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Review

Emerging therapeutic opportunities targeting nonclassical MHC-I molecules

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Nonclassical major histocompatibility complex class I (MHC-I) molecules, including human leukocyte antigen E (HLA-E), HLA-F, HLA-G, MHC-I-related protein 1 (MR1), and the CD1 family, constitute a conserved antigen-presenting system that regulates immune surveillance, tissue homeostasis, and tolerance through specialized interactions with innate and unconventional T cells. Although these molecules have long been implicated in cancer, infection, autoimmunity, and transplantation, their distinct immunobiology and therapeutic potential have largely been considered in isolation. Recent advances in structural immunology, single-cell and spatial profiling, engineered immune cell technologies, and early clinical studies have established nonclassical MHC-I pathways as tractable targets for immunotherapy. In this review, we synthesize the biology, disease-associated functions, and therapeutic targeting of these molecules, integrating immune checkpoint blockade, antibody-based therapeutics, and MR1- and CD1-restricted cellular immunotherapies into a unified framework. We further highlight shared immunological principles, emerging clinical translation, and opportunities for universal, off-the-shelf immune interventions.

Highlights

Nonclassical major histocompatibility complex class I molecules (human leukocyte antigen E, human leukocyte antigen F, human leukocyte antigen G, major histocompatibility complex class I-related protein 1, and the CD1 family) have emerged as key regulators of immune surveillance with limited polymorphism and specialized antigen repertoires.

Tumors and chronic infections exploit human leukocyte antigen E/natural killer cell group 2 member A and human leukocyte antigen G/leukocyte immunoglobulin-like receptor axes to suppress natural killer and $CD8^+$ T cell function, motivating new checkpoint blockade strategies.

Major histocompatibility complex class I-related protein 1- and CD1-restricted T cells, including mucosal-associated invariant T cells and invariant natural killer T cells, recognize conserved metabolite and lipid antigens and bridge innate and adaptive immunity.

Engineered cellular therapies, bispecific engagers, and combination strategies targeting nonclassical major histocompatibility complex class I pathways are advancing translational immunotherapy.

Nonclassical major histocompatibility complex class I molecules as emerging therapeutic targets

Major histocompatibility complex class I (MHC-I) molecules are central to immune surveillance, classically defined by the highly polymorphic human leukocyte antigen A (HLA-A), HLA-B, and HLA-C proteins that present peptide antigens to $CD8^+$ T cells. In parallel with these classical molecules, a distinct group of nonclassical and MHC-I-like proteins, including HLA-E, HLA-F, HLA-G, MHC-I-related protein 1 (MR1), and the CD1 family, has evolved specialized structural and functional properties (Table 1 and Figure 1). Although MR1 and CD1 are formally classified by the international immunogenetics information system as MHC-I-like, they are discussed in this review because they share structural similarities, restricted **polymorphism** (see Glossary), specialized antigen presentation, and emerging therapeutic relevance with nonclassical MHC-I pathways [28,29]. These molecules exhibit limited polymorphism, restricted antigen repertoires, and tightly regulated expression patterns. Unlike classical MHC-I molecules, which broadly sample intracellular peptides for surveillance by conventional $CD8^+$ T cells, nonclassical MHC-I molecules have evolved specialized immunological functions. For example, HLA-E presents conserved leader peptides to regulate natural killer (NK) and T cell activity, HLA-F can adopt open conformations with immunomodulatory properties, HLA-G delivers tolerogenic signals, and MR1 and CD1 present metabolite and lipid antigens, respectively [30–35]. These specialized antigen-presentation pathways allow nonclassical MHC-I molecules to sense cellular stress, infection, and tissue damage while modulating immune activation or suppression in a context-dependent manner. Through these features, they act as critical regulators at the interface between innate and adaptive immunity [36].

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Nonclassical MHC-I molecules play essential roles in maintaining immune homeostasis while enabling rapid responses to cellular stress. They contribute to maternal–fetal tolerance, immune privilege, and transplant acceptance, yet are also co-opted in pathological contexts. Viruses and tumors frequently exploit HLA-E and HLA-G to dampen cytotoxic lymphocyte activity [37–39], while altered MR1- and CD1-mediated antigen presentation shapes immune responses to infection, inflammation, and malignancy [40–42]. Their relatively conserved nature across individuals and their central positioning in immune regulatory circuits make these pathways particularly attractive therapeutic targets. Importantly, they function in coordination with, yet independently of classical immune checkpoints such as programmed cell death protein 1 (PD-1)/ programmed death-ligand 1 (PD-L1), offering opportunities for complementary or synergistic intervention [1–3].

In this review, we summarize the biology and immunological functions of key nonclassical MHC-I molecules and discuss their roles in cancer, infection, autoimmunity, and transplantation. We highlight emerging strategies targeting the HLA-E/natural killer cell group 2 member A (NKG2A) and HLA-G/leukocyte immunoglobulin-like receptor subfamily B member 1 (LILRB) axes, as well as advances in MR1- and CD1-directed cellular immunotherapies. Particular emphasis is placed on mucosal-associated invariant T (MAIT) cells and invariant natural killer T (iNKT) cells as central effector populations that recognize nonpolymorphic antigen-presenting molecules and hold promise for universal, off-the-shelf therapeutic applications.

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Table 1. Overview of major nonclassical MHC-I molecules

Molecules	Primary receptors	Key functions	Expression patterns	Therapeutic relevance	Refs
HLA-E	CD94/NKG2A (inhibitory), CD94/NKG2C (activating), some TCRs	NK cell regulation, presentation of leader peptides, stress-induced immunity	Low basal expression; upregulated by cellular stress, infection, tumors	Target of NKG2A blockade (monalizumab) in cancer; biomarker of immune escape	[1–4]
HLA-F	KIR3DS1, KIR3DL2, NK cell receptors	Regulation of NK cell activation; exists as peptide-bound and OC forms, the latter interacting with NK receptors	Mostly intracellular; expressed on activated lymphocytes, tumors	Emerging target in NK cell-based immunotherapy; potential role in cancer progression	[5–8]
HLA-G	LILRB1, LILRB2, KIR2DL4	Immune tolerance, inhibition of NK and T cells, DC modulation	Restricted tissue expression; induced in placenta, tumors, transplant settings	Target for LILRB1/2 inhibitors; immunosuppressive therapy; biomarker for tolerance	[9–11]
MR1	MAIT TCRs, TCRs of MR1-reactive T cells (likely cross-reactive conventional cells)	Presents vitamin B metabolites; activates MAIT cells; microbial surveillance	Ubiquitous low expression; increased during infection, metabolic stress	MR1-restricted TCR therapy in cancer; activation of MAIT cells for infection and tumors	[12–16]
CD1a	TCRs of lipid-specific T cells	Presents lipid antigens; activates skin-resident T cells	Surface-expressed; enriched on Langerhans cells, DCs	Target in skin immunity; inflammation and cancer therapeutic potential	[17,18]
CD1b	TCRs of NKT-like T cells	Presentation of microbial lipids (e.g., mycobacteria)	Endosomal expression; predominant on DCs	Target in tuberculosis vaccines; lipid-based immunotherapies	[19,20]
CD1c	TCRs of lipid-specific T cells	Detection of self- and microbial lipids	Surface-expressed; found on DCs, B cells	Cancer immunotherapy; antigen-specific T cell activation	[21,22]
CD1d	iNKT TCR	Activation of iNKT cells; cytokine burst; immune modulation	Broad hematopoietic expression; present on APCs including DCs, macrophages, B cells	Target for iNKT cell-based cancer therapy, adjuvants, and autoimmunity	[23–26]
CD1e	Assists in CD1b antigen processing	Facilitates lipid loading in endosomes	Intracellular localization; expressed in DCs	Supports lipid vaccine development; not a direct receptor target	[27]

Biology and immunological functions of nonclassical MHC-I molecules

HLA-E

HLA-E, also known as **HLA class Ib**, is a nonclassical MHC-I molecule characterized by very limited polymorphism and an invariant antigen-binding groove (Table 1 and Figure 1) [30]. Like classical MHC-I molecules, HLA-E's heavy chain associates with β2-microglobulin (B2M) and presents peptides on the cell surface [39]. Uniquely, HLA-E predominantly binds hydrophobic nonamer peptides derived from the N-terminal leader sequences of other MHC-I proteins, often called'Val-Leu-9' (VL9) peptides, with a consensus sequence such as VMAPRTLLL [43]. These leader peptides are generated during the synthesis of classical HLA-I molecules via signal peptide peptidases in the endoplasmic reticulum (ER) and are loaded onto HLA-E in the ER [44]. The presence of HLA-E loaded with these conserved self-peptides serves as a readout of classical MHC-I expression in cells.

Once at the cell surface, peptide-loaded HLA-E is specifically recognized by the CD94/NKG2 family of NK cell receptors (Box 1) [57,58]. In humans, this family consists of two key heterodimeric receptors that solely recognize HLA-E: the inhibitory CD94/NKG2A receptor and the activating CD94/NKG2C receptor [59]. Engagement of HLA-E by CD94/NKG2A delivers a dominant inhibitory signal to the NK cell, effectively protecting cells expressing HLA-E from NK cytotoxicity [60]. Conversely, engagement of the same HLA-E/peptide complex by CD94/NKG2C can transmit an activating signal that stimulates NK cell-mediated lysis [61]. In practice, the NKG2A pathway usually dominates because CD94/NKG2A has a higher affinity for HLA-E-peptide complexes than does CD94/NKG2C, and it induces a stronger inhibitory signal, thereby biasing toward NK cell tolerance under steady-state conditions [43,62]. This mechanism effectively allows NK cells to infer whether a target cell is maintaining normal MHC-I biogenesis and homeostasis, whereas altered peptide loading or reduced HLA-E stability can weaken inhibitory signaling and promote target cell killing. Although HLA-E primarily regulates NK cell function through CD94/NKG2 receptors, it can also present pathogen-derived and stress-associated peptides to HLA-E-restricted $CD8^+$ T cells [63]. These unconventional T cells have been implicated in antimicrobial and antiviral immunity and may also contribute to antitumor immune responses, highlighting an additional role for HLA-E in bridging innate and adaptive immunity [63].

HLA-F

HLA-F is a nonclassical MHC-I molecule distinguished by its limited polymorphism, intracellular localization, and unique structural properties (Table 1 and Figure 1). Accumulating clinical correlative and functional studies connect HLA-F to cancer biology, often aligning with an immune-evasive phenotype. In nonsmall cell lung cancer (NSCLC), elevated HLA-F expression has been associated with shorter overall survival in several patient cohorts, supporting its potential utility as a prognostic biomarker [31]. In hepatocellular carcinoma (HCC), elevated HLA-F expression within tumor lesions has been associated with shorter survival and with pathological features indicative of aggressive disease, including venous/lymphatic invasion and metastasis [5]. In glioma, experimental targeting of HLA-F with an anti-HLA-F antibody has been reported to suppress tumor cell proliferation, suggesting that HLA-F may contribute to tumor growth programs in addition to shaping immunity [6].

Several mechanisms have been proposed to explain the association between HLA-F expression and an immune-evasive phenotype in cancer. HLA-F may function as an inducible 'nonclassical checkpoint' on tumors because inflammatory cues such as interferon-gamma (IFN-γ) can upregulate HLA-F and increase the availability of **open conformer (OC)** species at the surface (Box 2). Depending on the receptor context, this shift can reshape NK and myeloid cell activation or inhibition thresholds [7,71].

Glossary

Antibody-drug conjugates: targeted cancer therapeutics that couple a monoclonal antibody specific to a tumor antigen with a cytotoxic payload, enabling selective delivery of the drug to malignant cells.
Atopic dermatitis: a chronic relapsing inflammatory skin disorder characterized by impaired skin barrier function, type 2 immune-mediated inflammation, intense pruritus, and recurrent eczematous lesions.
Cetuximab: a chimeric monoclonal antibody that targets the epidermal growth factor receptor, inhibits receptor signaling, and mediates ADCC.
Durvalumab: a human monoclonal antibody PD-L1 that enhances antitumor immunity by preventing PD-1-mediated T cell inhibition.
Extravillous trophoblasts: specialized placental cells that invade the maternal decidua and remodel uterine spiral arteries to establish adequate maternal–fetal blood flow during pregnancy.
Graft-versus-host disease (GvHD): a post-transplant immune complication in which donor-derived immune cells recognize and attack recipient tissues following allogeneic hematopoietic stem cell transplantation.
HLA class Ib: the nonclassical HLA-encoded MHC-I molecules—HLA-E, HLA-F, and HLA-G—which differ from the classical class Ia molecules (HLA-A, HLA-B, and HLA-C) in their limited polymorphism, restricted expression, and specialized roles in immune regulation.
Lymphangiogenesis: the formation of new lymphatic vessels from pre-existing lymphatic endothelium, often contributing to tumor progression and metastasis.
Lymphatic dissemination: the spread of tumor cells through lymphatic vessels to regional lymph nodes or distant sites.
Monalizumab: a humanized monoclonal antibody that targets the inhibitory receptor NKG2A and restores the cytotoxic function of NK cells and $CD8^+$ T cells by blocking HLA-E-mediated immune suppression.
Myelin: a lipid-rich insulating sheath produced by glial cells that surrounds neuronal axons, enabling the rapid and efficient transmission of electrical nerve impulses.
Open conformer (OC): a B2M-free MHC-I heavy chain that adopts an alternative surface-exposed

HLA-G

HLA-G is a nonclassical MHC-I molecule distinguished by limited polymorphism and a restricted tissue distribution compared with classical HLA-I molecules (Table 1 and Figure 1) [32]. Notably, the HLA-G gene produces seven distinct isoforms through alternative splicing, including four membrane-bound variants, HLA-G1 to HLA-G4, and three soluble forms, HLA-G5 to HLA-G7 [72]. HLA-G1 represents the full-length membrane-bound protein with α1–α3 domains paired with B2M, whereas HLA-G5 is an identical soluble counterpart lacking the transmembrane region [72]. The remaining isoforms lack one or more globular domains and generally do not associate with B2M [72]. HLA-G1 and soluble HLA-G5 are the most extensively studied due to their structural resemblance to classical HLA-I molecules. Functionally, HLA-G mediates immune inhibition through interactions with inhibitory receptors, primarily LILRB1 and LILRB2, and it has also been reported to interact with killer cell immunoglobulin-like receptor, two Ig domains and long cytoplasmic tail 4 (KIR2DL4) [73], thereby modulating the activity of NK cells and other immune cell populations.

HLA-G plays a central role in fostering immune tolerance in multiple contexts, with most mechanistic and functional studies focusing on membrane-bound HLA-G1 and soluble HLA-G5. HLA-G was first discovered at the maternal–fetal interface: **extravillous trophoblast** cells in the placenta abundantly express HLA-G, which protects the semi-allogeneic fetus from maternal immune attack by inhibiting lysis by uterine NK cells [32]. Outside of pregnancy, HLA-G expression remains highly restricted under healthy conditions to immune-privileged sites such as the thymus and cornea, but it can be induced in pathological situations. For instance, in patients

conformation capable of distinct receptor interactions compared to peptide-loaded MHC-I complexes.

Polymorphism: in MHC biology, polymorphism denotes allelic diversity among classical MHC molecules, a feature largely absent in non-classical antigen-presenting molecules such as MR1 and CD1d.

Psoriasis: a chronic immune-mediated inflammatory skin disease characterized by keratinocyte hyperproliferation, erythematous, scaly plaques, and dysregulated T cell-driven immune responses.

Riboflavin: a water-soluble vitamin (vitamin B2) that functions as a precursor of flavin cofactors essential to cellular redox reactions and microbial metabolism.

Tumor immunosurveillance: the process by which the immune system detects, monitors, and eliminates transformed or malignant cells to prevent tumor development and progression.

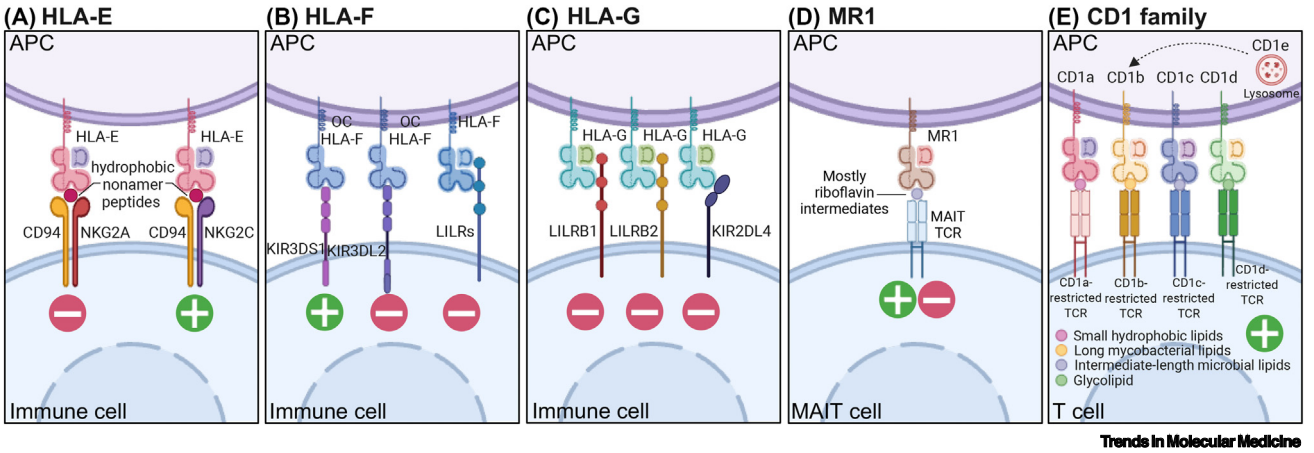

Figure 1. Structural and immune functions of nonclassical MHC-I molecules. (A) HLA-E preferentially presents hydrophobic nonamer leader-sequence peptides ('VL9') derived from classical HLA-I molecules and is sensed by CD94/NKG2 receptors on NK cells or CD8 T cells; engagement of CD94/NKG2A delivers dominant inhibitory signaling, whereas CD94/NKG2C can transmit activating signals depending on context and peptide repertoire. (B) HLA-F is depicted in open conformer (OC) and β2m-associated states. OC HLA-F is a high-affinity ligand for the activating NK receptor KIR3DS1 and is also recognized by the inhibitory receptor KIR3DL2, whereas β2m-associated (folded) HLA-F can engage inhibitory LILRs, together tuning NK activation thresholds during inflammation and disease. (C) HLA-G promotes immune tolerance by binding inhibitory receptors on lymphoid and myeloid cells, including LILRB1/ILT2 and LILRB2/ILT4, and can also engage KIR2DL4, collectively dampening cytotoxicity and antigen-presenting cell activation in settings such as pregnancy, transplantation, and tumor immune evasion. The schematic depicts the full-length membrane-bound HLA-G1 isoform, which contains α1, α2, and α3 extracellular domains and associates with β2-microglobulin. Other HLA-G isoforms arise through alternative splicing, including the soluble HLA-G5, the secreted counterpart of HLA-G1, and shorter isoforms that lack one or more extracellular domains. (D) MR1 presents small-molecule metabolites, most prominently intermediates from microbial riboflavin (vitamin B2) biosynthesis such as 5-OP-RU, to the semi-invariant MAIT TCR, thereby triggering rapid MAIT cell activation. In contrast, some MR1 ligands do not activate MAIT cells but instead competitively inhibit MAIT cell responses by blocking presentation of agonistic antigens such as 5-OP-RU. (E) CD1 isoforms exhibit distinct lipid-presenting specializations shaped by binding-groove architecture and intracellular trafficking. CD1a favors small 'headless' hydrophobic skin lipids, CD1b accommodates very long-chain mycobacterial lipids, CD1c presents intermediate-length microbial lipids, and CD1d presents glycolipids to iNKT cells. CD1e localizes to lysosomal compartments and acts as a lipid-processing factor that facilitates loading of complex glycolipids onto other CD1 molecules rather than directly presenting to T cells. Figure created using BioRender (https://BioRender.com). APC: antigen-presenting cell; HLA: human leukocyte antigen; ILT2: immunoglobulin-like transcript 2; KIR3DL2: killer cell immunoglobulin like receptor three Ig domains and long cytoplasmic tail 2; LILR:leukocyte immunoglobulin-like receptor; MAIT: mucosal-associated invariant T; MR1: major histocompatibility complex class I-related protein 1; NK: natural killer cell; NKG2: natural killer group 2; TCR: T cell receptor.

Box 1. Stress- and disease-induced changes in HLA-E peptide presentation shape NK cell responses

The repertoire of peptides presented by HLA-E, and the interactions of these peptides with NK cells, can be altered by cellular stress or disease. Notably, fragments of stress-induced proteins such as heat shock protein 60 (HSP60) can be loaded onto HLA-E [45,46]. These stress-related peptides do not engage NKG2A/CD94 effectively, leading to an abrogation of the inhibitory signal that would normally protect the cell [46]. As a result, the lack of NKG2A inhibition, combined with concurrent upregulation of ligands for activating NK receptors, shifts the balance in favor of NK attack, allowing the elimination of the aberrant cell.

During certain viral infections, pathogens exploit the HLA-E pathway to evade immune destruction. A well-studied example is human cytomegalovirus (HCMV), which encodes a peptide derived from the viral UL40 protein that mimics a host MHC-I leader sequence [47]. This viral signal peptide can bind to HLA-E and restore its expression on the infected cell, even as HCMV downregulates classical HLA-I molecules to avoid T cells [48,49]. The complex of HLA-E and viral peptide engages NKG2A/CD94 on NK cells, delivering an inhibitory signal that protects the infected cell from NK lysis. Interestingly, HCMV infection is also associated with an expansion of NK cells expressing the activating NKG2C receptor, suggesting that these complexes can drive NK cell activation in specific contexts [50].

In cancer, HLA-E is often dysregulated, influencing tumor immune evasion [51]. Many malignancies, such as colorectal cancer, lung cancer, and papillary thyroid cancer, upregulate HLA-E expression on the tumor surface to evade NK cell-mediated cytotoxicity, and hence tumor HLA-E expression is generally associated with a poor prognosis [52–54]. Mechanistically, HLA-E on tumor cells engages the NKG2A/CD94 heterodimeric receptors on both NK cells and certain $CD8^+$ T cells, thereby delivering an immunosuppressive signal that blunts antitumor cytotoxicity [2]. This interaction has led to interest in therapeutic strategies to disrupt this inhibitory axis. For example, in a Phase 2 clinical trial for unresectable stage III NSCLC, the addition of the anti-NKG2A monoclonal antibody monalizumab to durvalumab, which blocks PD-L1, provided additional clinical benefit [55]. In addition, HLA-E has emerged as a target for T cell-based immunotherapies, including vaccines designed to elicit HLA-E-restricted $CD8^+$ T cell responses against pathogen- or tumor-derived antigens [56]. Together, these complementary approaches highlight the therapeutic potential of HLA-E as both an immune checkpoint and an antigen-presenting molecule capable of directing protective T cell immunity.

with B-cell chronic lymphocytic leukemia, the proportion of leukemic cells expressing HLA-G ranged from 1 to 54%, and a lower proportion of HLA-G-positive cells was associated with longer progression-free survival [37,38]. Given this correlation, HLA-G is increasingly viewed as a novel

Box 2. HLA-F OCs encode immune activation signals for NK cells

Early biochemical and cell-biological work showed that HLA-F surface expression correlates with the presence of MHC-I heavy chains and is sensitive to perturbations that affect heavy-chain conformation, supporting the concept that HLA-F is tightly linked to OC biology and can participate in OC-rich membrane contexts [64,65]. HLA-F accommodates unconventional peptide lengths via an 'open-ended' groove, a feature attributed in part to a key substitution from arginine 62 to tryptophan (R62W) that reshapes the peptide-binding environment. Functionally, this adaptation supports the presentation of longer peptides and implies that HLA-F's sampling of cellular peptides may be biased toward atypical ligands generated during activation, stress, or infection [65].

HLA-F has been reported to interact with a subset of killer cell immunoglobulin-like receptors (KIRs), including the activating receptor KIR3DS1 and the inhibitory receptor KIR3DL2, although the functional significance of these interactions remains under investigation. OC HLA-F has been proposed to represent a particularly relevant ligand state. A previous study identified OC HLA-F as a high-affinity ligand for KIR3DS1 and demonstrated functional consequences: primary $KIR3DS1^+$ NK cells responded to HLA-F with degranulation/cytokine production and could inhibit HIV-1 replication *in vitro*, suggesting that the HLA-F–KIR3DS1 interaction may contribute to NK cell activation in specific contexts [66]. Complementary biophysical work further supported direct KIR3DS1–HLA-F binding and reinforced the concept that OCs of HLA-I, including HLA-F and other OC HLA-I heavy chains, provide a receptor landscape distinct from peptide-loaded HLA-Ia complexes [67]. Evidence for interactions between HLA-F and KIR3DL2 is more limited, although available studies suggest that HLA-F may participate in OC HLA-I-mediated regulation of NK cell education and activation during inflammation. Overall, although accumulating evidence supports interactions between HLA-F and selected KIRs, the receptor specificity, ligand requirements, and physiological significance of these interactions remain active areas of investigation [64].

Moreover, OC HLA-F can directly tune NK effector function by engaging KIR3D-family receptors, supporting a model in which tumors exploit OC-rich landscapes to bias NK signaling, particularly in settings where inhibitory KIR pathways dominate [7,8]. HLA-F may also suppress antigen-presenting cell programs through inhibitory leukocyte immunoglobulin-like receptors (LILRs) when in B2M-associated, peptide-bound conformations, potentially promoting tolerogenic dendritic cell (DC) and macrophage states that dampen downstream T cell priming [68,69]. Finally, consistent with broader OC biology, OC heavy chains can participate in *cis/trans* interactions that rewire membrane receptor organization, suggesting that HLA-F might modulate signaling not only as a ligand but also by organizing OC-enriched microdomains during tumor stress and immune engagement [8,70].

immune checkpoint in cancer, making the HLA-G pathway a potential target for immune checkpoint blockade therapies. Interventions such as antibodies that block HLA-G have been tested in two clinical trials (NCT04991740 and NCT04485013; see Clinician's corner).

MR1

MR1 is a nonpolymorphic antigen-presenting molecule specialized in the capture and presentation of small organic metabolites to an unconventional T cell population known as MAIT cells (Table 1 and Figure 1) [74–76]. Unlike classical MHC-I molecules, MR1 does not present peptide antigens and exhibits minimal polymorphism across human populations, highlighting its conserved immunological function [33,34]. A distinctive feature of MR1 is that many potent agonist ligands form a covalent Schiff base with Lys43 within the MR1 antigen-binding groove, stabilizing MR1 and promoting its cell-surface expression [77]. This highly conserved and essentially nonpolymorphic nature enables broad, shared recognition of MR1-presented antigens across individuals, making MR1 an attractive target for universal immunotherapeutic strategies.

Structurally, MR1 resembles classical MHC-I molecules, which consist of a heavy chain associated with B2M. However, its antigen-binding groove is uniquely adapted to accommodate small-molecule metabolites rather than peptides. The best-characterized MR1 ligands are vitamin B-derived antigens (VitBAgs), most prominently intermediates in the microbial **riboflavin** (vitamin B2) biosynthetic pathway [76,78–81].

Ligand binding within the ER stabilizes MR1 and promotes its trafficking to the cell surface. Once expressed on the plasma membrane, MR1–ligand complexes persist for several hours before being internalized into endosomal compartments. The majority of internalized MR1 molecules are subsequently targeted for degradation, while a smaller fraction may recycle back to the cell surface, potentially after exchanging ligands [82]. This dynamic trafficking behavior allows MR1 to integrate metabolic cues over time and to sample distinct intracellular ligand pools [82].

Engagement of MR1–ligand complexes by the MAIT cell T cell receptor (TCR) triggers rapid and robust MAIT cell activation, resulting in effector functions that include cytotoxic killing, cytokine production, and tissue repair responses [12,82–90]. During thymic development, VitBAgs derived from commensal or environmental microbes can reach the thymus and are presented by MR1 on double-positive thymocytes, driving the positive selection and maturation of the MAIT cell lineage [91]. In peripheral tissues, MR1-mediated presentation of microbial metabolites contributes to MAIT cell recruitment and functional programming at barrier sites, such as the skin and mucosa, where MAIT cells may participate in wound healing and immune surveillance [92–94]. In the context of cancer, MR1 can present tumor-associated metabolites that trigger MAIT cell cytotoxicity and cytokine secretion, implicating this pathway in **tumor immunosurveillance** [12,82–90].

Although MAIT cells typically express a semi-invariant TCR composed of a TCR alpha variable 1-2 joined with TCR alpha joining 33 (TRAV1-2–TRAJ33) (or, less commonly, TRAJ12 or TRAJ20) α chain paired predominantly with TRBV20 or TRBV6 family β chains, other MR1-restricted T cell populations with diverse TCR repertoires have also been identified, expanding the functional diversity of MR1-mediated immune recognition [95]. Multiple independent reports have identified atypical MR1-restricted T cells that recognize previously uncharacterized ligands derived from mammalian cells, including tumor-associated metabolites such as carbonyl-modified nucleobase adducts and vitamer derivatives [96,97]. Initial studies identified the MR1-restricted MC.7.G5 TCR as broadly cancer-reactive and selective for malignant cells [96]. However, subsequent studies demonstrated that MC.7.G5 recognition is largely dependent on the rare MR104

allomorph and is not strictly tumor-specific, as healthy B cells and monocytes expressing MR104 were also recognized [98,99]. Ligand studies further identified vitamin B6 metabolites, including pyridoxal and pyridoxal 5′-phosphate, as MR1-presented antigens that enhance MC.7.G5 reactivity, particularly with R9H (rare Arg31His mutation)-containing MR1 variants [100,101]. Nevertheless, additional MR1-restricted cancer-reactive T cells recognizing common MR1 allomorphs have since been described [102], supporting the therapeutic potential of MR1-targeted immunotherapy.

CD1 family (CD1a–e)

The CD1 family comprises a group of MHC-I-like antigen-presenting molecules that specialize in binding and presenting lipid and lipopeptide antigens, rather than peptides, to T cells (Table 1 and Figure 1) [103,104]. Unlike classical MHC-I molecules, which are highly polymorphic, CD1 molecules exhibit limited allelic diversity. Instead, antigenic diversity within the CD1 system occurs at the species level [35]. Different mammalian species express distinct numbers and combinations of CD1 isotypes, likely reflecting evolutionary adaptations for presenting structurally diverse lipid antigens encountered in specific ecological niches [35].

In humans, the CD1 family consists of five isoforms, including CD1a, CD1b, CD1c, CD1d, and CD1e, which differ in their intracellular trafficking patterns, tissue distribution, and lipid-binding properties. Collectively, CD1a, CD1b, CD1c, and CD1d molecules are capable of accommodating a wide range of lipid structures, from small monoacylated lipids and lipopeptides to large, complex tetra-acylated glycolipids [35]. CD1e is predominantly localized within intracellular compartments, particularly the lysosomes of antigen-presenting cells (APCs). Its primary function is to facilitate the processing of complex microbial lipids, such as phosphatidylinositol mannosides, and to promote their efficient loading onto CD1b molecules for subsequent presentation to CD1b-restricted T cells. This accessory role underscores the coordinated nature of lipid antigen processing within the CD1 system [35].

It is important to distinguish between CD1 ligands and CD1 antigens. While many self- and foreign lipids can bind CD1 molecules, not all CD1-associated lipids are capable of eliciting T cell responses. During biosynthesis, CD1 molecules often associate with endogenous self-lipids that function primarily as stabilizing placeholders, facilitating proper folding and transport of CD1 to the cell surface. These self-lipids can subsequently be exchanged for antigenic lipids either at the plasma membrane or during CD1 recycling through late endosomal and lysosomal compartments, where lipid-loading conditions are optimized [105,106].

Endogenous lipid antigens presented by CD1 molecules play important roles in tissue immunity and cancer immunosurveillance. CD1a presents self-lipids such as squalene and wax esters, which activate autoreactive T cells in skin barrier immunity and inflammatory disease [107]. In cancer, CD1c presents methyl-lysophosphatidic acids enriched in leukemia cells, enabling recognition by CD1c-restricted T cells; TCR engineering targeting these lipids has demonstrated antileukemic activity in xenograft models [21,108]. CD1d also presents endogenous lysophospholipids and glycosphingolipids that contribute to iNKT cell activation in inflammatory and malignant settings [109,110]. Collectively, endogenous lipid antigens represent promising targets for CD1-directed immunotherapy.

CD1-restricted T cells collectively constitute a notable fraction of the human αβ T cell compartment, although their frequencies vary depending on the CD1 isoform, tissue site, and detection method used. Among these, CD1a-autoreactive T cells are particularly abundant in peripheral blood and are further enriched in the skin [107,111], where they contribute to immune responses against self- and microbial lipids and have been implicated in inflammatory skin diseases,

including **psoriasis**, **atopic dermatitis**, and contact hypersensitivity, highlighting the therapeutic potential of targeting the CD1a axis [17,112,113]. CD1-restricted T cells are also distributed throughout other mucosal and peripheral tissues, consistent with the broad expression of CD1 molecules on antigen-presenting cells and the widespread availability of lipid antigens. In contrast, CD1d-restricted iNKT cells are comparatively rare in peripheral blood but represent the best-characterized subset of lipid-reactive T cells because of their potent immunoregulatory functions and well-defined antigen specificity [114–116].

NKT cells are a heterogeneous population that includes both type I (invariant) and type II (diverse) subsets. Type I iNKT cells express a semi-invariant TCR composed of a conserved Vα24-Jα18 chain paired predominantly with variable beta 11 (Vβ11) in humans (Vα14-Jα18 paired with Vβ8.2, Vβ7, or Vβ2 in mice) [114–116]. These cells are defined functionally by their ability to recognize α-galactosylceramide (αGalCer), a potent glycolipid agonist originally isolated from the marine sponge *Agelas mauritianus* and later identified for its strong antitumor activity in murine models. Engagement of αGalCer–CD1d complexes leads to rapid cytokine secretion and cytotoxic effector responses by type I NKT cells [117–119].

In contrast, type II NKT cells do not express a conserved TCR and instead utilize a diverse array of TCR α and β chains. These cells recognize a broader spectrum of lipid antigens, including sulfatide, a β-anomeric glycosphingolipid derived from **myelin** [120,121]. Type II NKT cells have been implicated in immune regulation and can exert functions distinct from, and sometimes opposed to, those of iNKT cells.

Nonclassical MHC-I molecules as therapeutic targets

Immune checkpoint blockade targeting the HLA-E/NKG2A axis

HLA-E serves as the principal ligand for the inhibitory receptor NKG2A, which is expressed on NK cells and subsets of $CD8^+$ T cells. Engagement of the HLA-E/NKG2A axis suppresses cytotoxic effector functions, thereby contributing to immune tolerance within the tumor microenvironment (TME) and in the peripheral circulation [1]. Importantly, this inhibitory pathway functions in parallel with the PD-1/PD-L1 axis, providing an additional and nonredundant mechanism of immune suppression.

Therapeutic blockade of NKG2A using the monoclonal antibody **monalizumab** has demonstrated the capacity to reinvigorate both NK cell and $CD8^+$ T cell responses (Figure 2A) [2]. André *et al*. first established that anti-NKG2A antibodies function as immune checkpoint inhibitors capable of unleashing antitumor immunity by restoring cytotoxic lymphocyte activity [1]. Monalizumab enhanced NK cell-mediated killing across multiple tumor models and restored $CD8^+$ T cell effector function, particularly when combined with blockade of the PD-1/PD-L1 axis. In addition, monalizumab potentiated NK cell-mediated antibody-dependent cellular cytotoxicity (ADCC) against antibody-coated tumor cells (Figure 2B) [1].

Clinical evidence supports the translational relevance of these findings. In a Phase 2 study evaluating monalizumab in combination with **cetuximab** in patients with previously treated squamous cell carcinoma of the head and neck, an objective response rate of approximately 31% was observed [1]. The therapeutic benefit was attributed primarily to enhanced NK cell-mediated ADCC driven by cetuximab, rather than direct effects on $NKG2A^+$ $CD8^+$ T cells. Similarly, a first-in-human dose-escalation study combining monalizumab with the anti-PD-L1 antibody **durvalumab** in patients with metastatic microsatellite-stable colorectal cancer demonstrated a manageable safety profile and encouraging clinical activity in a patient population historically resistant to PD-1/PD-L1 blockade [2].

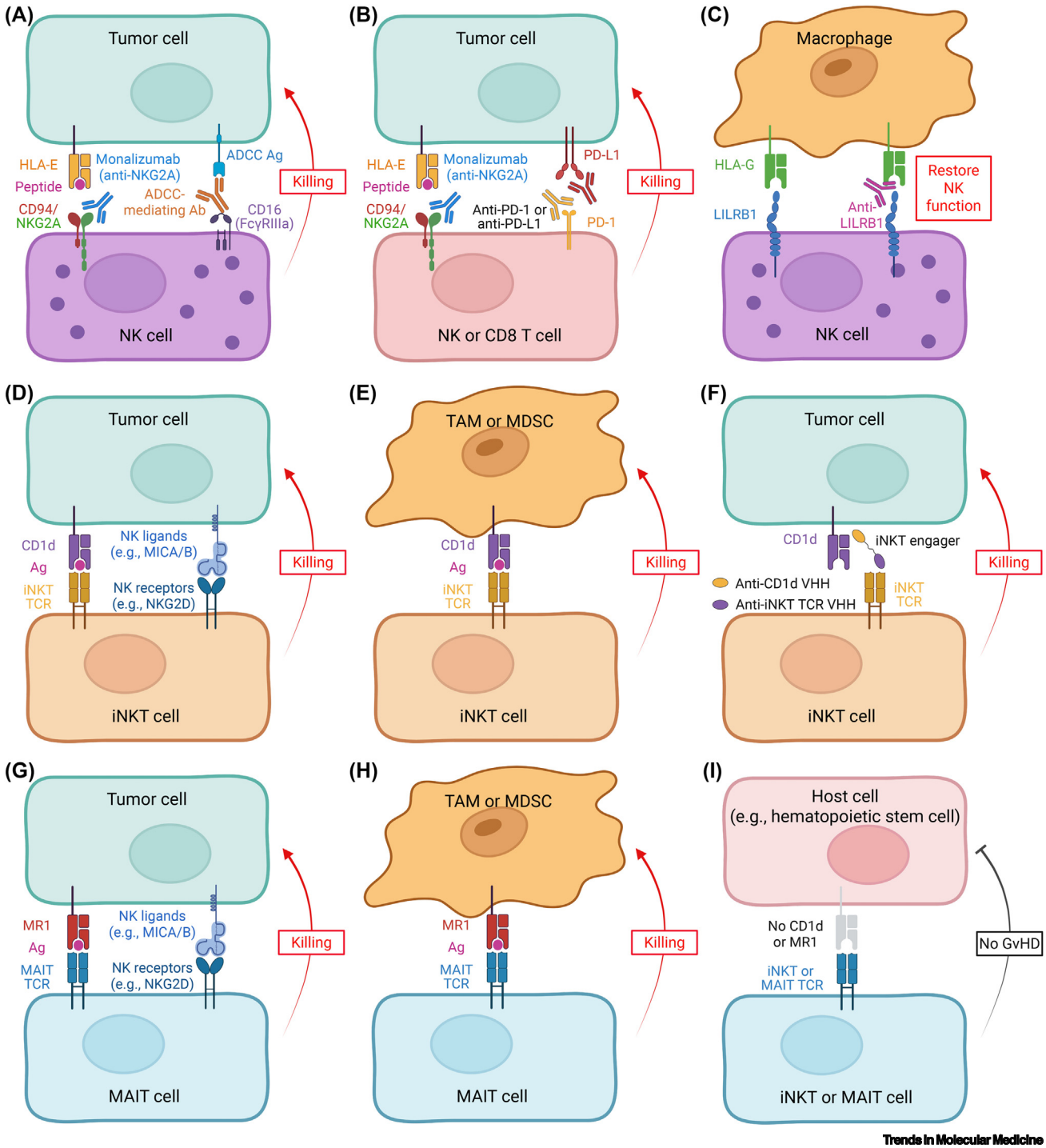

Figure 2. Immunotherapeutic strategies targeting nonclassical MHC-I molecules. (A) HLA-E/NKG2A blockade enhances NK cell antibody-dependent cellular cytotoxicity (ADCC). Tumor cells frequently overexpress HLA-E, which engages the inhibitory receptor NKG2A on NK cells and suppresses cytotoxic activity. Blockade of NKG2A with the monoclonal antibody monalizumab potentiates NK cell-mediated ADCC against antibody-coated tumor cells. (B) Combined checkpoint blockade of HLA-E/NKG2A and PD-1/PD-L1 pathways. The HLA-E/NKG2A axis operates in parallel with the PD-1/PD-L1 pathway to inhibit NK cell and $CD8^+$ T cell effector responses. Combined blockade of NKG2A and PD-1 or PD-L1 synergistically enhances cytotoxic lymphocyte-mediated tumor cell killing. (C) Targeting the HLA-G/

(Figure legend continued at the bottom of the next page.)

Beyond NK cell-mediated immunity, accumulating evidence highlights a critical role for NKG2A in regulating $CD8^+$ T cell responses. Montfoort *et al*. demonstrated that tumor-infiltrating $CD8^+$ T cells frequently express NKG2A, particularly in the inflamed TME and following therapeutic cancer vaccination [3]. High-dimensional immune profiling revealed that NKG2A defines a distinct effector $CD8^+$ T cell subset enriched for the tissue-resident marker CD103, yet lacking expression of conventional immune checkpoint molecules such as PD-1. Disruption of the NKG2A/Qa-1b axis through antibody-mediated blockade or genetic deletion of Qa-1b, the murine ortholog of HLA-E, significantly enhanced vaccine-induced antitumor immunity across multiple mouse tumor models, including those refractory to PD-1 blockade [3]. Notably, the therapeutic benefit in this context was mediated predominantly by $CD8^+$ T cells rather than NK cells, underscoring the cell-type-specific functions of NKG2A signaling.

Additional preclinical studies further support the relevance of targeting the HLA-E/NKG2A pathway. Liu *et al*. demonstrated that blockade of NKG2A using monalizumab enhances NK cell-mediated cytotoxicity against pancreatic ductal adenocarcinoma, irrespective of baseline HLA-E expression levels. Consistently, genetic knockdown of HLA-E markedly sensitized tumor cells to NK killing, reinforcing the functional importance of this inhibitory interaction [4].

Collectively, these findings indicate that NKG2A expression is frequently associated with adverse clinical outcomes and is often co-expressed with PD-1 on exhausted $CD8^+$ T cells, suggesting that NKG2A functions as an additional immune checkpoint whose blockade may enhance the efficacy of PD-1/PD-L1-targeted immunotherapy. Moreover, HLA-E has been reported to be more widely expressed than PD-L1 across multiple cancer types, highlighting its potential as a broadly applicable immunotherapeutic target [1,2].

Immune checkpoint blockade targeting the HLA-G/LILRB axis

During *Mycobacterium tuberculosis* infection, macrophages harboring intracellular bacteria exhibited high surface expression of HLA-G, which, in turn, induced upregulation and activation of LILRB1 on NK cells. Engagement of LILRB1 by HLA-G impaired NK cell effector functions through suppression of mitogen-activated protein kinase signaling mediated by recruitment of the tyrosine phosphatases Src homology region 2 domain-containing phosphatase-1 (SHP-1) and SHP-2 [9]. Importantly, antibody-mediated blockade of LILRB1 restored NK cell-dependent antimicrobial immunity in immuno-humanized mouse models, establishing the HLA-G/LILRB1 interaction as a functional immune checkpoint in tuberculosis [9] (Figure 2C).

LILRB1 immunosuppressive axis. HLA-G expressed by macrophages interacts with the inhibitory receptor LILRB1 on NK cells, leading to functional impairment and exhaustion. Therapeutic blockade of LILRB1 restores NK cell activity and promotes antitumor immunity. (D) Direct antitumor activity of iNKT cells. iNKT cells recognize CD1d-presented lipid antigens on tumor cells through their invariant TCR, leading to direct tumor cell killing. iNKT cells can additionally integrate signals from NK receptors, such as NKG2D, upon engagement with stress-induced ligands expressed by tumor cells. (E) iNKT cell targeting of immunosuppressive myeloid cells. Tumor-associated macrophages (TAMs) and myeloid-derived suppressor cells (MDSCs) express high levels of CD1d, enabling selective targeting and elimination by iNKT cells, thereby relieving immunosuppression within the tumor microenvironment. (F) iNKT cell targeting of tumor cells via engagers. Bispecific iNKT cell engagers simultaneously bind CD1d on tumor cells and the invariant TCR, stabilizing immune synapse formation and enhancing iNKT cell-mediated cytotoxicity. (G) MAIT cell-mediated recognition and killing of tumor cells. MAIT cells recognize MR1-presented metabolite antigens on tumor cells through their invariant TCR and contribute to tumor cell killing, supported by co-activation through NK receptors. (H) MAIT cell targeting of suppressive myeloid populations. MR1 expression on TAMs and MDSCs allows MAIT cells to selectively eliminate immunosuppressive myeloid populations, thereby promoting remodeling of the tumor microenvironment and enhancing antitumor immunity. (I) Allogeneic potential of iNKT- and MAIT-based therapies. Because CD1d and MR1 are nonpolymorphic and absent from healthy host tissues such as hematopoietic stem cells, iNKT- and MAIT cell-based therapies do not induce alloreactivity or graft-versus-host disease, supporting their use as allogeneic off-the-shelf cellular therapies. Figure created using BioRender (https://BioRender.com). GvHD: graft-versus-host disease; HLA: human leukocyte antigen; iNKT: invariant natural killer T cell; LILRB1: leukocyte immunoglobulin-like receptor subfamily B member 1; MAIT: mucosal-associated invariant T cell; MR1: major histocompatibility complex class I-related protein 1; NK: natural killer cell; NKG2A: natural killer group 2, member A; PD-1: programmed cell death protein 1; PD-L1: programmed death-ligand 1; TCR: T cell receptor; VHH: variable heavy domain of a heavy-chain antibody.

In oncology, aberrant expression of LILRB receptors and their ligand HLA-G has been documented across a wide range of hematological malignancies and solid tumors [10]. LILRB1 is expressed on several leukemia subtypes and diverse tumor cells, where it has been implicated in tumor cell survival and disease progression. For example, LILRB1 signaling has been shown to protect primary cutaneous T cell lymphoma cells from apoptosis and to promote gastric tumor growth. Conversely, in certain B cell malignancies, engagement of LILRB1 by HLA-G inhibits neoplastic cell proliferation, highlighting a context-dependent role for this pathway. Notably, blockade of LILRB1 on myeloma and lymphoblastic leukemia cells does not appear to impair NK cell-mediated cytotoxicity, suggesting that the functional consequences of LILRB1 signaling may vary depending on tumor type and cellular context [122].

LILRB2 expression has been reported in multiple solid tumors, including clear cell renal cell carcinoma and in stromal and myeloid cells within the TME [123]. Elevated LILRB2 levels correlate with poor tumor differentiation and increased metastatic potential. Mechanistically, LILRB2 signaling has been linked to the upregulation of vascular endothelial growth factor C, a key driver of **lymphangiogenesis** and **lymphatic dissemination** [124]. These findings support a role for LILRB2 in promoting tumor invasiveness and metastatic spread.

Consistent with these observations, HLA-G expression is frequently detected in malignant tissues but is largely absent from adjacent healthy tissues [125]. Experimental studies have demonstrated that HLA-G-expressing tumor cells exhibit enhanced growth in immunocompetent hosts, whereas in immunodeficient settings, they display widespread metastatic dissemination, underscoring the role of HLA-G in immune evasion [125]. Collectively, these data implicate the HLA-G/LILRB checkpoint in tumor progression and metastatic competence, supporting its exploration as an additional target for cancer immunotherapy.

Several HLA-G-targeted immunotherapeutic strategies are under development, including neutralizing monoclonal antibodies, **antibody-drug conjugates**, and HLA-G-specific chimeric antigen receptor-engineered T (CAR-T) cells [10,11]. However, clinical translation is challenged by the complex regulation and extensive isoform diversity of HLA-G. Alternative splicing generates multiple HLA-G variants lacking specific extracellular domains, limiting detection by currently available antibodies that predominantly recognize the α1 domain [126]. In contrast, the α3 domain is conserved across most tumor-associated isoforms and mediates binding to both LILRB1 and LILRB2. Targeting the α3 domain may therefore enable broader checkpoint blockade and more effective disruption of HLA-G–mediated immune suppression in cancer [10].

iNKT cell-based immunotherapies

Cell-based immunotherapies targeting nonclassical MHC-I molecules, particularly CD1d and MR1, have emerged as a promising therapeutic modality for a broad spectrum of diseases, including cancer, chronic viral infections, autoimmune disorders, and transplant-related complications (Box 3). iNKT and MAIT cells recognize lipid or metabolite antigens presented by CD1d and MR1, respectively, through semi-invariant TCRs characterized by high specificity and strong binding affinity [116,139,140]. These unique recognition properties enable rapid and potent effector responses that bridge innate and adaptive immunity. Importantly, CD1d and MR1 are expressed not only by subsets of tumor cells but also by immunosuppressive myeloid populations within the TME of several malignancies, including ovarian cancer and neuroblastoma, particularly on tumor-associated macrophages (TAMs) and myeloid-derived suppressor cells (MDSCs) [141–144]. This expression pattern provides a unique therapeutic opportunity to target both malignant cells and the immunosuppressive stromal compartment using iNKT- and MAIT cell-based approaches.

Box 3. Therapeutic applications of iNKT and MAIT cells beyond cancer

Beyond oncology, CD1d-targeted iNKT cell recognition of proinflammatory myeloid cells has important implications for nonmalignant diseases. In chronic viral infections, iNKT cells contribute to antiviral immunity by eliminating infected antigen-presenting cells and shaping adaptive immune responses [127–129]. In autoimmune diseases, selective targeting of pathogenic myeloid populations by iNKT cells can dampen excessive inflammation and restore immune balance [130]. Furthermore, in the context of allogeneic hematopoietic stem cell transplantation, iNKT cell-mediated elimination of inflammatory antigen-presenting cells has been shown to reduce GvHD while preserving graft-versus-leukemia effects [131,132]. In addition, a Phase 1/2 clinical trial investigating iNKT cell therapy for moderate-to-severe acute respiratory distress syndrome demonstrated that donor-unrestricted, allogeneic *ex vivo*-expanded iNKT cells could be safely administered to patients with coronavirus disease 2019-associated acute respiratory distress syndrome (ARDS) and were associated with induction of anti-inflammatory immune responses [133]. Collectively, these findings position CD1d-restricted iNKT cell-based therapies as a versatile immunotherapeutic platform with broad clinical applicability.

MAIT cells have been implicated in autoimmune and inflammatory diseases, although their precise roles remain complex and context-dependent. Both protective and pathogenic functions have been reported depending on the disease setting, tissue environment, and inflammatory milieu. Walkenhorst *et al*. demonstrated that TCR-mediated MAIT cell activation ameliorated disease severity in experimental autoimmune encephalomyelitis, a murine model of multiple sclerosis [134]. Blockade of MR1 exacerbated disease, whereas activation with the cognate antigen 5-OP-RU reduced inflammation, potentially through induction of amphiregulin, highlighting a tissue-protective function of MAIT cells in the central nervous system [134]. Similarly, Gnirck *et al*. revealed that tissue-resident MAIT17 cells in the kidney are activated and expanded in crescentic glomerulonephritis [135]. MAIT-deficient mice exhibited more severe disease, whereas mice with increased MAIT cell numbers were protected, suggesting a protective, anti-inflammatory role for MAIT cells in renal pathology [135]. However, other studies have demonstrated that MAIT cells can also promote inflammation through the production of IL-17, IFN-γ, and other proinflammatory mediators in target organs of autoimmune diseases [136–138], suggesting that MAIT cells may exert either protective or pathogenic effects depending on the immunological context.

In conclusion, iNKT and MAIT cell-based immunotherapies represent highly versatile and promising platforms for the treatment of cancer and immune-mediated diseases. Their invariant TCR specificity, restriction to nonpolymorphic CD1d and MR1, capacity to target both malignant and immunosuppressive cells, and suitability for off-the-shelf application position iNKT and MAIT cells as attractive next-generation cellular therapies with broad clinical potential.

Several hematologic malignancies and select solid tumors express CD1d, including acute myeloid leukemia, multiple myeloma (MM), chronic lymphocytic leukemia, and certain lymphomas. iNKT cells can directly recognize and eliminate CD1d-expressing tumor cells through TCR-mediated cytotoxicity driven by endogenous lipid antigens. In addition, iNKT cells express high levels of NK cell-associated activating receptors, including NKG2D and DNAX accessory molecule-1 (DNAM-1), enabling them to recognize stress-induced ligands such as major histocompatibility complex class I chain-related protein A and B (MICA/B) and CD155 expressed on tumor cells (Figure 2D).

Although most solid tumors lack CD1d expression, immunosuppressive myeloid cells within the TME express high levels of CD1d compared with other immune populations such as T cells, B cells, NK cells, and granulocytes [141,143,145]. This differential expression allows selective targeting of TAMs and MDSCs by iNKT cells (Figure 2E). *In vitro* studies using samples derived from patients with ovarian cancer demonstrated that iNKT cells selectively eliminate CD1d-expressing TAMs and MDSCs while sparing other immune subsets [146,147]. In a human MM xenograft model, CAR-iNKT cells efficiently depleted CD1d-expressing immunosuppressive cell populations, including M2-like macrophages, cytokine release syndrome-associated macrophages, and MDSCs, thereby remodeling the TME to favor antitumor immunity [119]. Consistent with these findings, Zhou *et al*. demonstrated that CAR-iNKT cells exhibit superior antitumor activity compared with conventional CAR-T cells. Mechanistically, CAR-iNKT cells eliminate CD1d-expressing suppressive macrophages, promote epitope spreading, and enhance endogenous T cell responses against tumor-associated neoantigens, collectively amplifying antitumor immune responses [148].

A biomimetic platform, termed the invariant NKT cell-targeted microparticle recruitment and activation system, has been developed as an *in vivo* ‘charging station’ for CAR-iNKT cells

[149]. This system presents CD1d loaded with α-GalCer to stimulate iNKT TCR signaling while simultaneously delivering interleukin-15 (IL-15) receptor alpha complexed with IL-15 to support survival and expansion. By providing localized chemotactic, activation, and cytokine signals, this platform enhances recruitment, activation, and persistence of CAR-iNKT cells, resulting in improved therapeutic efficacy in lymphoma and melanoma models [149].

In addition to cell-based approaches, the CD1d–iNKT cell axis has been exploited for the development of bispecific immune engagers designed to amplify antitumor immune responses (Figure 2F). Lameris *et al*. developed a series of single-domain antibodies (variable domain of heavy-chain-only antibodies, or VHHs) specific for human CD1d that selectively modulate the activity of CD1d-restricted T cells. Structural analysis of the ternary VHH1D12–CD1d–α-GalCer–iNKT TCR complex revealed that VHH1D12 simultaneously engaged both CD1d and the iNKT TCR, thereby stabilizing the immune synapse through intrinsic bispecific binding [150]. This enhanced molecular interaction translated into robust NKT cell activation and significantly increased iNKT cell-mediated cytotoxicity against CD1d-positive tumor targets. Building on this platform, subsequent studies engineered a bispecific T cell engager with expanded functionality by linking a CD1d-specific VHH to a Vδ2 TCR-specific VHH. This design generated a trispecific immune engager capable of simultaneously recruiting iNKT cells and Vγ9Vδ2 T cells to CD1d-expressing hematological malignancies [151]. Given the relatively low frequency of circulating iNKT cells in humans, the incorporation of Vγ9Vδ2 T cells may help overcome limitations in effector cell availability and enhance the overall therapeutic efficacy of this approach *in vivo* [151].

MAIT cell-based immunotherapies

MR1-restricted T cells, especially MAIT cells, are highly abundant in humans and can comprise 1–10% of circulating T cells and substantially higher proportions in tissues such as the liver, lung, and intestinal mucosa [74,152–154]. The development of MAIT cell-based immunotherapy is still in its early stages but has rapidly gained attention due to the unique biology of MAIT cells and their restriction to the nonpolymorphic MR1. MR1 is broadly expressed across multiple tumor types, including HCC [13,86,88], colorectal cancer [155–158], and lung cancer [14,87,159], as well as on immunosuppressive myeloid populations within the TME, providing a conserved and targetable platform for MAIT cell-mediated immune responses across patients.

Activation of MAIT cells through their invariant TCR using agonistic ligands such as 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil (5-OP-RU) markedly enhances their cytotoxic and cytokine-producing capacity (Figure 2G) [12–15]. Ruf *et al*. demonstrated that MAIT cells exert potent antitumor activity *in vivo* when activated by a combination of 5-OP-RU and the Toll-like receptor 9 agonist CpG [87]. Co-administration of these agents induced robust systemic expansion and activation of MAIT cells, characterized by elevated CD69 expression, acquisition of an effector memory phenotype, and increased production of IFN-γ, granzyme B, and perforin [87]. Activated MAIT cells mediated broad antitumor immunity across multiple murine models, including liver metastases, HCC, lung metastases, and subcutaneous tumors [87]. Notably, this antitumor effect was abrogated in MAIT-deficient *Mr1*$^{-/-}$ mice, confirming a central role for MAIT cells. Interestingly, deletion of MR1 in tumor cells did not fully abrogate MAIT cell-mediated tumor control, suggesting that MAIT cells can exert antitumor effects through additional mechanisms beyond direct MR1-dependent tumor recognition [87]. These may include indirect modulation of the TME, cytokine-mediated activation of other immune cells, or targeting of MR1-expressing stromal and myeloid populations.

Similar to CD1d, MR1 is highly expressed on TAMs and MDSCs compared with lymphoid populations such as T, B, and NK cells. *In vitro* co-culture assays using healthy donor-derived

Clinician's corner

The HLA-E/NKG2A and HLA-G/LILRB axes function as clinically relevant immune checkpoints that suppress NK and CD8^{+} T cell activity. Early-phase trials of NKG2A blockade, particularly in combination with PD-1/PD-L1 inhibitors or ADCC-inducing antibodies, show encouraging activity with manageable toxicity. These pathways may contribute to resistance to conventional checkpoint inhibitors and should be considered in patients with suboptimal responses.

Elevated tumor expression of HLA-E or HLA-G is frequently associated with immune evasion and poor prognosis. As diagnostic tools advance, evaluating nonclassical MHC-I expression in tumor tissue may help guide enrollment in clinical trials and inform combination immunotherapy strategies, especially in tumors with low PD-L1 expression.

Because iNKT and MAIT cells recognize nonpolymorphic CD1d and MR1, respectively, they are unlikely to induce graft-versus-host disease and are suitable for allogeneic platforms. Preclinical and early translational studies suggest that these cells can target both malignant cells and immunosuppressive myeloid populations. Clinicians involved in cellular therapy programs should monitor the ongoing development of CAR-iNKT and emerging MAIT cell-based therapies.

Nonclassical MHC-I molecules are central to immune tolerance in pregnancy, transplantation, and immune-privileged tissues. Therapeutic blockade may carry risks of immune-related adverse events or disruption of tolerance. Careful patient selection, especially in transplant recipients or patients with autoimmune disease, and close immune monitoring will be essential as these therapies advance into broader clinical practice.

Clinical development of nonclassical MHC-directed immunotherapies remains heterogeneous. Monalizumab targeting the HLA-E/NKG2A axis has progressed to randomized phase II evaluation in nonsmall cell lung cancer, although the added benefit of NKG2A blockade requires further validation. HLA-G antagonist TTX080 is

MAIT cells demonstrated efficient and selective killing of these MR1-expressing immunosuppressive myeloid cells, highlighting the capacity of MAIT cells to remodel the TME and relieve immune suppression (Figure 2H) [16].

A major advantage of MAIT and iNKT cell-based therapies is their restriction to nonpolymorphic antigen-presenting molecules, MR1 and CD1d, respectively. As a consequence, these cells do not mediate alloreactivity and are unlikely to induce **graft-versus-host disease (GvHD)**, enabling their development as allogeneic, off-the-shelf cellular therapeutics (Figure 2I). Compared with autologous cell therapies, this approach offers significant advantages in manufacturing scalability, reduced cost, and immediate availability for patients [160].

undergoing early-phase clinical testing, with preliminary evidence of immune activation but limited efficacy data. CD1d/iNKT-based therapies have demonstrated acceptable safety and immune activation, while CAR-iNKT approaches have shown promising early antitumor activity in neuroblastoma despite limited patient numbers and follow-up. In contrast, MR1/MAIT-targeted therapies remain largely preclinical because of unresolved issues surrounding ligand identity, tumor specificity, and MR1 allomorph dependence.

Concluding remarks and future perspectives

Nonclassical MHC-I molecules represent a versatile and still largely underexplored frontier in precision immunotherapy. While classical MHC-I has long dominated the clinical spotlight, molecules such as HLA-E, HLA-F, and HLA-G are finally transitioning from biological curiosities to actionable targets. Clinically, the field is already being shaped by early-phase efforts to disrupt inhibitory axes such as HLA-E/NKG2A, exemplified by the Phase 1/2 evaluation of monalizumab plus durvalumab in advanced solid tumors [161]. In parallel, HLA-G blockade is entering the clinic, such as the Phase 1 study of the HLA-G antagonist TTX080 for the treatment of refractory or resistant solid malignancies (NCT04485013). Importantly, cell therapies that leverage conserved antigen-presenting platforms are also advancing. Multiple CAR-NKT products are being tested in current clinical trials, underscoring a pivotal shift toward leveraging the rapid, potent effector functions of unconventional T cells to achieve more durable antitumor responses than with standard checkpoints alone [140,162–164] (Clinician's corner).

Despite this momentum, key challenges remain (see Outstanding questions). Looking forward, nonclassical MHC-I pathways offer a compelling route to bridge innate and adaptive immunity with target structures that are shared across patients. MAIT and iNKT cell-based therapies are particularly attractive for developing 'off-the-shelf' immunotherapies because MR1 and CD1d are effectively nonpolymorphic, supporting scalable, off-the-shelf strategies, whether via engineered effectors or stem cell-derived products and trial frameworks. Continued mechanistic dissection of ligand repertoires, receptor wiring, and tissue-specific tolerance circuits should enable safer, more precise interventions that extend beyond oncology into infection, autoimmunity, and transplantation.

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Declaration of interests

L.Y. is a scientific advisor to AlzChem and Amberstone Biosciences. None of these companies contributed to this study. The other authors declare no competing interests.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT to assist with improving the clarity, organization, and scientific language of the text. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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Outstanding questions

What are the endogenous and tumor-derived ligands presented by MR1 and CD1 molecules, and how do they differ across tissues and disease states?

How does context-dependent signaling through HLA-E/NKG2A and HLA-G/LILRB axes shape NK and $CD8^+$ T cell exhaustion versus protective immunity?

What mechanisms regulate isoform-specific expression and splicing of HLA-G in tumors, and how can therapies effectively target all immunosuppressive variants?

How do HLA-F open conformers reorganize membrane receptor networks, and what is their functional impact on immune synapse formation in cancer?

Can biomarkers be developed to stratify patients most likely to benefit from nonclassical MHC-I-targeted checkpoint blockade?

How can MAIT and iNKT cells be optimally engineered to enhance persistence, trafficking, and resistance to immunosuppressive tumor microenvironments?

What combination strategies (e.g., PD-1 blockade, epigenetic modifiers, and cytokine support) most effectively synergize with nonclassical MHC-I-directed therapies?

To what extent can MR1- and CD1-restricted cellular therapies be developed as universal, off-the-shelf products without inducing unintended immune pathology?

How do MAIT- and iNKT-based therapies compare mechanistically and clinically with conventional CAR-T and CAR-NK approaches in hematologic versus solid malignancies?

Can universal donor MAIT or iNKT cells be gene-edited to further reduce immunogenicity while maintaining anti-tumor function?

How can newly identified MR1-presented vitamin B6 metabolites and CD1-presented endogenous lipids be systematically validated for disease

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specificity, ligand abundance, TCR recognition, and healthy tissue reactivity before therapeutic translation?

How can ligand agonists, TCR-engineered cells, and bispecific engager platforms targeting MR1 or CD1 pathways be optimized for efficacy while preserving tissue-specific immune homeostasis?

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