and induces stem cell–like features in melanoma cells (Liu et al. 2017, Takeda et al. 2017). Interestingly, the development of the stem cell–like tumor phenotype seems to be driven metabolically by indoleamine 2,3-dioxygenase (IDO), an enzyme that degrades tryptophan. IFNγ-induced IDO activity in tumor cells depletes tryptophan from the microenvironment, thereby also efficiently inhibiting antitumor T cell responses (Uyttenhove et al. 2003).

REVERSING RESISTANCE TO ICB THROUGH MECHANISTICALLY BASED COMBINATION THERAPIES

The convergence of immune escape mechanisms in the MHC-I antigen-presentation pathway and in the interferon-signaling pathway suggests a means to overcome resistance. Loss of *B2M* and lack of surface expression of MHC-I molecules take away the main ligand for NK inhibitory receptors. Therefore, it would be hypothesized that following *B2M* knockout and MHC-I loss, cancer cells would be more sensitive to NK cells. This has led to the testing of the administration of a reformulated IL-2 to stimulate NK cells against *B2m*-knockout subcutaneous tumors in mouse models. The antitumor activity was mediated by not only NK cells but also $CD4^+$ T cells (Torrejon et al. 2020) (**Figure 3**). $CD4^+$ T cells were also involved in response to combined ICB in a different *B2m*-knockout model (Germano et al. 2021). The induced responses to *B2M*-knockout experimental tumors recapitulate the occasional observations of cancer cases with baseline *B2M* loss that respond to ICB, probably through the activation of non-$CD8^+$ effector immune cells (Benci et al. 2019, Germano et al. 2021, Grasso et al. 2020, Liu et al. 2019, Middha

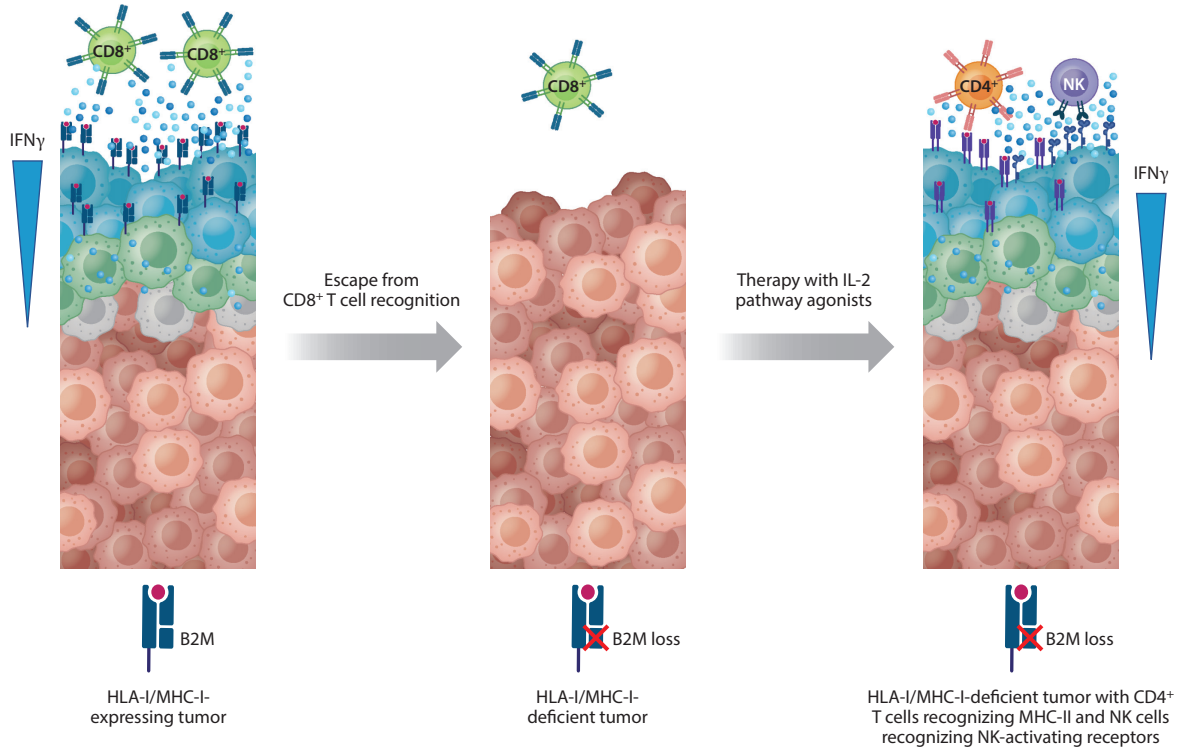

Figure 3

MHC-I/HLA-I-deficient tumors escape $CD8^+$ T cell recognition but can be controlled by therapy with reformulated IL-2, which mobilizes tumor-reactive NK cells and $CD4^+$ T cells. Abbreviations: B2M, β2-microglobulin; IL-2, interleukin-2; HLA-I, human leukocyte antigen class I; MHC-I, major histocompatibility complex class I; MHC-II, major histocompatibility complex class II; NK, natural killer cell.

et al. 2019, Nagasaki et al. 2020, Rizvi et al. 2018, Sade-Feldman et al. 2017). Despite these observations, it is clear that *B2M* loss is a mechanism of acquired resistance in the cases where it was not mutated at baseline, with deletion of one *B2M* allele and a deleterious mutation in the other allele.

Overcoming resistance to loss of IFNγ signaling can be achieved by inducing a strong type I interferon response in the tumor microenvironment, even when cancer cells themselves cannot respond to interferons due to *JAK* mutations. Intratumoral therapies such as Toll-like receptor (TLR) agonists, either CpG oligonucleotides or double-stranded RNA, can reverse resistance in mouse models with *Jak1* or *Jak2* knockout in the tumor cells (Kalbasi et al. 2020, Torrejon et al. 2020) (**Figure 4**). The clinical correlation of this benefit is shown in studies administering TLR9 or TLR3 agonists to patients with metastatic cancers that were previously progressing on anti-PD-1-based therapy, since a fraction of patients respond when receiving the combination of the intratumoral TLR agonist with systemic anti-PD-1 in injected and noninjected lesions (Márquez-Rodas et al. 2020; Ribas et al. 2018, 2021). Other potential means to induce intratumoral type I interferon responses include agonists of STING (Nicolai et al. 2020, Woo et al. 2014) and RIG-I-like receptors (Bald et al. 2014, Rehwinkel & Gack 2020, Such et al. 2020), as well as ADAR inhibitors if developed in the future (Ishizuka et al. 2019, Lawson et al. 2020).

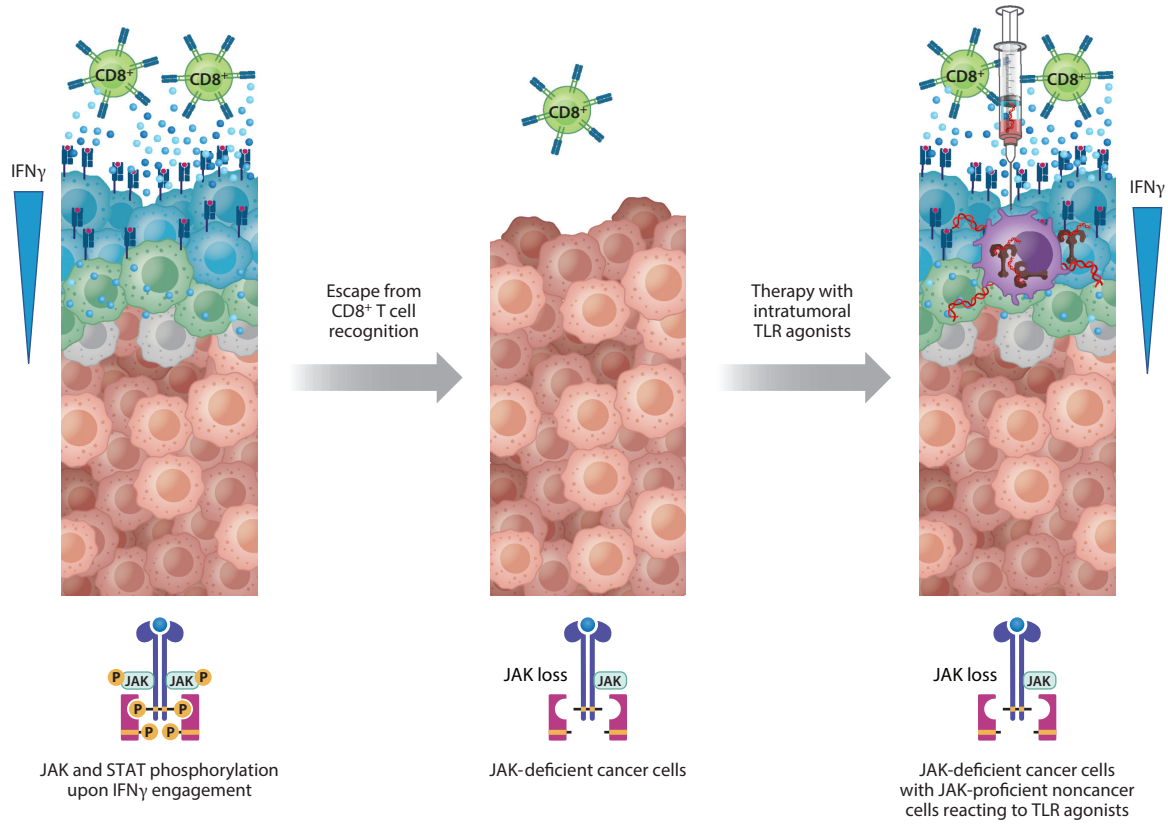

Figure 4

JAK1- or JAK2-deficient tumors escape CD8$^+$ T cell recognition by not amplifying the antitumor immune response with lack of IFNγ signaling. Upon injection of a Toll-like receptor (TLR) agonist, type I IFN signaling is started by noncancer cells in the tumor microenvironment, such as plasmacytoid dendritic cells (*purple*) when TLR9 agonists are injected, and reestablishes cancer cell recognition by CD8$^+$ T cells.

CONCLUSIONS

The mechanistic understanding of how cancer responds to and resists immune attack unleashed by ICB helps define the key pathways important in mediating antitumor activity. This mode of therapy requires that there be a certain level of baseline immune activation induced by the cancer and that the tumor microenvironment be permissive enough to allow T cells to exert antitumor cytotoxicity. Studies by several research groups using biopsies taken from patients at baseline, during response to ICB, and in occasional cases of acquired resistance point to the central role of the antigen-presentation pathway and the IFNγ signaling pathway. The knowledge of the specific means by which a cancer that was previously sensitive to therapy can become resistant has provided new hypotheses to test in preclinical models and in the clinic in order to develop combination therapies that address these resistance mechanisms. Additionally, there are other potential means to increase the antitumor activity of ICB, such as acting on additional immune checkpoints, modulating the tumor metabolism, inhibiting negative regulators in the tumor microenvironment, or altering the microbiome.

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Contents

Errata

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