Review Article

OMICS International

Overview of the Soluble and Membrane-bound Tumor Factors Limiting NK-mediated Immune Surveillance

Cristina Bottino[1,2], Mariella Della Chiesa[2], Alessandra Dondero[2*], Francesca Bellora[2], Beatrice Casu[2], Alessandro Moretta[2,3] and Roberta Castriconi[2,3]

[1]Istituto Giannina Gaslini, Genova, Italy

[2]Department of Experimental Medicine (DIMES), University of Genova, Italy

[3]Center of Excellence for Biomedical Research (CEBR), University of Genova, Italy

*Corresponding authors: Alessandro Moretta, Department of Experimental Medicine (DIMES); University of Genova, Italy, Email: alemoret@unige.it

Abstract

Many evidences suggest that NK cells are effective in patrolling for and eliminating tumors in their onset phase, but hardly limit the progression of large established solid tumors. Beside the transition of tumor cells towards a more aggressive phenotype, the NK cell efficacy might be limited by a complex immunosuppressive milieu present in the tumor microenvironment. Indeed, different mechanisms damping NK cell function have been shown in these last years. These include a plethora of tumor-derived immunomodulatory soluble factors (TGF-β, MIF, adenosine, L-Kynurenin, PGE2) as well as soluble ligands (MICA, ULBP-2, PVR, B7-H6) that compete with membrane-bound tumor ligands for binding to activating NK receptors. During NK-tumor cell contact the NK cell function can also be inhibited by the engagement on NK cells of different inhibitory receptors. The specific ligands might be either constitutively expressed at the tumor cell surface (HLA-I, B7-H3, PVR) or de novo induced/up-regulated (PD-Ls) by immunostimulatory factors (IFN-γ, TNF-α). These are largely released during the active phases of the immune responses and exert an unwanted side effect called "tumor adaptive immune resistance". This review aims to summarize the best-known molecular mechanisms that, at various times and in different ways, can limit the efficacy of the NK-mediated immune surveillance of tumors.

INTRODUCTION

Natural killer cells (NK) are crucial cytolytic effectors belonging to the family of innate lymphoid cell (ILC) [1,2]. Originally described as cells exerting a "natural" cytolytic activity due to their capability to kill the highly susceptible K562 eritroleukemia cell line, it is now well established that they require activation to exert optimal effector functions. Moreover, the susceptibility to NK-mediated killing of established tumor cell lines is superior to that of tumors *ex-vivo* isolated from patients, as occurs in bone marrow metastases that are much more resistant to NK-mediated aggression [3]. This supports the concept that an effective NK-mediated anti-cancer activity can't dispense with an optimal activation of endogenous or adoptively transferred NK cells. It is also crucial to understand which NK cell subset, once activated, can exert the most effective anti-cancer activity in a particular immunotherapeutic setting. Indeed, it has been recently stressed that a great heterogeneity in NK cell phenotype and functions exists that goes beyond the classical CD56^{dim} CD16^{high} and CD56^{bright} CD16[low/neg] NK cell dichotomy [4]. Beside the identification and description of CD56^{neg} NK cells [5] that are particularly abundant in peripheral blood of virus-infected donors, several studies highlighted the great heterogeneity of peripheral blood CD56^{dim} NK cells, which include subpopulations characterized by different capabilities of being activated by cytokines, antibodies or tumor contact [6,7]. Thus, adoptive transferred NK cell-based therapeutic protocols should combine optimal activation strategies, the selection of the best NK cell subpopulation, consider the *in vivo* persistence of the *in vitro* activated NK cells, and, last but not least, their chemokine receptor repertoire. Indeed, the scarce attitude of cytotoxic CD56^{dim} NK cells to reach and invade the tumor parenchyma represents a major obstacle for the effectiveness of NK cells, especially in the therapy of solid tumors [8,9]. This might occur also in a chemokine-rich tumor milieu, due to a defective expression in NK cells of the chemokine receptors involved in their migration toward peripheral tissues. This hypothesis matches with the scenario described in neuroblastoma patients whose peripheral blood CD56dim NK cells are characterized by an unusual reduced expression of CX3CR1, the fractalkine (CX3CL1) receptor [10]. Interestingly, CX3CR1 has been shown to be deeply down-regulated by transforming growth factor beta 1 (TGF-β1), a pleiotropic soluble factor released by most cell types including tumors that is capable of modulating pivotal effector functions of different immune cells [11,12]. TGF-β1 is one among the several soluble factors present in the tumor microenvironment that recently emerged as potent immune-modulators. Moreover, beside the classical HLA class I-mediated inhibition of the NK cell activity, several additional inhibitory signals have been recently described that, during NK-to-tumor contacts, limit the NK-mediated immune-surveillance. Thus, the choice of the most effective, activated NK cell subsets should not disregard their profile in terms of the cognate inhibitory receptor-ligand interactions. This review summarizes the best-known tumor-derived soluble factors and tumor-associated surface molecules exerting an immunomodulatory role in NK cells.

Soluble Immunomodulatory Mediators

MIF, adenosine, L-Kynurenin, PGE2

In the past few years, many different tumor-associated regulatory/suppressive mechanisms have been widely studied (Figure 1). Among these, molecules shed from the tumor cell surface, such as the soluble ligands of NKG2D [13-15], DNAM-1 [16] and NKp30 [17], activating receptors crucial for T- and/or NK cell-mediated immune-surveillance [18]. sMICA, sULBP-2, sPVR and sB7-H6 compete for binding with the ligands expressed on the tumor cell surface, thus hindering the efficient target recognition. Moreover, different tumor-secreted soluble mediators have been shown to clearly suppress the efficacy of the immune system. Among these, the macrophage migration inhibitory factor (MIF, also known as Glycosylation-Inhibiting Factor) [19-21] and adenosine, an endogenous purine nucleoside highly produced by tumors expressing CD39 and CD73, ectonucleotidases converting ATP to adenosine. Both MIF and adenosine have been demonstrated to inhibit cytotoxicity and cytokine production in human NK cells, the second mainly through the engagement of the adenosine receptor 2A (AdoR2A), which is coupled to adenylyl cyclase *via* Gs protein [22,23]. In the mouse model the use of AdoR2A antagonist reduced the metastatic potential of CD73+ tumors [24] that was further reduced by blockade of AdoR2B. Since *in vitro* AdoR2B blockade had no significant effects on NK cell cytotoxicity, the benefit observed in tumor-injected mice treated with AdoR2 antagonist might depend on both NK cell-dependent and -independent mechanisms. Along this line, in mice selectively lacking AdoR2 expression in myeloid compartment it has been shown that adenosine can indirectly suppresses T and NK cell-mediated antitumor activity by shaping the functions of different myeloid cells. In particular, in this mouse model, a reduced melanoma growth was associated with a significant increase in MHC class II expression and IL-12 release in tumor-associated macrophages (TAM), features fitting with an M1-like pro-inflammatory macrophage polarization. Moreover, AdoR2neg TAM, dendritic cells (DC), and myeloid-derived suppressor cells (MDSC) showed a clear reduction in IL-10 expression, a cytokine originally described as a negative regulator of IL-12 production in LPS-stimulated peripheral blood mononuclear cells [25]. To date IL-10 cannot be definitively included among the tumor-derived cytokines limiting NK cell activity since both stimulatory and inhibitory effects on NK cell functions have been described. On the contrary, a huge number of data have demonstrated the immunomodulatory function of L-kynurenine, the tryptophan catabolite derived from indoleamine 2,3-dioxigenase 1 (IDO1) pathway [26], and of Prostaglandin E2 (PGE2). Both factors deeply affect the cytokine-mediated upregulation of the expression and function of different activating NK receptors such as NKp46, NKp44 and NKG2D [27-29] a mechanism that seems to involve the c-Jun N-terminal Kinase (JNK) pathway [29]. The extent of *in vivo* suppression mediated by L-kynurenine and PGE2 might be considerable since these factors are released by several cell types colonizing tumor microenvironment, including the cancer-associated fibroblasts (CAF) [30,31], MDSC and DC [32]. In particular, IDO-expressing DC exert a deep immune-suppressive effect by affecting not only proliferation and effector function of NK cells, but also by inducing the conversion of CD4+ T cells into CD4+ CD25+ Foxp3+ regulatory T cell (Treg) [33]. Tumor-derived PGE2, *via* the EPA4 receptor [34,35] decreases human NK cells proliferation, granzyme B/perforin content [36] and drives NK cells towards apoptosis [37]. Moreover, *via* the EPA2 and EPA4 receptors, PGE2 induces the release of TGF-β1 by MDSC [38] that further inhibit NK cell activity. TGF-β1

represents a secretory immune-suppressive hallmark of several other cells in tumor microenvironment including Treg and TAM [39].

TGF-β1

TGF-β1 is the prototypic tumor-derived immunomodulatory soluble mediator, although many additional functions have been described over the years, highlighting its pleiotropic activity. Different studies reported a significant contribution of TGF-β1 in the epithelial to mesenchymal transition (EMT), a process that allows tumors of epithelial origin to acquire a less differentiated, invasive and pro-metastatic phenotype [40,41]. TGF-β1 has been shown to suppress the differentiation process and the effector functions of several immune cells [12]. In particular, it represses the development of human NK cells from CD34+ progenitors and inhibits differentiation of CD16^{pos} NK cells [42], TGF-β1 promotes the conversion of peripheral NK cells to a decidual NK-like phenotype [43] and, as shown in mouse salivary gland, it drives the differentiation of a particular ILC subpopulation sharing NK and ILC1 features [43]. Released as a large latent complex, TGF-β1 remains biologically unavailable until its activation in inflammatory sites such as the tumor microenvironment by signals including low pH, heat, proteases and members of the integrin receptor family [11]. Once activated, TGF-β1 shows a potent inhibitory effect on NK cells, both *in vitro* and *in vivo*, limiting the main NK cell effector functions including IFN-γ production and cytotoxicity [44-48]. In this context, *in vitro* conditioning of NK cells with recombinant TGF-β1 (rTGF-β1) caused severe downregulation of the surface expression of NKp30 and NKG2D, activating NK receptors cooperating in recognition and killing of several tumor histotypes [44]. The same occurred with rTGF-β2 [10], although knockout mice lacking TGF-β1 or TGF-β2 showed distinct phenotypic features suggesting that the two isoforms could also have specific, non-overlapping functions [49]. Interestingly, while the TGF-β1-mediated downregulation of NKp30 occurred at the transcriptional levels no significant changes in NKG2D transcript was observed in TGF-β1-treated NK cells [44]. Accordingly, it has been shown that the reduced NKG2D surface expression observed in the presence of TGF-β1 both *in vitro* and *in vivo*, is due to its capability of downregulating at transcriptional and translational level DAP10, the signaling subunit associated with NKG2D [50-52]. Very recently it has been shown that TGF-β1 also inhibits the IL-15-induced NK cell activation, particularly by selectively and quickly repressing the mTOR pathway [53], a crucial integrator of both pro- and anti-inflammatory signals. Recent data also suggest that the tumor-derived TGF-β1 might modify the migratory capability of NK cells. Indeed, it has been shown that neuroblastoma (NB) cell lines spontaneously release amounts of TGF-β1 capable of modulating the chemokine receptor repertoire of NK cells [10]. In particular NB-derived TGF-β1 increases CXCR4 and CXCR3 surface expression in all NK cells whereas it decreases that of CX3CR1 in the CD56^{dim} NK cell subset. Notably, unusual CX3CR1^{low} CD56^{dim} and CXCR3^{high} CD56^{bright} NK cell populations were observed in peripheral blood of patients with high risk NB (stage 4 or M) [10]. Thus, tumor-derived TGF-β1 can affect the expression of chemokine receptors that play a key role in the bone marrow homing, egress, interaction with endothelium and recruitment into peripheral tissues of NK cells. Recently, in a mouse model of pulmonary allergic responses, it has been shown that TGF-β1 is crucial for the generation of allergic response acting as chemotactic factor recruiting ILC2 and eosinophils [54]. Importantly, it has been demonstrated that TGF-β1 modulates in tumor cells, the expression of specific microRNAs and up-regulates B7-H3 (CD276) [55], a molecule belonging to the family of immune

checkpoint proteins, which acts as co-inhibitory factor and limits the function of NK cells. Overall data suggest that TGF-β1 antagonists, capable of overcoming or blocking its immunomodulatory effect might represent a valuable adjuvant therapy in the cure of different tumors. In this context, it has been recently shown that blocking of TGF-β1R in combination with antibodies targeting the NB-associated antigen GD2, potentiates the NK-mediated anti-NB activity leading to a reduced tumor growth and increased survival of mice injected with NB cell lines or patient-derived neuroblasts [56]. Moreover, ongoing clinical trials will evaluate the benefit of TGF-β or TGF-βR blockade in neoplastic patients (ClinicalTrials.gov NCT02452008; NCT02581787). It is of note however that, due to the wide homeostatic regulatory role of TGF-β, in order to obtain specific and restrained therapeutic effects, light should be made on the signaling pathways mediated by the different TGF-β isoforms and on the mechanisms regulating the immunomodulatory effects.

NK-To-Tumor Contact Immunomodulatory Signals

KIRs/HLA-I and NKG2A/HLA-E

The most powerful inhibitory pathway affecting the NK cell activity is represented by the interaction between HLA class I molecules (HLA-I) on target and specific inhibitory receptors on NK cells (Figure 1). These inhibitory receptors include Killer Ig-like Receptors (iKIRs), clonally distributed receptors distinguishing among allotypic determinants of the classical HLA-A, -B and - C, and the CD94/NKG2A heterodimer specific for HLA-E [57-59]. Mature NK cells can also express the activating counterpart of these HLA-I specific inhibitory receptors, i.e. activating KIRs (aKIRs) and CD94/NKG2C [60,61]. During NK cell maturation the engagement of inhibitory receptors by their self HLA-I ligands confers functional competence to NK cells, through a process referred to as "licensing" or "education" that has been explained by different models [62-64]. Based on the "rheostat" model, during NK cell education NK cell reactivity is tuned by the strength of the inhibitory signal induced by self-HLA-I molecules [65]. The inhibitory receptor repertoire acquired by NK cells during maturation guarantees that in normal conditions inhibitory signals prevail on the activating ones safeguarding HLA-I+ autologous healthy cells from NK cell-mediated killing. The NK cytolytic activity is unleashed in pathologic conditions such as virus infection or tumors where transformed cells increase the expression of ligands for activating NK receptors while downregulating that of HLA-I [66]. NK cell reactivity can be limited when tumor cells retain high levels of HLA-I expression, as occur in hematological malignancies such as acute lymphoblastic leukemia (ALL). However, it has been shown that in allogeneic settings such as in the context of haploidentical hematopoietic stem cell transplantation (haplo-HSCT), the differentiation of alloreactive NK cells from the donor, i.e cells expressing KIRs specific for HLA-I molecules absent in the recipient, strongly improve anti-leukemic surveillance [67,68].

The studies describing the beneficial graft versus leukemia (GvL) effect of alloreactive NK cells in haplo-HSCT has inspired the design of new immunotherapies aimed to enhance anti-tumor NK cell reactivity by blocking the interactions between HLA-I and iKIR or CD94/NKG2A. A fully human anti-KIR mAb (1-7F9, Lirilumab, IPH2101) that recognizes iKIRs (KIR2DL1, KIRDL2 and KIR2DL3) has been generated [69], which favors the NK cell-mediated killing of HLA-matched tumor cells as documented in vitro and in vivo in phase I/II clinical trials involving Acute Myeloid Leukemia (AML) and multiple

myeloma (MM) patients, [69,70]. Interestingly, Lirilumab has been successfully combined with the anti-CD20 rituximab to augment NK-mediated cytotoxicity against lymphoma cells in vitro [71]. Based on the broad expression of HLA-E on both solid and hematological malignances, a novel therapeutic approach has been designed to block the CD94/NKG2A-HLA-E interaction, by using the humanized anti-NKG2A Monalizumab, which is currently in a phase I/II clinical trial [72].
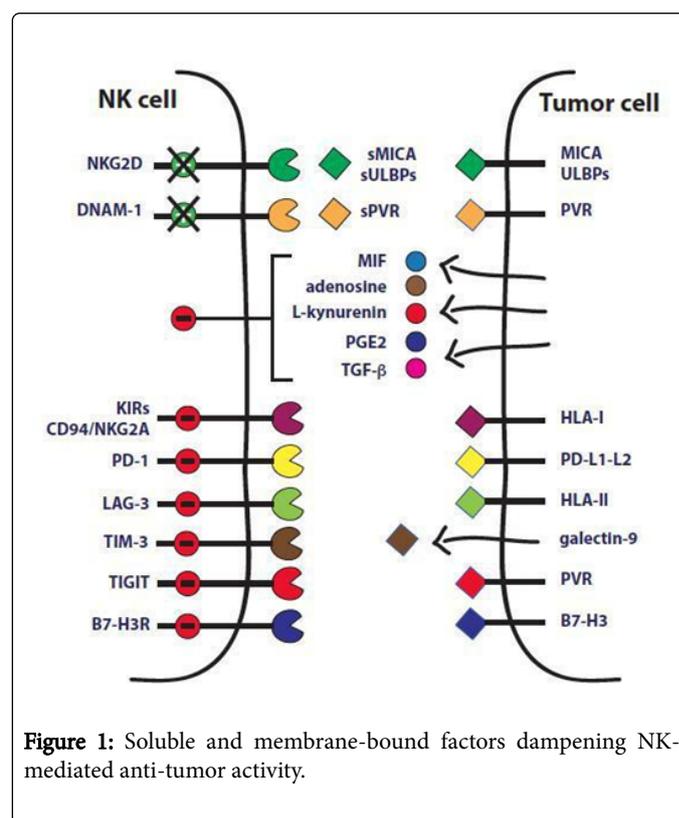

Figure 1: Soluble and membrane-bound factors dampening NK-mediated anti-tumor activity.

This novel approach was developed upon the observation that NKG2A+ NK cells predominate in the early period of immune reconstitution after HSCT, thus representing optimal targets to potentiate NK cell-mediated anti-leukemic activity [73]. In addition, NKG2A+ NK cells express higher levels of activating receptors such as NCRs as compared to more differentiated KIR+ NKG2A- NK cells [6]. The high expression level could compensate the low cytotoxic potential displayed by these less differentiated NK cells. KIRs, CD57 and LIR represent phenotypic hallmarks of terminally differentiated NK cells, which show a good cytolytic potential but poor responsiveness to cytokines [74]. Interestingly, in individuals exposed to pathogens such as cytomegalovirus (CMV), the mature NK cell population is characterized by a very high percentage of cells expressing CD94/NKG2C or aKIR [75-78], which show features similar to cells of adaptive immunity including clonal expansion capability, strong effector functions and longevity. This "memory-like" population represents a powerful candidate for adoptive NK cell transfer therapy in cancer patients [79]. Along this line it is crucial to define whether its efficacy might be limited during NK-to-tumor contacts by additional inhibitory or co-inhibitory signals, whose activity might be particularly relevant in the context of HLA-I^{low} or negative tumors.

PD-1/PD-Ls, LAG-3/HLA-II and TIGIT/PVR

The PD-1/PD-Ls axis is a well-known immune checkpoint, i.e. inhibitory pathways that physiologically maintain self-tolerance and limit the duration and amplitude of T cell immune responses, thus minimizing tissue damage [80-82]. The PD-1 receptor (CD279) has been demonstrated to limit T proliferation and switch off the T cell functions mostly in peripheral tissues. More recent reports show the presence of PD-1^{pos} NK cells in both cancer patients and in a relevant proportion of healthy donors who were serologically positive for human CMV [83-86]. PD-1 expression is confined to terminally differentiated NKG2A^{neg}KIR^{pos}CD57^{pos} NK cells and their antitumor activity can be partially restored *in vitro* by antibodies disrupting the interaction between PD-1 and its cellular ligands PD-L1 and PD-L2 [86,87]. PD-L1 (B7-H1, CD274) and PD-L2 (B7-DC, CD273) belong to the B7 family that consists of several members including B7-H3 (see below) (Figure 1). PD-L1 is expressed in several normal tissues, whereas PD-L2 is mainly restricted to antigen presenting cells (APC) [80,81]. Importantly, both inhibitory ligands can be expressed by tumor cells in response to immunostimulatory factors such as IFN-γ and TNFα that are released by activated T and NK cells [80,85,88]. This phenomenon called "adaptive immune resistance" [88] is a mechanism of escape by which cancer cells adapt their phenotype to the pressure of immune responses. Interestingly, it has recently been shown that PD-L1^{neg} metastatic cells purified from bone marrow aspirates of high risk NB patients have different capabilities of up-regulating PD-L1 in response to IFN-γ [85]. The lack of PD-Ls upregulation in some patient might contribute to a reduced clinical response to therapy with anti-PD-1. However, it should not be disregarded that the therapeutic efficacy of anti-PD1 antibodies has been observed also in patients who at the time of therapeutic decision carried PD-L1^{neg} tumors. Indeed, the clinical benefit might also depend on the strengthening of the crosstalk between PD-Ls+ APC and PD-1+ immune effectors including T or NK cells. IFN-γ is also a potent inducer of HLA class II molecules that can be recognized by LAG-3, an additional inhibitory mechanism that recently emerged in human NK cells together with TIGIT/PVR and TIM-3/galectin-9 interactions (Figure 1) [89-92]. Interestingly, the heterogeneous expression of the TIGIT inhibitory receptor observed in NK cells from healthy individuals inversely correlates with their capability of performing degranulation and IFN-γ release in response to IL-12 stimulation [91]. Moreover, low TIGIT expression has been described in CMV-induced terminally differentiated NK cells that appear more resistant to the inhibitory effect mediated by PVR^{pos} MDSC. Since studies explored the cytokine-induced expression of these co-inhibitory receptors mainly in long term-cultured NK cell lines such as NK92 [93], data on primary NK cells are required to better understand the relative contribution of these inhibitory pathways and the kinetic that regulates their emergence.

B7-H3R/B7-H3

Another interesting NK-to-tumor contact inhibitory pathway is mediated by the B7-H3 ligand, a tumor-associated surface molecule, also present in tumor-derived exosomes [94], which is endowed with both immune-regulatory and pro-tumoral functions. B7-H3 is capable of inhibiting the cytolytic activity of human NK cells against neuroblasts purified from bone marrow aspirates of high risk NB patients, which are characterized by reduced levels of HLA-I [18] and adhesion molecules (unpublished observation). The B7-H3-mediated inhibitory effect, which depends on its interaction with a still unknown inhibitory receptor, is particularly evident when using xenogenic B7-H3^{high} transfectants [95]. This suggests that this inhibitory pathway might require peculiar conditions to be unleashed, which could be represented by poor engagement of potent inhibitory NK receptors and/or by the presence of weak activating signals. It should be mentioned that in mouse B7-H3 has been described as a "friend" in tumor immunology [96]. In particular, intratumoral injection of an expression plasmid encoding mouse B7-H3 led to a complete NK- (and T-) mediated regression in approximately half of tumor-bearing mice [97]. Interestingly, the mouse *B7-H3* gene codes for a molecule characterized by two Ig-like domains while human cell tissues predominantly express a four Ig-like domains isoform resulting from exon duplication [98]. While in human B7-H3 is still an orphan ligand, an activating receptor has been identified in mice that is represented by TREM-like transcript 2 (TREML2, TLT-2), expressed by activated T cells and myeloid cells [99]. Importantly however, Leitner and co-workers who extensively faced this issue did not find evidence for B7-H3/TREML2 interaction in human [100]. Considering that B7-H3 belongs to the B7 family that includes members interacting with both activating and inhibitory receptors, it can't be excluded the existence of a complex scenario resulting from the capability of B7-H3 to engage receptors with opposite signal. However, to date most *in vitro* and *in vivo* data lean toward a B7-H3 inhibitory role in human and the B7-H3R/B7-H3 axis has been included among the immune checkpoints [81,101,102]. An adjuvant therapeutic strategy in cancer might be represented by antibodies disrupting the interaction between B7-H3 and its receptor/s. Different phase I Clinical trials are ongoing with humanized anti-B7-H3 mAbs (NCT02628535; NCT02982941; NCT02475213) [81] and encouraging results have been obtained in the first in-human intrathecal injection of radioiodinated anti-B7-H3 Ab (following standard therapy) in 21 neuroblastoma patients with recurrent Central nervous system (CNS) metastasis [103]. It is of note that the therapeutic efficacy of anti-B7-H3 mAbs might depend not only by the strengthening of the NK- (and T-) mediated anti-tumor responses, but also by the weakening of the direct pro-tumoral activity of B7-H3. Indeed, studies in tumor of different histotype showed that high B7-H3 expression drives tumor cell progression through different molecular mechanisms. These include promotion of migration and invasiveness [104] and reduction of sensibility to chemotherapy-induced apoptosis, as demonstrated in breast [105] and pancreatic carcinoma [106]. Accordingly, high expression of B7-H3 is a negative prognostic factor in several tumors including neuroblastoma [18,107-111]. In particular, in primary neuroblastoma high B7-H3 surface expression, in terms of both intensity and percentage of positive cells, has been correlated with poor event-free survival also in patients with localized disease (stage 1–3), suggesting that high B7-H3 expression might discriminate between low- and high-risk patients who need a more careful follow-up.

Conclusions and Future Perspective

NK cell-based immunotherapy is becoming a promising approach for the treatment of both hematological malignances and solid tumors. However, recent published data show that the complexity of the immune-suppressive milieu characterizing the tumor microenvironment can't be neglected. Indeed, different inhibitory mechanisms represented by soluble factors or by tumor-associated surface ligands could deeply reduce the NK cell activity against tumors. Importantly, malignant cells can constitutively express some of these ligands (HLA-I, B7-H3) or increase/de novo induce their expression (PD-Ls) as an adaptive defense mechanism promoted by

immunostimulatory factors (IFN-γ) that are released during effective NK and TH1 cell-mediated immune responses. Thus, future immunotherapeutic interventions should consider the possible onset in patients of multiple, different immunosuppressive mechanisms affecting the function of endogenous or adoptively transferred NK cells. Also the chemokine receptor repertoire acquired by NK cells during their *in vitro*-expansion needed for the adoptive transfer in patients should not be neglected. Along this line, it might be relevant to hinder the effects of factors (TGF-β1) capable of modifying, in endogenous or infused NK cells, the expression of chemokine receptors crucial for their extravasation and recruitment at the tumor sites.

Executive summary

NK cells and cancer immunotherapy

NK cells are crucial cytolytic effectors in anti-tumor immune responses.

NK cell-based immunotherapy is becoming a promising approach for the treatment of both hematological malignances and solid tumors.

Ready to kill or killers who require to be armed?

Originally described as cells exerting a "natural" cytolytic activity it is now well established that, to exert optimal effector functions, NK cells require activation *via* immunostimulatory cytokines and tumor contact.

NK cell population is heterogeneous and includes subsets characterized by different capabilities of being activated and different effector functions.

Once activated, NK cells need a chemokine receptor repertoire ideal to their recruitment in inflamed tissues such as tumors.

How tumors may dump NK cell function *in vivo*?

Tumor cells purified from patients are less susceptible to NK-mediated killing than established tumor cell lines commonly used *in vitro*.

A complex immunosuppressive milieu is present in the tumor microenvironment.

A plethora of tumor-derived immunomodulatory factors exists, either soluble or membrane bound, that might limit the NK-mediated immune-surveillance *in vivo*.

Soluble immunomodulatory mediators

Tumor cells may release soluble ligands (sMICA, sULBP-2, sPVR and sB7-H6) that compete with membrane-bound tumor isoforms for binding to activating NK receptors.

Different tumor-secreted soluble mediators (MIF, adenosine, L-kynurenine, PGE2, TGF-β1) have been demonstrated to inhibit NK cell function including proliferation, cytotoxicity and cytokine production. These factors can be released by tumor cells as well as by other cell types colonizing the tumor microenvironment (TAM, DC, MDSC, CAF).

TGF-β1 is the prototypic tumor-derived immunomodulatory soluble mediator. In NK cells, it represses development from CD34+ progenitors and differentiation, limits the main effector functions (IFN-γ production and cytotoxicity), reduces the expression level of activating receptors (NKp30, NKG2D) and alters the chemokine receptor repertoire.

NK-to-tumor contact immunomodulatory signals

NK cell reactivity can be limited when tumor cells retain high levels of HLA-I expression, as occur in hematological malignancies.

Tumor cells may express ligands (PD-L1, PD-L2, B7-H3) belonging to the immune checkpoint family, i.e. inhibitory pathways that limit the duration and amplitude of anti-tumor responses.

B7-H3 not only weakens the strengthening of the NK- (and T-) mediated anti-tumor responses, but also exerts a direct pro-tumoral activity.

Tumor cells can constitutively express inhibitory ligands or increase their expression in response to immunostimulatory cytokines (IFN-γ, TNF-α) that are released by activated NK (and T) cells, a phenomenon called "tumor adaptive immune resistance".

IFN-γ, besides inducing PD-Ls expression, is also a potent inducer of HLA- II that can be recognized by LAG-3, an additional inhibitory mechanism that recently emerged together with TIGIT/PVR and TIM-3/galectin-9 interactions.

Future directions

When designing immunotherapies, we can't neglect the complexity of the immune-suppressive milieu characterizing the tumor microenvironment. Thus, NK cell-based therapeutic protocols should combine optimal activation strategies, the selection of the best NK cell subpopulation, consider the *in vivo* persistence of NK cells, and their chemokine receptor repertoire. Valuable adjuvants may include the use of humanized mAb to disrupt immune checkpoints pathways and/or to hinder the effects of soluble factors that *in vivo* may dampen the NK cell activity against tumors.

Acknowledgment

Conflict Of Interest

A.M. is a founder and shareholder of Innate-Pharma (Marseille, France). The remaining authors declare no conflicts of interest.

References

1. Spits H, Artis D, Colonna M, Diefenbach A, Di Santo JP, et al. (2013) Innate lymphoid cells a proposal for uniform nomenclature. Nat Rev Immunol 13: 145-149.

2. Montaldo E, Vacca P, Moretta L, Mingari MC (2014) Development of human natural killer cells and other innate lymphoid cells. Semin Immunol 26: 107-113.

3. Castriconi R, Dondero A, Corrias MV, Lanino E, Pende D, et al. (2004) Natural killer cell-mediated killing of freshly isolated neuroblastoma cells: critical role of DNAX accessory molecule-1-poliovirus receptor interaction. Cancer Res 64: 9180-9184.

4.   Cooper MA, Fehniger TA, Turner SC, Chen KS, Ghaheri BA, et al. (2001) Human natural killer cells: a unique innate immunoregulatory role for the CD56 (bright) subset. Blood 97: 3146-3151.

5.   Brunetta E, Hudspeth KL, Mavilio D (2010) Pathologic natural killer cell subset redistribution in HIV-1 infection: new insights in pathophysiology and clinical outcomes. J Leukocyte Biology 88: 1119-1130.

6.   Béziat V, Descours B, Parizot C, Debré P, Vieillard V (2010) NK cell terminal differentiation: correlated stepwise decrease of NKG2A and acquisition of KIRs. PLoS One 5: e11966.

7.   Björkström NK, Riese P, Heuts F, Andersson S, Fauriat C, et al. (2010) Expression patterns of NKG2A, KIR, and CD57 define a process of CD56dim NK-cell differentiation uncoupled from NK-cell education. Blood 116: 3853-3864.

8.   Cantoni C, Huergo-Zapico L, Parodi M, Pedrazzi M, Mingari MC, et al. (2016) NK Cells, Tumor Cell Transition, and Tumor Progression in Solid Malignancies: New Hints for NK-Based Immunotherapy? J Immunology Research 13: 468.

9.   Carrega P, Bonaccorsi I, Di Carlo E, Morandi B, Paul P, et al. (2014) CD56(bright)perforin(low) noncytotoxic human NK cells are abundant in both healthy and neoplastic solid tissues and recirculate to secondary lymphoid organs via afferent lymph. J Immunol 192: 3805-3815.

10.   Castriconi R, Dondero A, Bellora F, Moretta L, Castellano A, et al. (2013) Neuroblastoma-derived TGF-beta1 modulates the chemokine receptor repertoire of human resting NK cells. J Immunol 190: 5321-5328.

11.   Worthington JJ, Klementowicz JE, Travis MA (2011) TGF-beta : a sleeping giant awoken by integrins. Trends Biochem Sci 36: 47-54.

12.   Seeger P, Musso T, Sozzani S (2015) The TGF-beta superfamily in dendritic cell biology. Cytokine Growth Factor Rev 26: 647-657.

13.   Groh V, Wu J, Yee C, Spies T (2002) Tumour-derived soluble MIC ligands impair expression of NKG2D and T-cell activation. Nature 419: 734-738.

14.   Salih HR, Rammensee HG, Steinle A (2002) Cutting edge: down-regulation of MICA on human tumors by proteolytic shedding. J Immunol 169: 4098-4102.

15.   Paschen A, Sucker A, Hill B, Moll I, Zapatka M, et al. (2009) Differential clinical significance of individual NKG2D ligands in melanoma: soluble ULBP2 as an indicator of poor prognosis superior to S100B. Clin Cancer Res 15: 5208-5215.

16.   Iguchi-Manaka A, Okumura G, Kojima H, Cho Y, Hirochika R, et al. (2016) Increased Soluble CD155 in the Serum of Cancer Patients. PLoS One 11: e0152982.

17.   Schlecker E, Fiegler N, Arnold A, Altevogt P, Rose-John S, et al. (2014) Metalloprotease-mediated tumor cell shedding of B7-H6, the ligand of the natural killer cell-activating receptor NKp30. Cancer Res 74: 3429-3440.

18.   Bottino C, Dondero A, Bellora F, Moretta L, Locatelli F, et al. (2014) Natural killer cells and neuroblastoma: tumor recognition, escape mechanisms, and possible novel immunotherapeutic approaches. Front Immunol 5: 56.

19.   Repp AC, Mayhew ES, Apte S, Niederkorn JY (2000) Human uveal melanoma cells produce macrophage migration-inhibitory factor to prevent lysis by NK cells. J Immunol 165: 710-715.

20.   Krockenberger M, Dombrowski Y, Weidler C, Ossadnik M, Hönig A, et al. (2008) Macrophage migration inhibitory factor contributes to the immune escape of ovarian cancer by down-regulating NKG2D. J Immunol 180: 7338-7348.

21.   Krockenberger M, Kranke P, Hausler S, Monika S, Hönig A, et al. (2012) Macrophage migration-inhibitory factor levels in serum of patients with ovarian cancer correlates with poor prognosis. Anticancer research 32: 5233-5238.

22.   Raskovalova T, Lokshin A, Huang X, Jackson EK, Gorelik E (2006) Adenosine-mediated inhibition of cytotoxic activity and cytokine production by IL-2/NKp46-activated NK cells: involvement of protein kinase A isozyme I (PKA I). Immunol Res 36: 91-99.

23.   Raskovalova T, Huang X, Sitkovsky M, Zacharia LC, Jackson EK, et al. (2005) Gs protein- coupled adenosine receptor signaling and lytic function of activated NK cells. J Immunol 175: 4383-4391.

24.   Beavis PA, Divisekera U, Paget C, Chow MT, John LB, et al. (2013) Blockade of A2A receptors potently suppresses the metastasis of CD73+ tumors.Proc Natl Acad Sci U S A 110: 14711-14716. Qin L, Thompson LF, Kuzel TM, Zhang B (2014) Requirement of NK cells for selective A2A receptor blockade to suppress CD73+ tumor metastasis. Immunotherapy 6: 19-21.

25.   D'andrea A, Aste-Amezaga M, Valiante NM, Ma X, Kubin M, et al. (1993) Interleukin 10 (IL-10) inhibits human lymphocyte interferon gamma-production by suppressing natural killer cell stimulatory factor/IL-12 synthesis in accessory cells. JEM 178: 1041-1048.

26.   Vacchelli E, Aranda F, Eggermont A, Sautès-Fridman C, Tartour E, et al. (2014) Trial watch: IDO inhibitors in cancer therapy. Oncoimmunology 3: e957994.

27.   Della Chiesa M, Carlomagno S, Frumento G, Balsamo M, Cantoni C, et al. (2006) The tryptophan catabolite L-kynurenine inhibits the surface expression of NKp46- and NKG2D-activating receptors and regulates NK-cell function. Blood 108: 4118-4125.

28.   Pietra G, Manzini C, Rivara S,Vitale M, Cantoni C, et al. (2012) Melanoma cells inhibit natural killer cell function by modulating the expression of activating receptors and cytolytic activity. Cancer research 72: 1407-1415.

29.   Song H, Park H, Kim J, Park G, Kim YS, et al. (2011) IDO metabolite produced by EBV-transformed B cells inhibits surface expression of NKG2D in NK cells via the c-Jun N-terminal kinase (JNK) pathway. Immunology letters 136: 187-193.

30.   Balsamo M, Scordamaglia F, Pietra G, Manzini C, Cantoni C, et al. (2009) Melanoma-associated fibroblasts modulate NK cell phenotype and antitumor cytotoxicity. Proc Natl Acad Sci U S A 106: 20847-20852.

31.   Li T, Yang Y, Hua X, Wang G, Liu W, et al. (2012) Hepatocellular carcinoma-associated fibroblasts trigger NK cell dysfunction via PGE2 and IDO. Cancer letters 318: 154-161.

32.   Löb S, Königsrainer A, Rammensee HG, Opelz G, Terness P (2009) Inhibitors of indoleamine-2,3-dioxygenase for cancer therapy: can we see the wood for the trees? Nat Rev Cancer 9: 445-452.

33.   Munn DH, Sharma MD, Lee JR, Jhaver KG, Johnson TS, et al. (2002) Potential regulatory function of human dendritic cells expressing indoleamine 2,3-dioxygenase. Science 297: 1867-1870.

34.   Holt D, Ma X, Kundu N, Fulton A (2011) Prostaglandin E(2) (PGE (2)) suppresses natural killer cell function primarily through the PGE(2) receptor EP4. Cancer Immunol Immunother 60: 1577-1586.

35.   Ma X, Holt D, Kundu N, Reader J, Goloubeva O, et al. (2013) A prostaglandin E (PGE) receptor EP4 antagonist protects natural killer cells from PGE2-mediated immunosuppression and inhibits breast cancer metastasis. Oncoimmunology 2: e22647.

36.   Hodge G, Barnawi J, Jurisevic C, Moffat D, Holmes M , et al. (2014) Lung cancer is associated with decreased expression of perforin, granzyme B and interferon (IFN)-gamma by infiltrating lung tissue T cells, natural killer (NK) T-like and NK cells. Clin Exp Immunol 178: 79-85.

37.   Li T, Zhang Q, Jiang Y, Yu J, Hu Y, et al. (2016) Gastric cancer cells inhibit natural killer cell proliferation and induce apoptosis via prostaglandin E2. Oncoimmunology 5: e1069936.

38.   Mao Y, Sarhan D, Steven A, Seliger B, Kiessling R, et al. (2014) Inhibition of tumor-derived prostaglandin-e2 blocks the induction of myeloid-derived suppressor cells and recovers natural killer cell activity. Clin Cancer Res 20: 4096-4106.

39.   Mantovani A, Sica A (2010) Macrophages, innate immunity and cancer: balance, tolerance, and diversity. Curr Opin Immunol 22: 231-237.

40.   Thiery JP, Acloque H, Huang RY, Nieto MA (2009) Epithelial-mesenchymal transitions in development and disease. Cell 139: 871-890.

41.   Xu J, Lamouille S, Derynck R (2009) TGF-beta-induced epithelial to mesenchymal transition. Cell Res 19: 156-172.

42. Allan DS, Rybalov B, Awong G, Zúñiga-Pflücker JC, Kopcow DH, et al. (2010) TGF-beta affects development and differentiation of human natural killer cell subsets. Eur J Immunol 40: 2289-2295.

43. Cerdeira AS, Rajakumar A, Royle CM, Lo A, Husain Z, et al. (2013) Conversion of peripheral blood NK cells to a decidual NK-like phenotype by a cocktail of defined factors. J Immunol 190: 3939-3948.

44. Castriconi R, Cantoni C, Della Chiesa M, Vitale M, Marcenaro E, et al. (2003) Transforming growth factor beta 1 inhibits expression of NKp30 and NKG2D receptors: consequences for the NK-mediated killing of dendritic cells. Proc Natl Acad Sci U S A 100: 4120-4125.

45. Laouar Y, Sutterwala FS, Gorelik L, Flavell RA (2005) Transforming growth factor-beta controls T helper type 1 cell development through regulation of natural killer cell interferon-gamma. Nat Immunol 6: 600-607.

46. Yu J, Wei M, Becknell B, Trotta R, Liu S, et al. (2006) Pro- and antiinflammatory cytokine signaling: reciprocal antagonism regulates interferon-gamma production by human natural killer cells. Immunity 24: 575-590.

47. Trotta R, Dal Col J, Yu J, Ciarlariello D, Thomas B, et al. (2008) TGF-beta utilizes SMAD3 to inhibit CD16-mediated IFN-gamma production and antibody-dependent cellular cytotoxicity in human NK cells. J Immunol 181: 3784-3792.

48. Mamessier E, Sylvain A, Thibult ML, Houvenaeghel G, Jacquemier J, et al. (2011) Human breast cancer cells enhance self tolerance by promoting evasion from NK cell antitumor immunity. JCI 121: 3609-3622.

49. Shi Y, Massagué J (2003) Mechanisms of TGF-beta signaling from cell membrane to the nucleus. Cell 113: 685-700.

50. Lee JC, Lee KM, Ahn YO, Suh B, Heo DS (2011) A possible mechanism of impaired NK cytotoxicity in cancer patients: down-regulation of DAP10 by TGF-beta1. Tumori 97: 350-357.

51. Park YP, Choi SC, Kiesler P, Gil-Krzewska A, Borrego F, et al. (2011) Complex regulation of human NKG2D-DAP10 cell surface expression: opposing roles of the gammac cytokines and TGF-beta1. Blood 118: 3019-3027.

52. Sun C, Fu B, Gao Y, Liao X, Sun R, et al. (2012) TGF-beta down-regulation of NKG2D/DAP10 and 2B4/SAP expression on human NK cells contributes to HBV persistence. PLoS Pathog 8: e1002594.

53. Viel S, Marçais A, Guimaraes FS, Loftus R, Rabilloud J, et al. (2016) TGF-beta inhibits the activation and functions of NK cells by repressing the mTOR pathway. Sci Signal 9: ra19.

54. Denney L, Byrne AJ, Shea TJ, Buckley JS, Pease JE, et al. (2015) Pulmonary Epithelial Cell-Derived Cytokine TGF-beta1 Is a Critical Cofactor for Enhanced Innate Lymphoid Cell Function. Immunity 43: 945-958.

55. Zhou X, Mao Y, Zhu J, Meng F, Chen Q, et al. (2016) TGF-beta1 promotes colorectal cancer immune escape by elevating B7-H3 and B7-H4 via the miR-155/miR-143 axis. Oncotarget 7: 67196-67211.

56. Tran HC, Wan Z, Sheard MA (2016) TGF betaR1 Blockade with Galunisertib (LY2157299) Enhances Anti-Neuroblastoma Activity of Anti-GD2 Antibody Dinutuximab (ch14.18) with Natural Killer Cells. Clinical cancer research : an official journal of the American Association for Cancer.

57. Moretta A, Bottino C, Vitale M, Pende D, Biassoni R, et al. (1996) Receptors for HLA class-I molecules in human natural killer cells. Annu Rev Immunol 14: 619-648.

58. Lanier LL (1998) NK cell receptors. Annu Rev Immunol 16: 359-393.

59. Parham P (2005) MHC class I molecules and KIRs in human history, health and survival. Nat Rev Immunol 5: 201-214.

60. Moretta A, Sivori S, Vitale M, Moretta L (1995) Existence of both inhibitory (p58) and activatory (p50) receptors for HLA-C molecules in human natural killer cells. J Exp Med 182: 875-884.

61. Braud VM, Allan DS, O'Callaghan CA, Söderström K, D'Andrea A, et al. (1998) HLA-E binds to natural killer cell receptors CD94/NKG2A, B and C. Nature 391: 795-799.

62. Kim S, Poursine-Laurent J, Truscott SM, Lybarger L, Song YJ, et al. (2005) Licensing of natural killer cells by host major histocompatibility complex class I molecules. Nature 436: 709-713.

63. Anfossi N, André P, Guia S, Falk CS, Roetynck S, et al. (2006) Human NK cell education by inhibitory receptors for MHC class I. Immunity 25: 331-342.

64. Brodin P, Karre K, Hoglund P (2009) NK cell education: not an on-off switch but a tunable rheostat. Trends Immunol 30: 143-149.

65. Joncker NT, Fernandez NC, Treiner E, Vivier E, Raulet DH (2009) NK cell responsiveness is tuned commensurate with the number of inhibitory receptors for self-MHC class I: the rheostat model. J Immunol 182: 4572-4580.

66. Long EO, Kim HS, Liu D, Peterson ME, Rajagopalan S (2013) Controlling natural killer cell responses: integration of signals for activation and inhibition. Annu Rev Immunol 31: 227-258.

67. Pende D, Marcenaro S, Falco M, Martini S, Bernardo ME, et al. (2009) Anti-leukemia activity of alloreactive NK cells in KIR ligand-mismatched haploidentical HSCT for pediatric patients: evaluation of the functional role of activating KIR and redefinition of inhibitory KIR specificity. Blood 113: 3119-29.

68. Ruggeri L, Aversa F, Martelli MF, Velardi A (2006) Allogeneic hematopoietic transplantation and natural killer cell recognition of missing self. Immunol Rev 214: 202-218

69. Romagne F, Andre P, Spee P, Zahn S, Anfossi N, et al. (2009) Preclinical characterization of 1-7F9, a novel human anti- KIR receptor therapeutic antibody that augments natural killer-mediated killing of tumor cells. Blood 114: 2667-2677.

70. Benson DM Jr, Bakan CE, Zhang S, Collins SM, Liang J, et al. (2011) IPH2101, a novel anti-inhibitory KIR antibody, and lenalidomide combine to enhance the natural killer cell versus multiple myeloma effect. Blood 118: 6387-6391.

71. Kohrt HE, Thielens A, Marabelle A, Sagiv-Barfi I, Sola C, et al. (2014) Anti-KIR antibody enhancement of anti-lymphoma activity of natural killer cells as monotherapy and in combination with anti-CD20 antibodies. Blood 123: 678-686.

72. Mcwilliams EM, Mele JM, Cheney C, Timmerman EA, Fiazuddin F, et al. (2016) Therapeutic CD94/NKG2A blockade improves natural killer cell dysfunction in chronic lymphocytic leukemia. Oncoimmunology 5: e1226720.

73. Ruggeri L, Urbani E, Andre P, Mancusi A, Tosti A, et al. (2016) Effects of anti-NKG2A antibody administration on leukemia and normal hematopoietic cells. Haematologica 101: 626-633.

74. Muccio L, Bertaina A, Falco M, Pende D, Meazza R, et al. (2016) Analysis of memory-like natural killer cells in human cytomegalovirus-infected children undergoing alphabeta+T and B cell-depleted hematopoietic stem cell transplantation for hematological malignancies. Haematologica 101: 371-381.

75. Della Chiesa M, Falco M, Bertaina A, Muccio L, Alicata C, et al. (2014) Human cytomegalovirus infection promotes rapid maturation of NK cells expressing activating killer Ig-like receptor in patients transplanted with NKG2C-/- umbilical cord blood. J Immunol 192: 1471-1479.

76. Della Chiesa M, Falco M, Podesta M, Locatelli F, Moretta L, et al. (2012) Phenotypic and functional heterogeneity of human NK cells developing after umbilical cord blood transplantation: a role for human cytomegalovirus? Blood 119: 399-410.

77. Foley B, Cooley S, Verneris MR, Curtsinger J, Luo X, et al. (2012) Human cytomegalovirus (CMV)-induced memory-like NKG2C(+) NK cells are transplantable and expand in vivo in response to recipient CMV antigen. J Immunol 189: 5082-5088.

78. Guma M, Angulo A, Vilches C, Gomez-Lozano N, Malats N, et al. (2004) Imprint of human cytomegalovirus infection on the NK cell receptor repertoire. Blood 104: 3664-71.

79. Della Chiesa M, Moretta L, Muccio L, Bertaina A, Moretta F, et al. (2016) Haploidentical Haematopoietic Stem Cell Transplantation: Role of NK Cells and Effect of Cytomegalovirus Infections. Curr Top Microbiol Immunol 395: 209-224.

80. Pardoll DM (2012) The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer 12: 252-264.

81. Shin DS, Ribas A (2015) The evolution of checkpoint blockade as a cancer therapy: what's here, what's next? Curr Opin Immunol 33: 23-35.

82. Zitvogel L, Kroemer G (2012) Targeting PD-1/PD-L1 interactions for cancer immunotherapy. Oncoimmunology 1: 1223-1225.

83. Benson DM Jr, Bakan CE, Mishra A, Hofmeister CC, Efebera Y, et al. (2010) The PD-1/PD-L1 axis modulates the natural killer cell versus multiple myeloma effect: a therapeutic target for CT-011, a novel monoclonal anti-PD-1 antibody. Blood 116: 2286-2294.

84. Terme M, Ullrich E, Aymeric L, Meinhardt K, Desbois M, et al. (2011) IL-18 induces PD-1-dependent immunosuppression in cancer. Cancer Res 71: 5393-5399.

85. Dondero A, Pastorino F, Della Chiesa M, Corrias MV, Morandi F, et al. (2016) PD-L1 expression in metastatic neuroblastoma as an additional mechanism for limiting immune surveillance. Oncoimmunology 5: e1064578.

86. Pesce S, Greppi M, Tabellini G, Rampinelli F, Parolini S, et al. (2017) Identification of a subset of human natural killer cells expressing high levels of programmed death 1: A phenotypic and functional characterization. J Allergy Clin Immunol 139: 335-346.

87. Ghiotto M, Gauthier L, Serriari N, Pastor S, Truneh A, et al. (2010) PD-L1 and PD-L2 differ in their molecular mechanisms of interaction with PD-1. Int Immunol 22: 651-660.

88. Taube JM, Anders RA, Young GD, Xu H, Sharma R, et al. (2012) Colocalization of inflammatory response with B7-h1 expression in human melanocytic lesions supports an adaptive resistance mechanism of immune escape. Sci Transl Med 4: 127ra37.

89. Anderson AC, Joller N, Kuchroo VK (2016) Lag-3, Tim-3, and TIGIT: Co-inhibitory Receptors with Specialized Functions in Immune Regulation. Immunity 44: 989-1004.

90. Sarhan D, Cichocki F, Zhang B, Yingst A, Spellman SR, et al. (2016) Adaptive NK Cells with Low TIGIT Expression Are Inherently Resistant to Myeloid-Derived Suppressor Cells. Cancer Res 76: 5696-5706.

91. Wang F, Hou H, Wu S, Tang Q, Liu W, et al. (2015) TIGIT expression levels on human NK cells correlate with functional heterogeneity among healthy individuals. Eur J Immunol 45: 2886-2897.

92. Komita H, Koido S, Hayashi K, Kan S, Ito M, et al. (2015) Expression of immune checkpoint molecules of T cell immunoglobulin and mucin protein 3/galectin-9 for NK cell suppression in human gastrointestinal stromal tumors. Oncol Rep 34: 2099-2105.

93. Sun H, Sun C, Xiao W (2014) Expression regulation of co-inhibitory molecules on human natural killer cells in response to cytokine stimulations. Cytokine : 33-41.

94. Marimpietri D, Petretto A, Raffaghello L, Pezzolo A, Gagliani C, et al. (2013) Proteome profiling of neuroblastoma-derived exosomes reveal the expression of proteins potentially involved in tumor progression. PLoS One 8: e75054.

95. Castriconi R, Dondero A, Augugliaro R, Cantoni C, Carnemolla B, et al. (2004) Identification of 4Ig-B7-H3 as a neuroblastoma-associated molecule that exerts a protective role from an NK cell-mediated lysis. Proc Natl Acad Sci U S A 101: 12640-12645.

96. Wang L, Kang FB, Shan BE (2013) B7-H3-mediated tumor immunology: Friend or foe? Int J Cancer 134: 2764-2771.

97. Sun X, Vale M, Leung E, Kanwar JR, Gupta R, et al. (2003) Mouse B7-H3 induces antitumor immunity. Gene Ther 10: 1728-1734.

98. Chapoval AI, Ni J, Lau JS, Wilcox RA, Flies DB, et al. (2001) B7-H3: a costimulatory molecule for T cell activation and IFN-gamma production. Nat Immunol 2: 269-274.

99. Hashiguchi M, Kobori H, Ritprajak P, Kamimura Y, Kozono H, et al. (2008) Triggering receptor expressed on myeloid cell-like transcript 2 (TLT-2) is a counter-receptor for B7-H3 and enhances T cell responses. Proc Natl Acad Sci U S A 105: 10495-10500.

100. Leitner J, Klauser C, Pickl WF, Stöckl J, Majdic O, et al. (2009) B7-H3 is a potent inhibitor of human T-cell activation: No evidence for B7-H3 and TREML2 interaction. Eur J Immunol 39: 1754-1764.

101. Janakiram M, Pareek V, Cheng H, Narasimhulu DM, Zang X (2016) Immune checkpoint blockade in human cancer therapy: lung cancer and hematologic malignancies. Immunotherapy 8: 809-819.

102. Picarda E, Ohaegbulam KC, Zang X (2016) Molecular Pathways: Targeting B7-H3 (CD276) for Human Cancer Immunotherapy. Clin Cancer Res 22: 3425-3431.

103. Kramer K, Kushner BH, Modak S, Pandit-Taskar N, Smith-Jones P, et al. (2010) Compartmental intrathecal radioimmunotherapy: results for treatment for metastatic CNS neuroblastoma. J Neurooncol 97: 409-418.

104. Lemke D, Pfenning PN, Sahm F, Klein AC, Kempf T, et al. (2012) Costimulatory protein 4IgB7H3 drives the malignant phenotype of glioblastoma by mediating immune escape and invasiveness. Clin Cancer Res 18: 105-117.

105. Liu H, Tekle C, Chen YW, Kristian A, Zhao Y, et al. (2011) B7-H3 silencing increases paclitaxel sensitivity by abrogating Jak2/Stat3 phosphorylation. Mol Cancer Ther 10: 960-971.

106. Zhao X, Zhang GB, Gan WJ, Xiong F, Li Z, et al. (2013) Silencing of B7-H3 increases gemcitabine sensitivity by promoting apoptosis in pancreatic carcinoma. Oncol Lett 5: 805-812.

107. Gregorio A, Corrias MV, Castriconi R, Dondero A, Mosconi M, et al. (2008) Small round blue cell tumours: diagnostic and prognostic usefulness of the expression of B7-H3 surface molecule. Histopathology 53: 73-80.

108. Roth TJ, Sheinin Y, Lohse CM, Kuntz SM, Frigola X, et al. (2007) B7-H3 ligand expression by prostate cancer: a novel marker of prognosis and potential target for therapy. Cancer Res 67: 78937900.

109. Zang X, Thompson RH, Al-Ahmadie HA, Serio AM, Reuter VE, et al. (2007) B7-H3 and B7x are highly expressed in human prostate cancer and associated with disease spread and poor outcome. Proc Natl Acad Sci U S A 104: 19458-19463.

110. Sun TW, Gao Q, Qiu SJ, Zhou J, Wang XY, et al. (2012) B7-H3 is expressed in human hepatocellular carcinoma and is associated with tumor aggressiveness and postoperative recurrence. Cancer immunology, immunotherapy : CII 61: 2171-2182.

111. Wang L, Zhang Q, Chen W, Shan B, Ding Y, et al. (2013) B7-H3 is overexpressed in patients suffering osteosarcoma and associated with tumor aggressiveness and metastasis. PloSone 8: e70689.