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REVIEW

Targeting NKG2A to elucidate natural killer cell ontogenesis and to develop novel immune-therapeutic strategies in cancer therapy

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Abstract

Natural Killer (NK) cells are innate immune cells with a primary role in the immune surveillance against non-self-cells. NK cell recognition of "self" relies on the surface expression on autologous cells of MHC class I (MHC-I) molecules. Either the absence or the down-modulation of MHC-I on target cells "license" NK cells to kill threatening tumor-transformed or virally infected cells. This phenomenon is controlled by a limited repertoire of activating and inhibitory NK receptors (aNKRs and iNKRs) that tunes NK cell activation and effector functions. Hence, the calibration of NK cell alloreactivity depends on the ability of iNKRs to bind MHC-I complex and these interactions are key in regulating both NK cell differentiation and effector functions. Indeed, the presence of iNKRs specific for self-MHC haplotypes (i) plays a role in the "licensing/education" process that controls the responsiveness of mature NK cells and prevents their activation against the "self" and (ii) is exploited by tumor cells to escape from NK cell cytotoxicity. Herein, we review our current knowledge on function and clinical application of NKG2A, a C-type lectin iNKR that binds specific haplotypes of human leukocyte antigens early during the NK cell maturation process, thus contributing to modulate the terminal maturation of NK cells as potent effectors against cancers cells. These NKG2A-mediated mechanisms are currently being exploited for developing promising immune-therapeutic strategies to improve the prognosis of solid and blood tumors and to ameliorate the clinical outcome of patients undergone allogeneic hematopoietic stem cell transplantation to treat high-risk hematologic malignancies.

KEYWORDS

cancer, immune-checkpoint, monalizumab, natural killer cells, NKG2A, stem cell transplantation

1 | INTRODUCTION

NK cells are innate lymphoid cells acting as immune sentinels against "non-self" threatening target cells. Peripheral blood NK cells represent about 10–15% of all circulating lymphocytes, but they can be also isolated in different tissues, including spleen, liver, lungs, and bone marrow (BM).[1–3] In adults, NK cells develop from CD34+ hematopoietic stem cells (HSCs) primarily in the BM niche, where they interact with stromal cells within a microenvironment enriched in cytokines and growth factors that support NK cell development and maturation.[4] From the bloodstream NK cells can then rapidly migrate to inflamed tissues or secondary lymphoid organs, where they perform immune surveillance against pathogens and tumors.

Abbreviations: ADCC, Ab-dependent cellular cytotoxicity; Allo-, allogenic; AML, acute myeloid leukemia; aNKR, activating NK cell receptor; BM, bone marrow; $CD56^{br}$, $CD56^{bright}$/$CD16^{neg\text{-}low}$; $CD56^{dim}$, $CD56^{dim}CD16^{pos}$; CLL, chronic lymphocytic leukemia; CTLA-4, cytotoxic T lymphocyte associated protein 4; DC, dendritic cell; DFS, disease-free survival; DNAM-1, DNAX accessory molecule-1; EGFR, epidermal growth factor receptor; GvHD, graft-versus-host disease; Haplo, haploidentical; HSC, hematopoietic stem cells; HSCT, hematopoietic stem cell transplantation; iNKR, inhibitory NK cell receptor; KIR, killer immunoglobulin receptor; LAG3, lymphocyte-activation gene-3; MSS-CRC, microsatellite-stable colorectal cancer; OS, overall survival; PD-1, programmed cell death; PD-L-1, programmed death ligand-1; SCCHN, squamous cell carcinoma of the head and neck; TIGIT, T cell immunoreceptor with Ig and ITIM domains; TIM-3, T cell immunoglobulin mucin-3; $uCD56^{dim}$, $CD56^{dim}CD16^{neg}$

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Unlike T and B cells, NK cell activation does not require a prior antigen sensitization, since their effector functions are controlled by a limited repertoire of activating and inhibitory receptors (aNKRs and iNKRs) that do not undergo somatic recombination.[5] Following activation, NK cells also produce and secrete chemokines and cytokines that play a key role in boosting inflammatory responses and in priming other cells of the immune system such dendritic cells (DCs) or macrophages, thus linking innate with adaptive immunity.[6–9]

2 | NK CELL SELF-TOLERANCE, ALLOREACTIVITY, AND LICENSING

NK cell activation relies on the ability to distinguish "self" from "non-self" via the recognition of Major Histocompatibility Complex (MHC) class I molecules by a large family of iNKRs. Indeed, the interaction of these receptors with specific haplotypes of Human Leukocyte Antigen (HLA) class I molecules on target cell surface are key in determining NK cell alloreactivity against "non-self" (missing-self hypothesis).[10] Currently, two classes of receptors mainly mediate the recognition of "self": the Killer Immunoglobulin Receptors (KIRs) and the C-type lectin receptors.

KIRs are an extremely polymorphic family of both iNKRs and aNKRs able to distinguish among different HLA-A, -B, and -C allotypes. Activating and inhibitory KIRs are highly homologous in the extracellular domain, but they differ in the cytoplasmic domain. Inhibitory KIRs are characterized by a long cytoplasmic tail containing the ITIM, while the short intracellular domain of activating KIRs interacts with the adaptor signaling molecule DAP-12, carrying the ITAM.[11–13]

C-type lectin HLA-I-specific receptors are represented by the CD94/NKG2 heterodimers of the C-type lectin receptor family: CD94/NKG2A is the inhibitory receptor and it contains an ITIM in the cytoplasmic domain, while, similar to activating KIRs, CD94/NKG2C is the activating receptor that lacks ITIM and it is associated to DAP-12 adaptor (Fig. 1). These receptors interact with the non-classical HLA-E molecules, expressed by almost all cell types and even up-regulated on tumor-transformed and viral-infected cells.[14] Indeed, to escape from NK cell recognition the human cytomegalovirus (HCMV) infection/reactivation mediates the up-regulation of HLA-E, that is then stabilized by the viral UL40 peptide, mimicking the leader sequence of HLA-I molecules. Furthermore, UL40 is emerging as a potential viral factor involved in shaping the NK cell phenotype, promoting the expansion of long-lasting "memory-like/adaptive" NK cells characterized by higher cytotoxicity upon a second stimulus.[15] These adaptive NK cells are characterized by an $NKG2C^{pos}NKG2A^{neg}$ phenotype and represent a host attempt to counteract HCMV immune-evasion through specific recognition of HCMV-infected cells by innate immune cells.[16]

Albeit NKG2A and NKG2C recognize overlapping set of HLA-E molecules,[17] the inhibitory receptor shows a higher binding affinity for its ligand, overcoming NK cell activation signals.[18] Thus, the expression of HLA-E is directly related to the number of HLA-I molecules in a given cell and NKG2A acts as a sensor to assess the net overall expression of HLA-I molecules on a target cell.[19] The expression of MHC-I-specific iNKRs plays a role not only in the self-tolerance but also in "licensing/educating" NK cell immune responses and in setting their tolerogenic threshold and alloreactivity.[20] Mature NK cells have to express at least one iNKR specific for a "self" HLA-I haplotype in order to recognize and kill target cells and to prevent NK cell activation against autologous cells.[21] The calibration of NK cell alloreactivity relies on the number of iNKRs expressed on NK cell surface and on the strength of their binding with MHC-I complex.[22,23] Indeed, the presence of redundant interactions is key in regulating both NK cell physiology and pathophysiology during the course of tumors and viral infections. Although this "at least one" model is widely accepted, it cannot be applied in the case of the lack of an adequate expression of self-HLA-I molecules. Indeed, in transporter associated with antigen

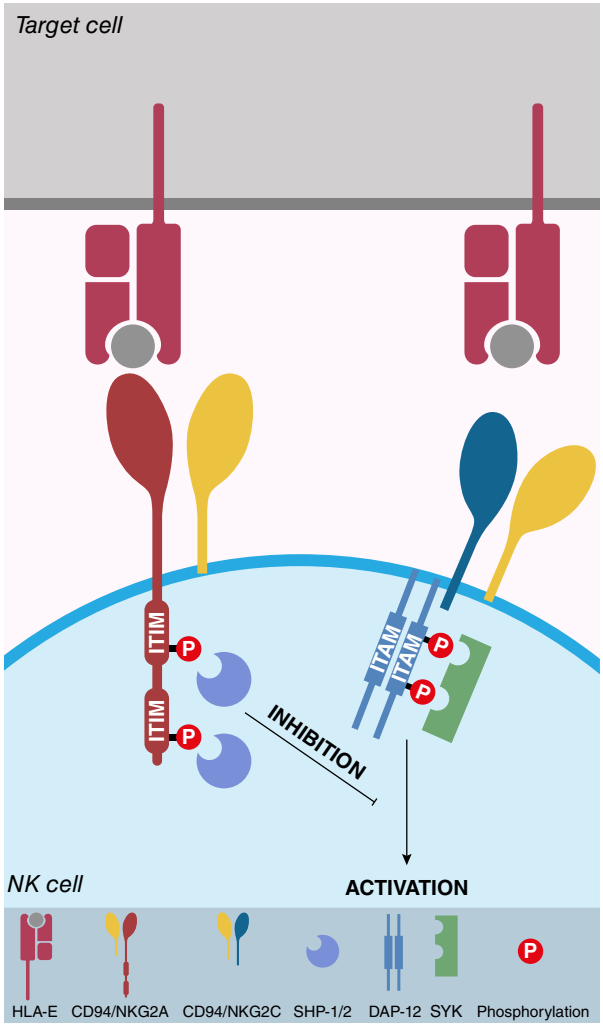

FIGURE 1 CD94/NKG2A and CD94/NKG2C signaling in NK cells. The inhibitory heterodimer CD94/NKG2A and its activating counterpart CD94/NKG2C recognize the non-classical MHC class I molecule HLA-E. NKG2C is characterized by a short cytoplasmic tail and a positively charged transmembrane domain, which interacts with the ITAM-containing adaptor molecule DAP-12. After the binding with HLA-E, DAP-12 binds to and activates the SYK family tyrosine kinases that trigger the downstream activation cascade. On the other hand, NKG2A has a long cytoplasmic tail containing ITIM motifs. After the binding with HLA-E, this receptor exerts its inhibitory function signaling through SHP-1/2 which is able to dephosphorylate multiple targets in the ITAM-activating pathway

processing (TAP)-deficient patients, showing very low levels of HLA-I molecules (approximately 10%), NK cells are not educated. However, NK cells from these TAP-deficient subjects express KIRs and high levels of CD94/NKG2A, keep their cytotoxic machinery, and are tolerogenic likely because their low expression of MHC-I molecules is sufficient to mediate the self-tolerance.[24–26] On the contrary, it has been extensively reported that under homeostatic conditions the lack of engagement of cognate MHC-I molecules renders NK cells hypo-responsive through a process called "disarming." Finally, uneducated NK cells could acquire a functional competence in an inflammatory context.[27]

Taken together, these experimental findings suggest that an inflammatory microenvironment (i.e., tumor or infections) could contribute in tuning NK cell education. In this context, the expression and the frequency of iNKRs on NK cell surface are currently being used as important therapeutic tools to cure solid tumors and to improve the prognosis of patients affected by high-risk hematologic malignancies and treated with hematopoietic stem cell transplantation (HSCT).[28]

3 | NK CELL ONTOGENESIS

While it is clear that NK cells derive from HSCs, many aspects of their differentiation steps are still a matter of debate. NK cell precursors have been found in different districts of human body, including thymus, spleen, liver, lungs, and BM, and many of these tissues also contain mature NK cells.[1–3] However, the current consensus states that the main site of NK cell differentiation and development in adults is represented by the BM niche.[29]

Classically, the human NK cell development has been subdivided in five distinct stages.[30] The first three stages are defined as NK cell precursors (stage 1 "pro-NK cells"; stage 2 "pre-NK cells"; and stage 3 "immature NK cells"), while the last two stages are identified by the differential expression of CD56 and CD16 surface markers.[31]

$CD56^{bright}/CD16^{neg\text{-}low}$ ($CD56^{br}$) NK cells, defined as stage 4, exert immuno-modulatory functions through the production of chemokines and cytokines and through the cross-talk with autologous DCs and monocyte/macrophages.[7–9,32,33] The cytoplasm of $CD56^{br}$ NK cell subset contains low levels of lytic molecules (i.e., perforin and granzymes) and this results in a poor cytolytic potential with respect to the more mature and terminally differentiated stage 5 $CD56^{dim}/CD16^{pos}$ ($CD56^{dim}$) NK cells.[34,35] $CD56^{br}$ cells are the predominant NK cell population in secondary lymphoid tissues where they actively interplay with DCs and T cells, while $CD56^{dim}$ NK cells are the main circulating NK cell subset that then migrate to peripheral tissues to act as immune sentinels.[36,37] Other than by the secretion of lytic granules in the context of above-mentioned mechanisms of "self" recognition, the effector functions of $CD56^{dim}$ NK cells are also ensured by the expression of CD16, which can mediate the so-called antibody-dependent cellular cytotoxicity (ADCC).[38,39]

Our current knowledge on the developmental relationship between $CD56^{br}$ and $CD56^{dim}$ NK cell subsets states that $CD56^{br}$ cells represent the precursors of $CD56^{dim}$ cells, as also suggested by the different telomeres length in these two NK cell subsets.[40] Moreover, also the surface expression of iNKRs is involved in the process of NK cell maturation. Indeed, CD94/NKG2A is the only HLA-specific iNKR expressed on $CD56^{br}$ NK cells. On the other hand, during NK cell reconstitution following allogenic (allo-) HSCT, circulating $CD56^{dim}$ NK cells soon express high levels of NKG2A that is then progressively lost over the time as soon as KIR^{pos} $CD56^{dim}$ NK cells start to expand.[41] This experimental evidence suggests that NKG2A plays a key role in the early stages of NK cell ontogenesis.

4 | NK CELL IMMUNE-RECONSTITUTION AND NKG2A IN HSCT

The disclosure of immune cell recovery after HSCT in patients affected by high-risk hematologic malignancies opened new important perspectives to better understand the ontogenesis, the tolerance mechanisms and the licensing of human NK cells. Indeed, the concept that NK cell licensing status can be tuned and functions as a rheostat is also supported by experimental evidence indicating that, following NK cell adoptive transfer from an HLA environment to another, NK cell functions can be reset.[42,43] This process becomes particularly relevant in the setting of allo- and haploidentical (haplo-) HSCT for the treatment of hematologic malignancies, where a state of immunologic tolerance between donor- and recipient-derived immune cells has to be achieved.[44–47] Moreover, our better understanding of the immune-reconstitution process in patients undergone HSCT is paving the ground to develop new biological therapies that improve the rates of overall survival (OS) and disease-free survival (DFS).

After conditioning therapy, HSCT patients undergo an aplastic phase that exposes immune-deficient recipient to a variety of side effects, including infections and tumor relapse. Thus, a fast engraftment of donor-derived HSCs and a vigorous recovery of immune cells are keys to endure a good clinical outcome of HSCT.[48] Innate immune cells recover quickly and early with respect to adaptive immune cells. NK cells are the first donor-derived lymphocytes to recover after HSCT prior B and T cells, thus representing the first line of defense in immune-compromised recipients.[49–52] Indeed, in the context of allo- and haplo-HSCT the presence of a mismatch between iNKRs on donor-derived NK cells and HLA alleles of the recipient cells induces a condition of alloreactivity that allows NK cells to prevent: (i) leukemic relapse by eliminating residual malignant cells, (ii) graft rejection by eliminating recipient immune cells that survived the conditioning regimens, and (iii) the onset of graft versus host disease (GvHD) by eliminating recipient antigen presenting cells that eventually present host antigens to donor T cells.[44,45,53,54]

Although NK cells reach normal levels and a complete donor chimerism within the first month after allo- and haplo-HSCT,[46,49–52] the maturation of NK cells takes longer. Indeed, the distribution of CD56 and CD16 on the surface returns similar to that of healthy donors only after few months from the transplant. Instead, the frequency of $CD56^{br}$ NK cells early after HSCT is remarkably higher than that of $CD56^{dim}$, thus further confirming the developmental relationship between these two NK cell subsets. Moreover, we recently reported that the low frequency of $CD56^{dim}$ NK cells in the first

weeks following HSCT is counterbalanced by the expansion of an unconventional NK cell subsets characterized by a $CD56^{dim}/CD16^{neg}$ phenotype ($uCD56^{dim}$).[51] The frequency of this latter NK cell population is very low under homeostatic conditions, although it is endowed with a remarkably high cytotoxicity.[55–58] In contrast, the $uCD56^{dim}$ NK cells reconstituting in the recipients early after haplo-HSCT are strongly impaired in their alloreactive effector functions due to the constitutive and transient high levels of NKG2A expression.[51,59] Hence, it is conceivable to hypothesize that $uCD56^{dim}$ NK cells could represent an additional[58] or an alternative[51] step in NK cell differentiation. Although the developmental relationship between $uCD56^{dim}$ and $CD56^{br}$ still remains to be elucidated, the high expression of NKG2A likely plays an important role in terminal NK cell maturation. Indeed, at later time points of immune reconstitution, NK cells lose the expression of NKG2A while acquire CD16 and KIRs together with high cytotoxic activity.[60]

The disclosure of potential alloreactive $uCD56^{dim}$ NK cells expanded early after allo- and haplo-HSCT has arisen new important questions. Indeed, the constitutive expression of CD94/NKG2A on almost all NK cells early after HSCT represents a novel targetable immune-checkpoint to boost NK cell alloreactivity early after transplant[51,61] in order to prevent acute and chronic GvHD, the onset of opportunistic infection and to ensure an optimal engraftment.[62,63]

5 | NKG2A IN TUMORS

NK cells are known to play a major role in the antitumor immune response as they can control both tumor progression and metastatization. However, malignant cells have the ability to hide from NK cell-mediated immune surveillance within the tumor microenvironment. Here, epithelial and/or stromal components release soluble factors (such as TGF-β1 and IL-10) or soluble form of NK cell receptor ligands that strongly affect NK cell function and maturation, thus supporting tumor progression.[64–68] Beside tumor microenvironment, the direct interaction between NK cell checkpoint inhibitors, such as the programmed cell death (PD)-1,[69,70] or several other immunomodulatory surface proteins, such as TIGIT, TIM-3, and LAG3, with their ligands expressed on cancer cells further hampers NK cell antitumor activities.[71]

Another strategy of tumor-transformed cells to escape to NK cell immuno-surveillance is the down-regulation of HLA,[72] a mechanism that is also used by several viruses to evade lymphocyte cytotoxicity.[73] Nevertheless, some types of cancer, including renal carcinoma, acute myeloid leukemia (AML), and hepatocellular carcinoma, do not completely lack HLA-I and the expression of non-classical HLA-E and HLA-G is even up-regulated.[74–76] A possible explanation is that the expression of HLA-I self-molecules on tumor cells impairs NK cell alloreactivity, thus rendering cancer cells resistant to NK lysis. Experimental evidence to support this working hypothesis indicates that renal carcinoma cells express HLA-E on cell surface and are able to inhibit the effector functions of tumor infiltrating NK cells via the up-regulation CD94/NKG2A heterodimer on NK cells.[77] It has been also reported that NK cells from AML patients show a decreased expression of the activating receptor NKp46 accompanied by an increased expression of NKG2A and impaired effector functions.[78] These phenotypic and functional abnormalities are likely mediated directly by tumor cells that do not only express HLA-E on their surface while releasing soluble molecules able to modulate the repertoire of NKRs.[68,75,77,78]

The increased expression of NKG2A in tumor-infiltrating NK cells is also emerging as a contributor in determining the poor prognosis of cancer. In this regard, the NKG2A expression in liver-resident and tumor-infiltrating NK cells correlates with NK cell exhaustion and it is associated with a shorter OS of patients affected by hepatocellular carcinoma patients.[79] Moreover, the up-regulation of NKG2A on NK cells in patients with lung cancer is a predictive factor of tumor metastatization.[80] In addition, the direct cross-talk between NK cells and intratumor stromal cells pathologically contributes to alter NK cell phenotype in lung carcinoma.[81] Similar results were obtained also in invasive breast cancer where NK cells show an increased expression of NKG2A and a decreased expression of the aNKRs NKp30, NKG2D, DNAM-1, and CD16.[82] In this context, the increased expression of HLA-E on tumor cells is associated with a higher incidence of relapse in breast cancer[83] and with a worst prognosis in colorectal adenocarcinoma.[84] Conversely, the lack of non-classical MHC-I molecule in colorectal cancer is directly associated with a better OS and DFS.[85]

6 | INHIBITION OF NKG2A CHECKPOINT AS NOVEL IMMUNOTHERAPY

It is well known that the survival of tumors is favored by the modulation of immune checkpoint that establishes an imbalance between immune surveillance and cancer cell proliferation.[86,87] In recent years, many immune-checkpoint inhibitors have been developed and they represent promising anticancer approaches that gave remarkable positive therapeutic results with limited side effects in several cancers refractory to conventional chemotherapy.[86]

The first efforts targeting immune-checkpoint inhibitors have been focused on T cell effector functions. In this regard, the two first monoclonal antibodies (mAbs) to be developed were ipilimumab and nivolumab that targeted the Cytotoxic T Lymphocyte Associated Protein 4 (CTLA-4) and the PD-1 immune-checkpoints, respectively.[86] Despite the clinical good results obtained in the treatment of a wide range of solid tumors including lung cancer, melanoma, and hematologic malignancies, many patients failed to give an optimal response to PD-1 and CTLA-4 checkpoint inhibitors. Therefore, there is a clinical need to find new alternative therapeutic targets that also take into consideration different effector cells, such as NK cells, with the final aim of developing new combined and more effective antitumor strategies. Indeed, the blockade of both CTLA-4 or PD-1 on T cells induce the release of IL-2, while the inhibition of PD-1 on NK cells boost the secretion of IFN-γ. The higher productions of these cytokines in response to the blocking of these checkpoint inhibitors are associated with a further activation of NK, T, and myeloid cells that exert more potent antitumor immune responses.[69,88]

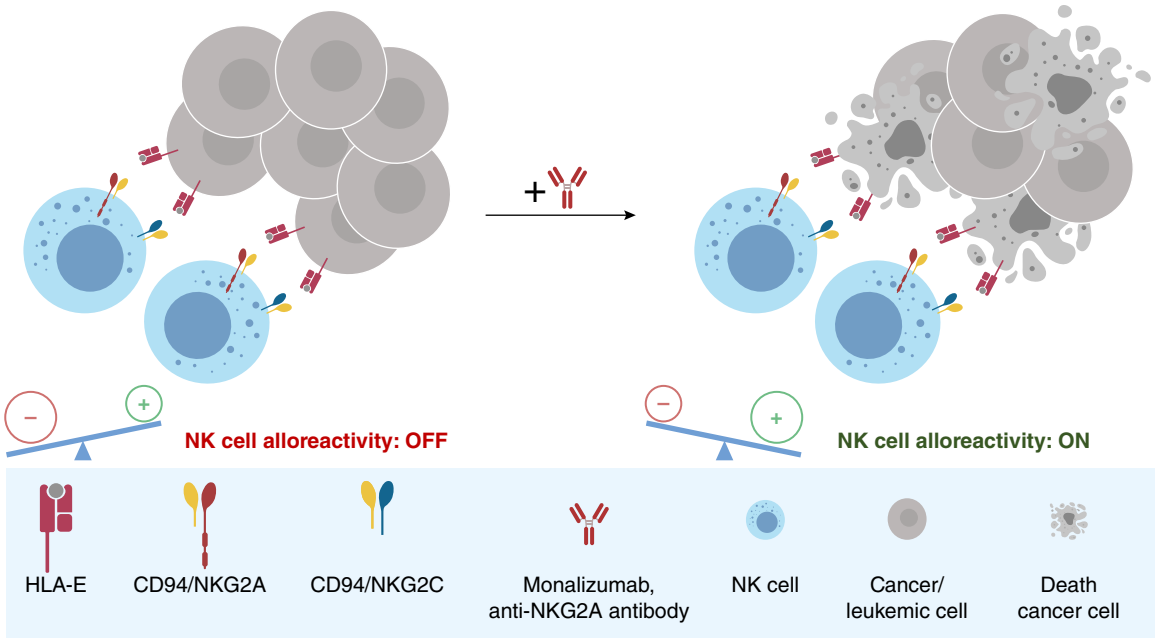

FIGURE 2 **Mechanism of action of anti-NKG2A therapy.** The over-expression of the non-classical HLA-E molecule by cancer cells allows protecting themselves from the killing mediated by NKG2A+ NK cells (left). The humanized anti-NKG2A mAb (monalizumab) is able to block the interaction between NKG2A and HLA-E, which results in the suppression of the inhibitory signaling by tumors on NK cells, allowing activating signals to overcome the inhibitory ones, thus unlocking NK cell alloreactivity against cancer cells (right)

An additional immune-therapeutic approach developed over the past recent years relies on the production of blocking mAbs against HLA-I-specific iNKRs. The rationale of this novel strategy is to increase NK cell alloreactivity, thus mimicking the "missing-self" response and engaging the action of aNKRs including NKp46, NKp30 (the two main activating NK receptors), and NKG2D.[89] In this regard, two IgG4 mAbs respectively blocking KIR2D/HLA-C (IPH2102, lirilumab, Innate Pharma) and NKG2A/HLA-E (IPH2201, monalizumab, Innate Pharma) have been recently developed for clinical application.

The blockade of NKG2A improves NK cell alloreactivity early after HSCT in cancers overexpressing HLA-E (Fig. 2). Since CD94/NKG2A can be also expressed by activated $\alpha\beta$ $CD8^{pos}$ T-cells, $\gamma\delta$ T cells, and NK-T cells,[90] the inhibition of NKG2A also activates different cytotoxic lymphocytes, thus boosting their antitumor potentials. An anti-NKG2A mAb had been first developed in mice,[91] and then humanized for clinical application in human (IPH2201, monalizumab). The impact of monalizumab had been first investigated in in vitro and in vivo studies on neoplastic cells and in humanized mouse models. The success of these preliminary investigations made it possible to develop both phase I and II clinical trials targeting hematologic and solid tumors. Moreover, the constitutive high expression of NKG2A on all reconstituting donor-derived NK cells early after haplo-HSCT would also justify the use of post-transplant monalizumab to boost NK cell alloreactivity in the first week after the infusion of HSCs to limit the onsets of opportunistic infection and of the GvHD and to improve engraftment.[51]

It has been first demonstrated that the tolerance of allogenic NK cells is due not only to KIR expression, but that NKG2A inhibition may play a major role in maximizing the killing of primary leukemia targets.[61,92] These data led to the development of a phase I clinical trial (*NCT02921685*) on the administration of monalizumab, 2 months after HLA-matched allo-HSCT. This study will determine the maximal tolerated dose and the recommended dose for phase II of monalizumab. In accordance, we recently demonstrated that the in vitro blockade of CD94/NKG2A early after haplo-HSCT is able to promote NK cell alloreactivity against B lymphoblasts expressing HLA-E.[51] These data support the concept that the administration of monalizumab in the first weeks after haplo-HSCT could efficiently strength NK cell alloreactivity without the need to search donor KIR-mismatched NK cell population to be infused as post-transplant adoptive cell therapies (i.e., donor lymphocyte infusion or DLI). Conversely, the timing of mAb administration is of critical concern in clinics and has to be further investigated, since the continuous blockade of CD94/NKG2A inhibitory receptor could induce an HLA-I negative environment that, in turn, might re-educate alloreactive donor-derived NK cells and reduce their effector functions.[42]

The potential clinical utility of monalizumab of the Chronic Lymphocytic Leukemia (CLL) has been first shown in 2016 by an in vitro study demonstrating that the blockade of NKG2A/HLA-E is able to restore the ability of $NKG2A^{pos}$ NK cells isolated from CLL patients to lyse otherwise resistant CLL B cells expressing HLA-E.[93] Moreover, in xenogeneic mouse models, the infusion of $NKG2A^{pos}$ NK cells pretreated with the NKG2A mAb was able to prevent the engraftment of AML cells.[14] These preclinical studies paved the ground for ongoing phase I/II clinical trial (*NCT02557516*) on the administration of monalizumab in combination with irutinib, a Bruton's tyrosine kinase inhibitor already used in the treatment of CLL, to determine a long-term clinical benefit for these patients.

Nowadays, monalizumab effectiveness is under investigation also in several ongoing clinical trials for the treatment of different solid tumors including head and neck cancer (*NCT02643550*,

TABLE 1 Ongoing clinical trials with monalizumab for the treatment of solid tumors

Clinical trial	Phase	Drug	Disease	Results
NCT02643550	I and II	cetuximab/monalizumab	Recurrent or metastatic SCCHN	Ex vivo: CD94/NKG2A+ NK and CD8+ T cells infiltrate the tumor and tumor cells express HLA-E. In vitro: NKG2A blockade potentiates cetuximab induced ADCC. In vivo: The administration of monalizumab plus cetuximab is safe in SCCHN and that the majority of treated patients have a stable disease or a partial response.
NCT03088059	II	afatinib/palbociclib/ monalizumab	Recurrent or metastatic SCCHN progressing after first-line platinum-based chemotherapy	N/A
NCT02459301	I	monalizumab alone	Gynecologic Malignancies	The administration of monalizumab (3 doses, every two weeks) is well tolerated, with no dose-limiting toxicities and minimal adverse events. About the 40% of patients have a short-term disease stabilization.
NCT02671435	I	durvalumab/monalizumab	Advanced solid tumors (recurrent or metastatic colorectal cancers)	Ex vivo and in vitro: Tumor infiltrating NKG2A+ and/or PD-1+ NK and CD8+ T cells are present in several cancer types; tumor cells express HLA-E in the large majority of solid tumors compared to PD-L1. Ex vivo, rodent model: NKG2A blockade potentiates PD-L1 inhibitors enhancing the killing ability of B cell lymphoma-infiltrating CD8+ T cells and IFN-g production. First-in-human study: The safety and toxicity profiles of monalizumab plus durvalumab in metastatic microsatellite-stable colorectal cancer, a non-responder cancer to single-agent anti-PD-1/PD-L1 therapy, show that this combined therapy is well tolerated, with no dose-limiting toxicity. Almost all patients treated give partial responses or have a stable disease.

NCT03088059), ovarian and endometrial cancer (*NCT02459301*), and metastatic colon cancer (*NCT02671435*).

The aim of N*CT02643550* phase I/II clinical trial is to investigate, in patients who prior received a systemic regimen for recurrent and/or metastatic head and neck squamous cell carcinoma (SCCHN), the safety of escalating doses of monalizumab in combination with cetuximab in order to simultaneously stimulate NK cell cytotoxicity and inhibit Epidermal Growth Factor Receptor (EGFR). In vivo experimental evidence demonstrated that both NK and $CD8^{pos}$ T cells expressing CD94/NKG2A infiltrate SCCHN and that tumor cells express HLA-E. Moreover, the in vitro NKG2A blockade was able to potentiate cetuximab induced ADCC (Table 1). The first in vivo preliminary results are encouraging and point out that the administration of monalizumab plus cetuximab is safe in SCCHN (Table 1) and that the majority of treated patients have a stable disease or a partial response (Table 1). Since tumor-associated NK cells have increased expression not only of NKG2A but also of PD-1, SCCHN response to monalizumab is under investigation in patients that are either naïve or resistant to PD-L-1 therapy (*NCT03088059*).

In the context of metastatic and recurrent gynecologic malignancies, monalizumab is being analyzed as single agent (*NCT02459301*). Preliminary data from the Canadian Cancer Trials Group indicate that the administration of monalizumab (three doses, every 2 weeks) is well tolerated, with no dose-limiting toxicities observed and minimal adverse events. Moreover, about the 40% of patients analyzed show short-term disease stabilization (Table 1).

The clinical trial combining monalizumab with durvalumab (MEDI4736, anti-PD-L-1 mAb) is also giving good results in advanced solid malignancies, especially in recurrent or metastatic colorectal cancer (*NCT02671435*). The rationale of this clinical trial is supported by ex vivo and in vitro preclinical results showing that tumor infiltrating $NKG2A^{pos}$ and/or $PD\text{-}1^{pos}$ NK and $CD8^{pos}$ T cells are present in several cancer types and that tumor cells express HLA-E in the large majority of solid tumors (Table 1). The ex vivo NKG2A blockade potentiates PD-L-1 inhibitors by enhancing the cytotoxicity of tumor-infiltrating $CD8^{pos}$ T cells and IFN-γ production in a rodent model of B cell lymphoma (Table 1). Moreover, the safety and efficacy of monalizumab in combination with durvalumab have been evaluated in metastatic microsatellite-stable colorectal cancer (MSS-CRC), a non-responder cancer to single-agent anti-PD-1/PD-L-1 therapy. The safety and toxicity profiles show that this combined therapy is well tolerated, with no dose-limiting toxicity. The preliminary data on the

efficacy are encouraging as almost all treated MSS-CRC patients gave partial responses or had a stable disease (Table 1).

7 | CONCLUDING REMARKS

The development of NK cell immunotherapy to boost the natural alloreactivity of NK cells against malignant cells is a fast-evolving field. Growing evidence indicates that classical and non-classical HLA molecules are expressed on the cell surface of a wide range of cancer cell types and that NK cell receptor expression can be modulated by tumor microenvironment. Therefore, the development of therapeutic strategies able to interfere with the HLA-I-specific iNKRs, thus mimicking the "missing-self" response could improve the prognosis of patients affected by several malignancies. In this context, NKG2A acts as a sensor of the net expression of HLA-I molecules on a target cell where this iNKR recognizes HLA-E molecules that are expressed on almost all human cells. Disclosing the impact of monalizumab on natural history of different malignancies and on NK cells will be also important to better understand the role of NKG2A both in NK cell ontogenesis and in NK cell clinical potential to fight and kill cancer cells. Moreover, the combination of different therapeutic strategies will advance our current knowledge in regard to the biology and heterogeneity of cancers.

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DISCLOSURES

The authors declare no competing financial interests.

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