# Natural Killer Cells in Myelodysplastic Syndrome

Subjects: Immunology

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Myelodysplastic syndrome (MDS) treatment remains a big challenge due to the heterogeneous nature of the disease and its ability to progress to acute myeloid leukemia (AML). The only curative option is allogeneic hematopoietic stem cell transplantation (HSCT), but most patients are unfit for this procedure and are left with only palliative treatment options, causing a big unmet need in the context of this disease. Natural killer (NK) cells are attractive candidates for MDS immunotherapy due to their ability to target myeloid leukemic cells without prior sensitization.

acute myeloid leukemia | cancer | immunotherapy | myelodysplastic syndrome | natural killer cells

## 1. Introduction

Myelodysplastic syndrome (MDS) is a group of heterogeneous clonal hematopoietic stem cell disorders characterized by cytopenias leading to ineffective hematopoiesis and increased blast production, resulting in bone marrow failure as well as risk of progression to acute myeloid leukemia (AML) [1][2][3]. MDS occurs more frequently in elderly people, which could relate to the increased inflammatory state that is associated with aging, promoting the clonal hematopoiesis that people see in patients [4][5][6]. MDS patients can be classified according to their disease risk; low-risk patients are characterized by bone marrow apoptosis and autoimmune disease manifestations, whereas high-risk patients present immune disfunction, less bone marrow apoptosis, and secondary DNA damage, accelerating the progression to AML [7][8]. On average, 30% of MDS patients progress into AML [9][10][11]. The heterogeneous nature of the disease requires a complex and personalized variety of therapeutic approaches. Among the choices for MDS treatment the only therapy with curative potential is allogenic hematopoietic stem cell transplantation (HSCT), with the remainder of therapeutic approaches involving palliative care. Allogeneic HSCT is associated with a high risk of serious complications such as infections and graft-versus-host disease (GVHD); and since MDS is a disease of the elderly, this option is unavailable to many patients [12]. Currently, the Food and Drug Administration (FDA) has only approved three drugs for the treatment of MDS, the immunomodulatory agent lenalidomide and the hypomethylating agents azacitidine and decitibine. No new drugs have been approved since 2006, and no drug has been approved for second-line treatments such as immunotherapy, occasioning a large unmet need for patients who do not respond to licensed treatments [13].

Natural Killer (NK) cells are attractive candidates for immunotherapy due to their ability to kill cancer cells without prior sensitization. They begin their development in the bone marrow and go through a step-wise process from hematopoietic stem cells (HSCs) to common lymphoid progenitors (CLPs) and NK cell precursors (NKPs) and finally into CD56+ circulating NK cells. CD122 expression whilst in the bone marrow is critical for lineage commitment. NK cells that express CD56 can also be classified according to their maturation status, characterized by the expression levels of CD56. $CD56^{bright}$ NK cells represent a more immature subset that can differentiate into $CD56^{dim}$ NK cells with the acquisition of the Fcγ receptor, CD16 [14][15][16]. NK cells belong to group 1 innate lymphoid cells and make up between 5 to 15% of the total population of circulating lymphocytes [17]. Once activated and triggered, NK cells display direct cytotoxicity against a variety of tumor targets by degranulation; the release of lytic granules containing perforins and granzymes into the synapse between NK and target cell or by induction of apoptosis through the death receptors FasL/TRAIL. Another mechanism is antibody-dependent cell-mediated cytotoxicity (ADCC), where an NK cell will bind to antibodies attached to target cells via the CD16 receptor. Cross-linkage of CD16 leads to perforin and granzyme release causing elimination of the target cell. Triggered NK cells can also secrete cytokines such as tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ) leading to a greater influence over immune responses (**Figure 1**) [18]. In the context of MDS, several studies have reported evidence of NK cell impairment, highlighting the importance of understanding their role in the context of preleukemic myelodysplasia [19][20][21][22].

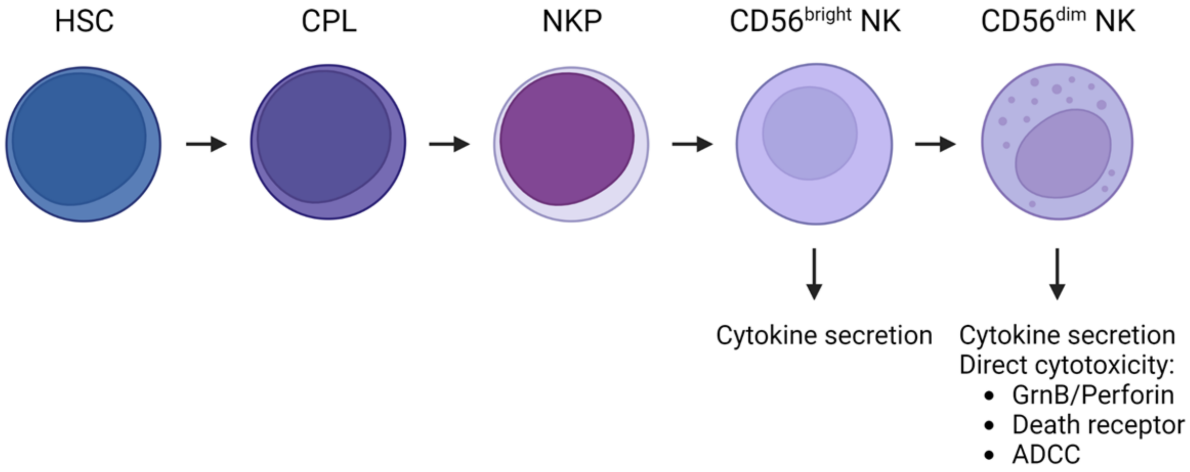


**Figure 1.** Stages of NK cell development and function. Schematic representation of the different stages of NK cell differentiation and maturation in humans. HSCs differentiate into CPLs and then into NKPs. The acquisition of CD56 will determine the last step into NK cells. $CD56^{bright}$ NK cells represent a more immature subset that can develop into $CD56^{dim}$ NK cells. Both subsets are capable of cytokine secretion, but $CD56^{dim}$ NK cells are more cytotoxic. Direct cytotoxicity can be exerted via granule secretion, death receptor ligation, or ADCC.

## 2. NK Cells in MDS

NK cell impairment is frequently reported in cancer to include a reduction in cell numbers, dysregulation of antigen expression, and a display of weaker functional abilities such as cytokine secretion and cytotoxicity. Although earlier

studies reported similar NK cell numbers in MDS patients compared to healthy donors [19], more recent reports show that MDS patients exhibit lower numbers of NK cells [22][23][24]. In most cases, this reduction in NK cell number does not correlate with a reduction in T cell lymphocytes. Instead, it is associated with higher risk subgroups of MDS patients according to the WHO and IPSS classification [22]. This is potentially informative, since it points to a particular association between NK cells and disease severity and not a general immunosuppression of broad lymphopenia. It suggests that NK cells are involved in the immune surveillance of dysplastic clones.

The dysregulation of NK cell antigen expression in cancer usually involves the downregulation of activating receptor expression and upregulation of inhibitory receptors, leading to weaker NK cell capacity for target cell recognition and killing. NK cell activation receptors include CD16, which mediates ADCC, and two families of natural cytotoxicity receptors (NCRs): the immunoglobulin superfamily of NKp30, NKp44, NKp46 and those that are C-type lectins, NKG2D and DNAM1. The C-type lectins are particularly important in leukemic cell targeting due to their recognition of MICA/B and ULBP1-6 and PVR (CD155) and Nectin-2 (CD112), respectively [25][26].

A hallmark of NK cells is the presence of killer cell immunoglobulin-like receptors (KIR) that bind to HLA-A/B/C molecules and mostly act as inhibitors of NK cell activation. NK cells also express the CD94/NKG2A complex that recognizes HLA-E and is the main inhibitor of NK activity. The KIR family of genes is highly polymorphic and four major inhibitory KIRs have been defined: KIR2DL2/3, KIR2DL1, KIR3DL1, and KIR3DL2. Immature NK cells will undergo a process called “education” or “licensing” by which they will acquire inhibitory receptors and become mature NK cells; NK cells lacking inhibitory receptors for HLA molecules will become hyporesponsive or anergic and be eliminated. The maturation of NK cells has been described as a gradual process characterized by the loss of NKG2A together with the acquisition of multiple KIR and CD57 [27][28].

Previous studies by Kiladjian et al. investigated NK cell receptor expression in MDS patients and reported comparable expression of NCRs in peripheral blood NK cells of MDS patients versus healthy controls [19]. In a more recent study by Carlsten et al., the downregulation of NKG2D and DNAM-1 expression in bone marrow-derived NK cells from MDS patients was reportedly associated with elevated blast counts and high-risk disease, but similar to Kiladjian et al. they did not see receptor changes in the surface of NK cells from peripheral blood [21]. In contrast, other studies investigating peripheral blood NK cells from MDS patients have reported loss of NKG2D, NKp30, NKp46, CD16, and CD161 expression [20][22][23][24]. Recent studies suggest that NK cell dysfunction in MDS may be attributed to the presence of NK cells with immature phenotypes characterized by an increase in the proportion of $CD56^{bright}$ NK cells, higher “early” KIR expression (KIR2DL2/3), and lower “late” KIR expression (KIR2DL1 and KIR3DL) in MDS patients [22][24][29]. The expression of NK cell ligands in bone marrow aspirates of MDS patients has also been studied since these receptor–ligand interactions are crucial for lysis of MDS blasts, but reports have yielded conflicting results. Epling-Burnette et al. reported that 30% of MDS blasts expressed the NKG2D ligands MICA/B [20]. In another study by Carlsten et al., NKG2D ligands MICs and ULBPs were found to be rarely expressed in the bone marrow of MDS patients. Instead, patients expressed the DNAM1 ligands, CD155 and CD112, showing an important role for DNAM1-CD155/CD112 interactions in the killing of MDS blasts [21].

These conflicting data on NK cell receptor expression in MDS may be attributed to the source of NK cells, whether obtained from peripheral blood or bone marrow, and timing of samples especially as it relates to the administration of chemotherapeutic agents, which are known to have an effect on the state of NK cells [30][31][32]. Taken together, these studies demonstrate an NK cell phenotype characterized by lack of maturation markers and the downregulation of NK cell activation receptors in MDS patients, which is associated with NK cell functional impairment.

Several studies have reported impaired or dysfunctional NK cell cytokine secretion or cytotoxicity in MDS patients. A reduction in MDS-NK cell secretion of TNF-α and IFN-γ was previously demonstrated in response to IL-2 and K562 stimulation [19][22]. NK cell cytotoxicity through perforin and granzyme release is associated with enhanced degranulation as measured by LAMP-1/CD107a expression or by the activation of death receptor-mediated pathways TRAIL and FasL [33][34]. Carlsten et al. reported lower degranulation in MDS-NK cells following exposure to K562 leukemic target cells relative to healthy controls [21]. Hejazi et al. showed no difference in the mobilization of CD107a, a molecule expressed on the NK cell surface after degranulation, when MDS-NK cells were exposed to K562. However, they showed a significant decrease in MDS-NK cell killing activity without any associated changes in CD107a expression compared to healthy donors. This observation prompted them to investigate whether degranulating MDS-NK cells are properly armed with cytotoxic molecules. They observed a substantial reduction in perforin and granzyme B loading of granules in MDS-NK cells, which explains why NK cells might still be able to mobilize CD107a to the cell surface without effective killing of target cells [22]. Similarly, other studies have shown impaired lysis of K562 and $CD34^+$ blasts by NK cells from MDS patients [19][20][21][22].

In a recent study, Tsirogianni et al. [35] analyzed peripheral blood NK cells from MDS patients who had been treated with azacytidine, and they tested their ability to lyse K562 leukemic target cells in vitro. Patients with higher lytic function showed significantly longer overall survival. Indeed, the association was so strong that the group was able to calculate a threshold of NK mediated lysis, which was predictive for survival beyond 2 years. Patients below the threshold showed a median overall survival of 18 months compared to those falling above the threshold with a median survival of 52 months. Collectively, these studies suggest that NK cell antitumor functions may be critical in the response to MDS. Their impairment, however, can be influenced by numerous factors in the tumor microenvironment that contribute to disease progression.

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