AJMB
eISSN: 2600-8173
https://doi.org/10.37231/ajmb.2024.8.2.749
https://journal.unisza.edu.my/ajmb
PENERBIT
Universiti Sultan Zainal Abidin

Asian Journal of Medicine and Biomedicine

Potential Role of Natural Killer Cell Group-2 D Receptor in Cancer Therapy

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Received: 14th February 2024 **Accepted: 20th June 2024** **Published: 27th October 2024**

Abstract

Immunological oncology has transformed cancer therapy, thereby increasing patients' chances of survival and quality of life. Natural killer (NK) cells, specifically, have come to prominence as powerful engines of the inherent immune system response, making them a prospective immunotherapy tool. One of the most crucial NK cell receptors namely NK-group 2, member D (NKG2D) has a pivotal role in both innate and adaptive immunity for establishing the degree of activation of NK cells. It serves as a pertinent activating receptor in the immunological identification and extermination of aberrant cells by natural killer cells and T lymphocytes, recognising a wide variety of ligands to offer comprehensive target specificity. This review aimed to emphasise a better understanding of the NKG2D receptor's structure, its signalling mechanism, and its potential and prospective implementation in a variety of medical contexts. A search for published material was carried out using some combinations of the terms "cancer", "immunotherapy", "natural killer" and “NKG2D” on PubMed, ScienceDirect, Scopus, and Google Scholar. All citations from the selected papers were examined. In light of the numerous studies conducted, it is deduced that NKG2D-mediated cancer chemotherapy offers an excellent prospect for usage as a type of chemotherapy soon; nevertheless, additional clinical trials are required before it can be employed in clinical settings. It is crucial to identify and comprehend the functions of several transcription factors that control the expression of NKG2D on the cell membrane by binding to its ligands. Furthermore, approaches centered on augmenting NKG2D expression in immune cells and elevating NKG2DL expression in cancer cells may efficiently trigger the antitumour immune response.

Keywords:

Cancer, Immunotherapy, Natural Killer Cell, NK Group-2 D Receptor

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Introduction

Cancer is the largest cause of mortality in the globe, accounting for over 10 million fatalities in 2020, or approximately one in every six casualties [1]. Smoking cigarettes, elevated body mass index, consumption of alcoholic beverages, inadequate intake of fruits and vegetables, and insufficient levels of regular physical activity account for almost one-third of all cancer-related fatalities. Several types of cancer are capable of getting cured if diagnosed at an early stage and addressed appropriately [2]. The most prevalent cancers in 2020 were breast, lung, colorectal and rectum, prostate, skin (non-melanoma), and gastric carcinoma. In 2020, the six most prevalent kinds of fatalities from cancer were pulmonary, colorectal, hepatocellular, gastric carcinoma, and breast cancer [3].

Immunotherapy has gained much attention and is becoming popular in developed countries. The most used immune cells for immunotherapy are T cells and natural killer (NK) cells**.** Current evidence suggests that immune responses independent of cytotoxic T cells, such as natural killer (NK) cells, play a crucial role in the efficacy of immunotherapeutic interventions. NK cells hold a distinct role in potentiating the innate immune response and activating the adaptive immune system [4-6]. Whether an NK cell remains silent or executes its killing capacity on malignant cells depends on the dynamic balance of stimulation events of two main structural classes of NK cell surface receptors, the killer cell immunoglobulin-like receptors (KIRs) and receptors of the C-type lectin-like family, which inhibit or activate signalling cascades. Natural killer group 2D (NKG2D) is one of the most critical activating receptors which play a pivotal role in NK cell tumour immunosurveillance and immune-mediated rejection of cancerous cells [7,8]. NKG2D receptor is one of the most widely studied immunoreceptors due to its significant relationship in activating immune responses against infected and cancerous cells. In this present review, we highlighted the molecular structure and signalling mechanisms of NK cell cytotoxicity via NKG2D receptor-mediated pathway as well as the potential of NKG2D to be used as a target for cancer chemotherapy. The selection of articles to be included in this review is illustrated in Figure 1.

Article selection flowchart

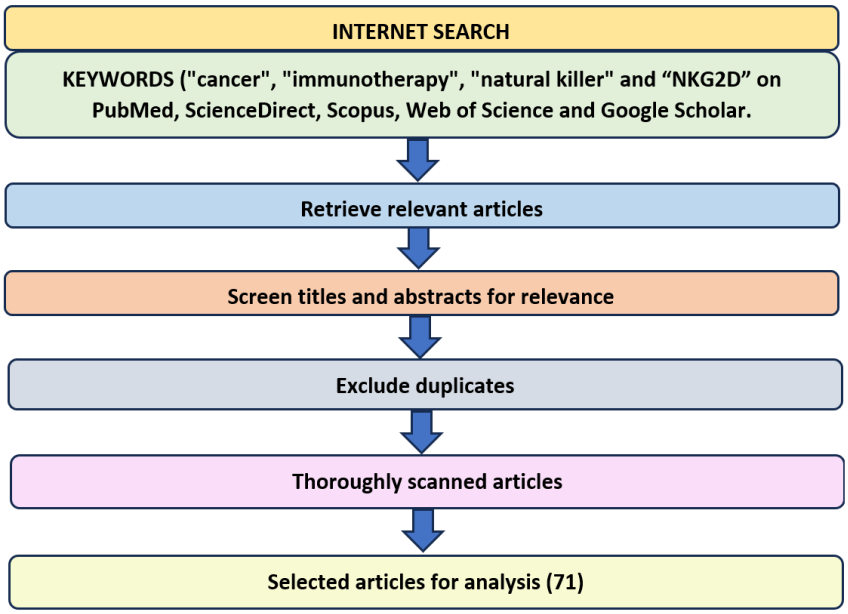

Figure 1: Flow diagram of the article selection process.

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NK cells and their receptors

Multiple rounds of investigations on cancer sufferers' cytotoxicity led to the discovery of NK cells in the early seventies. Depending on their physical characteristics, NK cells are killer cytotoxic lymphocytes that exhibit clusters of differentiation-56 (CD56) and CD16 cell-surface protein components, and they could eradicate cancerous and virus-infected cells without previous exposure. Considering the expression levels of surface marker proteins CD56 and CD16, two NK cell types have been established: CD56dim/CD16bright (high expression of CD16 and robust cytotoxic characteristics) and CD56bright/CD16dim (low expression of CD16 and strong immunoregulatory features). NK cells form roughly 10% of total lymphocytes that circulate in the peripheral circulation, with 90% of this subset consisting of CD56dim/CD16bright cells [9-11]. Whether an NK cell remains silent or executes its killing capacity on malignant cells depends on the dynamic balance of stimulation events of two main structural classes of NK cell surface receptors namely activating and inhibitory receptors. Inhibitory receptors in human NK cells include killer cell immunoglobulin-like receptors (KIRs), immunoglobulin-like transcripts (ILTs), and C-type lectin inhibitory receptors comprising CD94/NKG2A [12,13].

The activation types of receptors found in NK cells are considerably more intricate, although some of them are employed to identify molecules that are rarely expressed in normal circumstances but will be provoked by different types of distress. At least three activating receptor types have been identified: C-type lectin homodimer NKG2D, CD16, and the natural cytotoxicity receptors (NCRs). Three different NCRs have been observed, namely NKp46, NKp44, and NKp30 [14,15]. Furthermore, certain human activating cellular receptors, including distinct KIRs or NCRs, such as NKp30, NKp44, NKp46, and NKp80, convey the triggering signal via protein tyrosine kinase-dependent channels [16]. Major histocompatibility complex (MHC) class-I molecules are synthesised on the surfaces of healthy cells, acting as ligands for inhibitory receptors and contributing to NK cell self-tolerance. However, virus-infected or malignant cells tend to absent surface MHC-class-I protein manifestation, resulting in a diminished inhibiting signal in NK cells. Due to this absence, cells with low or no expression levels of MHC-class-I molecules will be efficiently lysed. Signals from activating receptors in NK cells shift the equilibrium towards NK cell excitement and ultimate destruction of target cells, either immediately via NK cell-mediated cytotoxicity or using the production of signalling molecules cytokines [17,18]. Different types of NK cell receptors are depicted in Figure 2.

NK cells serve a significant role in the cytotoxic approach because they exhibit a broad array of both activating and inhibitory receptors that attach to specific proteins in cancer cells. While certain inhibitory receptors were overexpressed in malignancy, the majority of activating receptors were suppressed. Therefore, modifications in NK cell receptors seen in cancer patients may help with the application of therapies that increase activating receptors or decrease inhibitory ones [19]. In general, the activation of the NKG2D receptor by its ligands produced by tumours initiates one of the critical signalling pathways that govern cancer immune monitoring [20,21]. Moreover, NKG2D activation on NK cells results in the exocytosis of cytotoxic granules and the generation of cytotoxic cytokines [22,23]. Furthermore, it has been discovered that NKG2D protective function prevents the body from developing cancer, and animals deficient in this receptor showed impaired immune surveillance of lymphoid and epithelial neoplasia [24].

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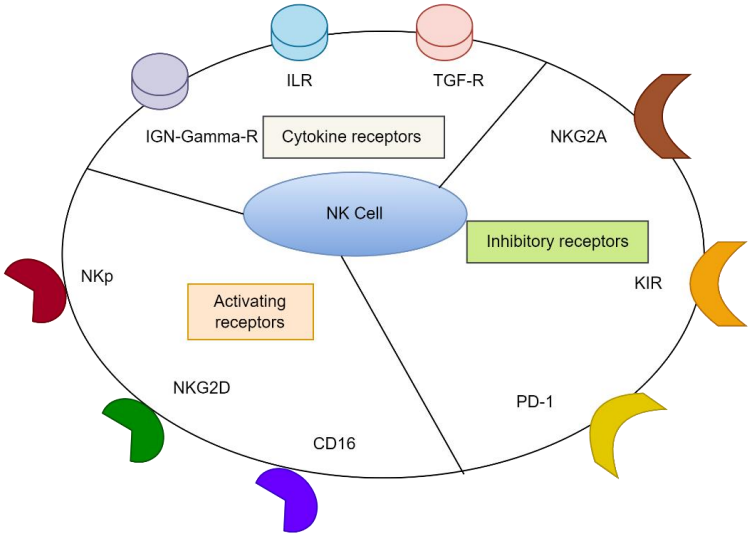

Figure 2: Schematic diagram depicting the different types of NK cell surface receptors. (ILR= Interleukin receptor, TGF-R= receptor for transforming growth factor, NKG2A and NKG2D= NK group 2 A & D receptor, CD16 = Cluster of differentiation-16, PD-1= Program cell death protein-1)

Structure, ligands and signalling mechanism of NKG2D

Within the NKG2 family of C-type lectin-like receptors, the NKG2D receptor is expressed by human NK cells, γδ T cells, and CD8+ αβ T cells. It is composed of 316 amino acids and the gene that transcribes it is KLRK1 (killer cell lectin-like receptor K1), which is located on chromosome 12p in humans and the NK-gene cluster on the sixth chromosome in mice. [25,26]. The NKG2D receptor exists as a homodimer made up of two transmembrane protein molecules connected by a disulfide that have modest intracellular areas devoid of signalling properties. The process of ligand attachment is carried out via its ectodomains. A dimer of DNAX-activating protein-10 (DAP10) is connected to every NKG2D monomer. An ionic interaction between a negatively charged aspartate in both transmembrane regions of the DAP10 dimer and a positively charged arginine in an NKG2D transmembrane segment maintains this association. As an adaptor protein, DAP10 recruits the p85 component of phosphatidyl inositol-3 kinase (PI3K) and the Growth factor receptor-bound protein-2 (Grb2)-Vav1 (proto-oncogene product) complex, which is responsible for additional downstream actions. This allows DAP10 to transduce the signal after ligand binding [27,28].

Eight functional "stress-induced ligands" for NK cells are present in the human genome. These include MHC class I chain-related protein-A (MICA), MICB, and UL16 binding protein-1 (ULBP1) to 6. All these proteins have been detected by NKG2D, which is found not only in NK cells, but also in cytotoxic T cells, and other T cell subsets. The NKG2D ligand/NKG2D-axis is a crucial regulator of anti-tumour action; nevertheless, clinical data regarding NKG2D ligand involvement in immune surveillance and escape appears inconsistent [29]. Induced-self protein molecules designated as NKG2D ligands are either absent or minimally distributed in the outermost layer of healthy cells, but they become excessively expressed in infected, changed, old, and distressed cells [30]. NKG2D selectively binds to the right ligands at different intensities to start downhill pathway signalling, which is essential for the immune system's responses against pathogens and malignancies [31].

The dynamic regulation of NK cells' response to abrupt activation is attributed to the interaction between their surface NKG2D and similar ligands of nearby cells in the microenvironment. This can attach to PI3K's

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https://journal.unisza.edu.my/ajmb

P85 subsections and draw in inositol-1,4,5-triphosphate, activating the downstream Grb-2 pathway [14]. Tyrosine phosphorylation is initiated by the interaction of Grb-2 and Vav. Following tyrosine phosphorylation, it can unite with Vav-1's Src homology-2 (SH2) domain to create a complex with a range of adaptor proteins, which in turn activates the JNK kinase and plays a crucial role in cytotoxicity, as seen in Figure 3.

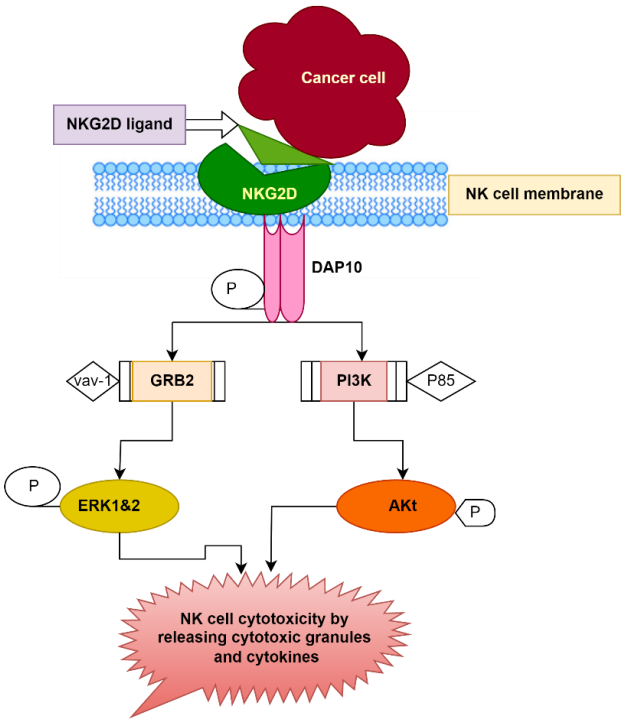

Figure 3: Schematic diagram demonstrating the signalling mechanism of NKG2D-mediated cellular cytotoxicity.

Additionally, it can cause cytotoxicity by releasing granulase via the phospholipase C signal pathway. Actin cytoskeleton reorganisation, transcription factor activation, and a rise in intracellular calcium concentration in NK cells are all caused by the activation of the PI3K and Grb2 signalling pathways [32]. Tumour cells and NK cells eventually create immunological synapses because of actin cytoskeleton recombination. Secretory vesicles containing active proteases in NK cells release perforin and granzymes that fuse with the membrane to cause tumour cell death [33,34]. Activation of transcription factors induces NK cells to express and secrete various cytokines, including FasL, tumour necrosis factor (TNF), and TNF-related apoptosis-inducing ligand, which kills tumour cells via the Fas/FasL pathway and the TNFα/TNF-receptor 1 (TNF-R1) pathway [35,36].

Recent advances in NKG2D as a cancer immunotherapy target

Numerous experimental animal models and clinical data have shown the importance of NKG2D/NKG2DL pathway regulation in modulating tumour growth and immunological responsiveness. These investigations brought to light the debate surrounding the function of NKG2D and its ligands in tumour immunity as well as the potential utility of the NKG2D/NKG2DL pathway in cancer therapy [37,38]. The use of NKG2D-mediated chimeric antigen receptors (CARs) in clinical research is currently one of the newest areas of interest. The

NKG2D ectodomain is typically fused to the CD3ζ cytoplasmic signalling domain to form NKG2D-based CARs. The NKG2D-CD3ζ CAR binds to endogenous DAP10 adaptors on its own. These CAR-modified cells produce more pro-inflammatory cytokines and exhibit improved cytotoxicity when they recognise NKG2DL+ tumour cells through NKG2D [39,40]. In a number of mouse tumour models for lymphoma, ovarian cancer, and multiple myeloma, NKG2D-CAR T cell infusion led to the removal of tumours and extended survival [41-43]. By secreting lethal cytokines, NKG2D-CAR-T cell infusion also transformed the cancer microenvironment from an immunosuppressive to an immunostimulatory milieu, and in certain circumstances, it even stimulated the establishment of tumour-specific memory T cell responses [44,45].

Clinical experiments with NKG2D-CAR-T cells have already begun [46]. A single dosage of NKG2D-CAR was administered to 12 patients suffering from multiple myeloma or acute myeloid leukaemia (AML) in a first-in-man clinical investigation. Notably, NKG2D-CARs were not shown to cause any serious negative effects [47]. Even though there was no objective tumour response, there were several instances of disease stability and one AML patient had temporarily better hematologic markers [48]. A lot of work is also done to look at the advantages of NK cells that express NKG2D-CAR. In vitro and in a mouse model of osteosarcoma, NK cells produced with a chimeric receptor comprising NKG2D, DAP10, and CD3ζ demonstrated promising outcomes. After prolonged activation of the receptor, the "responsiveness" was markedly enhanced by the expression of NKG2D-CAR. In a mouse model of osteosarcoma, engagement of NKG2D-CAR also resulted in increased cytokine production and a lower tumour burden, albeit not complete tumour elimination [49].

Increasing the expression of NKG2DL by tumour cells is one alternative approach to NKG2D cancer therapy, which should improve NKG2D-mediated tumour detection and removal. It has been demonstrated that a variety of small compounds, some of which are now being utilised in clinical settings, can alter the activity of NK cells and increase the expression of NKG2DL on tumour cells [50,51]. On a variety of cell lines, it has been demonstrated that inhibitors of histone deacetylases (HDACi) upregulate MICA, MICB, and ULBP2 [52,53]. NKG2D-mediated immunorecognition was enhanced by the HDACi valproate (VPA), which specifically increased the expression of MIC molecules on hepatocellular carcinoma (HCC) cell lines but not ULBPs. Notably, treatment to VPA did not induce the expression of NKG2DL in non-malignant primary hepatocytes. Notably, it was demonstrated that inducing NKG2DL by VPA improved the immune system's ability to recognise ovarian cancer cells using NKG2D-CAR, indicating that a combination therapeutic use would be highly advantageous [54]. MICA and MICB molecules were specifically induced to express on HCC by low dosages of bortezomib, but not on a primary hepatocyte [55].

It has been demonstrated that DNMTi 5-Aza-2'-deoxycytidine (decitabine), another possible chemical, can cause MICB expression in various cell lines [56]. Afterwards, it was shown that one unusual characteristic of AML cells was transcriptional downregulation of NKG2DL caused by hypermethylation of NKG2DL genes. Thus, AML cells treated with DNA methyltransferase inhibitors (DNMTi) expressed more NKG2DL on their cell surface and were more sensitive to NK cell line recognition [57]. It's interesting to note that DNMTi were also discovered to indirectly affect AML cells' production of NKG2DL by boosting the transcription of TIMP3, a protease inhibitor involved in NKG2DL shedding [58]. A previous investigation indicated that the combination of hypomethylating drugs, myeloid growth factors, and IFN-γ significantly enhanced the expression of NKG2DL on AML. In immunodeficient mice, the co-administration of decitabine and allogeneic NK cells enhanced the targeted killing of human AML cells, most likely through an increase in NKG2DL transcription [59]. Decitabine improved NKG2D ligand expression in glioma cells, especially for ULBP1 and ULBP3, and NKG2D-dependently restored NK-mediated lysis of mutant cells. HCC cells' transcriptional suppression of ULBP1 due to hypermethylation may be alleviated by inhibitors of the histone methyltransferase treatment [60,61].

https://doi.org/10.37231/ajmb.2024.8.2.749
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Several monoclonal antibodies (mAbs) that specifically target MICA/B have recently been developed. All these antibodies have different modes of action and are intended to increase the NKG2D signalling axis. An innovative method to make use of NKG2D ligands is to generate treatments that block MICA/B shedding. The inducement of antibodies in response to vaccination with the alpha 3 domain of MICA/B is one method to do this. These antibodies attach to MICA/B at the point of proteolytic cleavage, hence preventing shedding. In preclinical research, immunisation produced strong anti-tumour responses that were reliant on both NK and T cells [62,63]. Since CLN-619 is the only MICA/B-targeted mAb available in the clinic, Cullinan Oncology is taking a promising approach to targeting suppression of MICA/B shedding. CLN-619 is a humanised IgG1 antibody. By attaching to the alpha 3 domain, CLN619 stops MICA/B from shedding from cancer cells. It also has an active Fcγ1 domain that drives antibody-dependent cellular cytotoxicity [64]. The MICA/B genes are extremely polymorphic, as previously mentioned, yet CLN-619 has been shown to respond broadly to every allelic variation examined. Treatment with CLN-619 has been shown to reduce levels of shed MICA and enhance MICA on the surface of cancer cells concurrently. Crucially, the attached antibody does not impede the binding of MICA's alpha-1 and alpha-2 domains to NKG2D. Indeed, CLN-619 improves MICA's binding to NKG2D. Notably, in tumour xenograft models, CLN-619 has strong single-agent action at low dosages; however, its activity is significantly dependent on a functioning Fcγ1 domain [65,64].

Furthermore, NKG2DL expression can be promoted by two possible means, such as upregulation of the ligands by chemotherapeutic agents and inhibition of shedding of these ligands. The summary of possible ways to increase the expression of NKG2DL on tumour cells is listed in Table 1.

Table 1: List of some agents that promote NKG2DL expression in various types of cancer.

No.	Agents that induce NKG2DL expression	NKG2D ligand expressed	Cancer cell	References
1	histone deacetylase inhibitor sodium valproate	MICA and MICB	hepatocellular carcinoma cells	[66]
2	cisplatin	MICA and MICB	non-small cell lung cancer	[67]
3	Sunitinib	upregulated NKG2DLs, apoptotic genes, DNA damage repair genes	nasopharyngeal carcinoma cell and hepatoma cell line	[68]
4	Nutlin-3a	NKG2DL and DNAM-1 receptor	neuroblastoma	[69]
5	Inosine pranobex	multiple NKG2D ligands	human papillomavirus-associated warts	[70]
6	krüppel-like factor 4 (KLF4)	MICA	acute myeloid leukemia	[71]

The combined results above demonstrate unequivocally that NKG2D-mediated cancer therapy has great potential for use as a chemotherapeutic agent in the future; yet, several precautions must be addressed before it may be applied extensively in clinical practice. Additionally, NKG2D combined treatment plans may provide superior results compared to only one chemotherapy drug.

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Conclusions and Future Direction

NK cells are capable of eliminating tumour cells that exhibit a significant quantity of NKG2DLs; yet, in late-stage tumours, the triggered immune system response is minimal because the tumour cells' NKG2DL amounts have dropped. Consequently, approaches that concentrate on improving NKG2D transcription in immune cells, raising NKG2DL expression in tumour cells, and getting rid of soluble NKG2DLs may successfully trigger the immune response against cancer. Enhancing NKG2D transcription in immune cells can enhance the therapeutic benefits of NK-based immunotherapy. Therefore, it is crucial to recognise and comprehend the functions of several different transcription factors that influence the expression of NKG2D on the cell membrane employing its ligands. Apart from that, a deeper comprehension of the regulatory processes underlying stress-induced ligands at the post-transcriptional and post-translational levels may aid researchers in creating innovative chemotherapeutic interventions. Likewise, studies should aim to expand on our knowledge of how NKG2D ligands influence immune cell activity in both health and illness to regulate immune responses. This will pave the way for future applications of the NKG2D ligand axis in cancer treatment.

On top of that, it is challenging to merely interact with a single regulatory receptor to provide a therapeutic impact on natural killer cells due to the complex interactions between positive and negative regulatory receptors. NK cells in the immunosuppressive TME may need more than one activating signal, such as the concomitant engagement of both CD16A and NKG2D, to completely realise their destructive capabilities. When NK cell-activating medications are used in conjunction with other treatments that improve or supplement NK cell activation, such as lenalidomide, cytokines, or checkpoint inhibitors, their full therapeutic potential in the clinic may be realised. When combined with checkpoint inhibitors, the innate and adaptive immune systems can be activated simultaneously, potentially reducing the fatigue of PD-1-expressing T cells and NK cells. The potential of using the NKG2D receptor in therapeutic intervention will be demonstrated over the coming years, but we must continue to advance our understanding of the NKG2D ligand and receptor nexus as each novel discovery will enable us to tailor NKG2D-mediated treatments.

Conflict of Interest

All authors declare no conflict of interest.

Author's contribution

Each of the authors has made major contributions, and all authors acknowledge the article's final draft.

Funding

The authors obtained no financial assistance for this article's research, creation, and publishing.

Ethical approval

Not applicable

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