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FOCUSSED RESEARCH REVIEW

Current progress in NK cell biology and NK cell-based cancer immunotherapy

Raquel Tarazona[1] · Nelson Lopez-Sejas[1] · Beatriz Guerrero[1] · Fakhri Hassouneh[1] · Isabel Valhondo[1] · Alejandra Pera[2,3] · Beatriz Sanchez-Correa[1] · Nieves Pastor[5] · Esther Duran[5] · Corona Alonso[3,4,6] · Rafael Solana[2,3,4,6]

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Abstract

A better understanding of the complex interactions between the immune system and tumour cells from different origins has opened the possibility to design novel procedures of antitumoral immunotherapy. One of these novel approaches is based on the use of autologous or allogeneic natural killer (NK) cells to treat cancer. In the last decade, different strategies to activate NK cells and their use in adoptive NK cell-based therapy have been established. Although NK cells are often considered as a uniform cell population, several phenotypic and functionally distinct NK cells subsets exist in healthy individuals, that are differentially affected by ageing or by apparently innocuous viruses such as cytomegalovirus (CMV). In addition, further alterations in the expression of activating and inhibitory receptors are found in NK cells from cancer patients, likely because of their interaction with tumour cells. Thus, NK cells represent a promising strategy for adoptive immunotherapy of cancer already tested in phase 1/2 clinical trials. However, the existence of NK cell subpopulations expressing different patterns of activating and inhibitory receptors and different functional capacities, that can be found to be altered not only in cancer patients but also in healthy individuals stratified by age or CMV infection, makes necessary a personalized definition of the procedures used in the selection, expansion, and activation of the relevant NK cell subsets to be successfully used in NK cell-based immunotherapy.

Keywords Cancer · Immunosenescence · NK cell-based immunotherapy · Ageing · NK cells · PIVAC 19

Abbreviations

AML	Acute myeloid leukaemia
ADCC	Antibody-dependent cell cytotoxicity
Anti-TAA	Anti-tumour-associated antigens
APCs	Antigen-presenting cells
CAR	Chimeric antigen receptor
CMV	Cytomegalovirus
EGFR	Epidermal growth factor receptor
GMP	Good manufacturing practice
GvHD	Graft-versus-host disease
HCMV	Human cytomegalovirus
HLA	Human leukocyte antigen
HSCT	Hematopoietic stem cell transplantation
KIR	Killer cell immunoglobulin-like receptors
LAG-3	Lymphocyte-activating gene 3
MHC	Major histocompatibility complex
MCMV	Murine cytomegalovirus
MM	Multiple myeloma
NCRs	Natural cytotoxicity receptors
NHL	Non-Hodgkin lymphoma
NK	Natural killer
PD-1	Programmed death-1
TAA	Tumour-associated antigen
TIGIT	T cell immunoreceptor with Ig and ITIM domains
TIM-3	T cell immunoglobulin and mucin domain 3

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✉ Raquel Tarazona
rtarazon@unex.es

✉ Corona Alonso
corona_alonso@hotmail.com

✉ Rafael Solana
rsolana@uco.es

Extended author information available on the last page of the article

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Introduction

Since the discovery of murine and human natural cytotoxicity and natural killer (NK) cells in 1975, evidence supports that they play critical roles in the early control of viral infection and tumour immunosurveillance. In humans, low NK cytotoxicity correlates with increased risk for cancer as it was shown in an 11-year follow-up study [1]. The contribution of NK cells to cancer immunosurveillance is further supported by their role in hematopoietic stem cell transplantation (HSCT) inducing graft-vs-leukaemia effect. NK cells are the first lymphocytes to recover after HSCT [2], and besides their potent effect against leukaemic blasts, they exert a protective role against bacterial and viral infections. NK cells are the first lymphocyte population to reconstitute after HSCT. NK cell numbers usually reconstitute within the first 30 days, and they do not reach full functional competency until 6 months or more, depending on graft composition, immunosuppression, graft-versus-host disease (GvHD), or virus infections (in particular, CMV reactivation) [3, 4]. Early and robust NK cell recovery, over 150 cells/µl on day 30 post-HSCT, was associated with improved overall survival and less mortality related to transplantation, whereas low NK cell counts are associated with increased risk of human cytomegalovirus (HCMV) reactivation [5]. Selection of donor–recipient mismatches for killer cell immunoglobulin-like receptors (KIR) expressed by donor NK cells, and their ligands, human leukocyte antigens (HLA)-class I molecules, on the recipient, provide allogeneic anti-leukaemia effects that have been proven to be beneficial. Thus, an increase in survival and protection from relapse in acute myeloid leukaemia (AML) patients lacking HLA class I ligands for donor inhibitory KIR has been described in the course of allogeneic HSCT [6, 7].

Ageing is associated with an increased incidence of cancer including haematology malignancies. Indeed, age is the major factor that influences the health status and the immunosuppressed state of patients with cancer and has an impact on the selection of therapeutic procedures [8–10]. The use of older donors has increased consequently to the extension of allogeneic HSCT to older patients. Donor age ≥ 60 years has a significant negative impact on overall survival in patients receiving allografts for haematologic malignancies [11]. However, advanced donor age does not increase the risk of delayed engraftment or major long-term adverse effects [12].

In clinical trials, adoptive transfer of autologous NK cells to treat cancer has shown little benefits despite its lack of side effects [13]. Adoptive transfer of allogeneic NK cells has been demonstrated to be safe to treat solid and haematologic malignancies, and its use is being analysed in several clinical trials [14]. Allogeneic NK cell adoptive immunotherapy benefits from the short persistence of NK cells compared to T cells and their alloreactivity based on KIR-HLA mismatch [15]. In the last decade, different strategies to expand NK cells with high capacity to lyse tumour cells have been used. Harnessing NK cell activation represents a valuable strategy to be considered in cancer immunotherapy.

NK cell biology

Human NK cells are innate lymphoid cells characterized by the expression of CD56 and/or CD16. In peripheral blood, different subsets can be distinguished according to the expression of these markers (Fig. 1). $CD56^{bright}$ $CD16^{-}$ are considered immature cells that produce high levels of cytokines, whereas $CD56^{dim}$ $CD16^{+}$ subset represents mature NK cells with a high cytotoxic capacity, and $CD56^{-}CD16^{+}$ are considered a minor subset of dysfunctional NK cells expanded in several clinical conditions [16, 17]. The analysis of NK cells by mass cytometry using 28 markers [18] has shown an unexpectedly high degree of heterogeneity of NK cells and has helped to define two major separate clusters: a less mature cluster characterised by the expression of CD94 and NKG2A and a mature cluster defined by the expression of CD16 and CD57 markers. The development relationship between peripheral blood NK subsets is not fully established with evidence supporting either a linear model or a branched (nonlinear) model of NK cell differentiation (for review [19]).

Recognition and killing of target cells depend on a tune balance between NK inhibitory and activating receptors expressed on their surface that interact with their ligands on stressed cells (e.g. viral infected or malignant transformation) [20–22]. Among NK cell receptors, human NK cell function is principally regulated by KIR and NKG2A inhibitory receptors that interact with self HLA class I molecules. These receptors represent the major checkpoints of NK cell activation, although other non-HLA class I-specific inhibitory receptors have been identified on NK cells that also impact the final balance regulating NK cell cytotoxicity [23, 24]. These receptors include the T cell immunoglobulin and ITIM domain (TIGIT), PVR-related Ig domain (PVRIG, also termed as CD112R), lymphocyte-3 activation gene (Lag-3), T cell immunoglobulin and mucin domain containing-3 (Tim-3), Programmed death-1 (PD-1) and probably TACTILE (CD96). Activated CD4 and CD8 T cells and a subset of NK cells express Lag-3. In CD4 T cells, Lag-3 binds to Major Histocompatibility Complex (MHC) class II molecules on antigen-presenting cells (APCs), whereas in NK and CD8 T cells, Lag-3 binds to L-SECtin expressed in tumour cells. Tim-3 is expressed on

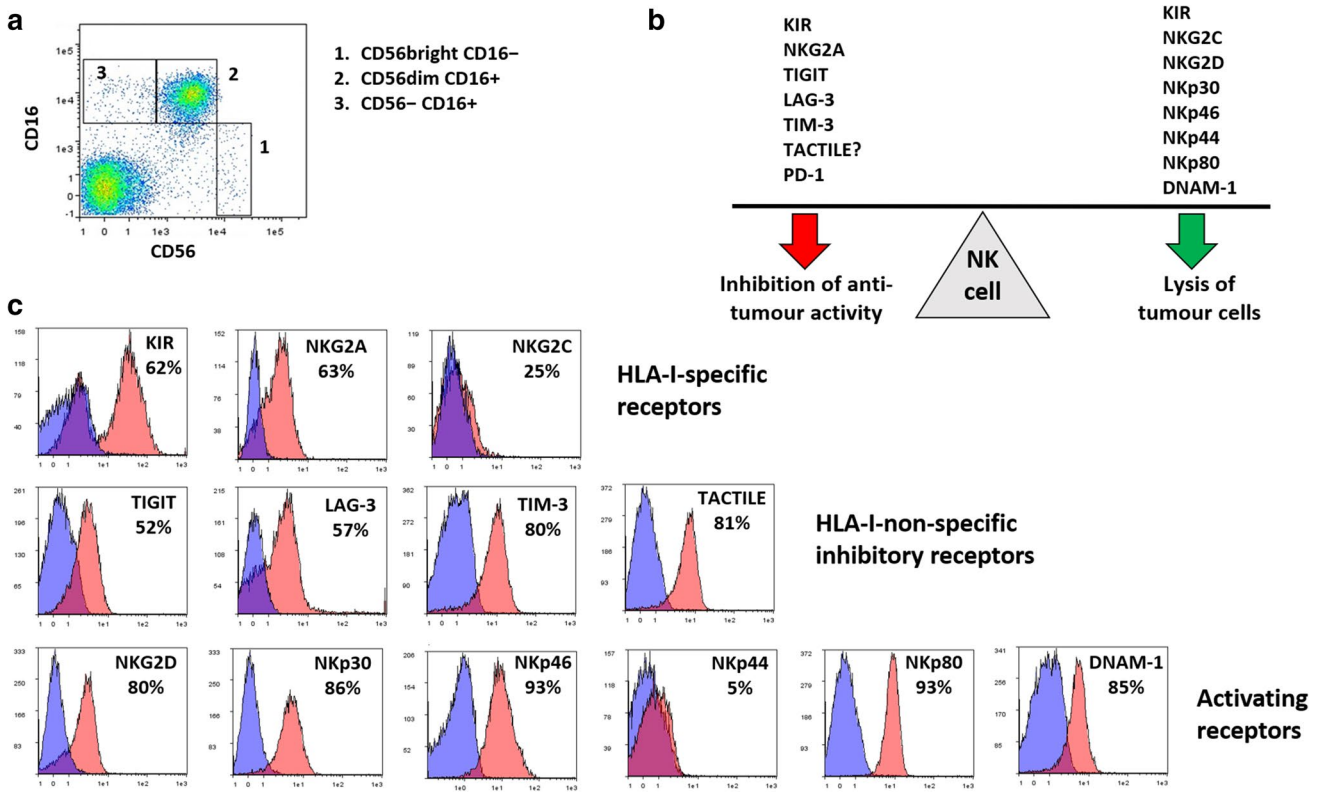

Fig. 1 Human NK cell subsets and receptors. **a** The expression of CD56 and CD16 defines 4 human NK cell subsets. **b** Human NK cells express a large panel of activating and inhibitory receptors. NK cell activation depends on the balance between inhibitory and activating receptors expressed on NK cell surface that interact with their ligands on tumour cells. The major inhibitory receptors recognize as ligands HLA class I molecules. Several non-HLA class I-specific inhibitory receptors are highly expressed on NK cells (e.g. TIGIT; TIM-3, LAG-3) and may represent checkpoints for NK cell activation. **c** A representative cytometry of peripheral blood NK cells in a healthy donor is shown

T cells, NK cells, and some APCs and its ligands include soluble ligands (galectin-9 and HMGB1) and cell surface ligands (Ceacam-1 and Phosphatidylserine) [25, 26]. TIGIT, PVRIG and TACTILE inhibitory receptors, together with DNAM-1 activating receptor, are part of an intricate ligand/receptor network. These receptors interact with Nectin and Nectin-like molecules. TIGIT shares both ligands CD112 and CD155 with DNAM-1, TACTILE binds to CD155 and PVRIG to CD112. These ligands are found overexpressed on tumour cells [24–26]. Together with inhibitory receptors specific for HLA class I molecules, KIR and NKG2A, these non-HLA I-specific inhibitory receptors have emerged as novel checkpoints in NK cell activation [23, 24].

Concerning the panel of activating receptors expressed by NK cells that orchestrates NK cell triggering and cytotoxic activity, NKG2D [27], the NCRs (NKp46 [28], NKp30 [29] and NKp44 [30]) and DNAM-1 [31] have been extensively analysed and are considered responsible of NK cell activation against many types of tumours upon interaction with their ligands. MICA/B (NKG2D ligands) and CD112 and CD155 (DNAM-1 ligands) are frequently expressed on tumour cells. We have previously shown that both NKG2D and DNAM-1 ligands are expressed in a high percentage of melanoma cell lines [32] and DNAM-1 ligands are frequently expressed on AML blasts [33]. Other NK cell receptors such as 2B4 (CD244), NKp80, NKG2C and activating isoforms of KIR may also contribute to NK cell activation against tumour cells [22].

NK cell subsets in healthy ageing

Healthy ageing is associated with a remodelling of NK cell subsets with different functional capacities (Fig. 2). Elderly individuals show a decrease in the percentage of $CD56^{bright}$ immature NK cells and an increase in the percentage of $CD56^{dim}$ NK cells expressing the CD57 marker [34]. It is well established that ageing is the major factor affecting the percentage of $CD56^{bright}$ NK cells [35, 36], whereas the expansion of $CD56^{dim}$ $CD57^{+}$ NK cells is mainly associated with CMV seropositivity not only in old but also in young individuals [35]. According to the linear model of NK cell differentiation (reviewed in [17]), peripheral blood

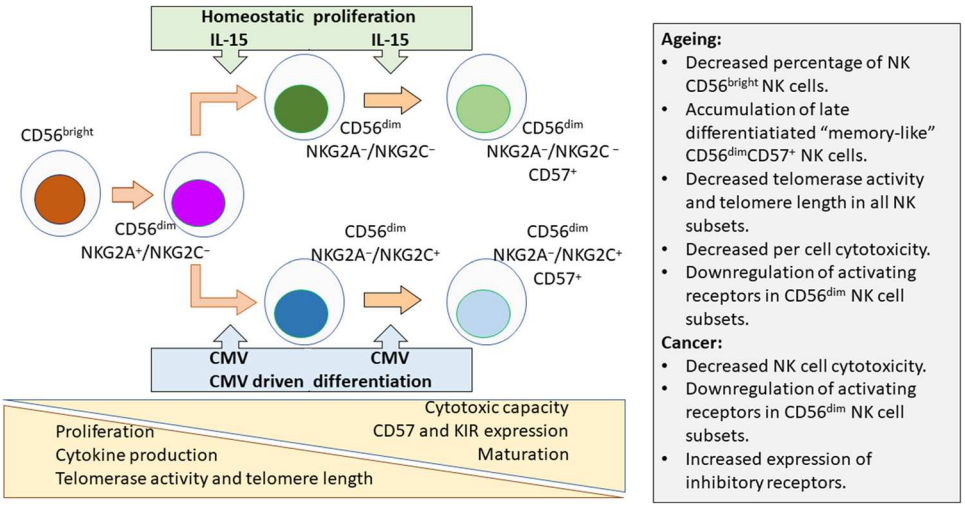

Fig. 2 Model of peripheral blood NK cell differentiation in healthy individuals and the effect of CMV. Altered expression of NK activating and inhibitory receptors and decreased NK cell cytotoxicity is often observed in cancer patients. Both ageing and cancer are associated with decreased cytotoxicity. Ageing is associated with decreased telomerase activity and telomere length in all NK cell subsets

$CD56^{bright}$ NK cells are immature NK cells that can be considered precursors of $CD56^{dim}$ NK cells, that, after activation and maturation can express the differentiation-associated marker CD57. A recent study analysing telomere length and telomerase activity of peripheral blood NK cell subsets supports a linear NK cell differentiation process of NK cells from immature $CD56^{bright}$ to $CD56^{dim}$ $NKG2A^{+}$ $NKG2C^{-}$, $CD56^{dim}$ $NKG2A^{-}$ $NKG2C^{-}$, $CD56^{dim}$ $NKG2A^{-}$ $NKG2C^{+}$ and late differentiated $CD56^{dim}$ $NKG2A^{-}$ $NKG2C^{+}$ $CD57^{+}$ subset [37]. All NK cell subsets presented a reduction in telomere length and telomerase activity with increasing subject age. Of interest, the telomeres of early differentiated NK cells were shorter in the elderly than in young individuals, reaching lengths equivalent to levels found in highly differentiated $CD57^{+}$ NK cells, considered as approaching senescence in young adults. These results highlight the overall ageing of all lymphocyte populations, including early compartments of lymphocyte differentiation, which may approach telomere-based cellular senescence in the elderly [37]. Thus, the study of NK cell subsets in elderly individuals supports an altered NK cell dynamic, with an age-associated decreased output of new $CD56^{bright}$ NK cells and a maintained or increased percentage of peripheral blood NK cells with markers of long-lived NK cells in the elderly ($CD56^{dim}$ $CD57^{+}$) [34]. However, if as suggested in recent studies [18], $CD56^{bright}$ NK cells and $CD56^{dim}$ NK cells are distinct lineages, these results indicate that they are differentially affected by ageing and by virus infection. Considering that ageing is associated with a severe decrease in the numbers of $CD56^{bright}$ NK cells, the debate over whether $CD56^{bright}$ NK cells are precursors of $CD56^{dim}$ NK cells or an independent lineage has important implications for novel approaches of NK cell-based immunotherapy in elderly donors.

NK cells from old individuals show an increased expression of CD57, a marker that defines a subset of mature $CD56^{dim}$ NK cells with cytolytic capacity and cytokine production, but a low proliferative response to cytokines [38, 39]. However, this expansion of $CD57^{+}$ $CD56^{dim}$ NK cells occurs mainly in HCMV seropositive individuals [38, 40, 41]. Although it has been proposed that HCMV is a major driving force of T and NK cell immunosenescence [42], recent advances support that HCMV infection triggers the maturation of a subset of lymphoid cells (NK, CD8 and CD4) phenotypically characterised by the expression of CD57 and a higher functional capacity to respond to different stimuli [43–46].

Murine NK cells and ageing

Murine NK cells are characterized by the expression of inhibitory receptors (e.g. Ly49A), involved in self-tolerance and licensing, and activating receptors (e.g. Ly49H, NKG2D, NKp46) responsible of triggering NK-mediated cytotoxic function and cytokine production. NK cells derive from lymphoid precursors that acquire these inhibitory and activating receptors and develop their effector functions after interaction with bone marrow stromal cells [47]. Several stages of NK cell maturation have been defined in mice, based on the expression of CD27 and CD11b: immature, $CD27^{+}$ $CD11b^{-}$, intermediate, $CD27^{+}$ $CD11b^{+}$, and mature, $CD27^{-}CD11b^{+}$, NK cells [48]. NK cells are essential for resistance to murine cytomegalovirus (MCMV) [49] and ectromelia virus, the agent of mousepox [50].

In aged mice, reduced NK cell function is associated with lower numbers of mature NK cells in peripheral tissues and the bone marrow. Ageing-related functional NK cell deficiency, associated with reduced numbers of mature NK cells in mice, has been well documented using different experimental models. This defect results in a decreased response to viral infections resulting in higher mortality. An intrinsic defect in the migration of mature NK cells and

an impairment of NK cell migration has been observed in aged mice after influenza [51] or ectromelia virus infection [52]. During influenza infection, aged mice have reduced NK cells infiltrating the lungs and significant reduction of their function [51, 53]. Defective NK cell trafficking has also been involved in age-dependent susceptibility to influenza virus infection due to both lower chemokine and integrin expression and delayed actin polymerization in response to influenza infection [54].

The reduced ability of NK cells from aged mice to produce IFN-γ after stimulation correlates with reduced numbers of mature, $CD11b^+$ $CD27^-$ NK cells in peripheral tissues and the bone marrow [51]. This reduced numbers of mature NK cells are the consequence of a defect in their terminal maturation since the percentages of immature NK cells are maintained in the bone marrow from aged mice [55]. Other age-related defects in NK cells, including reduced proliferation, defective maturation and dysregulated expression of activating and inhibitory receptors, a reduced capacity to eliminate tumour cells, and a decreased expression of T-bet and Eomes transcription factors have been defined [56]. The use of young-old mixed bone marrow chimaeras has demonstrated that the aged non-hematopoietic environment is responsible for the impaired maturation and function of NK cells [56]. Similar conclusions were obtained by using an adoptive transfer approach that showed that NK cell from both young and aged mice had a similar response to pathogen and maturation after being co-transferred into young mice, whereas the response and maturation from young mice were decreased after being transferred to aged mice [57]. Bone marrow from young and aged mice gave rise to similar percentages of $CD27^-$ mature NK cells in young mixed bone marrow chimeric mice. Although it has been proposed that age-related functional NK cell deficiency was completely reversed by injecting soluble IL-15/IL-15Rα complexes [57], other authors have shown that age-associated defects of NK cells are not restored by IL-15, suggesting that the defect in NK maturation is the consequence, at least in part, of altered maturational cues provided by bone marrow stromal cells [58].

Cytomegalovirus and adaptive "memory" NK cells

In the past decade, cumulative evidence obtained in mice and humans supports that under certain circumstances NK cells can acquire attributes of immunological memory. The generation of "memory" NK cells has introduced an additional degree of complexity in the understanding of NK cells. This term defines a long-lived population of NK cells that possess traits of adaptive immunity, such as clonal expansion, rapid proliferation, high cytotoxicity and cytokine production. In murine models, the discovery that NK cells expressing the Ly49H activating receptor, that interacts with the protein m157 encoded by the MCMV, expand in MCMV-infected mice and, after contraction, the remaining $Ly49H^+$ cells are shown to be long-lived NK cells with the capacity to undergo secondary expansion in response to viral challenge and conferred protective immunity [59–62] supporting their behaviour as "memory" NK cells. An analogous population of NK cells expressing the activating receptor NKG2C expands specifically in response to HCMV [63–65] and HCMV infected individuals have an enhanced response not only to HCMV (HCMV reactivation in HSCT [41]) but also to other viruses such as Hantavirus [66], Chikungunya virus [67], Hepatitis B and C virus [67] or Epstein–Barr virus acute infection [68]. In a recent study, it has been shown that chronic stimulation of adaptive NK cells through NKG2C results in proliferation and activation of $CD56^{dim}$ $CD57^+$ $NKG2C^+$ NK cells but also to the induction of the checkpoint inhibitory receptors LAG-3 and PD-1, resulting in dysfunctional cytotoxic capacity against tumour targets [69]. Thus, although it should be considered that $NKG2C^+CD57^+$ represent long-lived, "memory" NK cells in humans, with important implications for NK cell-based cancer immunotherapy, further studies are required to analyse the functional capacity of this NK cell subset, as, at least after in vitro stimulation, they may represent exhausted NK cells with limited cytotoxic activity against tumour cells.

Other murine model analysing the NK cell response to haptens or viruses also support the existence of NK cell memory subsets. Thus, hepatic NK cells expressing the chemokine receptor CXCR6 have been defined as memory NK cells [70]. A potentially similar population of long-lived NK cells expressing CXCR6 and other tissue residency markers that also exhibit recall responses to varicella-zoster virus, has been demonstrated in humans [71]. The observation of high frequencies of liver-resident NK cells expressing CXCR6 and NKG2C and KIR [72], suggest the possibility that the liver might be a site for NK differentiation to NK memory cells.

Taken together, these results support that the marked and persistent changes of NK cells observed in elderly and HCMV seropositive individuals may condition the NK cell response to tumours and the possibility to use these cells in NK cell-based immunotherapy.

NK cells in cancer patients

It can be appreciated significant phenotypic changes on NK cells of patients with cancer that can affect their functionality by reducing their lysis capacity and therefore limit NK cell-mediated tumour control (Fig. 2). In some instances, alterations observed in NK cells in young cancer patients may resemble the phenotype of these cells in elderly donors [8, 9].

Interactions between the repertory of KIRs and MHC class I are complex, as they can lead to a strong inhibition, weak inhibition or activation of NK cells [73]. In patients with leukaemia, it has been notified an increased expression of inhibitory KIR2DL1 (strong inhibition) and a lack of the inhibitory KIR3DL1 (weak inhibition) [74], as well as a lower level of the activating KIR2DS3 [75]. In non-small cell lung cancer, the expression of KIR2DL1, KIR2DL3, KIR2DL4 and KIR3DL1 were correlated with a poor prognosis [76].

We have previously described an altered NK cell phenotype associated with AML characterized by a decreased expression of DNAM-1, NKG2C and NCR receptors, NKp46 and NKp30. Fauriat et al. [77] correlated NKp46 expression with overall survival in AML patients and showed recovery of NK cell function after complete remission. Stringaris et al. [78] also reported downregulation of NKp46, upregulation of NKG2A and low cytotoxic capacity of NK cells from AML patients confirming previous results. Furthermore, in solid cancer such as prostate cancer, there was reported a decreased expression of several activating receptors (CD16, NKp30, NKp46, NKG2D and DNAM-1), and an increase in the inhibitory receptor CD85j [79]. NKG2A expression has been also linked with a poor prognosis in liver cancer [80].

In addition to changes related to cancer, cancer incidence increases with ageing [8, 9]. In elderly AML patients, the effect of age and cancer synergize and the downregulation of these activating receptors in NK cells is even more pronounced [33, 81]. In vivo culture with IL-15 can recover NK cell function in elderly AML patients [82] opening new possibilities for cancer immunotherapy based on autologous NK cells in elderly patients. An interesting aspect is that neither in ageing [83] nor in AML patients the expression of NKG2D seems to be significantly reduced [33]. NKG2D constitutes a major activating receptor in many cancer settings. Its ligands are frequently overexpressed in different types of tumours and consequently, strategies directed to improve NKG2D-mediated recognition of tumour cells should be considered in elderly cancer patients. However, shedding of NKG2D ligands is frequent in haematological cancer and solid tumours and affects NK cell function by blocking NKG2D recognition of its ligands on tumour cells [20, 84] and should be considered when designing strategies based on triggering NKG2D signalling.

AML blast as well as solid tumours such as melanoma express CD112 and CD155, ligands for the activating receptor DNAM-1, that are also recognized by the inhibitory receptors TIGIT (ligands CD112 and CD155) and PVRIG (ligand CD112), Thus, in cancer patients, the activating/inhibitory balance mediated by these paired receptors is threatened due to the downregulation of the activating receptor DNAM-1 and, even more, if the expression of the inhibitory receptors TIGIT and PVRIG is preserved. Further studies are required to establish the role of this axis in tumour control [24].

In addition to their direct cytotoxic capacity, NK cells can contribute to the elimination of tumour cells via antibody-dependent cell cytotoxicity (ADCC) in patients treated with anti-tumour-associated antigens (anti-TAA) that interact with CD16, the NK cell receptor for the Fc region of IgG (FcRγIII). The polymorphisms of CD16 have been implicated in the efficacy of anti-TAA mAb-mediated therapy (for review see [85]).

NK cell-based immunotherapies against cancer

As a consequence of the advances in our understanding of NK cell receptors and their ligands, the use of NK cell-based immunotherapy has reached a significance among the new anti-tumour therapeutic strategies. Both, blockade of inhibitory receptors (NK cell checkpoints) or triggering activating receptors have been considered as possible strategies to enhance NK cell cytotoxicity against tumour cells [86]. Besides, the demonstration of the role of allogeneic NK cells in the recognition and killing of AML blasts in HSCT has encouraged development of novel NK cell-based immunotherapeutic strategies for high-risk cancer patients [87] based on the adoptive transfer of autologous or allogeneic NK cells or genetically engineered NK cells, that have already been introduced in phase 1/2 clinical trials.

Enhancement of NK cell function

Due to the importance of NK cells in immunotherapy, different methods have been posed for enhancing NK cell activity toward tumour cells. Here we will briefly discuss some of them (Fig. 3).

Combination of cytokines and mAbs against tumour-associated antigens

Interleukins have been used in clinical trials to activate NK cells in vivo although severe adverse effects have been reported when IL-2 is administrated at high doses [88]. IL-15 is increased in the bone marrow of old individuals [89]. IL-15 plasma levels are significantly affected by health and lifestyle status in the elderly, with important decrease in sarcopenia [90] or associated with visceral adipose tissue [91], supporting the possibility to use IL-15 or the IL-15 super-agonist (ALT-803) to enhance NK cell function in a subpopulation of old patients.

However, administration of low doses of IL-2 used to enhance ADCC triggered by anti-TAA improves the

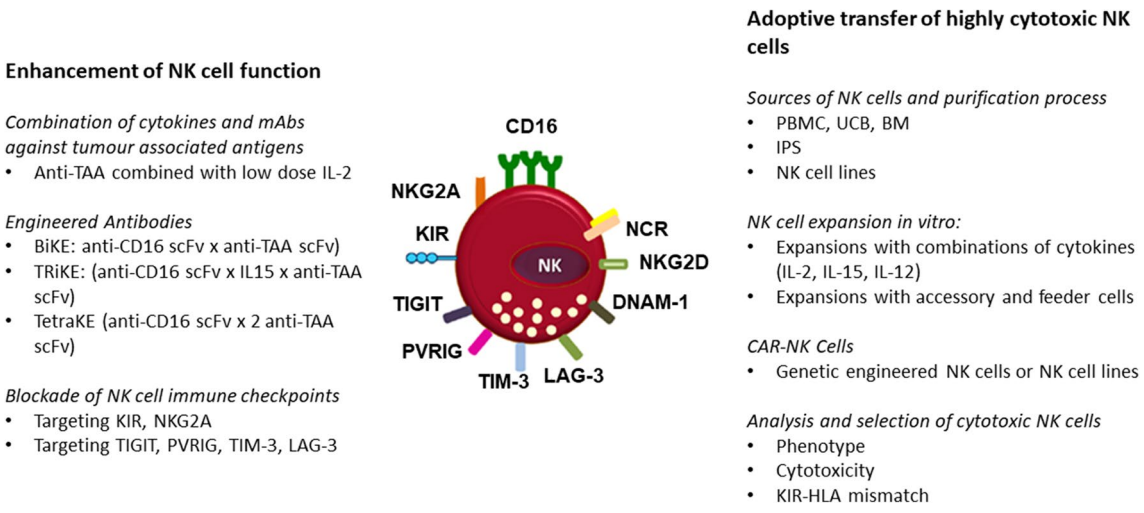

Fig. 3 Enhancement of NK cell cytotoxic capacity for adoptive immunotherapy. Different strategies can be used to enhance the capacity of NK cells to kill tumour cells. Cytokines such as IL-2 and IL-15 are used to activate and expand NK cells in vitro and in vivo. The promotion of ADCC represent a valuable strategy by using mAb targeting tumour antigens (TAA) alone or in combination with low-dose IL-2 or using killer engagers that link CD16 on NK cells to tumour antigens (BiKE), that can also include IL-15 (TRiKE, TetraKE). Checkpoint blockade using mAb directed to HLA class I-specific inhibitory receptors (KIR and NKG2A) and non-HLA class I-specific inhibitory receptors (e.g. TIGIT, PVRIG, TIM-3, LAG-3) can be used to block inhibitory signals and activate NK cells. (PBMC: peripheral blood mononuclear cells, UCB: umbilical cord blood; BM; bone marrow; IPSs: induced pluripotent stem cells)

anti-tumour response of these cytotoxic cells [92] and did not show relevant toxicity issues in phase1/2 clinical trials [93–95]. Other clinical trials have studied IL-12 also along with anti-TAAs, although they did not observe any improvement of NK cell activity [96, 97]. NKTR-214 (bempegaldesleukin) is a human recombinant IL-2 attached to polyethylene glycol at the region of IL-2 that contacts the CD25. After administration, it generates active cytokine species with limited binding to the IL-2Rα subunit, thereby activating $CD8^+$ T cells and NK cells, without expanding the T regulatory cells [98]. Its administration to cancer patients ($n=4$) in a first-in-human study is well-tolerated, shows evidence for activation of the immune response, and it is being combined with anti-checkpoint mAbs in ongoing clinical trials [99]. The use of IL-21 is also under evaluation in clinical trials, showing better results in combination with anti-TAA [100]. As described below, cytokines are also used in NK cell expansion protocols, which are critical for the success of therapy based on adoptive cell transfer [101].

Engineered antibodies

Bi-specific (BiKE), tri-specific (TRiKE), or tetra-specific killer engagers (TetraKE) are small engineered antibody molecules designed to create a bonding between the NK cell and the tumour cells. These engagers hold in one end of their structure an anti-CD16 antibody, which will bind CD16 to trigger NK cell cytotoxicity, and in the other end an antigen for the tumour cell. This connection between the effector and target cell will lead to enhanced cytotoxicity and cytokine production of NK cells. An example of BiKE is CD16xCD33 which enhances the NK activity against $CD33^+$ HL60 AML cell line in vitro [102]. TRiKE and TetraKE use IL-15 molecule as nexus between the antibodies and exhibited more cytotoxicity and generation of inflammatory cytokines than their BiKE ancestor [103]. Combined therapy using BiKEs or TriKEs with checkpoint blockade has been proposed to maximize NK cell anti-tumour response [103].

In addition, because glycosylation of Fc fraction influences antibody effector function, glycoengineering has provided to be useful to generate modified antibodies for use in immunotherapy [104].

Blockade of NK cell immune checkpoints

In the last decade, much attention has been given to the blockade of immune checkpoints for enhancing NK cell function against cancerous cells. The activity of NK cells can be modulated by the blockade of different NK immune checkpoints pathways, that constitute a promising therapeutic tool in immunotherapy [105].

It is known that HLA I inhibitory receptors are important modulators of NK cell activity and therefore interesting targets in this new therapeutic strategy. Both, KIRs and NKG2A blockade are under study, alone and in combination with other antibodies such as anti-PD-1 or rituximab (anti-CD20), to enhance NK cell activity [106, 107]. Anti-KIR antibodies, IPH2101 (1-7F9) and its replacement IPH

2102 (lirilumab, BMS-986015) that includes a modification in the hinge region that increases its in vivo stability have been analysed in clinical trials for haematological and solid tumours [108]. The antibody 1-7F9 blocks KIR signalling, therefore promoting NK cell-mediated lysis of HLA-matched AML blasts in vitro and in a xenograft model of AML [109]. However, when IPH2101 was tested in a clinical trial (NCT01248455) for multiple myeloma (MM) patients, negative results were obtained. The lack of effect of this antibody in vivo was probably due to antibody-induced hypo-responsiveness and contraction of the KIR2D$^+$ NK cell subset [110] and it has been proposed that IPH2101 alters NK cell function by inhibiting NK cell education [111]. It has been demonstrated that the use of the humanized anti-NKG2A antibody (Monalizumab) enhances tumour immunity by boosting both NK and CD8$^+$ T cell effector functions in mice and humans [107].

In patients with metastatic melanoma, Tim-3 signalling blockade with anti-Tim-3 antibodies resulted in NK cell function recovery [112]. This also was confirmed on a study conducted on lung adenocarcinoma patients, whose peripheral NK cells exhibited higher cytotoxicity and IFN-γ production after TIM-3 blockade [113].

A family of paired receptors that exert opposite functions after interaction with ligands of the Nectin/Nectin-like family has being considered of interest in novel NK cell-based immunotherapy approaches. Two of these ligands (CD112 and CD155) are frequently overexpressed on tumour cells. These receptors include DNAM-1, TIGIT, PVRIG and TACTILE. Whereas binding of DNAM-1 to CD155 or CD112 on target cells triggers NK cell-mediated cytotoxicity, TIGIT interaction with these ligands inhibits NK cell function. PVRIG is also an inhibitory receptor that interacts with CD112 [24]. Furthermore, a significant increase in NK cell-mediated cytolytic activity against tumour cells has been demonstrated following antibody-mediated blockade of TIGIT [114]. Additionally, TIGIT blockade drives enhancement of NK cell killing of tumour cells triggered by trastuzumab [115]. Several clinical trials based on checkpoint blockade using mAbs against TIGIT are ongoing [23, 24]. COM701, an anti-PVRIG mAb, is being tested in a phase I clinical trial (NCT03667716) in patients with advanced solid tumours either as monotherapy or in combination with Nivolumab [24]. It has also been reported an increase in IFN-γ production by NK cells and an improvement in the tumour control in different mouse models with lung metastases when CD96 (TACTILE) was blocked [116].

Adoptive NK cell-based immunotherapy

Advances in the knowledge of NK cell biology and new methods of cell processing under good manufacturing practice (GMP) conditions allow the possibility of a more accurate clinical use of these cells. NK cells are a promising tool for use in adoptive immunotherapy against different types of cancer due to their innate ability to recognize and lyse tumour cells through the rapid activation of a series of NK activating receptors without the need for prior sensitization [73, 117].

Sources of NK cells and purification process

Adoptive immunotherapy based on NK cells requires a high number of cells for its application from 1×10^6 to 8×10^7 CD3$^-$ CD56$^+$ NK cells per kilogram recipient body weight [118]. Therefore, as NK cells comprise a low percentage of cells (5–15% in peripheral blood), it is necessary to expand them before being used in adoptive immunotherapy. Autologous or allogeneic NK cells can be used for transfer and cells can be obtained from different sources: peripheral blood mononuclear cells (PBMC), umbilical cord blood (UCB), bone marrow (BM), induced pluripotent stem cells, (IPSs) and cell lines [119–125]. The expansion of large numbers of NK cells with high purity and viability are crucial factors for their use in therapy. Different protocols have been analysed for the expansion of clinical-grade NK cells observing GMP [126]. The expansion protocol can start from purified or enriched NK cells or NK cells expanded from apheresis products (e.g. PBMCs). Subsequently, NK cells are purified in the final stages of the process usually by magnetic separation to eliminate other immune cells such as T or B cells [127, 128]. This is crucial after an allogeneic HSCT where alloreactive T lymphocytes expanded from the donor can cause side effects to the patient such as GvHD [128–132].

Here we briefly review the protocols of expansion and ex vivo activation of NK cells from PBMCs. Safe and efficient production methods are essential to obtain a large number of functional NK cells ready for research and clinical application.

Induction of NK cell expansion by cytokines

The use of cytokines is a well-founded method to effectively expand NK cells and boost their activity, as they are known to mediate in their proliferation and activation. A wide array of cytokines such as IL-2, IL-15, IL-12, IL-18, IL-21 and type I IFNs are used for ex vivo expansion of NK cells [133–136]. IL-2 promotes survival, stimulates activation and improves cytotoxicity of NK cells in a relatively short time (24 h) after incubation. The stimulation of PBMCs with IL-2 results in the expansion of a population termed lymphokine-activated killer (LAK) cells that comprise a mixed population of NK and T cells with higher cytotoxic capacity against autologous tumour cells [137, 138]. However, the adoptive transfer of autologous NK cells combined with low doses of

IL-2 in cancer patients was safe but did not provide clinical benefits [88, 139, 140].

The versatility of expansion protocols using cytokines has been demonstrated; peripheral blood purified NK cells incubated with IL-15 and hydrocortisone, for 20 days, expanded 23 times their initial number, while NK cells stimulated with IL-2 and IL-15 alone or combined for 84 days produced an expansion of 1000 times their initial number [132, 141]. IL-2 and IL-15 are closely related cytokines, when used in combination increases proliferation, viability and favours the priming of NK cells. Only IL-15, and not IL-2, can maintain the cytolytic functions after the infusion of NK cells [101, 128, 142]. In vitro culture of NK cells with IL-2 and IL-15 induces the expression of activating receptors NKG2D, NKp44, NKp30 and NKp46 [40, 141, 143–146]. We have also shown that ex vivo stimulation of NK cells from AML patients with IL-15 and IL-2 increased the expression of NKp30, NKG2D, and DNAM-1 receptors and improved their cytotoxicity against tumour cell lines [82]. IL-12, also used in expansion protocols, can stimulate the production of IFN-γ by NK cells [147]. The combination of IL-12, IL-15, and IL-18 induces cytokine-induced memory-like (CIML) NK cells that are long-lived and highly cytotoxic cells with a high production of IFN-γ. CIML NK cells have been tested in adoptive immunotherapy in mouse tumour models and show promising effects against melanoma and lymphoma in vivo [148, 149].

NK cell expansion with accessory and feeder cells

Despite the activation and robustness of the cytotoxic response, the degree of expansion achieved by NK cells in culture with cytokines seems to be insufficient. The expansion of NK cells requires diverse survival, proliferation and activation signals. Thus, stimuli from accessory cells can be used to enhance the expansion of NK cells to obtain enough cells to be used in adoptive NK cell therapy [139, 150]. It has been described a better expansion of NK cells when the culture starts with the entire PBMCs fraction [151] than when purified NK cells are used, suggesting that other non-NK cells, included in the PBMCs fraction provide additional factors for the proliferation of NK cells. Co-culture of NK cells with autologous accessory non-NK cells or addition of growth-inactivated feeder cells are methodological strategies with pronounced effects on NK cell activation and expansion [152, 153].

Assays in healthy donors and patients with MM using the combination of IL-2 (500 U/ml) and anti-CD3 mAb has shown a wide range of NK cell expansions in PBMCs cultures from 190-fold in healthy donors [151] to 1600-fold in MM patients [154] after 20 days of culture. It should be noted that starting the culture from PBMCs leads to the expansion of unwanted $CD3^+$ T and NKT-like ($CD3^+CD56^+$) cells. However, the infusion of this heterogeneous population, containing potentially alloreactive T cells, did not cause side effects such as GvHD in a clinical trial [155]. This is probably due to the loss of T cell-mediated alloreactivity during extended expansion periods [152, 156]. Thus, the risks in allogeneic transplantation have to be considered [129].

Irradiated PBMCs (monocytes and/or autologous or allogenic B-lymphoblastoid cells) have been used as feeder cells in co-cultures of purified NK cells with the addition of IL-2 (1000 U/ml) with good results, from 25-fold to 30-fold increase [157, 158]. A combination of feeder cells (irradiated), IL-2, anti-CD3 mAb and recombinant human fibronectin fragment (RetroNectin) induces a powerful expansion of up to 4720 fold after 21 days, with more than 90% of NK purity [159].

The possibility of using as feeder cells genetically modified cell lines, such as K562-mb15-41BBL that express IL-15 and 41BBL to activate NK cells, is currently being tested with extraordinary results and with relatively low IL-2 concentrations (100 U/ml) with expansions ranging from 152-fold at day 14 to 277-fold increase at day 21 [160, 161]. Besides, they proved their effectiveness by eliminating tumour cells in mouse models of AML and against autologous and allogeneic tumour cells in vitro [161, 162]. Finally, despite the remarkable results in NK cell expansion with genetically modified allogeneic cell lines, these methods of expansion with tumour cell lines require the generation of a Master Cell Bank and certification and approval of strict regulations according to GMP for their safe use in adoptive immunotherapy [161, 163].

CAR-NK cells

Chimeric antigen receptor (CAR) is an artificial receptor initially designed to enhance T cell activity towards cancerous cells. CAR receptor can bind a wide range of molecules including proteins, carbohydrates and glycolipids. Treatment with anti-CD19 CAR-T cells has been shown successful [164], thus, becoming the most used CAR. It recognizes CD19, an antigen expressed in B cell leukaemia and lymphoma, as well as in B cells. CAR-T cells have shown positive results in haematological cancers in clinical trials [165, 166]. Nevertheless, CAR-T therapy requires to be personalized with autologous blood cells due to the need of the restricted HLA matching, and therefore plausible side effects such as GvHD, neurotoxicity, cytokine release syndrome, etc. Unfortunately, this is an expensive and time-consuming process [167, 168].

On the other hand, the use of CAR-NK overcomes some of the CAR-T cells disadvantages. As NK cells are not HLA restricted, there is no risk of GvHD making possible the production not only of allogenic CAR-NK cell but also

CAR-NK cell lines, which will reduce both the cost and time of the treatment. Thus, the NK-92 cell line is being used for the manufacture of CAR-NK cells, as it is a cell line that regenerates easily [125]. NK cells usually do not secrete the proinflammatory cytokines IL-1 and IL-6, which are involved in the initiation of the cytokine release syndrome [167, 169]. Moreover, CAR-NK cells can recognize other ligands different from the CAR receptor-ligand using their activating receptors NKp30, NKp44, NKp46, NKG2D or DNAM-1 [167]. For all these, CAR-NK cells emerge as a promising tool in immunotherapy against cancer.

Currently, CAR-NK cells are being tested in several clinical trials targeting different receptor in haematological and solid cancers, although they are still in early phases (NCT02944162, NCT03824964, NCT03690310, NCT03056339, NCT03692637, and NCT03692663).

Adoptive transfer of NK cells: results from clinical trials

The use of autologous NK cells in cancer immunotherapy was evaluated by Rosenberg et al. [88] when LAK cells were transferred to patients with metastatic renal cell carcinoma and melanomas. Although transferred NK cells persisted for a long time, no significant clinical benefit was observed. Similar results were observed in a posterior clinical study using autologous in vitro activated NK cells (NCT00328861) for the treatment of 8 patients with metastatic solid tumours (7 patients with metastatic melanoma and 1 patient with renal cell carcinoma). The transferred NK cells persisted in the peripheral circulation of patients from 1 to several weeks after infusion [13]. The results of these studies suggest that treatment with autologous NK cells alone is not effective and the role of the KIR-HLA interaction inhibiting the activity of NK cells may be at least partially responsible for these results [13, 124]. The use of combined therapies may open new possibilities for the use of autologous NK cells. A phase 1 clinical trial (NCT02481934) has investigated the safety and efficacy of multiple infusions of activated and expanded autologous NK cells in combination with anti-myeloma drugs in MM patients. NK cells expanded with K562-mb15-41BBL cells were administered to five patients with recurrent or refractory MM. Patients also received four cycles of chemotherapy treatment with two infusions of 7.5×10^6 NK/kg per cycle. Two patients developed chemotherapy-related neutropenia. After NK cell treatment, four of five patients showed stabilization of the disease and two patients showed a reduction in bone marrow infiltration and a long-term response. These results support that ex vivo expansion of highly cytotoxic autologous NK cells is viable and that multiple infusions are well-tolerated [94].

The adoptive transfer of allogeneic NK cells may confer a superior anti-cancer effect than autologous NK cells, due to the possibility of selecting donor NK cells according to KIR-HLA mismatch [124]. Several clinical studies have demonstrated that allogeneic infusions of NK cells are safe and effective in different cancer settings including haematological malignancies and solid tumours [15]. However, there is still poor evidence on the role of adoptive transfer of NK cells to treat cancer and, unfortunately, only a few completed clinical trials have been published so far (Table 1).

Many clinical trials with published results are in phase 1 and include a limited number of patients that impede to obtain accurate conclusions on the effect of adoptive transfer of NK cells on patient outcome. Thus, several clinical trials were developed to determine the safety and feasibility of haploidentical NK cell infusions in children with leukaemia. In childhood AML and ALL (clinical trials NCT00187096 and NCT00187096), NK cell infusion was well-tolerated, without GvHD, and successful engraftment of NK cells was observed [95, 170]. In one cohort the 2-year event-free survival estimate was 100%, supporting the feasibility and efficacy of this regimen [95]. However, in a phase 2 study (NCT00703820) designed to assess the efficacy of adoptive immunotherapy with haploidentical and KIR-HLA-mismatched NK cells in children with intermediate-risk AML, no difference was observed in event-free or overall survival. The authors suggest that in future clinical trials, repeated infusions of NK cells either during earlier phases of treatment to control tumour burden or during maintenance therapy could provide ongoing immune surveillance [171].

The effect of infusion of haploidentical NK cells before allogeneic stem cell transplant was analysed in phase 1 (NCT00402558) and phase 2 (NCT01390402) clinical trials that enrolled 21 patients with high-risk AML, MDS, or CML. Haploidentical alloreactive NK cell infusion before HSCT was well-tolerated without interfering with engraftment or affecting the rate of GvHD. Five patients showed durable complete remissions [172].

The results from another phase I/II clinical trial (EudraCT Number: 2011-003181-32), that enrolled patients with a median age of 64 years (range, 40–70 years) with treatment-refractory, high-risk MDS, MDS/AML or de novo AML treated with infusions of haploidentical NK cells, also support that MDS and MDS/AML and de novo AML, are susceptible to NK cell-based cancer immunotherapy [173]. Additionally, the results from a recent clinical trial (NCT02763475) with AML patients under 30 years old, support that the incorporation of NK cells to standard chemotherapy directly reduces the likelihood of relapse in these patients [174]. Together, these studies reinforce the use of NK cell-based immunotherapy strategies as a possible treatment approach both for haematologic malignancies and solid tumours.

Two clinical trials demonstrated that intravenous (NCT01385423) or subcutaneous (NCT02395822)

Table 1 Selected clinical trials with published results that use allogeneic NK cells to treat cancer patients

Disease	Patients	Treatment	NK cell dosage	Outcome	Phase	Clinical Trial Identifier	References
Relapse AML	10 children	*Conditioning regimen:* Fludarabina, Cyclophosphamide *Treatment:* Haploidentical KIR-HLA-mismatched NK cell infusion + rhIL-2	Range: $5–81 \times 10^6$ NK cells/kg	Treatment with KIR-HLA-mismatched NK cells after low-dose immunosuppression is well-tolerated and successful engraftment was observed	1	NCT00187096	Rubnitz et al. [95]
Relapse or refractory Leukaemia (ALL/AML)	15 children not HCT & 14 children relapse after HCT	*Conditioning regimen:* Clofarabine, Etoposide, Cyclophosphamide *Treatment:* Haploidentical NK cell infusion + rhIL-2	Range: $3.5–103 \times 10^6$ NK cells/kg	26 of 29 KIR-HLA-mismatched. NK cell infusions and IL-2 injections were well-tolerated. OS of patients without prior HCT was 0.36 and OS of patients with prior HCT was 0.25	1	NCT00697671	Rubnitz et al. [170]
High relapsing Multiple Myeloma	8	*Conditioning regimen:* Bortezomib alone or Cyclophosphamide, Dexamethasone, Fludarabine, Bortezomib *Treatment:* Haploidentical or autologous NK cell infusion + rhIL-2	Up to 1×10^8 NK cells/kg	Infusion of large numbers of NK cells generated with K562-mb15-41BBL was feasible and safe. Superior expansion and activity of fresh compared to cryopreserved cells. Two patients did not require additional treatment for 6 months, one of them had a partial response and the other showed a decrease in disease progression. In 5 patients, disease progression was not affected by NK cell infusion	2	NCT01313897 (IND 14560)	Szmania et al. [176]

Table 1 (continued)

Disease	Patients	Treatment	NK cell dosage	Outcome	Phase	Clinical Trial Identifier	References
Myeloid malignancies (AML, MDS, CML)	21 (2 children, 19 adults)	*Conditioning regimen:* Fludarabine, Busulfan, Tacrolimus, Methotrexate, GCSF *Treatment:* Haploidentical NK cell infusion (KIR-HLA mismatch in phase 1) before allogeneic transplant	Range: 0.02–8.32 × 10^6/kg	Adoptive transfer of NK cells before allogeneic transplant is safe and feasible. No toxicity occurred with the maximal cell doses used and efficacy was related to the number of infused NK cells Complete remission was observed in 5 adult patients	1–2	NCT00402558 NCT01390402	Lee et al. [172]
Poor prognosis refractory Non-Hodgkin Lymphoma (NHL)	15	*Conditioning regimen:* Cyclophosphamide, Pentostatin, Denileukin *Treatment:* Haploidentical NK cell infusion + Rituximab + rhIL-2	Range: 0.5–3.27 × 10^7 NK cells/kg	Treatment was well-tolerated and induced remission of 25% of highly refractory NHL patients. A short-term persistence of haploidentical NK cells and 28% overall response rate was observed	2	NCT01181258	Bachanova et al. [177]
Liver metastasis of gastrointestinal origin	9	*Conditioning regimen:* Fludarabine, Cyclophosphamide *Treatment:* Allogeneic NK cell infusion + rhIL-2 + Cetuximab	3 × 10^6, 8 × 10^6 or 12 × 10^6 NK cells/kg	Combined therapy using allogeneic NK cells administered via intra-hepatic artery, cetuximab and a high dose IL-2 is feasible, well-tolerated and can induce clinical responses. $FoxP3^+$ regulatory T cells and $PD\text{-}1^+$ T cells expanded in all patients, related to IL-2 administration	1	NCT 02845999	Adotevi et al. [178]
Advanced AML	42	*Conditioning regimen:* Fludarabine, Cyclophosphamide *Treatment:* Haploidentical NK cell infusion + rhIL-15	1.9 × 10^7/kg (those with rhIL-15 I.V) 1.2 × 10^7/kg (those with rhIL-15 S.C)	NK-cell infusions in combination with rhIL-15 induced remission in 32% of patients (rhIL-15 I.V.) and 40% patients (rhIL-15 S.C.). Cytokine release syndrome was observed in 56% of patients given subcutaneous rhIL-15	1	NCT02395822 NCT01385423	Cooley et al. [175]

Table 1 (continued)

Disease	Patients	Treatment	NK cell dosage	Outcome	Phase	Clinical Trial Identifier	References
Intermediate-risk AML in first complete remission	21 children	*Conditioning regimen:* Cyclophosphamide, Fludarabine *Treatment:* Haploidentical KIR–HLA-mismatched NK cells + rhIL-2	Range: 3.6–62.2 × 10^6 cells/kg	NK cell infusions are well-tolerated, and transient engraftment is observed. Adoptive transfer of NK cells did not improve event-free or overall survival rates in children with intermediate-risk AML	2	NCT00703820	Nguyen et al. [171]

administration of rhIL-15 in combination with haploidentical NK cell infusion demonstrated high rates of in vivo expansion of adoptively transferred donor NK cells after lymphodepleting chemotherapy and peripheral blood NK cells were more cytotoxic against K562 cells compared with those patients who did not receive rhIL-15. Cytokine release syndrome was observed in 56% of patients given subcutaneous rhIL-15 but not was observed with intravenous administration. NK-cell infusions in combination with rhIL-15 induced remission in 32% of patients (rhIL-15 I.V.) and 40% patients (rhIL-15 S.C.) [175].

A phase 2 clinical trial (NCT01313897) analysed the safety, persistence and activity of NK cells expanded and activated in vitro with the K562-mb15-41BBL cell line in high-risk relapsing myeloma. Autologous (5 patients) or haploidentical (3 patients) NK cells were infused after a preparative regimen followed by the administration of IL-2. No serious adverse events related to NK cell infusion were observed. It has been demonstrated superior expansion and activity of fresh NK cell products compared to cryopreserved products. Among the 7 evaluable patients, one had a partial response and in another, a decrease of disease progression was observed and neither patient required further therapy for 6 months. However, in the 5 remaining patients, disease progression was not affected by NK cell transfer [176].

In poor prognosis refractory Non-Hodgkin Lymphoma (NHL) patients, treatment with haploidentical NK cell infusion together with Rituximab and rhIL-2 was well-tolerated and induced remission of 25% of highly refractory NHL patients (NCT01181258). A short-term persistence of haploidentical NK cells and 28% overall response rate was observed. It should be highlighted that NHL patients have a suppressive environment that is associated with inferior clinical responses after donor NK cell infusions [177].

Compared to haematological malignancies, adoptive transfer of allogeneic NK cell in solid tumours remains elusive. A phase 1 clinical trial using allogeneic NK cells, infused via intra-hepatic artery, combined with anti-EGFR (cetuximab) and high dose IL-2 has enrolled 9 patients with liver metastasis of colorectal or pancreatic cancers. Clinical responses were observed in 3 patients who received donor NK cells with at least one KIR-ligand mismatch. Although the results showed that the combination was feasible, well-tolerated and clinical responses were observed in one-third of the patients, a detrimental expansion of regulatory T cells and PD-1^+ T cells related to administration of high dose IL-2 was observed in all patients [178].

Conclusions and perspectives

The development during the last decade of successful cancer immunotherapy strategies has represented a revolution in cancer treatment. The better understanding of the cellular and molecular interactions between the immune system and different types of tumour cells allows the rising number of approaches that are being actively exploited to manipulate cells of the immune system in novel cancer immunotherapy procedures. Since their discovery, NK cells have been proposed as effector cells in immunotherapy of solid tumours and haematological malignancies. NK cell-based cancer immunotherapy aims to selectively manipulate the mechanisms that regulate NK cell function to enhance NK cell cytotoxicity. Several strategies to trigger NK cell cytotoxicity by ADCC using either anti-TAA mAbs or engineered engagers, alone or in combinations with activating cytokines (IL-2 or IL-15) have been tested in clinical trials and proved to be safe and efficient. Besides, the technological advances to isolate, expand and activate NK cells ex vivo in GMP conditions, support the translation to clinics of NK-cell-based therapeutic approaches based on adoptive transfer of NK cells.

Considering the current knowledge on NK cell biology and that the immune system is frequently depressed in cancer patients and is affected by age or latent viral infections, we consider that a previous detailed analysis of NK cells and their activation and inhibitory receptors, should be required to define personalised NK cell-based cancer immunotherapy. This analysis will depend, not only on the immunotherapy protocol designed in each case (solid tumour vs. leukaemia, triggering host NK cells vs. adoptive therapy, etc.), but also on other aspects such as donors' age and CMV serostatus that affect NK cell subsets and their function.

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Compliance with ethical standards

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Affiliations

Raquel Tarazona[1] · Nelson Lopez-Sejas[1] · Beatriz Guerrero[1] · Fakhri Hassouneh[1] · Isabel Valhondo[1] · Alejandra Pera[2,3] · Beatriz Sanchez-Correa[1] · Nieves Pastor[5] · Esther Duran[5] · Corona Alonso[3,4,6] · Rafael Solana[2,3,4,6]

1 Immunology Unit, University of Extremadura, Cáceres, Spain

2 University of Cordoba, Córdoba, Spain

3 Instituto Maimónides de Investigación Biomédica (IMIBIC), Córdoba, Spain

4 Reina Sofia University Hospital, Córdoba, Spain

5 Department of Medicine, Faculty of Veterinary, University of Extremadura, Cáceres, Spain

6 Immunology Unit, IMIBIC-Reina Sofia University Hospital-University of Cordoba, Av. Menendez Pidal, 14004 Córdoba, Spain