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NK cell MHC class I specific receptors (KIR): from biology to clinical intervention

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The natural killer (NK) cell effector response towards infected cells or tumoural cells is guided by the integration of activating and inhibitory signals sensed by NK cell surface receptors. Major histocompatibility complex class I specific inhibitory receptors expressed by NK cells have two distinct roles: while allowing self tolerance, they are also needed for the acquisition of NK cell functional competence, a process termed education. In the context of allotransplantation, NK cell alloreactivity, arising from the expression on donor NK cells of inhibitory killer Ig-like receptors (KIRs) that do not recognize human leukocyte antigen from the patient, has shown clinical benefit for leukaemia patients. Based on these genetic studies, a blocking antibody directed against KIRs, as well as allogeneic NK cell infusions are now tested in clinical trials in various oncology indications. They offer promising immunotherapeutic approaches for the treatment of cancer patients.

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Introduction

Natural killer (NK) cells, at the frontier between innate and adaptive immunity, can kill cancer and infected cells and release cytokines that contribute to inflammation and immunoregulation. NK cells have been initially identified through their capacity to kill tumour cells (hence their name). Since then, the anti-tumour effect of NK cells has been documented in many aspects. *In vitro*, mouse and human NK cells can kill a broad array of tumour cells of haematopoietic and non-haematopoietic origin. *In vivo*, mouse NK cells can eliminate many transplantable and spontaneous tumours [1,2]. In the human, their role in the eradication of acute myeloid leukaemia (AML) in the situation of minimal residual disease after haematopoietic stem cell transplantation (HSCT) is strongly suggested [34] and has triggered interest in the manipulation of these cells for the treatment of cancer.

NK cell recognition of altered self

NK cells are regulated by a set of cell surface receptors that finely tune their potent effector functions. NK cells express various triggering receptors responsible for their activation. Natural cytotoxicity receptors, mostly restricted to NK cells and comprising NKp46, NKp30 and NKp44, play an important role in tumour cell killing. The cellular ligands recognized by these receptors are still elusive, with the exception of B7-H6, a ligand for NKp30 [3]. Other major receptors in NK cell-mediated recognition and killing of some tumours are NKG2D [4,5], whose ligands are the stress-inducible MICA/B and ULBP proteins [6,7], DNAM-1, specific for poliovirus receptor (PVR) and Nectin-2 [8], and CD16 that mediates antibody-dependent cellular cytotoxicity, an important mechanism of action of therapeutic monoclonal antibodies (mAbs). Collectively, NK cell activating receptors sense changes in the expression levels of their ligands induced by cellular stress. These ligands are self proteins, which are usually rare on normal cells, but are upregulated at the cell surface during either infection or tumoural transformation. As often seen in biological system, these potent activating signals are balanced by inhibitory receptors. Overall, NK cell activation is governed by the quantitative balance between activating and inhibitory signals.

The KIR family of MHC class I specific receptors

A group of inhibitory receptors interacts specifically with major histocompatibility complex (MHC) class I molecules. These receptors prevent NK cell-mediated attack against normal cells whereas cells with compromised MHC class I expression (e.g. by tumour transformation or viral infection) become susceptible to NK cell-mediated killing. In humans, there are two main types of inhibitory receptors for human leukocyte antigen (HLA) class I molecules: (i) killer Ig-like receptors (KIRs) that belong to the Ig superfamily and are specific for determinants shared by groups of HLA-A, HLA-B or HLA-C allotypes and (ii) CD94/NKG2A, a heterodimer related to C-type lectins that recognizes HLA-E, an HLA class Ib molecule loaded with peptides from leader sequence of classical MHC class I

Table 1

KIR molecules and their HLA ligands

Receptor	Number of alleles*	Ligand
KIR2DL1	43	HLA-C2: C*02, C*04, C*05, C*06
KIR2DL2	28	HLA-C1: C*01, C*03, C*07, C*08 Some HLA-C2: C*0501, C*0202, C*0401 Some HLA-B: B*4601, B*7301
KIR2DL3	34	HLA-C1: C*01, C*03, C*07, C*08 Some HLA-C2: C*0501, C*0202 Some HLA-B: B*4601, B*7301
KIR3DL1	73	Some HLA-A and HLA-B expressing Bw4 epitope HLA B*08, B*27, B*57, B*58 HLA-A: A*24, A*23, A*32
KIR3DL2	84	Some HLA-A: A*03, A*11
KIR3DL3	107	Unknown
KIR2DL5A and B	41	Unknown
KIR2DL4	46	HLA-G
KIR2DS1	15	HLA-C2: C*02, C*04, C*05, C*06
KIR2DS2	22	Unknown
KIR2DS3	14	Unknown
KIR2DS4	30	HLA-C: C*0501, C*1601, C*0202 Some HLA-A: A*1102
KIR2DS5	16	Unknown
KIR3DS1	16	Unknown

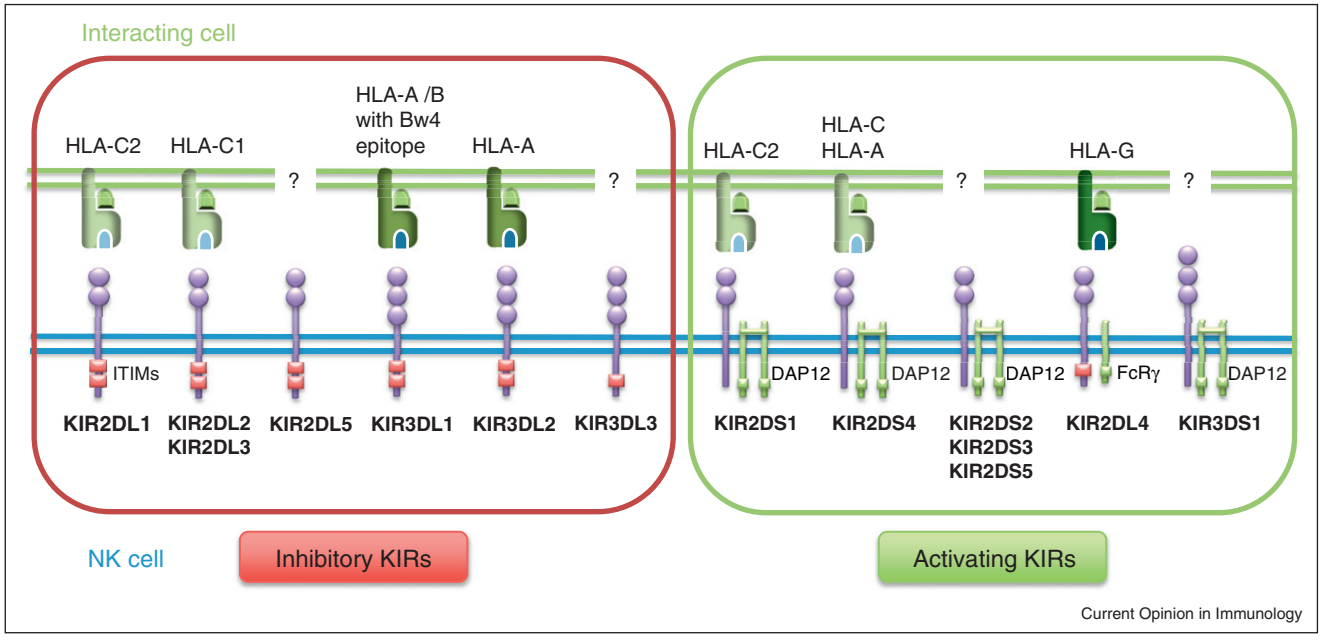
*Alleles can be found at http://www.ebi.ac.uk/ipd/kir/stats.html (update April 2011).

molecules [9]. Both type of receptors display immunoreceptor tyrosine-based inhibition motifs (ITIMs); ITIMs are tyrosine-phosphorylated upon crosslinking, and recruit tyrosine phosphatases that dephosphorylate activating adaptor molecules and shut down activation.

The human KIR family comprises polymorphic molecules expressed on NK cells and small subsets of $\alpha\beta^+$ and $\gamma\delta^+$ T cells. Inhibitory KIRs have long cytoplasmic tails (KIR-L) containing two ITIMs. Each KIR has as its ligand a subgroup of HLA class I allotypes, and each KIR displays two (KIR2DL) or three (KIR3DL) extracellular Ig-domains conferring specificity for HLA-C or HLA-A/B allotypes, respectively. KIR2DL1 and KIR2DL2/3 recognize distinct HLA-C allotypes, based on polymorphisms at positions 77 and 80 in the α1-domain of the HLA heavy chain. For example, KIR2DL1 binds HLA-Cw2, HLA-Cw4, HLA-Cw5 and HLA-Cw6 (called C2) whereas KIR2DL2 and KIR2DL3 bind to HLA-Cw1, HLA-Cw3, HLA-Cw7 and HLA-Cw8 (called C1) (Table 1, [10,11,12•,13]). Collectively, the inhibitory KIR2DL1, KIR2DL2 and KIR2DL3 receptors recognize all HLA-C allotypes. KIR3DL1 ligands are HLA molecules sharing the Bw4 epitope representing around 50% of human HLA-B alleles, and KIR3DL2 binds HLA-A3 and HLA-A11 [10] (Figure 1). While the specificity of KIR receptors has not been challenged since their initial discovery, some fine specificities have been revealed. KIR2DL1 has a high affinity for C2 allotypes, whereas KIR2DL2/3 has a high affinity for C1, but also for few members of HLA-B alleles, and a low affinity for C2 allotypes. The latter reactivity was found to be negligible in early studies, but was demonstrated to be functionally relevant with partial inhibition of KIR2DL2/3$^+$ NK cells against C2 expressing targets [14•,15•]. This partial cross-reactivity is consistent with the evolution of KIR2D in primates where an ancestor KIR2D having specificity for all modern C-type alleles predated a duplication in two members of KIR and C alleles with distinct specificities [15•]. An additional level of complexity is provided at the genetic level as the KIR locus is highly polymorphic for allele and gene content (Figure 2, Table 1). *KIR-L* genes are intermingled with *KIR-S* genes that are highly homologous in their extracellular domains but display a short cytoplasmic tail devoid of ITIM. KIR-S are associated with DAP12 signalling adaptor, via a charged amino-acid residue in their transmembrane domain, providing them with activating functions. A KIR-S version exists for virtually all KIR-L molecules. At the population level, KIR haplotypes can be defined with two major types (Figure 2). Haplotypes A and B share inhibitory KIR-L for HLA-C1 and HLA-C2 molecules, but differ strongly in their KIR-S content. Haplotype A, representing 50% of individuals, is devoid of most KIR2DS [16]. The function of KIR-S is still largely elusive. They probably recognize HLA molecules or modified HLA molecules, but only the specificity of KIR2DS1 for C2 alleles has been firmly established both biochemically and functionally (activating function against C1 bearing cells) [14•,17].

Although the definition of inhibitory receptors in other species is beyond the scope of this review, it is important to keep in mind that the CD94/NKG2A and MHC class I inhibitory systems are present in most mammals, and particularly in rodents. There are, however, major differences in rodents in the molecular structure of MHC class I

Figure 1



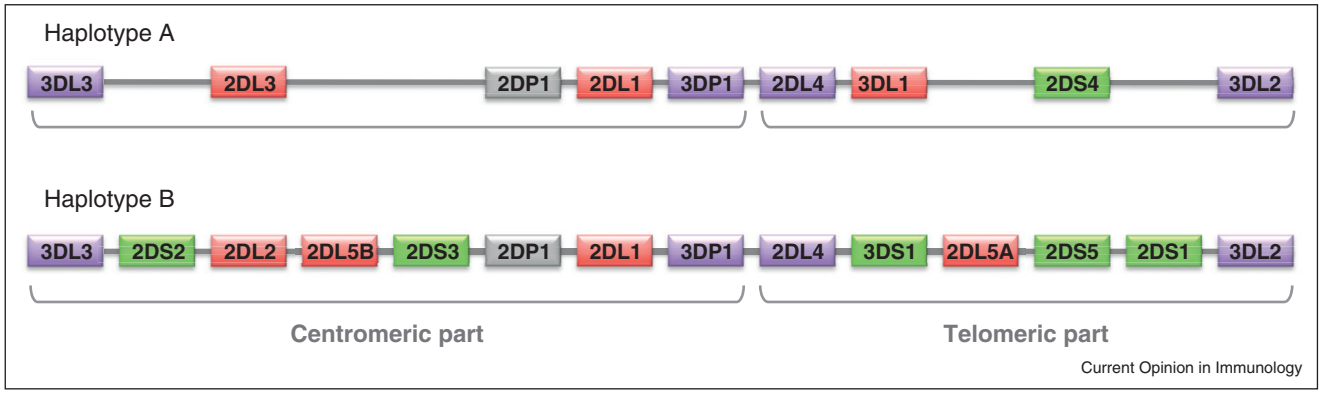
KIR receptors on human NK cells and their ligands. The KIR family comprises inhibitory receptors, with a long cytoplasmic tail containing ITIMs, and activating receptors that associate to activating adaptor molecules (DAP12 or FcRγ). KIRs have two or three extracellular Ig-like domains, conferring their specificity for HLA ligands. Detailed specificity for each KIR is described in Table 1.

specific inhibitory receptors that are lectin-like dimeric molecules of the Ly49 family [18].

Expression of KIRs and constitution of a functional repertoire of NK cells

During NK cell development, the expression of NKG2A and KIR-L and KIR-S is variegated, and diverse NK cell subsets displaying various combinations of inhibitory receptors emerge. During NK cell ontogeny, $CD56^{bright}$ NK cells expressing high density of NKG2A and few KIRs evolve to a set of terminally differentiated $CD56^{dim}$ $NKG2A^{-}$ KIR^{+} cells with potent effector functions [19]. NK cell subsets devoid of inhibitory receptors and NK cell subsets expressing one or several inhibitory receptors are present in a given individual and there is apparently no wholesale deletion of any subset. Nevertheless the HLA ligand content of an individual influences to a certain extent the relative proportion of the different subsets [12•,20•].

Figure 2



Schematic representation of haplotypes A and B at the KIR locus. KIR locus is located on the human chromosome region 19q13.4. Two examples of haplotypes A and B are depicted. Pseudogenes are indicated with grey boxes, activating receptor genes are in green, and inhibitory receptor genes in red. Conserved genes, which can encode activating or inhibitory receptor or be pseudogenes, are in purple boxes. Each centromeric haplotype fragment can combine with any telomeric haplotype fragment, giving rise to a high diversity of KIR haplotypes.

Such stochastic expression may lead to the generation of autoreactive cells. Indeed, cells that do not express any inhibitory receptor or cells that express a unique inhibitory receptor having no cognate ligand in a given individual (for example KIR2DL1 single positive NK cells in a C1/C1 homozygotes) do exist and could be potentially activated by normal self. Two mechanisms control this potential reactivity: first, normal cells express very few ligands for activating receptors, making unlikely that the threshold for NK cell activation is reached; second, a process referred to as education has been demonstrated both in humans and in mice [21–23]. Interaction of KIRs or Ly49 with MHC class I finely and quantitatively tunes NK cell reactivity so that NK cells expressing two inhibitory receptors for self MHC class I molecules display higher cytotoxic potential against a missing self target compared to NK cells expressing only one or no inhibitory receptor [24]. The precise molecular mechanism, timing and localization of this process remain largely unknown. However this education process was recently shown to be dynamic and reversible [25••,26••], and is associated with a reorganization of the plasma membrane resulting in the confinement of activating receptors in nanodomains in educated NK cells [27•].

NK cell education results in the generation of diverse populations of circulating NK cells with different potential effector functions against missing self but the final outcome of an NK cell response also depends on the density of activating ligands on target cells and on the cytokine milieu. Inhibitory receptor null cells, although hyporesponsive to missing self, exert potent antiviral activity presumably due to extensive cytokine production and high density of activating ligands on infected cells [28]. Apart from their expression on NK cells, KIR molecules are also expressed on a small subpopulation of CD8 T cells, and KIR-L have been demonstrated to inhibit TCR-mediated signals [29–31], in some cases in tumour infiltrating lymphocytes. Their *de novo* expression has been linked to chronic stimulation of T cells for example by recurrent viral infections. Their physiological role in T cell response dampening against infected or cancer cells or in the maintenance of memory cells remains to be established.

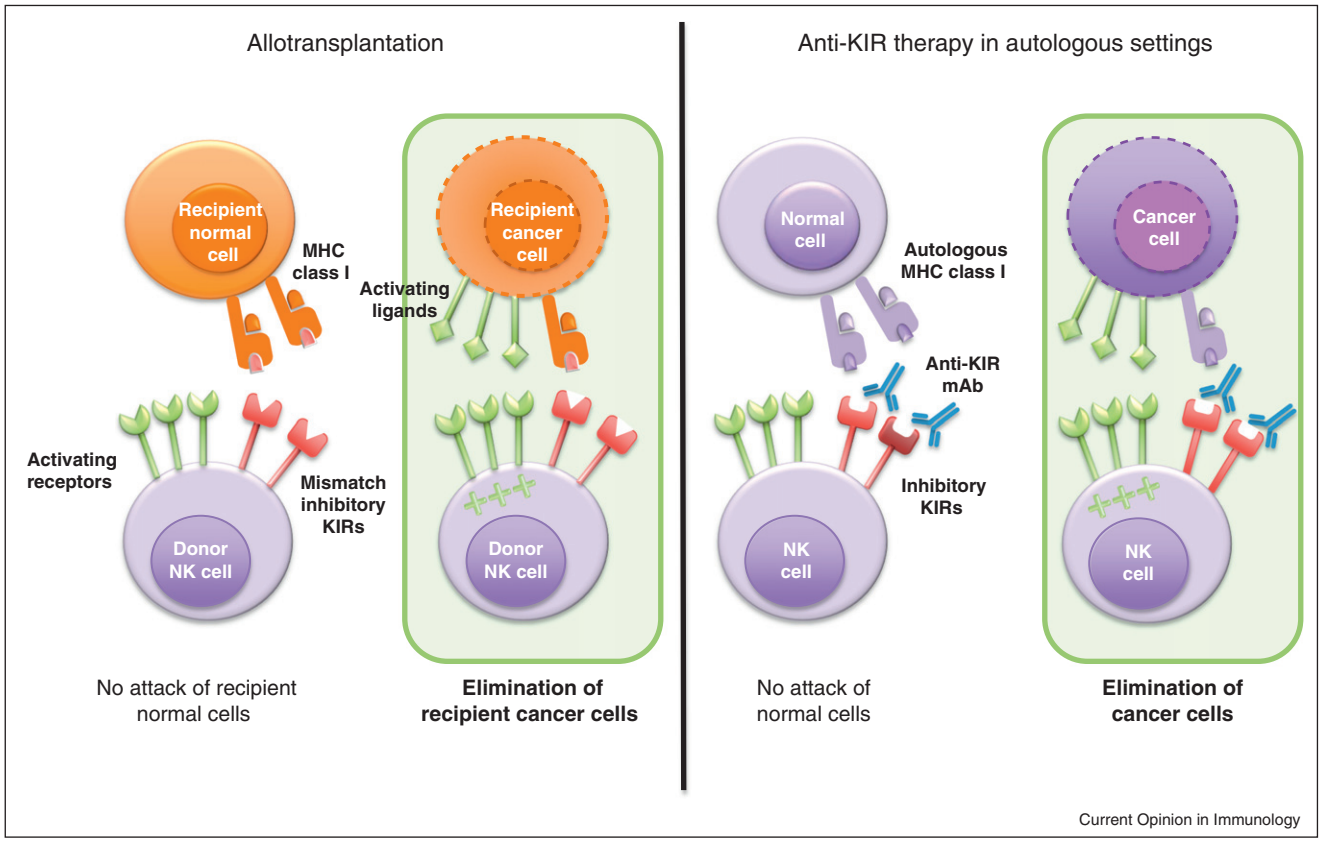
Therapeutic intervention in oncology based on the manipulation of the KIR system

Interest in the modulation of NK cell activity based on KIR manipulation has grown in recent years, arising from both preclinical and clinical studies showing that NK cell reactivity can be essential in tumour immunosurveillance and eradication of established haematological diseases [32]. In humans, allotransplantation has revealed an interesting role of inhibitory receptors, both in AML and multiple myeloma (MM) [33,34]. For certain donor recipient pairs, having genetic differences in MHC class I genes between the donor and the recipient, KIR expressing cells of the donor do not find their inhibitory MHC class I ligands in the recipient, leaving donor NK cells uninhibited (these donor NK cells are 'alloreactive NK cells') (Figure 3). As demonstrated *in vitro*, AML cells express ligands for NK cell receptors but are protected from NK cell lysis by MHC class I molecules. In haploidentical, MHC mismatch HSCT, clinical benefit correlates with the presence of alloreactive NK cells from the donor [34,35]. A role of KIRs in the outcome of unrelated transplantation for AML patients has also recently been shown at the genotype level, B haplotypes correlating with improved patient survival [36]. It remains to be seen if the particular context of allotransplantation provides NK cells with a favourable activation status (for example due to cytokines produced during immune reconstitution) necessary for their activity.

Although haploidentical procedures have shown very good results in AML, and now in paediatric acute lymphoblastic leukaemia (ALL, Locatelli *et al.*, personal communication), they are very complex as clinical procedures and are difficult to apply broadly. Most current allotransplantation settings are based on fully MHC-matched donor recipient pairs where KIR mismatch effect cannot be achieved. Injection of mature KIR-mismatched NK cells is currently being attempted in several clinical trials [37,38]. Initial attempts to work with preparations of purified NK cells led to promising results, although with a limited number of patients. In MM patients, autologous HSCT followed by injection of purified, short-term IL-2-stimulated, KIR-mismatched NK cells did not lead to graft failure; NK cells survived at least a few days *in vivo* and they were shown to kill MM blasts *in vitro* [39]. In a protocol not involving HSCT, purified, short-term IL-2-activated, haploidentical NK cells were injected into AML and other haematologic cancer patients after mild conditioning to avoid rejection of injected cells. In this study, injected NK cells survived in the host for a few days and were well tolerated [34]. Encouraging clinical signs in terms of disease free survival were seen in the above protocols, but Phase II clinical trials will be required to draw solid conclusions.

New drugs targeting NK cells are also in development that could provide more practicability for NK cell manipulation. The most advanced compound targeting NK cell population is a blocking anti-KIR monoclonal antibody. This antibody recognizes KIR2DL1/L2/L3 (a subset representing half of the overall NK cell population) and therefore blocks the inhibition imposed by all HLA-C alleles, allowing its use in all patients whatever their KIR and HLA genotypes. Preclinical data in mice with a surrogate antibody (anti-Ly49) demonstrated an anti-cancer effect in leukaemia models outside the context of transplantation [40]. Absence of toxicity and particularly of haematological toxicity was also demonstrated in these studies showing the existence of a therapeutic window for KIR blockade. In addition, studies in

Figure 3



Regulation of NK cell effector function. NK cells sense the density of expression of activating and inhibitory molecules at the surface of interacting cell. The integration by NK cells of these positive and negative signals leads either to tolerance to normal self or to NK cell activation and cytotoxicity. In the context of allotransplantation, donor NK cells can express KIRs having no HLA ligand in the recipient. These NK cells can therefore kill cancer cells that express high levels of activating ligands, while sparing patient normal cells that do not express sufficient amount of activating molecules. This situation can be mimicked in cancer patients by blocking inhibitory receptors on NK cells with a monoclonal antibody.

wild type and in KIR/HLA transgenic mice both demonstrated that the blockade of MHC specific inhibitory receptors for several weeks with antibodies did not abolish NK cell reactivity, suggesting that this treatment did not drastically interfere with NK cell education [41,42•].

Building on allotransplantation results in AML and MM, as well as on preclinical data showing reconstitution of NK cell lysis by anti-KIR mAb towards MHC class I^+ MM and AML blasts *in vitro* and in mouse models [43•], this anti-KIR2DL1/2/3 antibody has now been tested in Phase I clinical trials with good tolerability in both indications, confirming that the level of activated ligands on normal cells is not sufficient to induce toxicity (N Vey and D Benson *et al.*, manuscripts in preparation).

It remains to be seen if the administration of this blocking antibody to patients will provide clinical efficacy in Phase II trials without affecting NK cell development. Different treatment combinations providing additional activating signals to NK cells could synergize with anti-KIR mAb, like drugs that upregulate activating NK cell ligands on tumour cells (chemotherapy [44] or bortezomib [45]) or that boost NK cell activity (cytokines or therapeutic mAbs working through ADCC). Interestingly, lenalidomide, an approved drug for MM with immunostimulatory effects on NK cells [46], has an additive effect with anti-KIR mAb, *in vitro* and *in vivo* in preclinical models [47].

Concluding remarks

NK cells have emerged as a key component of the immune system with both a direct effect on cancer cells and infected cells and an effect on regulatory functions that drive and tune the immune response. The expression of different sets of inhibitory receptors leads to a repertoire of cells endowed with different potential to recognize the absence of MHC class I (missing self) during a maturation process that remains to be precisely dissected. KIR molecules have been shown to be key factors that influence the control of at least some tumours. Therapeutic tools are now available to manipulate those cells and these tools are currently being tested in the clinics.

The ontogeny and maturation of NK cells, their migratory properties and half-life will be important issues to address during these trials to take full advantage of these promising approaches.

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