Antigen presentation in cancer: insights into tumour immunogenicity and immune evasion

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Abstract | Immune checkpoint blockade, which blocks inhibitory signals of T cell activation, has shown tremendous success in treating cancer, although success still remains limited to a fraction of patients. To date, clinically effective CD8$^+$ T cell responses appear to target predominantly antigens derived from tumour-specific mutations that accumulate in cancer, also called neoantigens. Tumour antigens are displayed on the surface of cells by class I human leukocyte antigens (HLA-I). To elicit an effective antitumour response, antigen presentation has to be successful at two distinct events: first, cancer antigens have to be taken up by dendritic cells (DCs) and cross-presented for CD8$^+$ T cell priming. Second, the antigens have to be directly presented by the tumour for recognition by primed CD8$^+$ T cells and killing. Tumours exploit multiple escape mechanisms to evade immune recognition at both of these steps. Here, we review the tumour-derived factors modulating DC function, and we summarize evidence of immune evasion by means of quantitative modulation or qualitative alteration of the antigen repertoire presented on tumours. These mechanisms include modulation of antigen expression, HLA-I surface levels, alterations in the antigen processing and presentation machinery in tumour cells. Lastly, as complete abrogation of antigen presentation can lead to natural killer (NK) cell-mediated tumour killing, we also discuss how tumours can harbour antigen presentation defects and still evade NK cell recognition.

Major histocompatibility complex
(MHC). A locus that encodes several genes involved in antigen presentation and other related immune processes.

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CD8$^+$ T cells are the primary mediators of anticancer immunity, and modulation of the CD8$^+$ T cell response has been a central focus of immunotherapy to treat cancer[1]. When CD8$^+$ T cells specifically recognize antigenic peptides presented by the major histocompatibility complex (MHC; in vertebrates) or human leukocyte antigen (HLA; in humans) class I molecules on tumour cells, they become activated and kill the tumour cells. Owing to the presence of inhibitory signals at the tumour site, tumour-specific T cells are often dysfunctional. The removal of these signals by immune checkpoint inhibition (ICI) leads to the reinvigoration of T cells and clinical efficacy, although only in a fraction of patients[2]. The presence of other immunosuppressive factors and reduced antigen presentation by tumour cells could explain this limited activity[3]. Indeed, studies suggest that tumours have developed various means to limit HLA-I presentation of antigens and evade immune recognition.

Cancer rejection antigens are the targets of antitumour T cells. Candidates for such rejection antigens include tumour-associated antigens (TAAs; BOX 1), viral antigens and tumour-specific antigens (TSAs; BOX 1). Each of these categories has generated interest in the development of antigen-targeting onco-immunotherapies. With the advent of ICI, interest in TAAs has been reinvigorated using potent vaccine platforms in combination with ICI, or using rare high-affinity T cell receptors (TCRs) for adoptive T cell therapy or T cell-redirecting bispecific molecules[4–6]. Therapeutic vaccination approaches against human papillomavirus (HPV) have shown some promising results in HPV-induced cancers[7–9]. The majority of TSAs are neoantigens that can result from several types of genomic aberration in tumours. Neoantigens are present in most tumours and are associated with clinical response to ICI and adoptive T cell therapy[10–15]. Early clinical trial results of neoantigen-specific cancer vaccines suggest that neoantigen-specific T cell responses are elicited but it is unclear whether these responses can be clinically effective[16–20]. A challenge with targeting of neoantigens is that they are often unique to each patient and require the development of individualized approaches, called individualized neoantigen-specific immunotherapy (iNeST).

Box 1 | **Cancer rejection antigens**

Tumour-associated antigens

Tumour-associated antigens (TAAs) are self antigens encoded in the germline genome that are preferentially expressed in tumours[20,173]. They are generally weakly immunogenic owing to central tolerance and peripheral tolerance. Examples of TAAs are listed here.

- Cancer testis antigens, for example NY-ESO1, members of the MAGE, GAGE, XAGE, BAGE and PAGE families, SSX1, SSX2, and so on.
- Differentiation antigens, for example gp100, tyrosinase, melan-A/MART1, PSA.
- Overexpressed antigens, for example HER2, hTERT, CEA.
- Oncofetal antigens, for example PSA, AFP, WT1.
- Endogenous transposable elements such as human endogenous retroviruses. These have generated recent interest because they can be reactivated in cancers and are associated with response to immune checkpoint inhibition (ICI)[77,174–176]; they trigger an innate antiviral response[174,175] and have the potential to produce antigens that activate adaptive immunity[177,178].

Viral antigens

Being of foreign origin, viral antigens are highly immunogenic. Several viruses are implicated in carcinogenesis. However, these viruses are not necessarily tumour specific, and tumours may not necessarily express them at later stages of tumour development,for example human papillomavirus (HPV), Epstein–Barr virus (EBV) and Merkel cell polyomavirus (MCV).

Tumour-specific antigens

Tumour-specific antigens (TSAs) are highly tumour-specific and arise because of tumour-specific irregularities.

- The best characterized TSAs are neoantigens that result from several types of tumour-specific genomic aberrations, including single nucleotide variants, indels, gene fusions, aberrant splicing events and even integration of oncogenic viruses that may introduce novel chimeric transcripts. They can be recognized as foreign by the immune system and are highly immunogenic[10,14,15,179]. They are the main drivers of protective CD8$^+$ T cell response in ICI and tumour-infiltrating lymphocyte (TIL) therapy[10–15]. They are often unique to each patient and require the development of individualized approaches[180,181].
- TSAs can result from post-translational modifications, for example glycosylation or phosphorylation, that generate novel T cell and B cell epitopes[182–184]. Their contribution to antitumour immunity has yet to be demonstrated, and predicting these alterations is challenging.

Presentation of cancer rejection antigens by HLA-I is crucial for the success of immunotherapies aimed at stimulating antitumour CD8$^+$ T cell responses, including ICI and iNeST, and it is important to understand to what extent HLA-I presentation is defective in tumours. To elicit an effective antitumour response, antigen presentation has to be successful at two distinct events: first, cancer neoantigens have to be taken up by professional antigen-presenting cells (pAPCs), mostly dendritic cells (DCs), and cross-presented for priming of naive CD8$^+$ T cells[21]. Second, the neoantigens have to be directly presented by tumour cells for recognition and killing by primed CD8$^+$ T cells. Tumours develop multiple mechanisms to reduce antigen presentation at these two steps and escape immune recognition, including suppression of DC function, and downregulation of HLA-I expression by tumour cells by interfering with the antigen processing and presentation machinery (APM; FIG. 1). Although complete downregulation of HLA-I presentation can appear as an attractive escape mechanism for tumours to avoid immune recognition, the immune system has an important checkpoint to monitor loss of HLA-I presentation. Natural killer (NK) cells detect loss of HLA-I surface expression as a stress signal and target the stressed cell ('missing-self' recognition). Therefore, tumours have also evolved more subtle strategies of immune evasion without completely abolishing HLA-I surface expression. A better understanding of how tumours reduce HLA-I presentation of rejection antigens will provide insight into the design of novel approaches to overcome antigen presentation deficiency and ensure the success of immunotherapies that depend on antigen presentation. Tumours can also present antigens on HLA-II for recognition by CD4$^+$ T cells, and this pathway could also be subject to regulation for immune evasion. It is not reviewed here but is summarized in BOX 2.

In this Review, we briefly discuss the evidence for DC dysfunction that results in defective antitumour CD8$^+$ T cell response. Next, we review the evidence for defects in antigen presentation observed in human cancers, with a focus on understanding to what extent tumours abrogate, modulate or alter HLA-I presentation, and the importance of NK cells in monitoring and counteracting a reduction of antigen presentation. Finally, we discuss how these mechanisms of resistance inform the design of effective immunotherapies.

Dendritic cell defects

DCs play a central role in the initiation and maintenance of antitumour T cell immunity[21]. Upon taking up dying tumour cells that release danger signals, including molecules called damage-associated molecular patterns (DAMPs), DCs undergo maturation, migrate to the draining lymph nodes, and process and load cancer antigens onto HLA-I for presentation to CD8$^+$ T cells[22]. DCs also upregulate co-stimulatory molecules and produce pro-inflammatory cytokines that are essential for the adequate priming of naive T cells. Among the various DC subsets (BOX 3), type 1 conventional DCs (cDC1s) play a crucial role in antitumour immunity[23]. *Batf3*-knockout mice lacking cDC1s fail to mount an antitumour CD8$^+$ T cell response and respond to ICI[24–26]. Abundance of cDC1s in the tumour microenvironment (TME) is associated with T cell infiltration, overall survival in patients with cancer and response to ICI[22,27,28]. In addition to their role in the priming of naive tumour-specific CD8$^+$ T cells in the draining lymph nodes, cDC1s are essential for the reactivation of circulating central memory T cells and their differentiation into tissue-resident memory CD8$^+$ T cells in murine tumours and response to ICI[29]. Intratumoural cDC1s are also required for the recruitment of adoptively transferred or memory CD8$^+$ T cells to the tumour by producing CXC-chemokine ligand 9 (CXCL9) and CXCL10 upon activation via the stimulator of interferon genes (STING) pathway in tumour-bearing mice[30]. Intravital imaging studies of mouse tumours have revealed close contact between DCs and T cells in tumours, suggesting T cell–cDC1 crosstalk in the TME[27]. Indeed, cDC1s produce IL-12 upon sensing IFNγ released from T cells in the tumour bed in mice treated with anti-PD1 ICI[31]. IL-12 in turn augments CD8$^+$ T cell activation and function, presumably in the context of cross-presentation by cDC1s. Another study showed that tissue-resident

Human leukocyte antigen (HLA). In humans, MHC is also called HLA. HLA-I, or MHC class-I, includes classical HLA-Ia genes (*HLA-A*, *HLA-B* and *HLA-C*) and non-classical HLA-Ib genes. Classical HLA genes present peptides at the cell surface, while non-classical HLA gene products have several other functions including natural killer cell activation or inhibition, and presentation of metabolites, lipids, etc. In this Review, we use HLA-I to refer to the classical genes only. Similarly, HLA-II will be used to refer to classical MHC class-II genes. For most of the discussion, we use the term HLA instead of MHC.

Neoantigens
Mutated peptides presented on the tumour cell surface by HLA. They are specific to tumours, as they arise from somatic mutations, thus distinguishing them from self antigens.

Professional antigen-presenting cells
(pAPCs). Cells that specialize in presenting antigens on MHC molecules to prime and stimulate T cells. These include dendritic cells, macrophages and B cells.

Antigen processing and presentation machinery
(APM). Includes the peptide loading complex and also peptide processing machinery such as the proteasome.

memory CD8$^+$ T cells amplify antitumour immunity in mice by inducing the maturation and migration of DCs to the draining lymph nodes[32]. In addition, it was recently found that cDC1s are essential for CD4$^+$ T cell priming in a mouse fibrosarcoma engineered to express the model antigen ovalbumin[33]. cDC1s prime naive CD4$^+$ T cells by presenting tumour antigens on MHC-II. In turn CD4$^+$ T cells license cDC1s via CD40 signalling for the generation of an effective antitumour CD8$^+$ T cell response[33].These results contrast with the conventional model in which cDC2s prime CD4$^+$ T cells[34].

As cDCs play a major role in priming antitumour T cell response, they are trans-targets of tumours to escape priming of the adaptive immune system (FIG. 2). cDC1s are recruited to the tumour site by various chemokines from various cell sources depending on the tumour model, such as the CC-chemokine ligands CCL4[35] and CCL5 (REF.[36]) secreted by tumour cells, and CCL5 and XC-chemokine ligand 1 (XCL1, also known as lymphotactin) secreted by intratumoural NK cells[37]. Interestingly, cDC1s are sparse in the TME of early-stage tumours in comparison with the adjacent normal tissue, suggesting that inhibition of the recruitment of cDC1s or cDC1 precursors to the tumour site can be an early mechanism to limit the development of antitumour immunity[38]. cDC1 recruitment to the tumour can be inhibited through multiple mechanisms. Activation of the β-catenin signalling pathway in tumours prevents the recruitment of cDC1s to the tumour bed and confers resistance to ICI, by inhibiting tumour cell secretion of CCL4 or CCL5, depending on the mouse tumour model[35,36]. Similarly, activation of the β-catenin signalling pathway in human cancers is associated with low tumour infiltration of cDC1s and T cells[35,36]. Secretion of prostaglandin E_2 (PGE_2) produced in a cyclooxygenase 2 (COX2)-dependent manner by hypoxic murine tumour cells prevents the accumulation of cDC1s at the tumour site by impairing the viability and function of NK cells, and by inhibiting cDC1 responsiveness to chemokines[39,40]. Likewise in human cancers, COX2 expression inversely correlates with intratumoural NK cells and T cells[39]. PGE_2 potentially reduces cDC1 viability by targeting NK cells, because NK cells have also been shown to promote intratumoural cDC1 survival by producing Fms-like tyrosine kinase 3 ligand (FLT3L)[41]. Moreover, DCs exhibit a more mature phenotype in *Cox2* knockout tumours, indicating that PGE_2 suppresses DC maturation[39].

Upon taking up dying tumour cells and sensing associated DAMPs, immature cDC1s undergo a maturation process. They migrate to the draining lymph node and simultaneously deliver tumour antigens to the cross-presentation pathway for priming of naive CD8$^+$ T cells (BOX 3). The exact nature of tumour DAMPs that induce DC maturation is still not fully elucidated and seems to vary depending on the mechanism of tumour cell death[42–45]. Tumours have developed multiple pathways to suppress DC maturation and function. Necrotic tumour cells release high levels of PGE_2, which suppresses the immunostimulatory activity of DAMPs on macrophages and DCs in vitro[46]. Release of vascular endothelial growth factor (VEGF) by tumour cells affects DC differentiation and maturation[47,48]. The TME also actively produces cytokines that interfere with DC maturation, such as IL-6, transforming growth factor-β (TGFβ) and IL-10, and therefore promote the conversion of DCs into a tolerogenic phenotype[49–51]. A novel population of DCs with an immunoregulatory gene signature, called mature DCs enriched in immunoregulatory molecules (mregDCs), was recently identified in human and mouse tumours[52]. This immunoregulatory programme is associated with the uptake of dying tumour cells and appears to restrain cDC1 immunostimulatory function and limit T cell activation in the draining lymph nodes. This regulatory programme also appears to be present in normal tissues, suggesting that it is a homeostatic mechanism to regulate the strength of the T cell response. Secreted factors in the TME can hijack this regulatory programme to promote tumour escape. In addition to defects in maturation, studies have shown that intratumoural cDC1s can exhibit impaired cross-presentation. Elevated levels of oxidized lipid in DCs have been associated with defects in cross-presentation[53–56]. This phenomenon appears to be at least partially mediated by increased lipid uptake resulting from the upregulation of scavenger receptor MSR1 protein expression by intratumoural DCs[53].

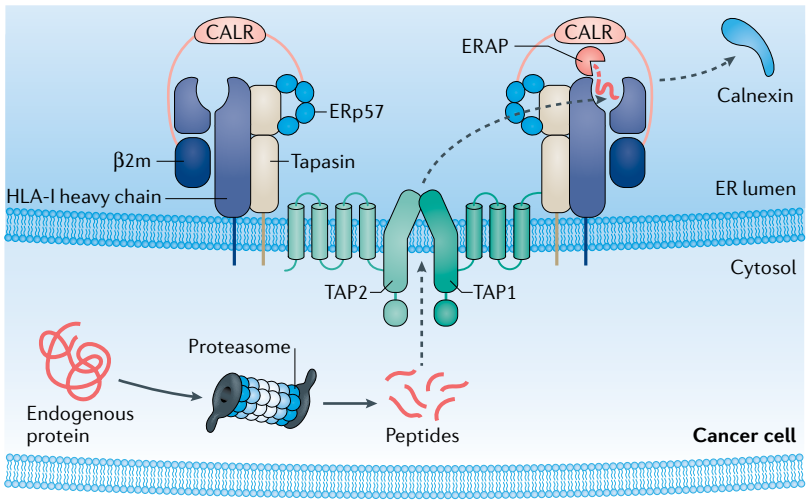


Fig. 1 | **Antigen processing and presentation machinery.** The HLA-I complex displayed on the surface of cells consists of the class I human leukocyte antigen (HLA-I) heavy chain, β2-microglobulin (β2m) and the antigenic peptide. The antigen presentation pathway is summarized here. Endogenous proteins are degraded by the proteasome into peptides. Some of these peptides are further trimmed by cytosolic proteases[164,165]. The peptides are then translocated through the transporters associated with antigen processing (TAP) into the endoplasmic reticulum (ER) where they may get further trimmed by ER aminopeptidase (ERAP) either as free peptides or after loading onto HLA-I. Loading onto HLA-I is a multistep process facilitated by the peptide loading complex (PLC), which consists of the TAP and ER chaperones[166]. First, calnexin promotes the initial folding and the assembly of HLA-I heavy chain, then the HLA-I heavy chain assembles with β2m in the absence of a peptide. This empty complex is highly unstable for most HLA-I alleles, and it is stabilized by association with the core PLC consisting of the chaperone tapasin in complex with ERp57 and TAP, via calreticulin (CALR). A single TAP heterodimer associates with two empty HLA-I complexes. Tapasin then proofreads peptides for stable binding in the groove formed by the HLA-I heavy chain α_1 and α_2 domains[167,168]. Binding of high-affinity peptide induces the dissociation of HLA-I complex from the PLC and subsequent trafficking to the cell surface. Adapted from REF.[168], CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/).

Box 2 | **HLA-II antigen presentation modulation in tumours**

The role of class II human leukocyte antigen (HLA-II) expression on tumours remains largely unknown, although there is mounting evidence that it plays an important role in antitumour immunity[185]. A recent study found that the presence of $CD4^+$ T cells with a cytotoxic signature was associated with response to PDL1 blockade in bladder cancer[186], and these cells were capable of directly killing tumour cells. Defects in the HLA-II antigen processing and presentation machinery have been observed in tumours and have been reviewed by Seliger et al.[187]. In addition, HLA-II expression can be modulated at the transcriptional level. IFNγ induces HLA-II expression by upregulating the HLA-II transactivator (CIITA) in many tumour types[188].

Another study found that oxidized lipids are produced by DCs upon reactive oxygen species (ROS) activation in an ovarian cancer model[55]. Oxidized lipids activate the endoplasmic reticulum (ER) stress response factor, X-box binding protein 1 (XBP1), which induces lipid synthesis and aberrant accumulation of lipid droplets. The environmental signals leading to ROS activation in cDC1s have yet to be identified. Recent data suggest that oxidized lipids block cross-presentation by sequestering the chaperone HSP70 and precluding the translocation of the MHCI–peptide complexes to the cell surface in mice[56].

Thus, tumours can modulate their antigenicity in *trans* by altering tumour-associated DC function. Instead of allowing maturation of DCs and a DC-mediated antitumour response, DCs in tumours can be redirected towards a dysfunctional, tolerogenic or even immunosuppressive phenotype. By targeting DCs away from the immunosuppressive tumour environment, cancer vaccines can help overcome some of these defects and promote effective T cell priming. Alternatively, in situ vaccines are also being developed and show promising results. The delivery of potent adjuvants such as polyinosinic:polycytidylic acid (poly(I:C)), a Toll like receptor (TLR) 3 ligand, or a STING agonist that can overcome some of the immunosuppressive signals of the tumour site and induce DC maturation, has shown benefit in preclinical cancer models[57–59]. VEGF blockade therapy could also help promote DC differentiation and function[60]. In tumours with low DC infiltration, this treatment may have to be combined with FLT3L treatment to increase the number of intratumoural cDC1s, and with radiation therapy, chemotherapy or even adoptive T cell therapy to enhance tumour cell death and provide tumour antigens to cDC1s, for efficacy[25,26,61,62]. As another approach to increase intratumoural cDC1s, the delivery of CCL4 to the tumour was found to recruit cDC1s and improve response to ICI in preclinical models[63].

In situ vaccines
The delivery of an innate stimulus to dendritic cells (DCs) at the tumour site. Unlike conventional vaccines, which co-deliver antigens and innate stimulus to DCs to stimulate antitumour T cell immunity, in situ vaccines rely on the antigens released by dying tumour cells as a source of tumour antigens for DCs. Examples of innate stimuli evaluated in the clinic are TLR agonists (TLR7/8 ligands, TLR9 ligands, the TLR3 ligand poly(I:C)), STING agonist and anti-CD40 agonist antibody.

Peptide loading complex
(PLC). Includes the core set of proteins in the endoplasmic reticulum (ER) that mediate peptide transport into the ER and subsequent loading of peptide onto HLA-I. These include TAP1, TAP2, tapasin, ERp57, calnexin, calreticulin, ERAP1, ERAP2, HLA-I and β2m.

Defective HLA-I presentation in tumours

Following priming by cDC1s, $CD8^+$ T cells migrate from the draining lymph nodes to the tumour, where they recognize antigens directly presented on HLA-I by the tumour cells for killing. In addition to alterations in DC function to limit T cell priming, alterations in the antigen presentation pathway exist in tumours, sometimes leading to escape from recognition by $CD8^+$ T cells. A multitude of processes and players are involved in the presentation of a peptide by HLA-I. The HLA-I complex displayed at the surface of cells consists of three components: the HLA-I heavy chain, β2-microglobulin (β2m) and the 8–12 amino acid peptide derived from an endogenous protein. The HLA-I complex itself is a component of the APM, involved in the process of peptide presentation by HLA-I[64] (FIG. 1). The proteasome is the principal enzymatic complex that processes cellular proteins into peptides that are then transported by the ATP-binding cassette transporters TAP1 and TAP2 (or collectively, TAP) into the ER. Loading of peptides on HLA-I occurs in the ER and is aided by several accessory proteins and chaperones in the peptide loading complex (PLC). Once a stable peptide–HLA-I complex is formed, it is translocated to the cell surface. Although all nucleated cells constitutively display peptide–HLA-I complexes on their surface, antigen presentation can be upregulated in an inflammatory environment, mainly through the cytokine IFNγ produced by activated lymphocytes[65]. IFNγ receptor signalling through the JAK–STAT signalling pathway leads to the induction of several APM components, including the HLA-I heavy chain and β2m, either in *cis* or in *trans*[66,67] (FIG. 3a). This feedback loop allows for amplification of the immune response and increased recognition and killing of the target cells[68].

As human cancers develop in an immunocompetent host, tumour development is shaped by the host's immune system via a process called cancer immunoediting, and eventually tumours develop the capability to escape antitumour immune responses[69]. There are several mechanisms by which tumours reduce antigen presentation on their surface, including antigen depletion, reduced surface expression of HLA-I through genetic alterations, modulation of transcription, and alteration of the HLA-I peptidome repertoire through mutagenesis of the HLA-I genes and other components of the APM.

Antigen depletion

Depletion of cancer rejection antigens can be an attractive immune escape mechanism for tumours, especially if the antigen is a by-product of tumorigenesis, and not functionally critical for tumour cell survival. Several TAAs are a by-product of dysregulation of expression of their encoding genes, and the majority of neoantigens are derived from passenger mutations, which can also be dispensable for the tumour. Antigen depletion in tumours can occur by copy number loss at the genomic level, by RNA expression downregulation via epigenetic mechanisms or by post-translational mechanisms (FIG. 3a). In the case of subclonal neoantigens, which are present only in a subpopulation of tumour cells, antigen loss can be mediated by $CD8^+$ T cell killing of the entire subclonal cell population[70]. TAA loss has also been observed between different lesions from the same patients[71,72]. There is emerging evidence that tumours can lose neoantigens, both at the DNA level and the RNA level, under immune pressure. Tumours of untreated patients with non-small-cell lung cancer (NSCLC) show enriched hypermethylation at promoters of genes encoding non-expressed neoantigens, compared with the wild-type form of the same genes in other purity- and ploidy-matched tumours[73]. Moreover, neoantigen-encoding genes were less likely

Polymorphism at the HLA locus
HLA is the most polymorphic locus in humans, with more than 19,000 alleles documented. HLA-I consists of three genes, and since both alleles of each gene are expressed, up to six different HLA-I proteins or allotypes may be expressed in an individual, with each allotype presenting its own set of peptides. As different HLA-I allotypes may present a distinct set of peptides, the total repertoire of peptides presented by HLA-I (also called the HLA-I ligandome) is highly diverse.

to be expressed in tumours with high levels of immune infiltrates in these patients. Of 88 early-stage NSCLC tumours from untreated patients 43 (48.9%) showed evidence of copy number loss of clonal neoantigens, based on multi-region sequencing. In another study, neoantigen loss was associated with acquired resistance to ICI in four patients with NSCLC[74]. Disappearance of subclonal mutations, as well as the loss of clonal mutations, which are early mutations in tumour evolution and present in most of the tumour cells, was mediated by chromosomal deletions and loss of heterozygosity (LOH). Many of the eliminated mutations were recognized by the patients' T cells, further suggesting that immune pressure shaped the tumour neoantigen landscape. In addition to modulation of gene expression, tumours can also modulate neoantigen presentation by regulating protein turnover. Mutant proteins can be prone to misfolding and thereby higher turnover via the proteasome, leading to increased antigen presentation[75]. Recently, Jaeger and colleagues[76] made the intriguing finding that tumours can 'hide' mutated proteins from the antigen presentation pathway, by stabilizing mutated proteins with HSP90.

As effective neoantigens are not necessarily functionally important for the tumour, loss of such antigens can present as a refractory mechanism to antitumour immunity, and it poses a challenge to iNeST. The fact that ICI is effective in several patients with high tumour mutation burden suggests that neoantigen depletion as a mechanism for immune evasion may not be comprehensive, likely because immune escape mechanisms such as upregulation of immune checkpoints may remove the necessity of comprehensive neoantigen depletion. It is vital that iNeST should aim at a 'killer blow' upfront, by targeting a variety of clonally distributed neoantigens at once, to avoid falling into a trap of targeting subclonal neoantigens that are more likely to undergo depletion.

Box 3 | **Dendritic cell subsets and functions**

Dendritic cells (DCs) are a highly heterogeneous population of cells. They can be divided into three major subsets.

- Plasmacytoid DCs (pDCs) originate from both myeloid DC progenitor and lymphoid progenitors. They are poor at internalizing and presenting exogenous antigens but have a unique ability to produce massive amounts of type I interferon[189]. Their role in antitumour immunity is yet to be fully explored but intratumoural pDCs appear to exhibit impaired type I interferon production and immunosuppressive properties.
- Conventional DC1 and DC2 subsets (cDC1 and cDC2) originate from myeloid progenitors. They have distinct phenotypes and functions[190–192].
  - cDC1s are specialized in cross-presentation of exogenous antigens on class I human leukocyte antigen (HLA-I) for $CD8^+$ T cell priming. cDC1s have developed unique biological properties to promote uptake of cell-associated antigens and favour the processing of antigens for cross-presentation, including the use of the lectin CLEC9A (also known as DNGR1) to shuttle material from dead cells into endocytic compartments specialized in cross-presentation[193,194]. More recently, WDFY4 was identified in a CRISPR screen as crucial for the cross-presentation of dead-cell-associated antigens. The exact function of WDFY4 remains to be determined, although preliminary studies suggest that it may promote antigen trafficking to the cross-presentation pathway[195].
  - cDC2s are specialized in presentation of exogenous antigens on HLA-II for $CD4^+$ T cell priming. In turn $CD4^+$ T cells engage with cDC1s via CD40 signalling to 'license' them to cross-prime $CD8^+$ T cells[196,197], although a recent study challenges this view[33]. In patients with melanoma, an intratumoural cDC2 signature is associated with tumour-infiltrating $CD4^+$ T cells and response to immune checkpoint inhibition, and preclinical studies show that cDC2s drive protective antitumour CD4 immunity[34].

Genetic alterations in HLA-I and B2M

The HLA-I heavy chain and β2m are the core components of the peptide-presenting HLA-I complex. Cells can express up to six different HLA-I alleles from three genes (*HLA-A*, *HLA-B* and *HLA-C*) each presenting a unique set of peptides. Both mutations and loss of copy number leading to reduced or complete loss of peptide presentation have been described in their encoding genes. These genetic events can have different impacts on the extent and diversity of HLA-I presentation. As β2m is a component of all HLA-I allotypes, a heterozygous deleterious mutation or LOH at *B2M* can lead to reduced surface expression levels of HLA-I. Deleterious mutation on one allele coupled with LOH, or complete copy number loss of *B2M* is needed for complete loss of surface HLA-I. On the other hand, loss of a subset of HLA-I allotypes is likely to reduce the diversity of the presented peptide repertoire, as different HLA-I allotypes present a different repertoire. Understanding the prevalence of these events is important to estimate how often antigen presentation may be lost in different cancer indications, and to design appropriate immunotherapy strategies in cancers that do not respond to or become resistant to current immunotherapies.

Mutation identification at the HLA locus is challenging owing to the high degree of polymorphism at the HLA locus. Careful analysis of HLA-I gene mutations with customized alignments for HLA-I genes from the TCGA data set identified non-synonymous mutations in 3.3–4% of more than 7,500 samples of tumour tissue paired with corresponding normal tissues[77,78], although this frequency varies by cancer type and can be as high as 14%, as in stomach cancer. *B2M* non-silent mutations, on the other hand, appear less frequently (0.86%) and are most frequent in stomach cancer (5.7%) in the same sample sets. The lower frequency of *B2M* mutations compared with HLA-I mutations could be partly due to the fact that the β2m protein is smaller (119 amino acids) compared with the HLA-I heavy chains (362–366 amino acids). Most of the *B2M* mutations were heterozygous. Interestingly, HLA-I and *B2M* mutations were enriched in tumours that show high T cell cytolytic activity, suggesting selection pressure. Moreover, loss-of-function mutations (nonsense, frameshifts and splice site mutations) were enriched among HLA-I as well as *B2M* mutations across tumours, consistent with a tumour suppressor-type role for HLA-I and β2m[78].

It is noteworthy that some cancers exhibit abnormally high *B2M* mutation rates. In lymphomas, including diffuse large B cell lymphoma (DLBCL) and Hodgkin lymphoma, *B2M* is mutated or lost in more than 25% of patients, the mutations are enriched for inactivating frameshift indels or truncations, and about half of the patients carrying β2m aberrations show bi-allelic inactivating aberrations[79,80]. Because these cancers originate from B cells, which are pAPCs, immune surveillance might play an important role during early tumour development, and hence abrogation of antigen presentation may be imperative in driving tumour growth.

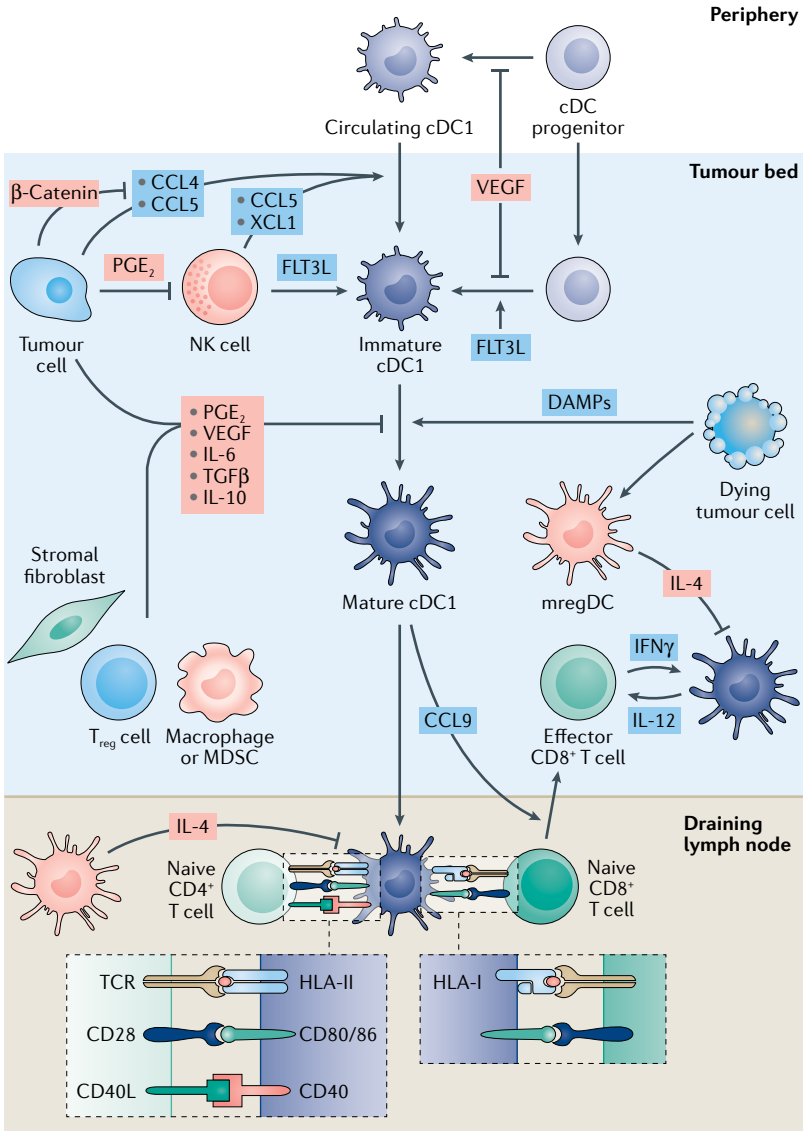
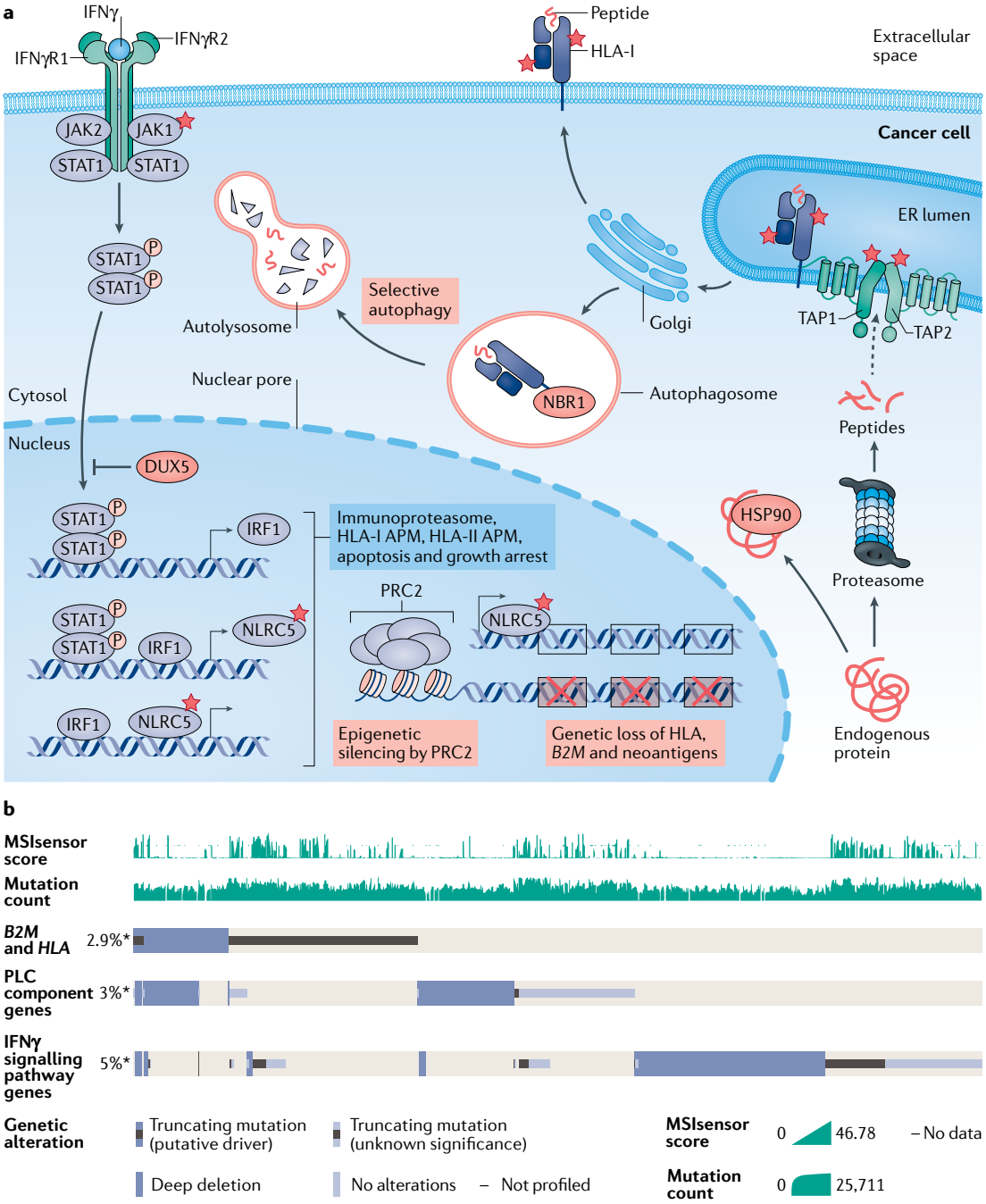


Fig. 2 | **Dendritic cells in antitumour immunity.** Type 1 conventional dendritic cells (cDC1s) are recruited into the tumour by chemokines. Fms-like tyrosine kinase 3 ligand (FLT3L) supports the cDC1 differentiation and survival. cDC1s take up dying tumour cells and undergo maturation upon the release of damage-associated molecular patterns (DAMPs), they migrate to the draining lymph nodes, and process and load cancer antigens onto class I human leukocyte antigen (HLA-I) and HLA-II for presentation to $CD8^+$ T cells and $CD4^+$ T cells, respectively. Naive $CD4^+$ T cells are primed first and in turn license cDC1s to prime $CD8^+$ T cells through CD40–CD40L signalling. In addition, intratumoural cDC1s produce the chemokines CXC-chemokine ligand 9 (CXCL9) and CXCL10 to recruit effector $CD8^+$ T cells. In the tumour microenvironment cDC1s produce IL-12 upon sensing IFNγ released from T cells, which in turn augments $CD8^+$ T cell activation and function. This feedback loop allows for amplification of the immune response. Tumours have developed multiple mechanisms to alter DC functions and escape the immune system. Vascular endothelial growth factor (VEGF) prevents the differentiation of DC precursors into cDC1s and limits cDC1 maturation. Activation of the β-catenin pathway in tumours prevents the recruitment of cDC1s to the tumour bed by inhibiting the chemokine secretion. Prostaglandin E_2 (PGE_2) prevents the accumulation of intratumoural cDC1s by altering their recruitment and their survival, and suppresses DC maturation. Various cell types including tumour cells, regulatory T cells (T_{reg} cells), M2 macrophages, myeloid-derived suppressor cells (MDSCs) and stromal cells, produce cytokines that interfere with DC maturation, such as IL-6, transforming growth factor-β (TGFβ), IL-10 and promote the conversion of DCs to a tolerogenic phenotype. A novel population of DCs enriched in immunoregulatory molecules, called mregDCs, restrain cDC1 immunostimulatory functions and limit T cell activation. In addition, induction of endoplasmic reticulum stress in intratumoural cDC1s leads to impaired cross-presentation. CCL, CC-chemokine ligand; TCR, T cell receptor; XCL1, XC-chemokine ligand 1.

Interestingly, these cancers also downregulate CD58, which is involved in activation of both T cells and NK cells, suggesting the need for escape from T cell as well as NK cell recognition[79]. Microsatellite instability-high (MSI-H) colorectal cancers (CRCs) also carry frequent *B2M* mutations (24% of 182 patients)[81], the majority of them being truncating mutations that would result in loss of function. Mutations in HLA-I and *B2M* are highly enriched in MSI-H cancers in general[82]. These cancers have an exceptionally high mutation rate and therefore a high neoantigen burden, so downregulation of antigen presentation could present an important and frequent mechanism to escape the immune system. Alternatively, it is plausible that the high frequency of *B2M* mutations in these cancers is a by-product of coding microsatellites found in *B2M*.

Besides non-silent mutations, genetic losses can also occur at HLA-I and *B2M* loci. The clinical impact of *B2M* loss has been examined mainly in the context of melanoma response to ICI treatment in several studies. Somatic mutation or LOH at *B2M* coupled with confirmed loss of protein expression[83] was found in 5 of 17 patients with melanoma who were non-responders or who progressed on ICI treatment. In other ICI cohorts of patients with melanoma, *B2M* LOH was enriched in non-responders at ~30% frequency and was associated with poor survival[83–85]. Complete loss of *B2M* was found only in non-responders. Recent studies have also started to examine HLA-I LOH in cancer and its impact on antitumour immunity. Studies of small cohorts of patients with cancer suggest a high prevalence of LOH at HLA-I loci, at ~40% (36 of 90 patients) in NSCLC[86] and in 83% (30 of 36) of thymic epithelial tumours[87]. Montesion and colleagues[88] assessed HLA-I LOH in a large pan-cancer clinico-genomic data set of 83,644 patient samples. They found that HLA-I LOH was common and detected in 17% of the patients, although it varied across tumour types (2–42%). Our understanding of the prevalence of LOH at HLA-I in patients with cancer is still developing, partly because of a lack of reliable computational tools, complicated by the polymorphic nature of the HLA-I locus. Thus, LOH at *B2M* and at HLA-I in patients with cancer appears to be frequent. Higher frequency of *B2M* and/or HLA-I LOH compared with complete loss, while naturally expected (complete loss requires two genetic events whereas LOH needs one), is also consistent with



the notion that tumours probably do not need complete downregulation of antigen presentation to escape immune recognition. Furthermore, at HLA-I loci, partial genetic loss is likely to reduce the diversity of peptides presented.

In conclusion, complete downregulation of surface HLA-I expression due to genetic loss and/or mutation in *B2M* or HLA-I heavy chain is rare, but as it is irreversible, HLA-I-independent therapies are needed in these scenarios unless HLA-I presentation can be restored by engineering tumour cells. Such approaches include NK cell-based therapy (see NK cell recognition of tumours section below), redirection of tumour-associated macrophages to become tumoricidal[89], and synthetic immunology strategies, such as CD3-bispecific antibodies or T cell engineering of chimeric antigen receptor (CAR), that redirect T cells to recognize surface tumour antigens independently of HLA-I, which have shown promising clinical responses especially in the treatment of haematological malignancies[90–92]. The recent discovery that a TCR that exhibits pan-cancer cell recognition via the invariant MR1, a HLA-I-like molecule, also has the potential to offer additional therapeutic opportunities[93].

Defects in other APM components

Aberrations in genes encoding other APM components besides HLA-I and β2m are not widespread, with the exception of certain cancers. Focusing on likely functional alterations, including homozygous deletions and truncating mutations, our analysis of the TCGA database indicates that ~3% of 10,967 analysed

◀ Fig. 3 | **Modulation of antigen presentation in cancer. a** | Processes responsible for antigen presentation in tumour cells are shown in shades of blue, while tumour-associated mechanisms resulting in reduced surface expression of class I human leukocyte antigen (HLA-I) are shown in salmon. Tumour-specific downregulation of molecules is indicated by a red star. In an inflammatory environment, T cells, natural killer cells and macrophages produce IFNγ, which induces signalling via the IFNγ receptor (a heterodimer of IFNγR1 and IFNγR2). This signalling leads to transcription of the HLA-I antigen processing and presentation machinery (APM) components and immunoproteasome subunits, as well as apoptosis and growth arrest. In some tumours, transcriptional reprogramming may induce expression of DUX5, which interferes with IFNγ signalling. NLRC5, a transactivator of expression of several APM components, is a frequent target of copy number loss in cancer. Other mechanisms of genetic dysregulation include epigenetic silencing by polycomb repressive complex 2 (PRC2) or genetic loss of HLA-I genes and of the antigen-encoding gene itself. HSP90 can sequester unfolded mutated protein to prevent its degradation by the proteasome and subsequent HLA-I presentation. Among the peptide loading complex (PLC) components, mutations in TAP have been rarely identified, but mutations have been identified at varying rates in HLA-I and in *B2M*. Cancer cells can also redirect peptide–HLA-I complexes to degradation by selective macroautophagy, mediated by NBR1. **b** | An oncoprint plot showing genomic alterations in genes encoding the PLC and IFNγ signalling components across several TCGA subjects. cBioportal was used to generate the plot. *B2M* and HLA are shown separately from genes encoding other PLC components, owing to their direct involvement in the HLA-I complex and extensive mutations compared with other PLC component genes. Genes encoding other PLC components include *TAPBP* (tapasin), *PDIA3* (ERp57), *CANX* (calnexin), *CALR* (calreticulin), *TAP1*, *TAP2*, *ERAP1* and *ERAP2*. The IFNG gene set includes *IFNGR1*, *IFNGR2*, *JAK1*, *JAK2*, *STAT1* and *NLRC5*. To focus on alterations that would induce loss of function, we selected possibly homozygous deletions (deep deletions) and truncating mutations (frameshift indels, nonsense mutations, nonstart mutations, etc. that are expected to truncate the protein). Only the subjects showing any of these alterations in the selected genes are shown, amounting to ~9% (956) of 10,967 patients in the TCGA data set. Green barplots at the top show a microsatellite instability score (MSIsensor score[169]) and total somatic mutation counts. Percentages in front of a gene set indicate the percentage of the total number of TCGA subjects that carry the selected alterations.

patients with cancer have these alterations (aberrations in PLC; FIG. 3b). Myeloproliferative neoplasms (MPNs) provide a particularly interesting insight into mutations in APM. They are associated with recurrent activating mutations in *JAK2* (110 of 151 patients) and frameshift mutations in the gene encoding calreticulin (*CALR*) (26 of 151)[94] in a mutually exclusive manner. Potential loss of functional calreticulin owing to these *CALR* mutations would impede recruitment of HLA-I to the PLC and reduce surface expression of HLA-I[95]. Truncating mutations in *CALR* were also found, although at a low frequency, in the TCGA cohort, which does not include MPNs (Supplementary Fig. 1). Most of these mutations are heterozygous and likely only reduce HLA-I surface expression. Moreover, cancers with frameshift mutations in *CALR* can be sensitive to ICI, suggesting that immune evasion in these tumours may be attributed to checkpoint upregulation and T cell exhaustion[96]. Nevertheless, the frequency of these alterations is high, and as in lymphomas, NK cell activity modulation may be important in MPNs. Indeed, MPNs show reduced activity and numbers of NK cells[97,98].

Reversible modulation of HLA-I expression

While genetic alterations in APM can result in irreversible loss or modulation of antigen presentation, several non-genetic means of loss of surface HLA-I expression have been observed in tumours, including modulation of cytokine signalling-mediated HLA-I upregulation, baseline transcription of APM, epigenetic regulation of HLA-I and post-translational modulation of surface HLA-I.

IFNγ-mediated HLA-I expression regulation. In an inflamed TME, activated immune cells release IFNγ, a cytokine with pleiotropic functions[99]. IFNγ signalling is a key pathway regulating HLA-I presentation (FIG. 3a) and HLA-II presentation (BOX 2), and disruption thereof is a mechanism for tumour immune escape. CRISPR screens performed in mouse tumour models[100] and in human melanoma cell lines[101] identified that the knockout of genes encoding components of IFNγ signalling is associated with resistance to ICI or impaired recognition of tumour cells by T cells. Specifically, the signalling components found to be necessary for immune surveillance were STAT1, JAK1, IFNγR2, IFNγR1 and JAK2, all directly involved in IFNγ signalling (FIG. 3a). Consistent with these results, loss of IFNγ pathway components in tumours was found to be associated with resistance to anti-CTLA4 therapy in patients with melanoma[102].

JAK1 and *JAK2* loss-of-function mutations have been reported in patients with cancer, and especially, *JAK1* loss-of-function mutations occur frequently, with the highest frequency observed in endometrial cancer in ~8% of patients[103] (Supplementary Fig. 1). Recurrent loss-of-function mutations in *JAK1* also correlate with a reduced IFNγ response signature. However, in several patients whose tumours harbour *JAK1* recurrent mutations, reduced expression of this signature has not been observed, which is likely due to heterozygous mutations and not homozygous loss of *JAK1*. Further insights about the clinical relevance of *JAK1* or *JAK2* mutations can be obtained from ICI clinical trials. Analysis of four patients with melanoma who acquired resistance to ICI showed that two of these patients had acquired *JAK1* and/or *JAK2* mutations after treatment[85]. Another study examining somatic mutations in IFNγ pathway genes in patients whose cancers were refractory to PD1 blockade identified one high-allelic-frequency *JAK1* mutation that was detected in 1 of 23 patients with melanoma, and another homozygous *JAK1* mutation in 1 of 16 patients with colon cancer[104]. Although low-allelic-frequency mutations were also found in other genes such as *JAK2*, *IFNGR1*, *IFNGR2* and *STAT1*, disease in patients carrying these heterozygous (or subclonal) mutations was not refractory to the therapy. This observation further fortifies the argument that heterozygous mutations in genes involved in antigen presentation can lead to reduced antigen expression in tumours instead of complete loss of antigen presentation. Interestingly, although maintenance of IFNγ signalling in tumours can potentially enable immune recognition, IFNγ signalling also has pro-tumour effects[105] and can enhance fitness of tumour cells in an inflamed microenvironment[106]. Presumably, in tumours in which IFNγ signalling functions in this manner, the APM might be intact, but immune recognition might be evaded by other means, including PDL1 upregulation.

Transcriptional and post-translational downregulation of HLA-I expression. Direct transcriptional regulators of HLA-I genes are frequently affected in tumours.

Analysis of tumour samples from patients with bladder cancers (specifically, transitional cell carcinoma) showed that tumours from 11 of 72 (15.3%) patients had total HLA-I loss based on immunohistochemistry analysis and mostly showed loss of HLA-I gene expression at the transcriptional level[107]. Besides HLA-I, other APM component genes were also downregulated in a coordinated manner, suggesting that a master regulator of APM gene expression might have been modulated in the respective samples[107]. NLRC5 (also known as MHC class I transactivator, CITA) is an IFNγ-inducible nuclear transactivator of several genes involved in the HLA-I antigen-presentation pathway[108,109] (FIG. 3a), and its encoding gene is a major target of copy number losses, found in 28.6% of 7,730 patients with cancer pooled across several cancer types in TCGA, while ~2% of 9,061 patients with cancer have somatic mutations in *NLRC5* (REF.[110]). In addition, the transcription factor DUX4, normally expressed in embryonic development, is reactivated in many cancers and blocks IFNγ-mediated induction of HLA-I gene expression. This mechanism was also found to be associated with resistance to anti-CTLA4 immunotherapy in patients with metastatic melanoma[111].

Reduction of HLA-I gene expression can also be achieved by epigenetic means, via hypermethylation of HLA-I genes (FIG. 3a). In patients with gastric cancer, tumour-specific hypermethylation at the promoters of at least one of the HLA-I genes was found in 87.23% of the patients, and the hypermethylation correlated with decreased expression of the corresponding genes in the majority of those patients[112]. Similarly, 70.1% of 87 patients with oesophageal cancer have hypermethylation of the promoter of at least one of the HLA-I genes[113]. A recent genome-wide CRISPR screen identified that polycomb repressive complex 2 (PRC2) epigenetically repressed several genes encoding APM components as well as *NLRC5*, both at the basal level and in response to IFNγ signalling[114], using the erythroleukaemia line K-562. This is a normal developmental process in stem cells and in neural progenitors, and tumours arising from these lineages can use this machinery to modulate antigen presentation. For example, the prevalence of both HLA-I and HLA-II downregulation in DLBCL is further expanded beyond mutation-based mechanisms by epigenetic downregulation, wherein *EZH2* mutations in germinal centre B cell-like subtype of DLBCL are associated with downregulation of both HLA-I and HLA-II[115].

Besides transcriptional downregulation of HLA-I, tumours show inhibition of surface expression of HLA-I by post-translational mechanisms. Macroautophagy is one such mechanism to regulate surface expression levels of HLA-I. In a recent discovery, pancreatic ductal adenocarcinoma (PDAC) cells were found to reduce HLA-I surface expression by selective autophagy of HLA-I complexes via the cargo receptor NBR1 (REF.[116]) (FIG. 3a). HLA-I was enriched in lysosomes of human PDAC cell lines and in PDAC tumours from patients (in all nine patients who were assessed). Autophagy inhibition in mouse tumour models was associated with increased T cell infiltration and ICI sensitization of the tumours, which were otherwise insensitive to single-agent ICI.

Thus, tumours can exploit wide-ranging mechanisms for modulating HLA-I expression at several levels, including at the signalling level by modulation of IFNγ signalling, at the transcription level by downregulating master regulators of HLA-I expression and other APM components or by epigenetic silencing, as well as at the post-translational level by redirecting HLA-I to the autophagy pathway. Possibly there are other mechanisms yet to be discovered. Several of these HLA-I downregulation mechanisms are reversible and amenable to therapeutic intervention. Combination strategies that result in upregulation of HLA-I expression are of potential benefit to patients. Targeting IFNγ remains a promising option, despite its dual role in cancer. Earlier cancer trials using IFNγ have not been successful, but it is now being investigated in combination with ICI. Radiation has been shown to induce HLA-I expression in mouse pancreatic cancer cell line models, possibly via NLRC5, which also gets upregulated after radiation[117]. In vivo, these models, which are normally resistant to ICI, showed increased susceptibility to ICI in combination with radiation. In cell lines where PRC2 has been shown to be responsible for epigenetic modulation of APM gene expression, strategies such as using EZH2 inhibitors have been shown to restore HLA-I expression[115].

In conclusion, the sum of somatic mutations and copy number loss events in genes encoding APM components combined with non-genetic mechanisms, although not comprehensively investigated across all cancer types, substantiates an important role of loss of HLA-I presentation as a tumour immune evasion strategy in a subset of patients. Around 9% of patients across TCGA carry homozygous loss or truncations in PLC and IFNγ-signalling pathway components (Supplementary Fig. 1). This is not the full picture of modulation of antigen presentation though. It does not account for LOH, which is present in a higher proportion of patients, or missense mutations, which may be a mixture of loss- or gain-of-function mutations not necessarily modulating antigen presentation. Nevertheless, the loss-of-function truncations provide interesting insights. MSI-H cancers carry truncating mutations in several PLC and IFNγ-signalling components, suggesting attenuation of antigen presentation in these tumours of high mutation burden (Supplementary Fig. 1). Although these truncating mutations can be a mechanical consequence of microsatellite instability, some of the genes encoding APM components seem to be particularly susceptible to these mutations. For example, *B2M* is 10-fold shorter than *JAK1*, but more frequently carries truncating mutations than *JAK1*, and genes encoding proteasomal and immunoproteasomal catalytic units are less likely to carry truncating mutations (Supplementary Figs 2,3). Combinations of APM defects can induce a more pronounced attenuation of antigen presentation compared with heterozygous defect in one of the APM genes[118]. Irreversible loss of antigen presentation seems to be rare, while a mixture of losses mediated by non-genetic mechanisms and heterozygous genetic defects is potentially more prevalent. An explicit investigation of this hypothesis is needed.

Macroautophagy
A catabolic pathway that degrades cytosolic components including proteins and organelles. Autophagosomes capture these cytosolic materials and fuse with lysosomes to mediate their degradation.

Box 4 | **The proteasome and its siblings**

Peptide processing is primarily performed by the proteasomal complex, which is responsible for turnover of cellular proteins, generating peptide fragments that eventually get presented on class I human leukocyte antigen (HLA-I). There are several types of proteasome, the most common one being the constitutive proteasome, which is expressed in the vast majority of tissues under normal conditions[198]. The other major type of proteasome is the immunoproteasome, which is primarily expressed under inflammatory conditions in response to IFNγ in tumours but constitutively in professional antigen-presenting cells. The immunoproteasome replaces three catalytic subunits (β1, β2 and β5) of the constitutive proteasome with IFNγ-induced alternative catalytic units (β1i, β2i and β5i), which have different cleavage specificities. Hybrid forms of the proteasome, called intermediate proteasomes (see the comprehensive review by Morozov and Karpov[199]), have also been found in some tissues and tumour cell lines[200,201]. The repertoire generated by the intermediate proteasome largely matches that of the immunoproteasome[202].

Changes in HLA-I peptide repertoire

Alterations in components of the APM in tumours can potentially change the repertoire of peptides that can be presented by a certain HLA-I complex instead of reducing HLA-I surface expression. Although an altered peptide repertoire might increase immunogenicity, DCs with an unaltered APM do not necessarily present this altered repertoire. Thus, T cells might not be primed against this altered repertoire and tumour immune evasion can occur. This question has not been formally addressed yet, to our knowledge.

Qualitative change of the peptide or antigen repertoire can result from alterations in peptide processing or peptide preferences of HLA-I. The proteasome, and related complexes, such as the immunoproteasome and intermediate proteasome, are the main enzymatic complexes responsible for peptide processing (BOX 4). The main function of the immunoproteasome is to increase the efficiency of processing of proteins into HLA-I-compatible peptides, by producing peptides with hydrophobic C-terminal residues that are preferentially transported by TAP into the ER and loaded onto HLA-I[119–121]. In agreement with this idea, it was found that the expression of two immunoproteasome subunits in tumour samples from patients with melanoma (472 patients from TCGA data) correlates with better survival[122]. Overexpressing these subunits in melanoma cell lines derived from patients induced better T cell responses from autologous tumour-infiltrating lymphocytes (TILs) from these patients, compared with the unaltered versions of the cell lines[122]. Can tumours modify the constitutive proteasome and immunoproteasome or alter their relative expression and potentially escape immune response by reducing presentation of rejection tumour antigens? Evidence of large-scale peptide repertoire changes by tumour-specific alterations in the proteasomal complex is currently lacking. Anecdotal studies suggest that some tumour epitopes can be differentially processed by the constitutive proteasome and the immunoproteasome, including some epitopes that are destroyed by the immunoproteasome[123–125]. Such a qualitative change in peptide repertoire is an issue particularly for T cell therapies targeting a single epitope and it is crucial for these approaches to confirm the presentation of candidate epitopes under normal and inflammatory conditions.

ERAP
ERAP1 and ERAP2 are endoplasmic reticulum-resident aminopeptidases that may trim peptides that bind to HLA-I. ERAP1 and ERAP2 are collectively referred to as ERAP.

Endoplasmic reticulum aminopeptidase 1 (ERAP1, also known as ERAAP in mice) and ERAP2 are aminopeptidases in the ER involved in trimming peptides to the optimal length for binding to HLA-I[126]. ERAP gene deletion has limited impact on HLA-I surface expression, but results in a qualitative change in the peptide repertoire by altering the peptide length spectrum[127,128]. An appreciable proportion of the peptide repertoire remains unaltered[128] between ERAAP-sufficient and ERAAP-deficient dendritic cells from mice, as ~75% of total peptides detected using mass spectrometry on peptides eluted from HLA-I were shared between them (actual overlap could be higher owing to the sensitivity limits of mass spectrometry). Although polymorphisms in the ERAP genes are related to several diseases[129,130], and also predisposition to or severity of cancer[131], *ERAP1* and *ERAP2* are rarely mutated in patients with cancer (0.6–0.8% of tumours in TCGA)[132]. Moreover, studies examining expression of the ERAP genes in patients with cancer have shown that they are expressed in all tumour samples examined, but they can vary widely in their expression levels[132,133]. Thus, qualitative alterations in peptide repertoire due to ERAP gene aberrations are possible, but rare. It could be partly due to the complex role of ERAP enzymes, as they can also over-trim and destroy tumour rejection epitopes[134], and modulation of ERAP might be beneficial to patients. Indeed, in some cancers such as the luminal subtype of bladder cancer, lower expression of *ERAP2* is associated with better overall survival of patients[135] treated with ICI.

Somatic mutations in the gene region encoding the peptide-binding pocket of the HLA-I heavy chain could potentially alter the binding preference — an interesting possibility that has not yet been fully explored. Single amino acid missense mutations have been detected in gene regions encoding the peptide-binding grooves in the α1 and α2 domains of HLA-I in tumour tissue samples from patients with several cancers[78,82]. These missense mutations are not recurrent compared with the hot spots of nonsense mutations and frameshift indels in the signal peptide and the α3 domain, and their functional impact on peptide binding has not yet been investigated to our knowledge. In silico analysis predicts that single amino acid substitutions in the peptide-binding groove can impact peptide repertoires, depending on both the position that is substituted and the chemicophysical properties of the amino acids involved[136]. Similarly, HLA-B*57:01 and HLA-B*57:03, which differ by only two amino acids in the peptide-binding cleft, exhibit different peptide-binding motif preferences[137]. Analysis of the ligandomes presented by mutated HLA-I alleles could help address this hypothesis.

Surprisingly, preclinical studies suggest that cells with an altered HLA-I repertoire due to ERAAP or TAP deficiency still elicit strong T cell responses[138,139]. ERAAP-sufficient wild-type mice can mount a strong T cell response to ERAAP-deficient cells[139]. Treatment of tumour-bearing mice with ERAP inhibitors that act broadly on both DCs and tumour cells led to induction of protective antitumour immunity in the CT26 colorectal carcinoma mouse model[140]. In melanoma cell lines, ERAP1 inhibition increased availability of the tumour

antigen MART1, resulting in higher levels of T cell responses using MART1-specific T cells[141]. Similarly, TAP gene silencing in tumours, although it partially reduces HLA-I surface expression, can also alter the repertoire of presented antigens by allowing the presentation of cryptic antigens and has been shown to increase tumour immunogenicity in a 4T1 breast carcinoma mouse model. When the deficiency is tumour specific, how an immune response is mounted against antigens that are in theory only generated by tumour cells is not fully understood, as APCs not carrying the deficiency may not process the antigen in the same manner as the tumour. A possible mechanism that explains this is cross-dressing, that is, the transfer of intact HLA-I–peptide complexes from tumour cells to DCs as shown in mice[142,143]. The existence of this pathway remains to be validated in humans.

Qualitative alterations in the antigen repertoire displayed by tumours pose a challenge to iNeSTs that rely on computational neoantigen predictions, as predicting such qualitative changes is currently not doable and difficult to achieve. Our understanding of qualitative alterations in antigen presentation in tumours is limited, and further exploratory studies of this topic are certainly needed.

NK cell recognition of tumours

NK cells, although part of the innate immune system, share a lot of features reminiscent of adaptive immunity, such as fine-tuned processes of education, licensing and activation, as well as the generation of memory phenotypes[144]. The potential of NK cell-focused cancer therapies, their arsenal of activating and inhibitory receptors, as well as concepts of NK cell memory formation have been discussed elsewhere and are beyond the scope of this review[144–148]. Here, we focus on the importance of NK cells in monitoring and counteracting a reduction in antigen presentation, or their role in modulating tumour immunogenicity. In principle, HLA-I-loss phenotypes should result in NK cell-mediated killing of tumour cells, but there are several factors that likely co-determine the efficiency of this process: first, the differential distribution of NK cell subsets across different tissue types; second, their abundance in the tumour; third, tumour immune evasion strategies modulating NK cell effectiveness, activation or missing-self recognition (FIG. 4); and fourth, the immunogenetic composition of a patient's genome. All of these points are relevant to how much selective pressure NK cells can exhibit on a tumour in a given tissue, ideally resulting in the preservation of functional antigen presentation.

Dogra et al.[149] have recently provided a comprehensive overview on the distribution of NK cell subsets across a large variety of healthy tissues. Besides significant differences in total abundance, they demonstrated that CD56^{dim}CD16^{+} NK cells with strong cytolytic effector function are relatively common, for example, in bone marrow or blood, but less prevalent in tonsils, gut and lymph nodes. There is also significant variability

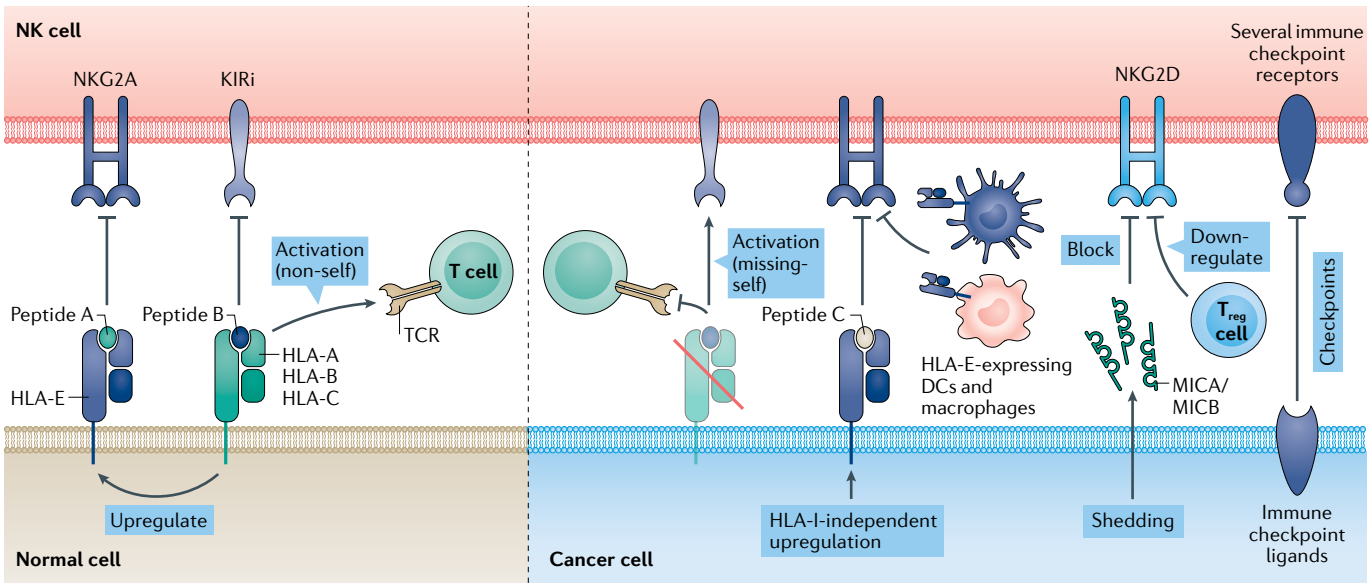


Fig. 4 | **Effects of HLA-I loss on natural killer cell activity and examples of tumour immune escape.** In normal cells and in the case of normal class I human leukocyte antigen (HLA-I) expression, T cell activation can be achieved by the interaction of a T cell receptor (TCR) with its ligand (HLA-I molecule presenting an antigen), but the presence of HLA-I inhibits natural killer (NK) cell activation via interaction with inhibitory killer cell immunoglobulin-like receptor (KIRi). Also, HLA-E is supplied with HLA-I signal peptides, expressed on the cell surface, and inhibits NK cell activation via interaction with NKG2A. In a tumour with HLA-I loss (for the sake of simplicity, we here assume a total loss of HLA-I expression), T cell activation is inhibited, but NK cells can be activated via 'missing-self' recognition, the loss of the HLA-I–KIRi interaction. However, other immune escape mechanisms have been described that achieve downregulation of NK cell activity. Examples include HLA-I-independent upregulation of HLA-E as well as its expression on dendritic cells (DCs) and macrophages, the blocking of the activating receptor NKG2D via shedding of MICA and MICB from the tumour cell or its downregulation via regulatory T cells (T_{reg} cells), and an upregulation of immune checkpoints including PD1 and TIGIT. Similarly to T cells, NK cells also express a variety of other immune checkpoints that were found to be upregulated in various cancer types, including PD1, TIGIT, CD112R/PVRIG and CD96/TACTILE. Several tumours overexpress ligands for these inhibitory receptors, including nectins and nectin-like molecules such as CD155/NECL5 and CD112/Nectin 2 (REFS[148,170,171]). Similarly, tumours may express HLA-G, which may inhibit NK cells via its receptors ILT2 and KIR2DL4 on NK cells[172].

of NK cell infiltration in solid tumours of patients with cancer[150]. Intratumoural NK cell abundance was shown to be prognostic across several cancer types in patients[151]. In patients with melanoma, NK cell frequency was predictive of response to anti-PD1 treatment and overall survival[41], and a curated gene signature predicted NK cell infiltration and better outcome[152].

Several tumour escape strategies have been described[153] that interfere with the effectiveness of NK cells in cancer, as summarized in FIG. 4. Furthermore, suppressive immune cells in the TME contribute to an exhausted phenotype[154]. *HLA-E*, a non-classical HLA gene, the protein product of which presents peptides derived from leader sequences of other HLA-I molecules and thus serves as a sensor for HLA-I expression, provides an inhibitory signal to NKG2A on NK and also $CD8^+$ T cells. This inhibitory signal has been shown to be strengthened in various cancer types. High levels of HLA-E have been shown in diverse cancer types, and research in patients with early breast cancer has demonstrated that its expression can be decoupled from HLA-I expression in tumour cells[155]. Its receptor, NKG2A, is expressed on a large fraction of cytotoxic lymphocytes[153]. In addition, other suppressive factors have been described, involving exosomes, hypoxia and suppressive cytokines[154], as well as the shedding of ligands for activating receptors[156,157] and checkpoints as summarized in FIG. 4.

Lastly, there is mounting evidence that patients' genetic configuration of germline HLA-I and killer cell immunoglobulin-like receptor (*KIR*) gene variation might be a modifier of antitumour activity upon HLA-I downregulation. Cytomegalovirus (CMV)-responsive memory-like NK cells ($CD57^+NKG2C^+$) in various non-tumour tissues were more prevalent in carriers of HLA-Bw4, HLA-C1 and HLA-C2 allotypes, which interact with inhibitory KIR on NK cells[149] and significantly contribute to their education[158]. Patients with NSCLC heterozygous for HLA-C1 and HLA-C2, which have different KIR specificities, showed increased NK cell infiltration compared with homozygous allele carriers[73].

In summary, tumours successful in downregulating antigen presentation may have devised several strategies to overcome the 'missing-self' surveillance by NK cells. We believe gaining better understanding of these characteristics of the tumour will enable development of better therapeutics by using the untapped potential of NK cells and better biomarker strategies. Current NK cell-focused therapies are at an exploratory stage, and mainly use allogeneic NK cell infusion or focus on NK cell enhancers, while other broader approaches include engaging activating receptors, checkpoint inhibitors and targeting the TME[147,159–163].

Conclusion

Cancer immunotherapy has shown promising success, but many patients are still not responding to this treatment. Understanding the biological mechanisms associated with efficacy of, or escape from, immunotherapy agents in cancers will inform the design of novel immunotherapy approaches, combination therapy and identification of biomarkers for patient selection. Many immunotherapies rely on $CD8^+$ T cell recognition and killing of tumour cells. These immunotherapies are dependent on both endogenous T cell priming by intratumoural DCs and the presentation of tumour antigens by HLA-I displayed on tumour cells. Although not comprehensively investigated across all cancer types, the reports published so far suggest a widespread modulation of HLA-I surface expression through a broad range of mechanisms. Furthermore, early evidence suggests that tumours may combine multiple mechanisms to reduce HLA-I presentation. Their exact role in resistance to immunotherapy remains to be carefully analysed. Enrichment of HLA-I and *B2M* LOH and other alterations in patients who do not respond to ICI suggest a role for HLA-I reduction in resistance. However, the fact that ICI can still work in patients with some of these same alterations suggests that, especially for tumours with high mutation burden, such manipulation of antigen presentation by tumours may not be complete, and antigen presentation acts as an Achilles' heel of the tumour. For patients with complete and irreversible damage to antigen presentation, although relatively rare yet important to address, $CD8^+$ T cell-dependent immunotherapy may not be a viable option. These patients may be directed to other onco-immunotherapies independent of HLA-I expression, such as activation of NK cells or tumoricidal macrophages, synthetic immunity such as CAR-T cell therapy and CD3 bispecific antibodies, or other traditional cancer treatments. For the other patients with reversible downregulation of antigen presentation, the design of strategies aimed at restoration of antigen presentation on tumour cells is needed, and these strategies might be of benefit in combination therapy with ICI.

Data availability

The data that support the findings of this study are available as Supplementary Figures and in cBioportal: https://www.cbioportal.org/.

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L.D. and S.J. contributed equally to the manuscript as a whole. C.H. led the NK cell topic.

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