Review article

Adaptive natural killer cell expression in response to cytomegalovirus infection in blood and solid cancer

Suruthimitra Okpoluaefe [a,c], Ida Shazrina Ismail [a,b], Rafeezul Mohamed [a,b], Norfarazieda Hassan [a,b,c,*]

[a] Department of Biomedical Science, Advanced Medical and Dental Institute, Universiti Sains Malaysia, 13200 Bertam, Kepala Batas, Penang, Malaysia
[b] Breast Cancer Translational Research Program, BCTRP@IPPT, Advanced Medical and Dental Institute, Universiti Sains Malaysia, 13200 Bertam, Kepala Batas, Penang, Malaysia
[c] Emerging Infectious Disease Group, Advanced Medical and Dental Institute, Universiti Sains Malaysia, 3200 Bertam, Kepala Batas, Penang, Malaysia

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ABSTRACT

Natural Killer (NK) cells are conventionally thought to be an indefinite part of innate immunity. However, in a specific subset of NK cells, recent data signify an extension of their "duties" in immune surveillance and response, having characteristics of adaptive immunity, in terms of persistence and cytotoxicity. These cells are known as the adaptive or memory-like NK cells, where human cytomegalovirus (HCMV) infection has been shown to drive the expansion of adaptive NKG2C+ NK cells. HCMV is a ubiquitous pathogen whose prevalence differs worldwide with respect to the socioeconomic status of countries. The adaptive NK cell subpopulation is often characterized by the upregulated expression of NKG2C, CD16, and CD2, and restricted expression of NKG2A, FCεRγ and killer immunoglobulin-like receptors (KIR), although these phenotypes may differ in different disease groups. The reconfiguration of these receptor distributions has been linked to epigenetic factors. Hence, this review attempts to appraise literature reporting markers associated with adaptive or memory-like NK cells post-HCMV infection, in relation to solid cancers and hematological malignancies. Adaptive NK cells, isolated and subjected to *ex vivo* modifications, have the potential to enhance anti-tumor response which can be a promising strategy for adoptive immunotherapy.

1. Introduction

Human cytomegalovirus (HCMV) is a type-5 β-herpesvirus that belongs to the family Herpesviridae. HCMV infection catalyzes the large-scale expansion of immune cells, classically involving CD8+ T cells [1,2], as well as NKG2C+ NK cells; a unique subset of adaptive or 'memory-like' NK cells [3–5]. In healthy people, HCMV infection is manifested sub-clinically, with mild symptoms ranging from fatigue to fever. On the contrary, the immunocompromised cohort is at high risk and tends to exhibit the infection symptomatically, with the infection often leading to end-organ disease or fatality [1,6,7]. For example, Gerna et al. (2012) [8], iterate that in post-solid organ transplantation patients, HCMV infection can be discerned asymptomatically or lead to HCMV disease.

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* Corresponding author.
 *E-mail address:* fara.hassan@usm.my (N. Hassan).

Globally, HCMV burden is believed to range from 60% to 90% [1]. More accurately, a population's seroprevalence is attributed to a country's socioeconomic status [9], where higher incidence is observed in countries with low socioeconomic backgrounds, and vice versa, a potential vindication of the cleanliness hypothesis. For example, the Centers for Disease Control and Prevention (CDC) quotes that approximately 40% of Americans tested positive for HCMV serology by the age of 40 [10], whereas about 100% of individuals from the African continent are believed to be HCMV hosts [11]. In addition to the socioeconomic status hypothesis, the chances for HCMV-positive serology tend to rise as a population age [12].

Sharing similarities with other viruses in Herpesviridae, once HCMV infection is established, the virions are maintained indefinitely during the host's lifespan, alternating between lytic and latent cycles. Quiescence is often established in CD34+ myeloid progenitor cells or primary myeloid cells or CD14+ monocytes [13]. According to Noriega et al. (2012) [14], reactivation is often triggered by immune suppression, inflammation, and stress. HCMV can undertake both lateral and horizontal routes of transmission, where infectious particles can be transferred through the exchange of bodily fluids such as blood [15], urine [16], saliva [17,18], breast milk [19,20], and sex fluids [21,22] (Fig. 1A). Viral particulates can also penetrate the mucosal membrane, thus, enabling placental penetration [20,23] (Fig. 1B).

In recent years, HCMV has shown a growing potential to be grouped as an oncovirus, being exclusively found in more than 90% of cancers, while the surrounding non-cancerous tissues remain HCMV-free [24]. HCMV has been linked to causing breast cancers through the generation of polyploid giant cells by initiating the giant cell cycle [25–27]. In addition, congenital HCMV is known to cause abnormalities in areas of growth, hearing, and neurology in fetuses [28–30]. The incubation period of HCMV is thought to extend up to 8 weeks in adults [31] and up to 21 weeks in congenital infections [32], which therefore, enables an asymptomatic manifestation of the infection in immunocompromised folks.

The virus escapes the immune defense mechanism through processes involving the manipulation of major histocompatibility complexes, MHC-I and MHC-II antigen presentation [14], and host gene regulation through the production of microRNAs [33]. Nauclér et al. (2019) [24] reported that HCMV-encoded lytic proteins, US2-US11, are responsible for the downregulation of HLA class-I and class-II presented peptides to T cells, which in turn negatively affect CD4+ T cell effector functions, antibody production, and CD8+ T cell-associated cytotoxicity towards virus-infected cells. Furthermore, to evade NK cell-induced cell death, UL18, an HCMV-encoded HLA class-I homolog, binds to NKG2A/CD94 on NK cells, instigating their inhibition, alongside upregulating HLA-E to avert the activation of conventional NK cells. Things get interesting when HLA-E, a non-classical type-I MHC binds to leader peptides of class I MHCs, for self-recognition. During HCMV infection however, the upregulated HLA-E is presented bound to an HCMV-encoded peptide, UL40, which stimulates the NKG2C/CD94 ligand on NK cells and leads to the expansion of an NKG2C+ adaptive or memory-like NK cells. This rearrangement of the NK cell receptor repertoire (Table 1) arises due to epigenetics with the expansion of the NK cells expressing NKG2C being its hallmark modulation [3,34,35] (Fig. 2).

NKG2C and NKG2A are respectively the activating, and inhibitory surface receptors found on NK cells. The number of charges generated in any interaction defines the effector function, activation, or inhibition of NK cells. Both receptors bind to HLA-E, which in a virus-free condition, presents self-peptide from class-I MHC in the form of nonamers; composed of 9 amino acids [36], whereas during HCMV infection HLA-E is stabilized by HCMV-derived UL40 [37]. Adaptive NKG2C+ NK cells recognize the UL40 peptide, which in addition to pro-inflammatory signals, regulates the population expansion and differentiation of NKG2C+ NK cells [38] (Fig. 3). The HLA-E/UL40 axis is, thus, important to the control of HCM. In a general sense, NK cells are classified as CD3-/negative CD56+/positive. It is believed that during NK cell maturation, they downregulate CD56 marker expression, changing their phenotype from CD56bright to CD56dimCD16bright or CD56-CD16+. CD56 and CD16 are important mutually exclusive markers, that allow us to have a vague understanding of NK cell behavior, before dwelling into their other associated receptors. CD56 is a neural cell adhesive molecule (NCAM), which belongs to the IgG superfamily. They are responsible for mediating homophilic adhesion. CD16, on the other hand, is a low-affinity receptor for the Fc portion of IgGs. CD16-IgG interactions lead to a display of antibody-dependent cell cytotoxicity (ADCC) by the CD16-activated NK cell. NK cell function, and are therefore, typed based on their distribution of CD56 and CD16. NK cells which are CD56bright tend to have regulatory functions, being renowned for their cytokine secretion, while
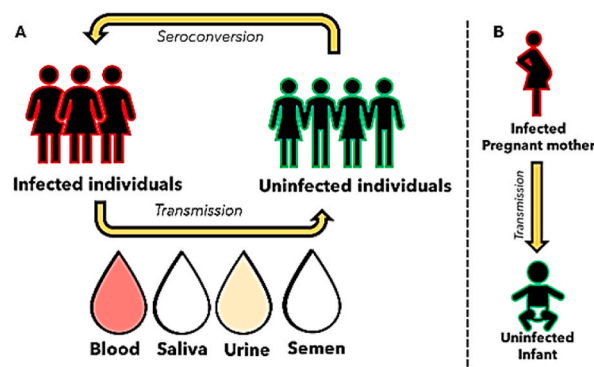

Fig. 1. HCMV is transmitted through the dissemination of bodily fluids such as blood, saliva, urine, and sex fluids. HCMV can undergo horizontal transmission of disease, from infected individuals to non-infected individuals (Fig. 1A), and lateral transmission of disease, from infected mother to uninfected fetus (Fig. 1B).

Table 1

NK cell receptors and their ligands in humans, and relative to HCMV-derived peptides. NK cell receptors, NKG2C, NKp30, NKp44, NKp46 and CD16 are activating receptors, while NK cells receptors NKG2A. KIR2DL1, KIR2DL2/3, KIR3DL1, and KIR3DL2 are responsible for the inhibition of NK cells.

| NK cell surface receptor	Ligands	Ligands relative to HCMV
CD94/NKG2C	HLA-E presenting leader peptides from HLA-A, HLA-B or HLA-C (nonamers)	HLA-E presenting HCMV-derived peptide UL44
NKp30	pp65, B7–H6, viral hemagglutinin, heparan sulfate glycosaminoglycans	pp65
NKp44	Heparan sulfate glycosaminoglycans	Not reported
NKp46	Heparan sulfate glycosaminoglycans	Not reported
CD16	Fc portion of IgG antibodies	Fc portion of IgG antibodies
CD94/NKG2A	HLA-E presenting leader peptides from HLA-A, HLA-B or HLA-C (nonamers)	HLA-E presenting HCMV-derived peptide UL44
KIR2DL1	HLA-C2 (HLA-C with lysine at position 80)	Not reported
KIR2DL2/3	HLA-C1 (HLA-C with asparagine at position 80)	Not reported
KIR3DL1	HLA-Bw4	Not reported
KIR3DL2	HLA-A3, HLA-A11, HLA-A26	Not reported
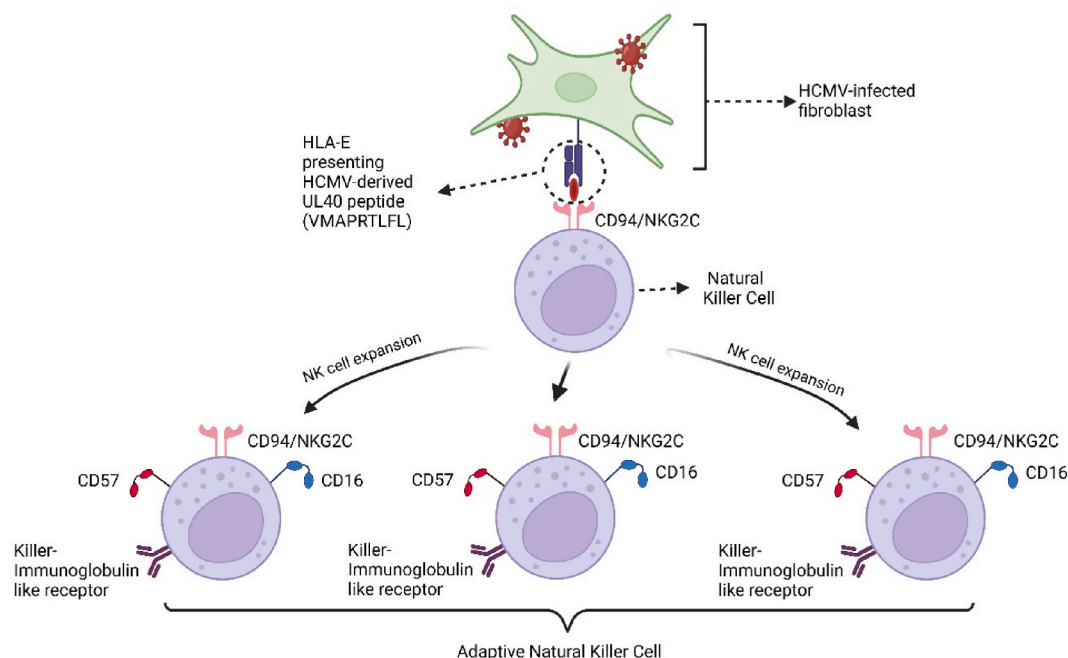
Fig. 2. The illustration depicts the activation and subsequent expansion of the adaptive NK subset after its initial interaction with HLA-E that presents a viral nonamer, UL40, with NKG2C/CD94 coupled receptor on NK cells.

CD16bright/+ NK cells are regarded as cytotoxic NK cells due to their enhanced ability to kill virus-infected or cancerous cells through ADCC (Fig. 4). Among these classes of NK cells, the use of NKG2C+ NK cells for the treatment of primary tumors and metastasis would be advantageous due to their higher level of cell cytotoxicity, long-liveness, memory, and capacity to be transplanted.

Markers such as CD16 and CD57 are associated with NK cell maturation, while loss of NKG2A, FcεRγ, and PLZF mark HCMV-driven NK cell maturation [39,40]. NKG2C/CD94 is found to be significantly higher in CD56dimCD16+ and CD56-CD16+ NK cells in individuals with HCMV-positive serology [41]. NK cells co-expressing NKG2C and CD57 are described to have the ability to 'remember' and respond with memory-like function against HCMV-infected cells [42]. Numerous studies have shown a selective expansion of NKG2C+CD57+ NK cells, and NK subset devoid of FcεRγ in HCMV seropositive individuals. Adaptive or memory-like NK cells have several other additional features such as restricted expression of killer immunoglobulin-like receptors (KIRs), and downregulation of NKG2A and cytotoxic T cell late differentiation marker, CD57 [43].

For NKG2C gene expression to occur on NK cells, having only one allele from either parent is sufficient. Studies show that NKG2C copy number is the highest in the double positive haplotype, NKG2C+/+, and is significantly lower in the single positive haplotype, NKG2C+/−, whereas NKG2C was undetected in people with a double deletion haplotype, NKG2C−/− [44,45]. However, in people with a NKG2C double deletion haplotype, NKG2C−/−, a recent study has shown an expansion of a CD2+ adaptive NK cell subset, displaying similar characteristics as NKG2C+ NK cells, in terms of epigenetic changes, terminal differentiation, and synergistically elevated levels of CD16, ERK, and S6RP [46].

NK cell receptor expression also changes with age. In terms of CD94, NKG2C/CD94, and NKG2A/CD94, studies have reported a correlation between HCMV infection and age [12,41,47]. CD56+NKG2C+ and CD56+CD16+ NKG2C+ NK cells increase with age, whereas CD56+NKG2A+ NK cells decrease with age in HCMV+ people [12,47]. The IFN-γ robustness of the adaptive NK subset is not
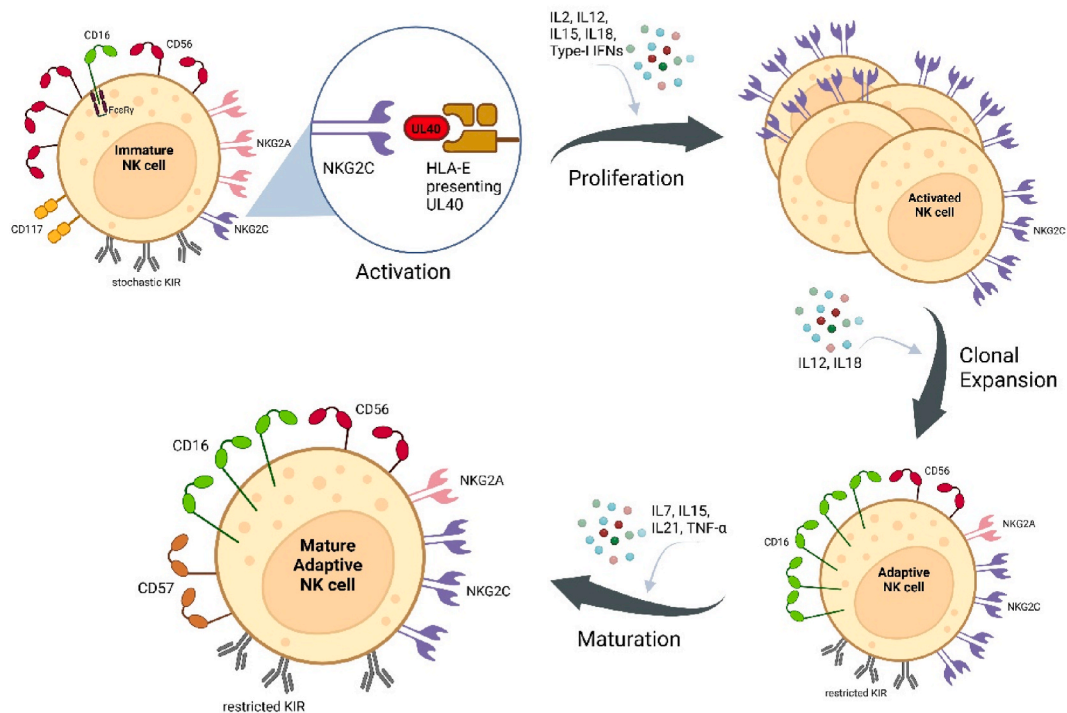
Fig. 3. Activation, proliferation, clonal expansion and maturation of NK cells following HCMV infection. The interaction between NKG2C and HLA-E presenting the HCMV peptide UL40, along with proinflammatory signals by IL-2, IL-12, IL-15, IL-18 and type-I interferons, results in the activation og the immature NK cell, leading to the proliferation of a large subset of NKG2C+ NK cells. These cells then undergo clonal expansion, where CD16bright/+ CD56dim/- NKG2Adim/- NKG2C+ NK cells are selectively expanded. With signals from IL-7, IL-15, IL-21 and TNF-α, the adaptive NK cell matures, presenting CD57.
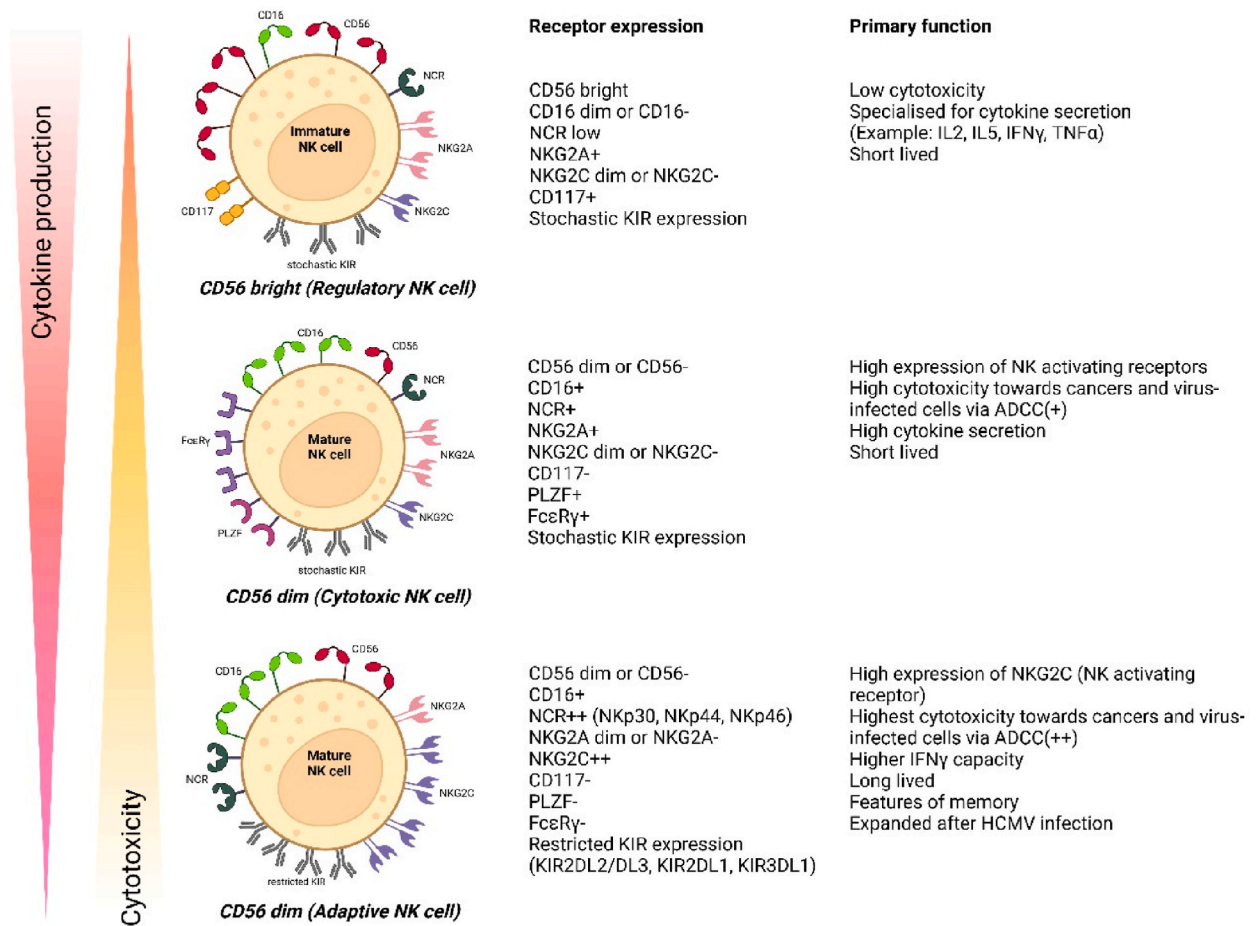
affected by age [47].

As mentioned, the loss of FcεRγ is a common marker for NK cell receptor reconfiguration in response to HCMV infection and is often associated with the expansion of NKG2C+ NK cells. FcεRγ- and NKG2C+ NK cells form the adaptive NK cell subset, although, these two markers can dissociate from one another. In hematopoietic cell transplant patients (n=196), the lack of at least one signaling molecule amongst FcεRγ, SYK and EAT-2 in the CD3- CD56dim NK subset correlated with HCMV serostatus independent of gender and age, with it being detected in 50.4 % of HCMV+ donors and 10.1% of HCMV- donors, with CD3- CD56dim FcεRγ-being the larger subset [34]. Muntasell et al. (2016) [44] found that FcεRγ-preferentially accumulates in NKG2C+ NK cells. They further iterated that the highest proportion of CD3- CD56dim FcεRγ- NK cells expressed high NKG2C and diminished NKG2A. The lack of FcεRγ, SYK and EAT-2 in CD3-CD56dim NK cells correlated with NKG2C expression, whilst deficiency of FcεRγ correlated significantly with lessened levels of NKp30, an immunoglobulin-like activating NK receptor. This reflects the necessity of FcεRγ in NKp30 production at the cellular level [34].

NKG2C-CD56dim express a variegated and stochastic distribution of activating and inhibitory KIRs [40], while the adaptive NKG2C+ CD56dim NK subset has a more restricted range of inhibitory KIRs where KIR expression is limited to one or two types. Single KIR clones are reported to produce more resilient NKG2C+ NK cells than adaptive NK cells with polyclonal KIRs [48]. Of note, in most cases, the adaptive NKG2C+CD56dim NK phenotype expresses self-HLA class-I specific KIRs, involving KIR2DL2/DL3, KIR2DL1, and KIR3DL1, in that order [42,44,48,49]. Therefore, a restricted co-expression of HLA-C-specific KIR describes the expansion of the adaptive NKG2C+ NK cells, with a positive association with KIRDL2/3 and KIR2DL1, while association varies for KIR3DL1 and KIR3DL2.

This review examines recent literature that focuses on the expansion of adaptive or memory-like NK cells in HCMV-infected individuals with blood or solid cancers. Even though HCMV infection evokes an expansion of T and NK cells as aforementioned, we are focusing on the latter due to its lack of stringency and augmented potential anti-tumor activity. We are exploring these adaptive NK cell subsets in diseased individuals to identify discrepancies, which then have the potential to be used for corrective treatment measures.

1.1. The role and effect of human cytomegalovirus in cancer

Recent research has uncovered a connection between viral infections and the development of cancer at different locations. It is widely believed that approximately 10–15% of viral infections are linked to the occurrence of malignancies [50–52]. Comparable to known cancer-causing viruses like Human papillomavirus-16 [53], Human papillomavirus-18 [54], John Cunningham Virus [55], and

Receptor expression

CD56 bright
CD16 dim or CD16-
NCR low
NKG2A+
NKG2C dim or NKG2C-
CD117+
Stochastic KIR expression

Primary function

Low cytotoxicity
Specialised for cytokine secretion
(Example: IL2, IL5, IFNγ, TNFα)
Short lived

CD56 bright (Regulatory NK cell)

CD56 dim or CD56-
CD16+
NCR+
NKG2A+
NKG2C dim or NKG2C-
CD117-
PLZF+
FcεRγ+
Stochastic KIR expression

High expression of NK activating receptors
High cytotoxicity towards cancers and virus-infected cells via ADCC(+)
High cytokine secretion
Short lived

CD56 dim (Cytotoxic NK cell)

CD56 dim or CD56-
CD16+
NCR++ (NKp30, NKp44, NKp46)
NKG2A dim or NKG2A-
NKG2C++
CD117-
PLZF-
FcεRγ-
Restricted KIR expression
(KIR2DL2/DL3, KIR2DL1, KIR3DL1)

High expression of NKG2C (NK activating receptor)
Highest cytotoxicity towards cancers and virus-infected cells via ADCC(++)
Higher IFNγ capacity
Long lived
Features of memory
Expanded after HCMV infection

CD56 dim (Adaptive NK cell)

Fig. 4. NK cell subset classification based on the expression of classical surface receptors CD56, CD16, NKG2C, NKG2A, natural cytotoxicity receptor (NCR), and KIRs. NK cells are divided into regulatory, cytotoxic and adaptive phenotypes, based on the expression of these receptors, and having distinct functional properties.

Epstein-Barr Virus [56], HCMV is also seen to have proto-oncovirus properties in various cancers, such as observed in breast cancer [27,57,58], colorectal cancer [55], and gliomas [59].

Biopsies of triple-negative breast cancer, a highly invasive kind of breast cancer linked to poor prognosis, with HCMV strains B544 and B693 infection, unveiled that these HCMV-infected/transformed cells express a hybrid epithelial-mesenchymal phenotype and had an increase in cancer stemness, a variable that is measured by an increase in stemness markers *SSEA-4, Nanog, Oct4, SOX2, CD44,* and *CD49f* [27]. The hybrid epithelial-mesenchymal phenotype is a biological process in which epithelial cells acquire the properties of mesenchymal cells; increased motility, invasiveness, and resistance to apoptosis, a quality often reflected in aggressive cancers. This change in phenotype was also reported *in vitro* assays using HCMV-infected colorectal cancer cell lines, HT29 and SW480 [60].

In addition to this, high percentages of colorectal polyps' biopsies were also found to exhibit HCMV-related pro-cancer proteins, IE1-72 (82%), and pp65 (78%), whilst absent in surrounding non-neoplastic tissue [61]. Several other reports further confirm this claim [62] finding HCMV DNA, immediate early and pp65 proteins in borderline ovarian tumors (BOT) [63], high-grade serous ovarian adenosarcomas [64], and alveolar soft part sarcomas [65], with Rahbar et al. (2021) [63] going further to show that in BOTs, HCMV infection correlated with 5-lipoxygenase expression, an enzyme that activates antiapoptotic pathways. The authors also showed that HCMV prevalence and IgG titer were significantly elevated in patients with BOTs (83.3%) as compared to the control group consisting of age-matched women (65.6%). Harkins et al. (2002) [61] further iterate that *in vitro* infection of HCMV in, Caco-2, a colorectal cancer cell line, resulted in the induction of Bcl-2 and COX-2 proteins, with both markers linked to the progression of colorectal cancer. These proteins are responsible for cellular functions such as cellular proliferation, angiogenesis, and metastasis, whilst some suppress the ability of the immune system to respond locally, all being the trademarks of the big C ("cancer").

Similarly, Cobbs et al. (2002) [59] iterate that 100% of malignant glioma biopsies (WHO grades II to IV) were HCMV positive, with multiple HCMV proteins, notably IE1-72 found in all samples, with varying frequencies of pp65 tegument protein, M_r 76,000 early protein and M_r 52,000 delayed early protein, being expressed in them. A meta-analysis by Farias et al. (2019) [66] which consisted of data from 1871 glioma patients and 319 non-glioma patients revealed the association of pp65 and gB nucleic acids, with *IE1-72* with

the strongest association with glioma incidence, which is supported by Xing et al., 2016 [67] who linked HCMV infection to glioma disease progression through the overexpression of the angiogenesis marker, endocan.

1.2. Hematological malignancies and hematopoietic stem cell transplantation

Shafer et al. (2013) [68] found that in patients with follicular lymphoma (FL), a non-Hodgkin's lymphoma, low levels of NK cells in venous blood was the single determining factor that was associated with poorer prognosis. The study showed that even with similar treatment regimens, 24% of FL patients with low NK cell levels favored mortality, whereas only 2% of patients with normal or high NK cell levels passed away because of their illness [68]. This is supported by the findings of a clinical trial using anti-CD20 monoclonal antibodies, Obinutuzumab and Rituximab, for the treatment of FL and Diffuse large B-cell lymphoma (DLBCL), which reported that low peripheral NK cell counts lead to shorter progression-free survival in FL and DLBCL, and shorter overall survival in FL [69].

Another study went on to show that NK cell activating receptors decreased in cases of acute myeloid leukemia relapses [70], enabling disease progression as the neoplasm can escape NK cell surveillance. Hofland et al. (2019) and Huergo-Zapico et al. (2019) [71,72], on the other hand, reported hyporesponsiveness in the overall NK cell population and linked it to the downregulation of NKG2D, but found that NK cell functionality is retained in chronic lymphoblastic leukemia (CLL), provided they receive adequate activating signals via their activating receptors, unlike the T cell compartment. This shows that an impaired NK cell function could lead to cancer progression and a poorer prognosis.

When looking more specifically at adaptive NK cell marker expression, in chronic lymphocytic leukemia (CLL) patients with HCMV-positive serology, several studies associate elevated NKG2C levels [72,73], with lowered frequencies of NKp30 on CD56dim CD16+ and CD56- CD16+ without respect to CLL, while CD57 expression is not associated with HCMV serology [72]. Hofland et al. (2019) [72] further iterate that the memory NK cell phenotype that arises in the HCMV+ CLL patients displays a NKG2C+ NKG2A- KIR+ p75-phenotype. Intriguingly, contrary to these studies Puiggros et al. (2021) [74] found that independent of HCMV serology and NKG2C zygosity, CLL and CLL-like monoclonal B-cell lymphocytosis patients exhibited reduced levels of NKG2C+ NK cells than healthy people. Reduced expansion of CD94/NKG2C NK cells in chronic lymphocytic leukemia and CLL-like monoclonal B-cell lymphocytosis was not related to increased human cytomegalovirus seronegativity or NKG2C deletions.

In advanced acute leukemia patients (n=60) who were receiving myeloablative treatment and haploidentical transplantation from HCMV+ donors, adaptive NK cells that were defined by robust IFN-γ responsiveness, and overexpression of NKG2D, KIR and CD57 with stagnant PD-1 levels, were elevated in patients who showed no progression of disease in the first 90 days following transplantation, in addition to remaining heightened 3 years post-transplant, irrespective of HCMV reactivation incidence [75]. Interestingly enough, they also found that recipients who received grafts from donors with higher adaptive NK cell levels had better chances of disease-free progression [75,76], as also reported in autologous HSCT patients with multiple myeloma [77].

In HSCT patients, the adaptive CD56dimCD57+NKG2C+ NK cell phenotype expanded in specific response to HCMV reactivation, which led to lowered leukemic relapse (26%), and heightened disease-free survival (55%) in patients who underwent reduced intensity myeloablative treatment regimens, independent of age and graft rejection [76]. In this group, Schlums et al. (2015) [34] further iterate significant frequencies of CD3- CD56dim NK cells lacked the signaling proteins, EAT-2, SYK, and FCεRγ in a mutually exclusive manner, from 0.5 to 1 year following transplantation. Similarly, in another study involving HCMV reactivation in patients who received HCMV naïve grafts, the expansion of distinct populations of CD57+NKG2C+, CD57+ FCεRγ-, and CD57+ EAT-2- NK cells were reported in CD3-CD56dim NK cell populations [78].

In a study examining graft sources, umbilical cord blood, and allogeneic siblings, for patients undergoing HSCT, a significant increase in CD57+NKG2C+ NK cell subset expansion was observed post-transplant in individuals with reactivation and those previously sensitized to HCMV [42], while NK cells expressing NKG2A decreased concurrently in this cohort. In support, Rashidi et al. (2019) [79] described a KIR+NKG2A-adaptive NK cell phenotype in HSCT recipients, with the highest levels of these cells being present in patients with HCMV reactivation, followed by HCMV+ serology without reactivation, and HCMV- serology without reactivation, in disease groups acute myeloid leukemia, acute lymphoblastic leukemia, chronic myeloid leukemia, myelodysplastic syndrome, chronic myelomonocytic leukemia, and myelofibrosis. They postulate that these phenotypes of cells express NKG2C since NKG2A and NKG2C are mutually exclusive. Regarding the distribution of KIR types, individuals with HCMV seropositivity exhibited a preference for KIR2DL2/3, and KIR2DL1 [42]. These expanded NKG2C+ NK cells exhibit enhanced degranulation [80], while CD57+NKG2C+ NK cells are declared to confer protection against leukemia relapse through the control of neoplasm reactivation, independent of age and graft versus host disease [76].

Berrien-Elliot et al. (2022) [70] were able to induce a memory-like NK cell population using cues from IL-12, IL-15, and IL-18. They describe this memory-like NK cell population expressing CD56, NKG2A, NKp30, NKp44, NKp46, and DNAM-1, as being distinct from conventional NK cells, able to self-replicate, persisting for months in an immune-compatible setting, and have enhanced cytotoxicity. These cytokine-induced memory-like NK cells share similarities with the adaptive NK cell subset that expands post-HCMV infection [76], where they too were able to degranulate myeloid leukemia cells.

1.3. Solid organ cancers

At the time of this review, limited studies are reporting adaptive NK cell expansion or the associated markers in HCMV-infected individuals with solid organ cancers. This may be partly because NK cells exhibit limited tumor infiltration. However, our literature search showed that in some solid organ cancers, the adaptive or memory NK cell subpopulation showed tissue residency traits and their associated markers [81,82].

Bordignon et al. (2023) [83] iterated that 72% of HCMV+ human epidermal growth factor receptor-2 positive (HER2+) breast cancer patients had an adaptive NK cell population that is characterized by NKG2C+ FCεRγ-. Interestingly, they further exclaimed that although this subset of cells produced more IFN-γ than NKG2C- FCεRγ+ NK cells, IFN-γ production was only enhanced in NK cells that expressed NKG2C+ and FCεRγ-in chorus, while the presence of a single NKG2C marker was sufficient to elicit higher functionally in healthy individuals.

Table 2
Clinical trials utilizing NK-cell-based therapies for treatment of advanced cancers.

Clinical Trial	Phase	Cancer type	NK-Based Therapy	Description	Method	Outcome
UMIN UMIN000007527 [87]	I	Advanced digestive cancer	Autologous NK cells	Development of a novel technique capable of expanding large quantities of highly activated clinical-grade NK cells, and the determination of their safety during mono-treatment.	Patients (refractory to standard treatment) were intravenously given autologous NK cells 3 times per week in a dose-escalating manner.	NK cells expanded approximately 4720 times and were highly lytic *in vitro* with strong expression of NKG2D and CD16. Therapy was well tolerated with no severe adverse reaction.
NCT03056339 [88]	I and II	CD19-positive Lymphoid Tumors (relapsed or refractory non-Hodgkin's lymphoma or chronic lymphocytic leukemia)	CAR-Transduced NK cells	Development of CAR-NK cell-based therapy to counteract substantial toxic effects of CAR-T cell therapy that has shown incredible clinical efficacy in treating B-cell cancers.	HLA-mismatched anti-CD19 CAR-NK cells (derived from cord blood) were administered to patients at 1×10^5, 1×10^6 or 1×10^7 per kilogram of weight after lymphodepleting chemotherapy.	Patients responded quickly to treatment, with the infused CAR-NK expanded and maintained at low levels for a minimum of 12 months. The trial ended with most patients having responded to treatment without the development of major toxic effects.
KCT0003973 [89]	I	Advanced Hepatocellular Carcinoma (HCC)	Autologous NK cells + hepatic arterial infusion chemotherapy (5-fluorouracil and cisplatin)	Determination of the feasibility and safety of NK cell therapy in HCC, along with the evaluation of the synergistic effect of locoregional high-dose NK cell therapy in combination with hepatic arterial infusion chemotherapy	Patients (refractory to standard treatment) were given activated NK cells through hepatic arterial infusion (5-fluorouracil and cisplatin) for 5 days in a row in a dose-escalating manner.	Therapy was well tolerated with no adverse reaction. The trial concluded with a 63.6% objective response rate, and 81.8% disease control rate. The medians for progression-free survival and overall survival were 10.3 and 41.6 months, respectively.
NCT03958097 [90]	Pilot study	Advanced non-small lung cancer (type IIIB/IIIC and IV)	Autologous NK cells + Sintilimab	Evaluation of the safety and efficacy of a combination therapy comprising autologous NK cells and PD-1 antibodies.	Patients (refractory to first-line platinum-based therapy) were given sintilimab and constant NK cell quantities once every 3 weeks.	Therapy was tolerated with no unexpected adverse reaction. The trial concluded with an objective response rate of 45 %, and an overall survival at 17.1 months. The 6-month and 12-month overall survival was 95% and 80%, respectively.
NCT03068819 [91]	I	Acute myeloid leukemia (AML)	Donor Memory-like NK cells (ML-NK cells) + fludarabine, cytarabine and filgrastim	Development of a novel immunotherapy platform for the treatment of AML that has relapsed after allogeneic hematopoietic cell transplantation (HCT), and to the evaluation of donor ML NK cells robustness, persistence and antileukemic potency in the absence of exogenous cytokines.	Pediatric/young adult patients with post-HCT relapsed AML were administered fludarabine, cytarabine and filgrastim. Two weeks later they were administered donor lymphocytes and ML NK cells from their original HCT donor via infusion.	No significant toxicity was recorded during the trial period. The NK cells expanded and showed persistence for more than 3 months with leukemia-triggered IFN-γ production. The trial ended with 50% of patients achieving complete remission, where 25% maintained a durable remission for more than 3 months, while 12.5% maintained remission for more than 2 years.

Similarly, in people with glioblastoma multiforme (GBM), NKG2C+ NK cells are present in higher frequencies in the HCMV infected than in healthy cohorts [84,85]. Dominguez-Valentin et al. (2016) [85] further iterated that in GBM patients, HCMV was the sole contributor to the rearrangement of NK cell receptors to express high levels of CD16, NKG2D, NKG2C, and KIR2DS4 (*KIR2DS4*00101*), with KIR2DS4 (*KIR2DS4*00101*) being exclusively found in HCMV+ patients, extending their survival and conferring reduced risk in healthy people in developing GBM. They also found that the detection of KIR2DS4 indicates a naturally milder form of the disease. Liu et al. (2018) [86], on the other hand, affirmed that although patients with GBM had a suppressed humoral response to HCMV, treatment-responsive patients with relatively high anti-HCMV IgG had improved overall survival. Murad et al. (2022) [84] concluded their study by iterating that the expanded NKG2C+ NK cells due to stimuli from feeder cells, eliminate primary glioblastomas, and therefore, have potential therapeutic value for treating GBMs. Several NK cell-based clinical trials with positive outcomes have already been carried out to treat various cancers (Table 2).

In contrast, in suspected lung cancer patients, Brownlie et al. (2021) [81] described a distinct tissue-resident memory NK cell (trmNK) in the lung and blood, with the markers CD94a+ NKG2C+ inhibitory self-KIR+ in CD56bright CD16- NK cells, that are phenotypically distinct from the NKG2C+ self-HLA class I KIR+ CD56dim CD16+ adaptive NK cells often described in peripheral blood. They further iterated that although the expansion of the adaptive peripheral NK cells was restricted to HCMV+ patients, the trmNK cells were detected in a large proportion of HCMV+ patients, indicating that HCMV may not be the sole determining factor of their expansion. These trmNK cells showed hyperresponsiveness to target K562 cells in terms of TNF and CD107a degranulation.

A similar trmNK profile was reported in intrahepatic NK cells, where Marquardt et al. (2015) [82] described an intrahepatic CD49a+ trmNK cell phenotype in the cancer-free biopsies of patients with primary or metastatic liver cancer as CD56brightCD16-NKG2C+, expressing high levels of NKp46, NKp30, NKG2D and DNAM-1. These trmNK cells exhibit low levels of perforin, Granzyme A, and high levels of Granzyme B compared to liver-resident conventional NK cells. On the contrary, Rennert et al. (2021) [92] reported an adaptive NK cell phenotype that is similar to peripheral blood adaptive NK cells, in hepatocellular carcinoma. These cells expand in HCMV+ HBV-associated hepatocellular carcinoma patients and exhibit low expression of the tissue-resident markers, CD49a, CXCR6, and CD69, unlike their CD56bright counterparts found in hepatocellular carcinoma lesions. However, they found that these CD56dim FCεRγ-adaptive NK cells have a limited anti-tumoral response, in terms of CD107a degranulation, reduced IL-12 and IL-18 responsiveness via macrophage inflammatory protein-1 (MIP-1) and IFN-γ, and CD8+ T cell immunoregulation.

1.4. Role of human cytomegalovirus in solid organ transplantation

In lung transplant recipients (LTRs), Calabrese et al. (2019) [93] reported a dual phenotypic expansion of NKG2C+ NK cells in peripheral blood and bronchoalveolar lavage respectively, with the former exhibiting a relatively more mature CD16+ NKG2A- KIR+ Ki67bright phenotype, while the latter, exhibiting a more immature CD16-NKG2A+ KIR+ Ki67dim phenotype. Ki67 is a marker that denotes proliferative capacity. The authors noted the elevation of NKG2C+ NK cells were in response to HCMV and reported that the presence of NKG2C+ NK cells in bronchoalveolar lavage was indicative of viremia or HCMV reactivation, where patients had an increased possibility of chronic lung allograft dysfunction (CLAD), and mortality [93]. Vietzen et al. (2021) [94] went on to show that HCMV UL40 variants had a role to play, with the VMTPRTLIL variant being substantially overrepresented in individuals developing CLAD, compared to the VMAPRTLIL, VMAPRTLVL, and VMAPRTLLL UL40 variants, which were present significantly in non-CLAD patients.

Adding to the work of Calabrese et al. (2019) [93], they reported that the VMTPRTLIL variant increased the proliferation of NKG2C+ NK cells the most [94]. On the contrary, Vietzen et al. (2023) [95], disclosed that UL40 variants VMAPRTLIL and VMTPRTLVL, were substantially overexpressed in LTRs with donor-specific antibodies (DSA) and antibody-mediated rejection (ABMR), where they found that both variants were associated with the enhanced proliferation of proinflammatory and cytotoxic CD16+NKG2C+ NK cells. Interestingly, patients with NKG2C WT/WT genotypes were reported to be protected from HCMV reactivation compared to LTR patients with NKG2C WT/DEL genotypes [96].

In kidney transplant recipients, the expansion of NKG2C+ adaptive NK cell subset is recorded to have a protective role against HCMV reactivation, whereas, in HCMV disease groups, significantly lower levels of NKG2C+ NK cells with associated adaptive markers CD57+, ILT2+, or FCERΓ-, NKG2C+CD57- and NKG2C+ILT2- were observed [97,98]. In contrast, Redondo-Panchón et al. (2017) [43] reported high NKG2C+ NK cells in KTR patients with HCMV viremia. In liver transplant patients, Forrest et al. (2023) [99] demonstrated that HCMV+ donor livers with CD2+NKG2C+ adaptive liver resident NK cells were able to prevent post-transplant HCMV viremia. They also demonstrated increasing frequencies of peripheral blood adaptive NK cells defined by CD2, with lowered levels of Siglec-7, NKG2A, and FCεRγ.

2. The role of HCMV on other viral infections

2.1. SARS-CoV-2

At the time of this review, the role of HCMV in SARS-CoV-2 infection remains underexplored. Albeit Jaiswal et al. (2022) [100] reported that Coronavirus disease-2019 (COVID-19) patients with HCMV viremia or reactivation showed a lower prognosis, where survival in patients with viremia was 25%, compared to 80% survival in those without reactivation. They further showed that although the NKG2C+ NK cells were significantly reduced in survivors and the deceased at day 15 post-infection, at day 30, their levels were elevated significantly in survivors while remaining at the lower threshold in the dead. They also noted a trend that patients with lower

adaptive NK cell levels had a higher possibility for HCMV reactivation (p=0.07).

2.2. Hepatitis infection

Among the HCMV+ cohort with hepatitis B virus (HBV) and Hepatitis C virus (HCV) infection, a significant increase in CD56dim NKG2C+ NK cells were observed in the majority of the HCMV+ individuals [49]. In unison, Malone et al. (2017) [101] reported this adaptive NK cell subpopulation is strongly linked to HCMV infection in HCMV+ individuals with concurrent chronic HBV, HCV, or Hepatitis Delta Virus. The expanded NKG2C+ NK cells had polyfunctional responses through antibody-dependent cell cytotoxicity and infected cells but showed poor response to IL-12 and IL-18 stimulation [3,34,80,101]. In HCMV+ HCV+ individuals, KIR2DL1 and KIR3DL1 were substantially lowered, whereas KIR2DL3 remained constant [101]. The CD56dim NKG2C+ NK cell subset was found to exhibit significantly lessened levels of NKG2A, CD161, Siglec-9, and NKp30, while ILT2, CD57, and CD2 were commonly found [49].

2.3. Human immunodeficiency virus infection

NKG2C+ NK cells increase proportionally with HCMV co-infection in HIV+ individuals [45,102–104], with Heath et al. (2016) [103] stipulating that NKG2C expression being highest in HCMV+HIV+ individuals, followed by HCMV+HIV-, HCMV-HIV+ and HCMV-HIV- groups. This shows that despite HIV being able to cause the expansion of adaptive NK cell repertoires, HCMV infection grants a more robust expansion. CD57 and KIR expression were reported to be unaltered between HIV+ and HIV- controls [102]. They further reveal that their HIV+ group presented NK cells with elevated levels of CD85j+KIR+ and NKG2A+, while NK cells in their HIV- control group exhibited NK cells with lowered levels of KIR+NKG2A+. Interestingly, even in HIV+ individuals with NKG2C DEL/DEL genotype, HCMV infection drives the expansion of an adaptive subset of NK cells often portrayed by the expression of CD2 or co-expression of CD2 and CD16 [39]. Thomas et al. (2012) [105] however, found that NKG2C deletion is a risk factor in HIV infection.

2.4. Adaptive NK cells potential in cancer immunotherapy

This review focuses on the adaptive NK cell marker expression in blood and solid cancers in people who tested positive for HCMV by serological assays. The NKG2C+ NK subset expands as a response to HCMV infection to limit viral replication [37]. Although HCMV is showing growing potential to be classified as an oncovirus [25–27], the expansion of immune cells, particularly the NKG2C+ NK cell subset, proves to be an asset in cancer prognosis and treatment. Not only is the expanded subset highly cytotoxic, but they are also persistent with the expanded repertoire often existing for up to a few years without exhaustion. Albeit being strongly associated with the progression of malignancies as discussed in the previous section, studies show that HCMV infection also causes the immunomodulation of cytotoxic T cells and NK cells towards higher effector functions that are directed with NKG2C specificity [106,107].

Liu et al. (2017) [108] iterate that polyclonal NK cells cultured with HLA-E expressing feeder cells, the corresponding ligand for NKG2C, led to the expansion of an adaptive subset of NK cells which had heightened alloreactivity against HLA class I mismatches. Understanding this relationship and harnessing important features of these transformed immune cells might be useful in designing therapies that work alone or hand-in-hand with conventional cancer treatment such as surgery, chemotherapy, and radiotherapy. The adaptive NK cell subset can be generally described in terms of the expression of NKG2C or CD2, loss of NKG2A and FCεRγ surface receptors, persistence, and long-liveness.

These adaptive NK cells are highly responsive to target cell stimuli following subsequent HCMV exposure and linked to improved prognosis in diseases like follicular lymphoma, diffuse large B cell lymphoma [69], acute myeloid lymphoma [70], autologous HSCT with multiple myeloma [77] and grafts from donors with high adaptive NK cell levels [75,76]. According to Diermayr et al. (2008) [109], NK cells expressing a single type of KIR are highly cytotoxic against HLA type-I mismatches in acute myeloid leukemia (AML) blasts and are effective killing agents against AML [108]. However, our review does find that the expansion of the adaptive NK cell subset welcomes unfavorable outcomes in post-lung transplant individuals [93,94]. It is our opinion that more research needs to be done to study the adaptive NK cell subset that arises in tissues. This data may enable us to widen the range of diseases/conditions that can be potentially treated with adaptive NK cell-based therapies. The effect of adaptive NK cells may vary based on the specific characteristics of HCMV infection, the type/subtype of diseases such as cancer, an individual's immune status, and epigenetic factors. What may be useful in one circumstance may be harmful in another, thereby generalizing the effect of adaptive NK cells not possible.

Contrary to peripheral adaptive NK cells, trmNK cells that are characterized by the expression of CD49a+, a marker that shows organ infiltration, are phenotypically different and are believed to arise from different cues [81]. Despite their hyperresponsiveness via CD107a degranulation and TNF-α secretion in response to target cells, this population of trmNK cells were also found in a portion of HCMV seronegative patients, suggesting the potential role of other virus infections alongside HCMV for their expansion. due to their tissue-specific nature and heightened responsiveness, trmNK cells found in the human lung could serve as a viable option for treatment regimens addressing solid tumors [81]. *In vivo* studies should be done to investigate if HCMV alone can initiate the expansion of these cells, as current data is insufficient to conclude in that regard.

It is pertinent to note here, that the field of NK cell biology and the interaction between NK and HCMV cells in the cancer context is complex and evolving. Literature that researched or enhanced the role of NK cells in this regard is few and requires more facts and exploration. Continuous research is needed to fully understand the nuances of these interactions and their effects on health and disease. As aforementioned, individual variations in immune responses can contribute to different outcomes in different people, and disease groups. The chronic activation of NK cells due to persistent HCMV infection/reactivation may lead to unprecedented levels of inflammation or warrant an immunosuppressive effect causing a depletion of immune cells, which will be favorable for cancer evasion.

The response of adaptive NK cells in such situations may be associated with inflammatory processes, with prolonged inflammation harmful and contributing to tissue damage and other health issues.

3. Conclusion

The purpose of this review was to study recent literature that reported on the expansion of adaptive or memory NK cells in blood and solid cancer patients due to CMV infection in humans for a potential adoptive or cancer immunotherapy. The expressions of KIR repertoires, NKG2C, and FCεRγ were also studied due to their interrelation with peripheral and tissue-resident NK cells. All the chosen articles have exhibited evidence of the expansion of NK cells in individuals with HCMV and provided sufficient affirmation that NKG2C is indeed one of the markers of HCMV infection, regardless of the site of NK cell isolation. However, the expansion of tissue-resident NKG2C+ NK cells in response to HCMV infection is understudied, requiring additional research to be done.

Data availability statement

All data supporting the findings of this study are derived from sources which are cited appropriately in the manuscript. The datasets and resources referenced are available through respective publications, databases, or repositories. Specific data can be accessed by referring to the cited articles in the reference list. No new data were created or analyzed in this study.

CRediT authorship contribution statement

Suruthimitra Okpoluaefe: Writing – original draft. **Ida Shazrina Ismail:** Writing – review & editing. **Rafeezul Mohamed:** Writing – review & editing. **Norfarazieda Hassan:** Writing – review & editing, Visualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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