Monitoring NK cell activity in patients with hematological malignancies

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Natural killer (NK) cells are lymphocytes of the innate immune system that can recognize and kill various types of malignant cells. Monitoring the activity of peripheral NK cells in patients affected by hematological malignancies may provide prognostic information or unveil ongoing tumor-specific immune responses. Moreover, further insights into the biology of NK cells might also promote the development of novel strategies for stimulating their anticancer activity. Here, we review the main methods to monitor phenotypic and functional NK cell properties in cancer patients, focusing on individuals affected by multiple myeloma, a hematological malignancy currently treated with immunomodulatory drugs.

Introduction

The classification of cancers deriving from the hematopoietic system has become increasingly complex with the advent of novel techniques of molecular and cellular biology that can be used to precisely characterize malignant cell clones.[1] Nonetheless, hematological neoplasms can be roughly classified into lymphomas and leukemias. The former are lymphoid tumors initially confined to peripheral lymphoid organs and extranodal tissues, while the latter include both lymphoid and myeloid malignancies that originate in the bone marrow but generally invade the peripheral blood. All hematological cancers are therefore exposed very early during oncogenese and throughout tumor progression to effectors of the immune system. Thus, the immunological microenvironment should be taken into particular consideration to fully understand and treat hematological malignancies.

The term "cancer immunosurveillance" is generally employed to describe the process whereby the immune system eliminates newly formed malignant cells. After an initial debate on the physiological relevance of this progress, it is now widely accepted that the interaction between malignant cells and immune cells is one of the most prominent parameters determining disease outcome in cancer patients. In line with this notion, Hanahan and Weinberg have recently added two novel features that highlight the complex interplay between developing tumors and the immune system to the six hallmarks of malignancy that they had originally proposed in 2000.[2] These novel hallmarks are the ability of neoplastic cells to avoid immune destruction, and the ability of chronic inflammation to promote tumor progression.[3] As a result, pharmaceutical companies are now developing several anticancer drugs that operate via the immune system, both in its innate and adaptive components.

Natural killer (NK) cells are innate lymphocytes recently reclassified as members of the group 1 of innate lymphoid cells (ILC1).[4] NK cells are defined by their capacity to kill target cells upon recognition through a set of activating and inhibitory receptors. In the course of immune responses, NK cells are rapidly activated by monocytes[5] and dendritic cells[6] trans-presenting the immunostimulatory cytokine interleukin-15 (IL-15). This rapid (6–12 h) process primes NK cells to kill their targets mainly through the polarized release of cytotoxic granules that contain the pore-forming factor perforin, granzymes, and several other proteins. NK cells also secrete interferon γ (IFNγ) and other cytokines upon stimulation, in particular when this is mediated by the combination of IL-12 and IL-18.

NK cells play an important role in the early defense against intracellular pathogens.[7] Within lymphoid organs, they are strategically positioned in the proximity of sentinel macrophages that line the lymphatic sinus, where they can efficiently respond to cytokine signals emanated from pathogen-sensing phagocytes by secreting IFNγ.[8] NK cells have been shown to kill not only infected cells, but also malignant cells of various origin, in vitro and in vivo. This latter property underpinned their discovery in the 1970s and drew considerable interest from tumor immunologists. Subsequently, it was found that NK cells are capable to sense the absence of MHC class I molecules on the surface of target cells through inhibitory receptors of the killer cell immunoglobulin-like receptor (KIR) family in humans and Ly49 in mice.[9] Such an absence of MHC class I molecules, which is often referred to as "missing-self," characterize many cancers, in particular of hematological origin, and is thought to originate from a step of T cell-dependent selection. NK cells are also equipped

with a variety of activating receptors that altogether contribute to their ability to recognize and kill neoplastic cells. The prototypical NK-cell activating receptor is killer cell lectin-like receptor subfamily K, member 1 (KLRK1, best known as NKG2D), which recognizes various proteins expressed on the surface of target cells in response to several forms of cellular stress, including DNA damage, infection and oncogenic stress.[10] In humans, many malignancies of hematopoietic or non-hematopoietic origin (but not healthy tissues) also express natural killer cell cytotoxicity receptor 3 ligand 1 (NCR3LG1, best known as B7-H6) on their surface, which can be recognized by the NK-cell activating receptor natural cytotoxicity receptor 3 (NCR3, also known as NKp30).[11]

Taken together, these observations suggest that NK cells are an important component of the endogenous arsenal of anticancer defenses, especially at early stages of oncogenesis and tumor progression. In this context, NK cells might indeed detect and kill transformed cells, in turn favoring the activation of tumor-associated antigen (TAA)-specific T and B lymphocytes. Later on, NK cells might also play an important effector functions, in particular in the presence of TAA-targeting antibodies. In fact, human NK cells are believed to be among the most prominent executor of antibody-dependent cellular cytotoxicity (ADCC), owing to a robust expression of Fc fragment of IgG, low affinity IIIa, receptor (FCGR3A, also known as CD16a).[12] Nonetheless, NK cells are often insufficient to mediated tumor regression, and a general decrease of NK-cell functions is frequently observed in cancer patients.[13–15] Presumably, this originates from tumor-derived mediators that negatively regulate NK-cell activity, such as transforming growth factor β (TGFβ), or to the intense chemotherapeutic regimens that are often employed in cancer patients.

Here, we review the rationale and the methods for monitoring the function of circulating NK cells in individuals affected by hematopoietic malignancies. In addition, we summarize the results of a series of studies on NK-cell function in multiple myeloma (MM) patients, to illustrate what principles can be learned from such a monitoring and what questions should be addressed in the near future.

Why Monitoring the Activity of NK Cells in Cancer Patients?

NK-cell activity as surrogate marker of general immunological functions

Jérôme Galon's group has defined the immune contexture as the type, functional orientation, density and location of adaptive immune cells within distinct regions of solid neoplasms.[16] They then quantified all these parameters in patients with colon cancer to delineate an "immunoscore" that turned out to have a better prognostic value than the classical scoring system relying on histological and anatomical features of neoplastic lesions.[17] This study highlighted not only the critical importance of immunological functions in tumor rejection but also the value of monitoring the immunological status of individuals affected by cancer. Of note, the immunoscore in its present form is not particularly adapted to tumors of hematological origin, as these

grow within lymphoid organs that are naturally infiltrated with immune cells. Moreover, as T cells infiltrating solid tumors are generally believed to be TAA-specific, their level is supposed to reflect the intensity of antitumor immune responses. In the circulation, the situation is completely different, as TAA-specific T cells are diluted within a huge amount of T cells with unrelated specificities. Thus, the assessment of immune responses against hematological cancers must proceed by alternative techniques.

Circulating immune cells have been monitored for a long time for different purposes, as the peripheral blood is rapidly accessible without the need for invasive procedures. Often, the status of circulating immune cells is argued not to reflect that of their tumor-infiltrating counterparts. In the case of hematological malignancies, however, the blood de facto constitutes the tumor site or a site of intense cancer cell transit. Evaluating circulating immune cells is therefore highly relevant for many types of hematological tumors. But, what should be evaluated? How? Why? We believe that monitoring the activity of circulating NK cells is useful for the following reasons. First, this can be performed with simple assays (see below), as most NK cell subsets (in spite of their notable repertoire) can respond to malignant cells (be them nude or coated with antibodies) in a few hours. Second, it may provide indirect insights into the general proficiency of the immune system, as often the two parameters are correlated with each other. For example, in the course of chronic infection[18] or during metastatic tumor dissemination,[19] the activity of NK and T cells often decreases concomitantly, a phenomenon that may reflect a significant degree of interdependence between innate and adaptive components of the immune system. Third, it could give indications on tumor immunogenicity. In fact, the activation of NK cells may represent a hint of ongoing antitumor immune response. By analogy to the immunoscore approach, it would therefore be interesting to consider the prognostic value of the activity of circulating NK cells in patients with hematological malignancies. This parameter has already been shown to influence disease outcome in patients affected by other types of cancer.[20]

Predicting or monitoring the effect of anticancer (immune) therapy

Monitoring the function of circulating NK cells is obviously essential when patients are treated with drugs that are supposed to directly target NK cells. IPH2101 is a human monoclonal antibody specific that blocks the interaction between common KIRs (including KIR2DL1, KIR2DL2, and KIR2DL3) and their ligands, thus potentiating the cytotoxic activity of NK cells against autologous cancer cells in vitro.[21] At least theoretically, IPH2101 might be beneficial for the treatment of various cancers, provided that it really increases the cytotoxic activity of NK cells in patients. Phase I clinical trials involving IPH2101 have already been conducted in patients affected by refractory MM, a setting in which IPH2101 significantly increased the cytotoxic activity of NK against autologous myeloma cells ex vivo.[22]

The efficacy of several new anticancer agents relies (as a whole or in part) on the elicitation of anticancer immune responses. These therapeutic agents include an increasing large panel of tumor-targeting monoclonal antibodies. Among them, rituximab as well as second-generation anti-CD20 antibodies are

now commonly used for the treatment of B-cell malignancies, based on their ability to stimulate ADCC. However, a subset of patients does not respond well to anti-CD20 antibodies, some of them because of a polymorphism in the FCGR3A-coding gene that lowers the affinity of the receptor on NK cells for IgG_1.[23] Monitoring the activity of NK cells against autologous target cells coated with anti-CD20 antibodies prior to use would hence be helpful to identify those patients that have the highest chances to respond to therapy (an example of personalized medicine). A number of strategies are also under investigation to stimulate ADCC in patients receiving tumor-targeting therapeutic antibodies. In this context, monoclonal antibodies are often combined with immunostimulatory cytokines such as IL-2 and granulocyte macrophage colony-stimulating factor (GM-CSF) or immunomodulatory drugs (IMiDs). It will therefore be important to correlate the ability of peripheral NK cells to mediated ADCC with the therapeutic efficacy of these combinatorial regimens.[24]

Along similar lines, many conventional chemotherapeutic agents exert anticancer effects that depend on the immune system, especially when given at "metronomic," non-toxic doses.[25] Monitoring the activity of circulating NK cells in this setting may lead to potentially important discoveries. Vice versa, it will be important to understand which of the immunosuppressants that are currently employed in the clinics inhibit NK-cell function.[26] Indeed, as immunosuppressants are generally given to inhibit adaptive autoimmune responses, they should ideally spare the innate immune system, leaving patients with an important barrier against infections.

Understanding the impact of cancer cells on immunological functions

Why can NK cells function normally in cancer patients or, more often, be significantly impaired in their activity? Obtaining precise insights into the cell-intrinsic and cell-extrinsic circuitries that regulate the function of NK cells in physiological and pathological settings may lead to the development of novel strategies to stimulate for therapeutic purposes. It is therefore essential to monitor the activity of NK cells with appropriate protocols prior to the initiation of in-depth analyses.

Techniques to Monitor the Activity of Circulating NK Cells

Effector functions

For several decades, the activity of NK cells has been measured using the ^{51}Cr release assay. In this assay, target cells (most often the erythroblastic leukemia K562 cells) were loaded with ^{51}Cr and then co-cultured for 4 h with peripheral blood mononuclear cells (PBMCs). Eventually, the release of ^{51}Cr in culture supernatants was quantified as a marker of target cell death. This simple assay has several drawbacks. First, its results are highly dependent on the ratio between effector and target cells. Thus, limited extents of target cell death may originate from a low percentage of NK cells in the PBMC sample as well as from a poor cytotoxic activity of individual NK cells. Second, it does not provide insights into the activity of individual NK cells relative to

other parameters, such as cytokine secretion. For these reasons, the activity of NK cells nowadays is preferentially measured by means of the so-called lysosomal-associated membrane protein 1 (LAMP1, best known as CD107a) assay.

The LAMP1 assay measures the release of secretory lysosomes (cytotoxic granules) by NK cells that enter in contact with target cells, a phenomenon commonly referred to as degranulation. The membrane of cytotoxic granules contains proteins, such as LAMP1, that are transiently exposed on the surface of NK cells upon fusion of granules with the plasma membrane. Thus, the staining of NK cells maintained in non-permeabilizing conditions with fluorochrome-labeled LAMP1-specific antibodies coupled to flow cytometry identifies cells that have recently undergone degranulation. Importantly, degranulation has been shown to correlate with the cytotoxic activity of NK cells,[27] and occurs rapidly after their contact with target cells (usually within an hour). The frequency of degranulating NK cells also turned out to be less dependent on the effector to target cell ratio than lysis of target cells (TW et al., unpublished observations). Moreover, the measurement of degranulation by flow cytometry can be combined with the evaluation of cytokine/chemokine expression levels through an intracellular staining with specific antibodies.[28,29] Upon ex vivo stimulation, NK cells mainly secrete IFNγ, tumor necrosis factor α (TNFα), chemokine (C-C motif) ligand 3 (CCL3), CCL4, and CCL5.[30] A low fraction of activated NK cells may also secrete GM-CSF, IL-10, and IL-13.[31] Flow cytometry allows for the precise identification of NK cells expressing one or more cytokine/chemokine(s). Usually, all cytokine-expressing cells also show signs of degranulation. Of note, a wide range of stimuli can be used to stimulate NK cells to degranulate and secrete cytokines/chemokines. For example, transformed cells including K562 cells, which do not express MHC class I molecules on their surface, and lymphoma B cells coated with anti-CD20 antibodies induce robust NK cell responses.

Expression of cell surface receptors and intracellular proteins

Multiparametric flow cytometry now allows for the measurement of up to 12 fluorescent signals in a single tube. Hence, even with limited blood samples, multiple phenotypic and functional parameters of circulating NK cells can be analyzed (**Table 1**). Mean fluorescence intensity is generally used to quantify the level of expression of each receptor. If both the cytofluorometer and the staining procedure are well calibrated, data from patients analyzed on different occasions can be compared with accuracy. This information can relate to:

NK-cell activation

Upon stimulation with cytokines such as IL-2 or IL-15 or interaction with target cells, NK cells express increased levels of CD25, CD69, CD25, NCR2 (also known as NKp44), and granzyme B. Recent reports have also demonstrated that in the course of various infectious diseases, NK cells upregulate expression the expression of KLRC2 killer cell lectin-like receptor subfamily C, member 2 (KLRC2, also known as NKG2C).[32,33]

NK-cell receptors and maturation

NK cells are equipped with a complex set of activating and inhibitory receptors that critically regulate their function in

Table 1. Phenotypic and functional parameters of NK cells that can be assessed by flow cytometry

Parameter	Flow cytometry-compatible marker
Cytotoxicity	CD107a, GZMA, GZMB, PRF1
Cytokine secretion	GM-CSF, IFNγ, IL-10, IL-13, TNFα
Chemokine secretion	CCL3, CCL4, CCL5, XCL1
Activation	CD25, CD69, GZMB, KLRC2, NCR2
Maturation	CD56, CD57, KIR, KLRC1, SELL
Activating receptors	CD16, CD226, CD244, KLRK1, NCRs, KIR-S
Inhibitory receptors	KIR-L, LILRB1

Abbreviations: CCL, chemokine (C-C motif) ligand; GM-CSF, granulocyte macrophage colony-stimulating factor; GZM, granzyme; IFNγ, interferon γ; IL, interleukin; KIR, killer cell immunoglobulin-like receptor; KLRC, killer cell lectin-like receptor subfamily C; KLRK1, killer cell lectin-like receptor subfamily K, member 1; LILRB1, leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 1; NCR, natural cytotoxicity triggering receptor; PRF1, perforin 1; SELL, selectin L; TNFα, tumor necrosis factor α; XCL1, chemokine (C motif) ligand 1.

response to putative target cells, including malignant cells. In addition, NK cells express various cytokine and chemokine receptors, which also profoundly influence their activation and effector functions. Tumors often alter the expression of these receptors on the surface of NK cells, a process that may favor the escape of malignant cells from immunosurveillance.[34] Upon development in the bone marrow, NK cells operate a maturation process during which they stop proliferating, acquire effector functions, and express a peculiar profile of receptors (including activatory and inhibitory, as well as cytokine/chemokine receptors) on their surface. Also the maturation of NK cells is often inhibited by neoplasms.[35] The monitoring of this process by flow cytometry has been recently refined on the basis of several markers including selectin L (SELL, also known as CD62L)[36] and killer cell lectin-like receptor subfamily C, member 1 (KLRC1, also known as NKG2A).[37]

Repertoire of NK cells

Multiparametric flow cytometry and epitope-specific antibodies also allow for the study of the NK-cell repertoire. Using a panel of 5 antibodies against inhibitory receptors of the KIR and NKG2D family and a boolean gating strategy, Björkström et al. have recently demonstrated that 27 different NK-cell populations can be identified that express different combinations of the 5 receptors analyzed.[38] How the repertoire of NK cells evolves in the course of tumor progression is currently unknown.

Intracellular mediators

Intracellular staining procedures can be implemented to measure not only the expression level of cytokines and chemokines, but also that of proteins involved in the cytotoxic functions of NK cells, such as granzyme A, granzyme B, and perforin. Recently, several antibodies specific for transcription factors that regulate the activity of NK cells have also been generated. In particular, this is the case of T-box 21 (TBX21, best known as T-bet) and eomesodermin (EOMES), 2 transcription factors that are essential for the development and maturation of murine NK cells. The expression of these transcription factors by cytotoxic lymphocytes is altered in the course of chronic infection,[39] and

could therefore be deregulated also in the context of oncogenesis and tumor progression.

Gene expression

Microarrays are valuable tools to measure global gene expression profiles and identify molecular pathways that are altered by tumors. So far, only a few studies have investigated global changes of gene expression in NK cells isolated from cancer patients. One study reported no difference in the gene expression profile of NK cells obtained from healthy individuals and melanoma patients.[40] Conversely, signs of activation were detected in NK cells infiltrating non-small cell lung carcinoma lesions as compared with NK cells located in the healthy lung parenchima of the same patient.[41] Clearly, this type of approach, which is not extensively used yet, will help deciphering the role of NK cells in cancer immunosurveillance and will open avenues to manipulate the antineoplastic functions of these cells.

Monitoring NK Cells in Multiple Myeloma Patients

Phenotype of NK cells in MM patients

MM is a malignant disease of plasma cells that, despite major therapeutic progresses, is incurable. Like many neoplasms, MM is associated with a marked deficiency in general immunological function, which presumably contributes to the escape of cancer cells from immunosurveillance.[42] The role of NK cells in the control of MM has been intensively investigated. A seminal study by Carbone et al. demonstrate that NK cells could kill autologous MM cells in a NKG2D- and NCR-dependent manner.[43] Moreover, MM cell lines derived from early-stage patients were found to express low levels of MHC class I molecules and high levels of the NKG2D ligand MHC class I polypeptide-related sequence A (MICA), while cell lines obtained from late-stage patients displayed the reverse phenotype, suggesting the existence of an NK cell-mediated pressure in the course of MM progression.[43]

Table 2 summarizes the results of several studies[44–52] that monitored the number, phenotype and functional activity of circulating NK cells in patients affected by MM at different stages of the disease, as compared with NK cells from the blood of healthy individuals. These studies suggest that patients with active MM have an increased number of circulating NK cells.[44,46,52] Of note, such an increase is also detectable in patients affected by monoclonal gammopathy of undetermined significance (MGUS), an asymptomatic pre-malignant disorder characterized by the proliferation of a plasma cell clone in the bone marrow that may precede overt myeloma. The expansion of NK cells in MM patients is not associated with their activation, as no increase in CD25, CD69, and HLA-DR levels has been observed in this setting.[45] In line with this notion, two studies reported a decrease in NKG2D expression on the surface of NK cells from MM patients.[49,51] MM progression has been shown to correlate with increased levels of soluble MICA in the circulation, an event that may promote the downregulation of NKG2D on cytotoxic lymphocytes.[49] However, no correlation between the levels of NKG2D on the surface of NK cells from MM patients and the concentration of circulating soluble MICA has been detected thus far.[51]

Table 2. Variations in phenotypic and functional NK-cell parameters as detected in the circulation of multiple myeloma patients

Ref.	NK cell number	Activation status	Activating receptors	Cytotoxicity	Notes
44	Increased			Unchanged	Increased NK also in MGUS
45	Unchanged	Unchanged			
46	Increased			Decreased	Increased NK also in MGUS
47	Increased in BM				Increased NK also in MGUS
48			Decreased CD16 Decreased CD244 Unchanged KLRK1 Unchanged NCR		Trend toward reduced KLRK1 levels in MM
49			Decreased KLRK1		Bortezomib increased MICA levels on MM cells
50				Decreased	Stage-dependent decrease
51			Decreased KLRK1		Not linked with soluble MICA levels
52	Increased also in BM				

Abbreviations: BM, bone marrow; KLRK1, killer cell lectin-like receptor subfamily K, member 1; MICA, HC class I polypeptide-related sequence A; MGUS, monoclonal gammopathy of undetermined significance; MM; multiple myeloma; NCR, natural cytotoxicity triggering receptor; NK, natural killer.

Irrespective of its cause, the downregulation of NKG2D may contribute to the escape of MM from immunosurveillance as well as to downregulation of MICA levels on the surface of MM cells. Presumably, this is not the only strategy whereby MM cells evade recognition by NK cells. Indeed, Fauriat et al. have observed a downregulation of the activating receptors CD244 (also known as 2B4) and CD16a on NK cells from MM patients.[48] Moreover, Benson et al. have reported that NK cells from MM patients express the inhibitory receptor programmed cell death 1 (PDCD1), while normal NK cells do not.[53] A therapeutic antibody that blocks the interaction between PDCD1 and its ligand CD274 (best known as PD-L1), which is expressed by MM cells, has been shown to enhance the activity of NK cells against autologous MM cells, through effects on both NK-cell trafficking and cytotoxicity. Alterations in the phenotype of NK cells commonly observed in MM patients might also be associated with a decrease in cytotoxic activity. This has been demonstrated in 2 studies based on the ^{51}Cr assay,[46,50] one of which even reported a tumor stage-dependent decrease in NK-cell functions.[50] The capacity of NK cells from MM patients to secrete cytokines and chemokines has not yet been addressed.

In summary, while the number of circulating NK cells increases in MM patients, these cells express an altered pattern of activating and inhibitory receptors and exhibit functional defects that impair their ability to control oncogenesis and tumor progression.

Effect of diverse treatments for MM on the phenotype of NK cells

A wide range of anticancer agents have positive effects on the immune system that de facto contribute to their therapeutic efficacy.[25] In particular, several novel treatments against hematological malignancies have an impact on NK cells.[54] In the case of MM, the therapeutic efficacy of IMiDs (such as lenalidomide) is believed to originate, at least in part, from the activation of antitumor immune responses. Thus, the administration of lenalidomide to PBMCs ex vivo activated the cytotoxic activity of NK cells (against both MHC class I-deficient cell lines and antibody-coated targets).[55] Such an activation is rather indirect, as it involves the secretion of IL-2 by CD4$^+$ T cells[56] as well as the production of specific cytokines by dendritic cells.[57] However, a clear effect of lenalidomide on NK cells in vivo remains to be formally demonstrated. In a murine model of severe immunodeficiency (SCID mice), the combination of rituximab and lenalidomide inhibited the growth of mantle cell lymphoma cells,[58] but the nature of the specific immune cell subset controlling tumor growth was not addressed. In fact, in the mouse, myeloid cells preferentially mediate ADCC, not NK cells.[12] The status of NK cells has been monitored in MM patients that underwent allogenic stem cell transplantation followed by several cycles of lenalidomide-based chemotherapy, both before and after each cycle of treatment. A transient slight increase in NCR2$^+$ NK cells[59] as well as a prolonged increase in their cytotoxic activity was observed,[60] suggesting a positive effect of lenalidomide on NK-cell activity. However, these studies fail to clarify whether the increase in NK-cell activity is due the administration of lenalidomide or to the suspension of prophylactic immunosuppressive treatments. As a matter of fact, many immunosuppressants impair the activity of NK cells. For example, dexamethasone has been shown to profoundly inhibit NK-cell function, even when administered in combination with lenalidomide.[61,62]

Other treatments against MM could also indirectly stimulate the activity of MK cells. For example, bortezomib (an inhibitor of the proteasome) has been shown to increase the levels of expression of MICA on the surface of MM cells, thus promoting NK-cell activation.[49]

Conclusions and Future Directions

It has now become clear that immunological functions must be taken into attentive consideration in the context of anticancer therapy. Usually, tumor-specific immune responses are indeed an indicator of good prognosis, and a wide range of conventional chemotherapeutics and novel immunomodulatory drugs can boost such responses. Many different aspects of the immunobiology of NK cells can now be monitored by flow cytometry, including their cytotoxic potential, their ability to secrete cytokines and chemokines, their activation status and their expression levels of activatory and inhibitory receptors. Monitoring phenotypic and functional parameters in circulating NK cells suggested that the activity of these cells is often reduced in patients affected by various neoplasms, including MM. Additional studies are now needed to characterize, with increasing precision, individual NK-cell responses. Notably, it will be interesting to see which cellular responses are mostly affected in the course of tumor progression, how the activity of circulating NK cells correlate with relapse or complications, how such an activity might be improved, and how various anticancer agents currently employed in the clinical routine impact on NK-cell function. The challenge of harnessing immunological monitoring to improve our understanding of antitumor NK cell responses (be them spontaneous or elicited by therapy) includes (1) to select appropriate cohorts of patients and (2) to take into account all the clinical parameters that may influence the correct interpretation of results. Moreover, high-content studies such as those based on microarrays, next-generation sequencing, and mass spectrometry should be performed to elucidate how hematological cancers impair the activity of NK cells and translate these findings into novel anticancer therapies.

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