Use of NK cells in haematological cancer therapy

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Abstract
NK cells play an important role in the defence against certain virus infections and cancers. They are considered a part of the innate immune system and have already shown their potential in the treatment of several haematological malignancies.

Here I present an overview of the characteristics of NK cells and their functions, including their activation and regulation, their killing mechanisms and cytokine production. Their most important receptors and ligands are mentioned. Finally, the potential treatment of haematological malignancies with NK cells is revised, including the use of autologous and allogeneic NK cells, the use of monoclonal antibodies and treatment with CAR NK cells.

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Methods
A PubMed search was conducted to find relevant articles, following the recommendations of my supervisor.

1. Introduction
NK cells were discovered approximately 40 years ago based on their ability to spontaneously kill tumor cells in vitro. They have later been shown to contribute to the defence against certain viral infections and cancers in vivo. NK cells are the object of significant interest and their usage may significantly improve cancer treatment.

2. The immune system
The immune system distinguishes between self and non-self. It protects us from pathogens and may eliminate cancer cells. The immune system can be divided into adaptive and innate parts, although their functions are tightly interwoven and mutually dependent on each other.

2.1 The adaptive immune system
The adaptive immune system comprises B- and T-cells. B cells mediate humoral immunity, while T cells mediate cell-mediated immunity. B and T cells recirculate between blood and lymphoid organs in their search for pathogens. If a lymphocyte encounters its antigen it may proliferate and mature in secondary lymphoid tissues (e.g. lymph nodes). Both B and T cells express highly diverse receptors that are clonally distributed. The receptor diversity is partly due to gene rearrangement. B cells express the B cell receptor (BCR). Upon binding to antigen it may induce B cells to mature into plasma cells and memory B cells. This process is also dependent on T cell help. Plasma cells produce and secrete large amounts of antibodies that help the immune system
eliminate pathogens. The survival of long-lived memory B cells ensures efficient responses to secondary challenge with the same pathogen.

T cells mature in the thymus and can be distinguished from other cells by their expression of T cell receptors (TCR). In the thymus, only T cells that interact with self MHC molecules presenting self-peptides survive. T cells with too high affinity for self MHC molecules are eliminated (negative selection). T cells with lacking affinity for self MHC molecules are neglected. When T cells exit the thymus they are naïve, but mature. In response to antigen stimulation by dendritic cells, naïve T cells proliferate and differentiate into armed effector lymphocytes. These effector T cells may kill pathogen infected cells and tumors, or produce cytokines. When pathogens have been cleared, most effector T cells die (contraction phase). A small subpopulation of T cells survives and lives on as memory cells. (Sun et al. 2009)

The presence of memory T cells increases the frequency of cells responding to the immunizing antigen 100-1000 fold compared to initial levels. There are two types of memory T cells: effector memory and central memory cells. Effector memory T cells rapidly mature into effector T cells and secrete large amounts of IFN-γ, IL-4 and IL-5 early after re-stimulation. These cells lack CCR7, but express inflammatory chemokine receptors suggesting that they enter inflamed tissues. Central memory T cells express CCR7 and recirculate. Upon antigen stimulation they lose CCR7 and differentiate into effector memory cells.

It is possible to distinguish several subtypes of T cells; such as cytotoxic, helper and regulatory cells. Cytotoxic T cells express CD8. They kill pathogen infected cells and tumor cells. Helper T cells express CD4. They produce cytokines and stimulate other cells. Helper T cells contribute to the maturation of B cells as well as activation of T cells, macrophages and NK cells. Regulatory T cells are also called Tregs. There are many subtypes of Tregs and they control the immune response. Most Tregs express the transcription factor FoxP3 and may express CTLA-4. They may inhibit other cell types by competing for cytokines such as IL-2, produce inhibitory cytokines (e.g. IL-10, TGF-β) or induce contact-dependent inhibition through CTLA-4.

2.2 The innate immune system:
Most pathogens are eliminated by the innate immune system without expansion of antigen-specific lymphocytes. There is no need for clonal expansion of innate lymphocytes since each receptor is expressed on a relatively large subpopulation of cells. The innate immune system use a limited set of germ-line encoded receptors and secreted proteins. There is no rearrangement of genes in the innate immune system. (Lanier 2005) Traditional memory responses have not been considered a part of the innate immune system although lately NK cells have been shown to display memory responses against viruses, haptenes and allogeneic cells. (Von Andrian 2011, Nabekura et al 2014, Sun et al 2009, Peterson et al.1999)

It is possible to divide the innate immune defence into cellular and non-cellular types. Physical barriers, such as skin and mucous tissue, and antimicrobial substances that destroy or inhibit microbes, such as lysozyme in tears or lactoferrin in breast milk, are two examples of non-cellular defence mechanisms in the innate immune system. Complement factors also contribute
to the innate immune defence. These factors are found in the serum and are mainly produced in the liver. Among other effects, they attract macrophages and neutrophils and opsonize pathogens. Normal flora, as we find on the skin and in the digestive tract, is also protective by competing with pathogenic bacteria. (Bogen B. 2002)

The cellular innate immune responses are mediated by phagocytic leukocytes (macrophages, neutrophils, eosinophils and basophils), dendritic cells, innate lymphoid cells (ILCs) and natural killer cells (NK cells).

Dendritic cells play an important role both in the innate and adaptive immune system. They engulf, digest and present antigenic peptides to T cells and are exclusively able to activate naïve T cells. Macrophages also act as antigen presenting cells, although their main area of responsibility is eliminating debris, microbes and cancer cells.

Neutrophils, eosinophils and basophils may be distinguished by different staining properties of their granules. They are produced in increased numbers during immune responses, when they leave the blood to sites of infection or inflammation. Neutrophils are found in high numbers and take up a variety of micro-organisms by phagocytosis. Eosinophils and basophils are less abundant, but like neutrophils they have granules containing a variety of enzymes and toxic proteins, which are released when the cells are activated. Eosinophils and basophils are thought to be important mainly in the defence against parasites. They may also contribute to allergic inflammatory reactions, in which their effects are damaging rather than protective. (Nairn R. 2006)

NK cells have many different functions that are presented later, including killing of virus infected cells, tumor cells, and antibody coated cells (ADCC) and allogeneic cells.

2.3 Interplay between the adaptive and the innate immune system.

Dendritic cells are the most important antigen-presenting cells (APCs). They have critical roles both in the adaptive and innate immune system. They present antigenic peptides on MHC class I and II molecules to cytotoxic and helper T cells respectively. They also provide additional signals needed to activate naïve T cells (Nairn R. 2006)

It is well known that antibody binding to a microbe activates complement and opsonizes them to killing by NK cells, neutrophils and macrophages (Bogen B. 2002)

Cytokines produced by cells in the innate immune system activate cells in the adaptive immune system and vice versa. Cytokines produced by macrophages attract antigen-specific lymphocytes and bring the adaptive immune response into play. Another example is the production of IL-2 by T cells that activate NK cells.

NK cells can also modulate the activity of other leukocytes, such as dendritic cells and T cells, through cytokine secretion and various receptor-ligand interactions, influencing not only the
strength, but also the quality of T cell responses. This may favour the development of Th1 responses that enhance anti-tumor and virus defences. (Moretta et al 2014)

3. NK cells

3.1 Description
NK cells originate from hematopoietic stem cells and undergo maturation primary in the bone marrow. They are considered innate immune cells. They recognize and kill transformed cells, such as tumor cells and virally-infected cells. They are instantly cytotoxic and produce cytokines, allowing for a much faster immune reaction than T cells. They have a lifespan of up to two weeks and are found in the blood, spleen, lung, bone-marrow, tonsils, lymph nodes, placenta and gut-associated lymphoid tissues. (Moretta et al 2014)

Morphologically, NK cells are large granular lymphocytes with abundant cytoplasm and azurophilic cytoplasmic granules. Cell surface markers detectable by flow cytometry and immunohistochemistry are used to identify NK cells. Human NK cells are defined by the presence of CD56 and lack of T-cell receptor CD3. (Davies et al 2014)

3.2 Subtypes of NK cells
Several subsets of NK cells have been identified based on the expression of markers such as CD56. The most important subsets are the CD56dim and CD56bright cells that differ in function and anatomical distribution. The CD56dim subset generally produces less cytokines than the CD56bright subset. The CD56dim NK cells are highly cytotoxic. The production of chemokines and cytokines by the CD56bright NK cells is triggered by cytokine stimulation. This is in contrast to the CD56dim population that is triggered by target cell interactions. Most CD56bright NK cells are found in secondary lymphoid tissues, while CD56dim cells are present in the blood and spleen. (Geller M. 2011)

3.3 NK cells compared to other leucocytes.
NK cells have characteristics in common with other leucocytes and may be considered a transitional lymphocyte bridging the innate and adaptive immune system. They do share common killing mechanisms with cytotoxic T cells, such as the use of perforin and granzymes. Similarly to T cells, they also produce IFN-γ. (Lanier 2005). Unlike T cells, they are not able to produce IL-2, important for NK cell activation and proliferation.

NK cells differ from naïve T cells in that they are ready for an immediate action and they do not depend on APCs to be able to recognize their targets as T cells do. They do not require selection or expansion of specific clones in order to exert their functions. It has recently been found that some NK cells develop into memory NK cells, a feature that was thought to be exclusive for T and B cells. (Sun et a. 2009)

3.4 NK cell functions
NK cells have several tasks, such as the elimination of infected and transformed cells. They also produce high amounts of cytokines. In order to avoid auto-immunity it is important that NK
cells are self-tolerant. One theory explaining the ability to kill foreign and transformed cells, while leaving “self” cells unharmed is the “missing self” hypothesis.

3.4.1. The “missing self” hypothesis
MHC class I molecules are present on most nucleated cells in the body. They present peptides to cytotoxic T cells and may also be recognized by NK cell receptors.

The human MHC molecules are called HLA because they were first discovered on leukocytes (human leukocyte antigens). They can be divided into HLA class I and class II molecules based on differing structure, peptides presented and immune function. There are two types of HLA class I molecules: classical (HLA-A, -B and -C) and non-classical (HLA-E, -F, -G, -H). (Geller M. 2011)

NK cells express inhibitory receptors recognizing MHC class I molecules. These interactions inhibit NK cell cytotoxicity and cytokine production. Cells expressing these ligands are spared from NK cell lysis. In the absence of these ligands, NK cells may become activated. Such activation is dependent on activating NK cell receptors recognizing target cell ligands.

According to the 'missing self' hypothesis, one function of NK cells is to recognize and eliminate cells that fail to express the full set of self MHC-class I molecules. The “missing self” - hypothesis was proposed by Karre in 1985. Karre proposed that NK cells kill cells that have lost their ability to express self MHC class I molecules or express allogeneic MHC molecules, not able to engage the inhibitory NK cell receptors. The loss of MHC class I molecules is a common event in cancers and virally-infected cells. NK cells can in this way eliminate cells that are insensitive to cytotoxic T cells.

3.4.2 Integration of inhibitory and activating signals
NK cells must integrate activating and inhibitory signals in order to respond properly. When activating NK receptors are engaged, NK cells may become activated and kill surrounding cells if not controlled by the inhibitory class I receptors described above. The inhibitory receptors usually have a dominant effect over the activating receptors. Infected cells may up-regulate ligands for activating receptors while MHC class I expression is reduced. This activates NK cells and the infected cells are eliminated.

3.4.3 Stress-induced self recognition
NK cells express activating receptors that recognize stress-induced ligands. These ligands are expressed at very low levels (or not at all) under normal conditions but are up-regulated by various forms of cellular stress such as viral infections and cancer development. (Vivier E 2012)

The activating receptors primarily involved in this recognition are NKG2D and DNAM-1. (Davies et al 2014)

3.4.5 Cytokine activation
In addition to the mechanisms cited above, cytokines play a crucial role in NK-cell activation. There is a host of different cytokines that contribute to the activation and development of NK cells. Some of the best known are IL-2, secreted by T-cells, IL-12, produced by macrophages and dendritic cells and IL-18 produced by activated macrophages and Kupffer cells. They all activate
NK cells. IL-15 regulates NK cell activation and proliferation, and is involved in NK cell development. IL-15 is mainly produced by DC, monocytes and macrophages.

3.4.6 Killing mechanisms
NK cells kill target cells mainly by inducing apoptosis. This is similar to cytotoxic T cells. The release of cytotoxic granules containing pore-forming perforin and granzymes play a dominant role in this process. (Bogen B. 2002)

NK cells may also induce apoptosis by TNF α-related apoptosis inducing ligand (TRAIL). TRAIL induces apoptosis of target cells by interacting with death receptors present on activated cells and tumor cells. NK cells may in this manner eliminate tumor cells and limit the presence of activated cells, such as neutrophils and T cells at the site of infection (Schuster I S et al 2014) TNF-α released by NK cells may also induce apoptosis of target cells by triggering caspase 8. In addition, NK cells express Fas ligand. Fas ligand may engage Fas receptor on target cells (present on most cells) and induce apoptosis.

3.4.7 Cytokine production
NK cells respond to signals from other innate immune cells, including dendritic cells, macrophages, and pathogen-infected tissue cells. These signals are relayed in the form of cytokines such as IL-1, IL-10, IL-12, IL-15, and IL-18. NK cells respond by secreting cytokines such as tumor necrosis factor-α (TNF-α) and interferon γ (IFN-γ). In this way, following the triggering of innate immune cells by pattern recognition receptors, NK cells can relay and amplify cytokine signals.

When TNF-α is released it may induce apoptosis of the target cells, while IFN-γ activates macrophages for phagocytosis and pathogen lysis. IFN-γ also induces MHC class I expression on tumor cells sensitizing them to CD8+ T cell killing. The combination of TNF-α and IFN-γ may also induce senescence in tumor cells. (Markus et al 2014) It has been shown that NK cells secrete several other factors, including immunoregulatory cytokines such as IL-5, IL-10, IL-13 as well as the growth factor GM-CSF, and the chemokines MIP-1α, MIP-1β and IL-8. (Fauriat 2010)

3.5 NK cell receptors and their ligands
As stated above, NK cells distinguish between normal and abnormal cells by using a complex array of cell surface receptors that control their activation, proliferation and effector functions. NK cell receptors are expressed on relatively large subpopulations of cells making them well suited for early immune responses since clonal expansion is not needed. (Lanier 2005)

The main groups of receptors are as follows:
- Killer Cell Immunoglobulin-like receptors (KIRs)
- Natural cytotoxicity receptors (NCRs)
- CD94/NKG2 receptors
- NKG2D receptor
- CD16 receptors
- DNAM-1 receptors
3.5.1 Killer Cell Immunoglobulin-like receptors
Most killer cell immunoglobulin-like receptors (KIRs) are inhibitory and recognize MHC class I molecules. Ligand binding by these receptors result in suppressed cytotoxicity and cytokine secretion by both NK and T cells. (Lanier 2005).
KIRs belong to the Ig superfamily of receptors. They are type I transmembrane glycoproteins and are named according to the number of extra-cellular immunoglobulin domains (2D or 3D) and the length of their intracellular tails. This tail determines whether they are activating (short) or inhibitory (long). (Lanier) KIRs recognize certain HLA-A, -B and -C molecules. The function of the activating KIRs is more uncertain, although it has been shown that the activating KIR2DS1 receptor recognizes HLA-C2 alleles and contributes to NK alloreactivity. (Pende et al. 2009) It has been shown that the presence of donor KIR2DS1 partially protects against leukemia relapse after hematopoietic stem-cell transplantations. (Venstrom et al. 2012)

3.5.2 NCR (Natural cytotoxicity receptors)
NCRs belong to the immunoglobulin (Ig) superfamily of receptors. This group of receptors trigger NK mediated killing and cytokine secretion. The NCR family includes NKp46 and NKp30 that are expressed on both resting and activated NK cells, and NKp44 that is expressed only on activated NK cells. The NCRs have been shown to interact with a wide spectre of cellular, bacterial, parasitic and viral ligands. Many aspects of the ligand binding are still unclear. These receptors also recognize ligands on tumor cells. (Kruse et al. 2014)

3.5.3 CD94/NKG2 receptors
These receptors recognize the non-classical HLA-E molecule. HLA-E presents leader fragments from other HLA class I molecules. CD94/NKG2 heterodimers are formed by the CD94 glycoprotein bound to either NKG2A or NKG2C. CD94/NKG2A binding to HLA-E inhibits NK-cell cytotoxicity and cytokine production. The CD94/NKG2A receptor is thought to contribute to self-tolerance of the NK cells. The CD94/NKG2C activates NK-cells. (Bogen B. 2002)

3.5.4 NKG2D receptor
The activating receptor NKG2D interacts with self-molecules that are up-regulated on stressed cells. The NKG2D receptor is constitutively expressed on most NK-cells and recognizes the stress-induced MHC class I-like molecules MICA/B, as well as ULBPs, commonly expressed on tumor cells and virally infected cells. (Geller M. 2011)

3.5.5 CD16
The activating CD16 receptor (FcyRIIIa) on NK cells recognizes antibody-coated target cells and mediates antibody-dependent cellular toxicity (ADCC). This low affinity Fc receptor is present on essentially all CD56dim peripheral blood NK cells. These receptors bind to the Fc portion of IgG1 and IgG3 antibodies. Upon recognition of antibody-coated cells, NK cells are rapidly activated and degranulate, resulting in target cell killing. (Geller M. 2011)

3.5.6 DNAM-1 receptors
DNAM-1 belongs to the Ig-superfamily of receptors. It is constitutively expressed on all NK cells and has two different ligands, CD155 (PVR) and CD112 (Nectin-2). Both ligands are present on many human cancer cells making them sensitive to NK cell mediated killing. DNAM-1 associates with LFA-1 on the cell surface and mediates activating signals. (Ferrari de Andrade
DNAM-1 controls NK cell cytotoxicity and IFN-γ production in response to a wide range of cancer cells and infected cells. Its ligands, CD112 and CD155, have been found in different pathological conditions, and recent evidence suggests that their expression is up-regulated by cellular stress.

4. How cancers escape immune surveillance

Tumor cells are known to use various strategies to escape immune control. NK cells are perhaps the best studied mediators of the innate immune defence against cancers. Their activity may be affected in several ways by the developing tumors. NK cells may be rendered unresponsive, their numbers may be reduced or they may fail to recognize tumor cells. Most of these effects can be attributed to either changes in the tumor microenvironment or the tumor cells themselves. (Moretta et al 2014)

4.1 Changes in the microenvironment

The secretion of immunosuppressive cytokines such as IL-10 by tumor or stromal cells results in the down-regulation of NKp30, NKp44 and NKG2D. Down-regulation of these activating receptors reduces NK cytotoxicity and cytokine production. Another immunosuppressive cytokine, TGF-β, has been shown to inhibit NK function during chronic interaction with tumor cells. TGF-β antagonizes IL-15 which is important for NK cell proliferation and activation. (Markus et al 2014)

Shedding of soluble ligands is another mechanism by which tumors inhibit NK cell function. Shedding of ligands may down-regulate or block activating NK cell receptors. Chronic ligand-induced stimulation may also render NK cells unresponsive. Many tumor cells release soluble NKG2D ligands (MICA/B and ULBPs). These soluble ligands have been identified in the sera of patients with various cancer types including glioma, neuroblastoma, breast, lung, colon, and ovarian carcinomas as well as in AML patients. (Markus et al 2014)

4.2 Changes in tumor cells

Some tumor cells escape T-cell mediated control by decreasing their expression of MHC class I molecules. This down-regulation however may increase their susceptibility to NK mediated killing, although tumor cells often exhibit other transformations that protect them from NK cell lysis.

Other cancer cells, such as AML, may display increased levels of MHC class I molecules, inhibiting NK cell function. This may lead to NK cell anergy due to the chronic interaction with inhibitory NK cell receptors.

CML patients often express high levels of MICA inducing weak NKG2D expression. This may also result in escape from NK cell killing.

There may also be a selection for cancer cells that have lost their NK-activating ligands. NK susceptible tumor cells are killed while resistant tumor cells expand. In this manner NK cells can contribute to immune-editing of cancer cells. (Markus et al 2014). All these mechanisms and several others contribute to tumor escape from NK cell surveillance.
5. Haematological cancer
The NK cell function is often reduced in haematological cancer patients. Both qualitative and quantitative defects have been reported, although the qualitative effects seem to dominate. NK cells may show inhibited cytotoxicity, impaired activation and impaired differentiation. (Farnault et al. 2012)

5.1 Types of haematological cancer
In leukaemia, there is a rapid proliferation of a subpopulation of leukocytes that causes displacement of normal blood cells leading to life threatening complications such as infections, anaemia and bleeding. (Evensen S.A. 2008)
Leukaemia is usually classified according to the maturity of the cancer cells. Acute leukaemia is characterized by the proliferation of relatively immature hematopoietic cells while chronic leukaemia consists of more mature cells. In addition to clinical examination and lab tests, the classification and subtyping of leukaemia is based on cytogenetic tests and immunophenotyping. This is important since various leukaemia subtypes require different treatment. There are four main types of leukaemia: acute lymphoblastic leukaemia, acute myeloid leukaemia, chronic lymphocytic leukaemia and chronic myeloid leukaemia. (Evensen S.A. 2008).

Haematological malignancies and their response to therapy have been linked to KIR expression. There is also evidence that other NK cell receptors affect leukaemia development.

Acute lymphoblastic leukaemia or ALL is common in children. ALL can be subdivided into B- and T-cell ALL. ALL is relatively resistant to NK cell killing. This resistance does not appear to involve inhibitory mechanisms, but rather deficient NK cell activation, (Romanski et al. 2005). This may in part be caused by the impaired production of IL-2 and IFN-γ that accompanies ALL. ALL cells also lack MICA/B and ULBP expression. (Farnault et al 2012).

The most common leukaemia in adults is acute myeloid leukaemia (AML). Here there are more than 20 % leukaemia blasts in bone marrow and/or blood. AML patients who receive stem cell grafts from KIR2DS1 donors have a lower risk of relapse after allogeneic HSCT. (Davies et al 2014). In addition to down-regulation of NCRs and NKG2D, down-modulation of DNAM-1 and CD94/NKG2C has been described in AML. (Farnault et al 2012). This may protect tumor cells from NK cell lysis.

In chronic lymphocytic leukemia or CLL, B-lymphocytes grow uncontrolled and accumulate in the bone marrow and blood. In advanced stages, CLL results in swollen lymph nodes and hepato/splenomegaly. CLL blasts do not express MICA/B or ULBP ligands and are insensitive to NK cell killing.

Chronic myeloid leukemia, CML, is a bone marrow stem cell disorder with proliferation of mature granulocytes and their precursors. Its hallmark is a chromosomal translocation called the Philadelphia chromosome. CML is now treated with tyrosine kinase inhibitors, such as Imatinib mesylate, which has led to improved long term survival. (Evensen S.A. 2008). The abnormally high levels of MICA and weak expression of NKG2D reported in CML can be reversed by Imatinib. (Davies et al 2014)
It has been observed that CML patients expressing KIR2DS1 display reduced responses to Imatinib and worse overall survival. Another tyrosine kinase inhibitor, Dasatinib, may help these patients. (Davies et al 2014)

5.2 Hematopoietic stem cell transplantation
Hematopoietic stem-cell transplantation (HSCT) is used primarily for haematological and lymphoid cancers and involves the intravenous (IV) infusion of autologous or allogeneic hematopoietic stem cells. HSCT results in more cures and remissions than alternative treatment protocols, but there is a risk of morbidity and mortality. Approximately 15-20% of patients with advanced cancer undergoing allogeneic HSCT die from transplant related complications. Reducing the toxicity of the preparative regimen is critical to improve the safety of HSCT. (Copelan 2006)

This may be achieved by combining NK cell-based immunotherapy with reduced intensity conditioning (RIC) of the patients. In addition to the toxicity of the preparative regimen, the patients treated with HSCT may suffer from GvHD, an immunological disease caused by T cells in the graft. In order to avoid or minimize GvHD and the risk of graft failure, it is important to find a donor that matches the HLA molecules of the host. HLA molecules are highly polymorphic and they are encoded by the major histocompatibility complex (MHC). HLA class I molecules (HLA-A, -B and -C) are expressed by almost all nucleated cells, while HLA class II molecules (HLA-DR, -DQ, and -DP) are expressed by antigen presenting cells. Activated cells may also express HLA class II molecules. Ideally, donors and recipients are matched at all MHC loci (etc HLA identical siblings). Unrelated donors are matched on HLA-A, -B, -C, -DRB1, -DQB1 and possibly -DPB1. The best possible match is referred to as a 10/10 (12/12) match. (Ferrara et al 2009).

5.3 Graft versus host disease and Graft versus leukaemia effects
Graft versus host disease (GvHD) is a well-known complication of hematopoietic stem cell transplantations and may be life threatening. GvHD is an immune-mediated disease caused by donor T cells responding to host HLA molecules and peptides. Donor T cells attack and destroy host tissue and organs, causing a range of symptoms including dermatitis, hepatitis, and enteritis. In acute GvHD, symptoms appear within 100 days of the allogeneic HSCT, while chronic GvHD describes a more diverse syndrome developing after day 100. Other transplantations, such as non-irradiated blood products and transplantation of solid organs containing lymphoid tissue may also cause GvHD. The seriousness of the GvHD is directly related to the degree of HLA mismatch between host and donor. (Ferrara et al 2009)

Graft versus leukemia (GvL) effects after HSCT are however desirable. As has been shown by the pioneering work by Velardi et al., NK cells have the ability to mediate GvL effects without GvHD. Their results resulted in a new era for the use of NK cells in cancer treatment. (Davies et al 2014)

The noticeable beneficial effect of alloreactive NK cells from haploidentical donors (family donors that share only one HLA haplotype with the recipient), was first assessed in adult AML patients, but has later been reported also in children with ALL. The probability of leukaemia relapse in these patients was very low and the survival rate was at least as good as that of patients receiving HSCT from HLA-matched siblings or unrelated donors. (Moretta et al 2014)
5.4 Immunotherapy with NK cells

Some of the therapies that exploit the properties of NK cells are autologous NK cell therapy, allogeneic NK cell therapy and monoclonal antibodies. Cytokines may improve the outcome of autologous and allogeneic HSCT by expanding NK cells in the recipient. Hydrocortisone and IL-15 has been used satisfactorily to \textit{ex vivo} activate and expand allogeneic NK cells before NK cell infusion (Geller M. 2011). Lately, the use of modified NK cells has been tested. These NK cells express a chimeric antigen receptor (CAR) and are termed CAR NK cells.

5.4.1 Autologous NK cell therapy

In autologous NK cell therapy the patient’s own NK cells are extracted and manipulated \textit{in vitro} to enhance their response to tumor cells. The manipulated cells are then re-infused into the patient.

This procedure has had little clinical benefit probably due to inhibitory KIRs recognizing self MHC class I on the tumor cells (Geller M. 2011). An important finding from these trials was that in order for donor cells to expand in the recipient, it was necessary to create space for the graft. This requires elimination of recipient leucocytes that would otherwise compete for growth factors and cytokines. (Geller M. 2011)

5.4.2 Allogeneic NK cell therapy

Allogeneic NK cell therapy refers both to donor lymphocyte infusions, i.e. transfer of mature allogeneic NK cells and transplantation with hematopoietic stem cells that eventually develop into mature allogeneic NK cells. These allogeneic NK cells are capable of mediating GvL effects. Haploidentical transplantation is one form of allogeneic HSCT. This treatment protocol was originally developed to overcome the lack of HLA-matched donors. In haploidentical transplantation the related donor and recipient share only one HLA haplotype. The patients receive high doses of hematopoietic stem cells depleted of T cells in order to avoid GvHD. They are also subject to strong cytotoxic and immunosuppressive conditioning regime to prevent graft rejection. This procedure has shown the potential of NK cells in cancer treatment. (Vivier 2012)

The beneficial effects of this procedure may be explained by the missing-self hypothesis. Subsets of donor-derived NK cells that are not restrained by MHC class I molecules in the patient may have GvL potential. There is no risk of GvH reactions since recipient cells, apart from the leukaemia cells, lack activating ligands for donor NK cells. (Vivier E 2012)

The importance of KIRs in HSCT has recently been shown. Patients receiving grafts from donors with the KIR2DS1 genotype had a reduced risk of relapse from AML after both unrelated and matched sibling donor allogeneic transplant (Davies et al 2014)

5.4.3 Monoclonal antibodies

Many monoclonal antibodies (mAbs), such as rituximab, bind tumor cells. The low affinity Fc receptor CD16 present on NK cells efficiently induc antibody-dependent cellular cytotoxicity (ADCC) of cells coated with IgG1 and IgG3 antibodies. (Davies et al 2014).

Another monoclonal antibody that makes use of ADCC by NK cells is Alemtuzumab. It is an IgG1-type mAb directed against CD52 on tumor cells. Alemtuzumab is used in the treatment of
refractory CLL. This antibody kills target cells by complement-activation and/or ADCC, but seems also capable of inducing direct apoptosis via caspase-dependent and -independent mechanisms. (Keating et al. 2002)

The monoclonal human antibody IPH-2101 of the IgG4 isotype has been tested in patients with myelomatosis. This antibody blocks interaction of KIR2D receptors with HLA-C. This should theoretically enhance NK cell cytotoxicity (Korde et al 2014), but no clinical response was observed. Several monoclonal antibodies are now in clinical trials. Their number has been growing constantly in the last ten years and they offer an interesting and promising field of development in the treatment of many different types of cancer.

5.4.4 Use of cytokines
Rosenberg’s group reported in the 1980s on the successful use of lymphokine activated killer (LAK) cells to treat patients with advanced cancer. LAK cells were generated by culturing peripheral blood mononuclear cells (PBMCs) with high doses of IL-2. This resulted in a mixture of activated NK and T cells. In these early studies, LAK cells were generated in vitro from the patients own PBMCs and they were re-infused later together with high doses of IL-2. High doses of IL-2 however lead to vascular leakage and hypotension. (Rosenberg et al 1985)

Also IL-12, IL-15 and IL-18 have been used alone or in combination with histamine dihydrochloride, to enhance NK- and T-lymphocyte proliferation and cytotoxicity. This protocol enhanced NK cytotoxicity and tumor control with significant improvement in leukaemia-free survival. (Farnault et al 2012)

While IL-2 is an important cytokine and currently heavily used, IL-15 may be a better way of expanding NK cells since it does not stimulate Tregs. Stimulation of Tregs is not a desirable since they suppress NK proliferation and effector functions. A possible solution could be selective depletion of Tregs in protocols that make use of IL-2. (Geller M. 2011)

5.4.5 CAR NK cells
Chimeric antigen receptor (CAR) cells express genetically modified receptors. T or NK cells are transfected with these receptors in order to endow them with a distinct specificity. The chimeric receptor may contain parts of a monoclonal antibody reacting to a specific tumor antigen.

There are ongoing discussions about the advantages of using NK cells as compared to T cells as CAR vectors. One of the features that might make NK cells preferable is their relatively short lifespan. It may give a better control over possible side-effects. Allogeneic NK cells are expected to induce an immune response and be rejected after a few days, and even autologous NK cells should disappear relatively rapidly. (Klingemann 2014)

NK cells have additional advantages over T cells because they are not exclusively depended on the CARs. As mentioned earlier, NK cells display spontaneous killing of tumor cells by several different mechanisms such as NKP30, NKP44, NKP46, NKG2D and DNAM-1. They also express CD16 rendering them capable of killing antibody-coated tumor cells.
Fast and strong production of IFN-γ and GM-CSF could also enable CAR-NK cells to induce anti-tumor responses by surrounding cells. *(Klingemann 2014)*

6. Conclusions

Better knowledge about tumor cell transformations and their influence on the immune system is needed. This may lead to tailored cancer treatments targeting the various transformations and their effects on the immune system.

Further studies of KIRs and their ligands may allow for better donor selection to patients in need of allogeneic HSCT or NK cell infusions. The sensitivity of tumor cells to NK cells killing may also be increased by blockade of inhibitory receptor-ligand interactions. Also, the combination of reduced intensity conditioning with NK cells therapy has great clinical potential.

The genetic engineering of NK cells, such as CAR NK cells, is opening a whole new field of possibilities.

Some of the conclusions drawn above must be interpreted with caution. The heterogeneity of the study populations and sometimes small study groups makes it hard to draw certain conclusions.

Further research is needed to help uncover the potential of immunotherapy in general, and NK cells in particular, in the treatment of haematological cancer.
7. References

3. Davies et al. «Opportunities and limitations of natural killer cells in adoptive therapy.»