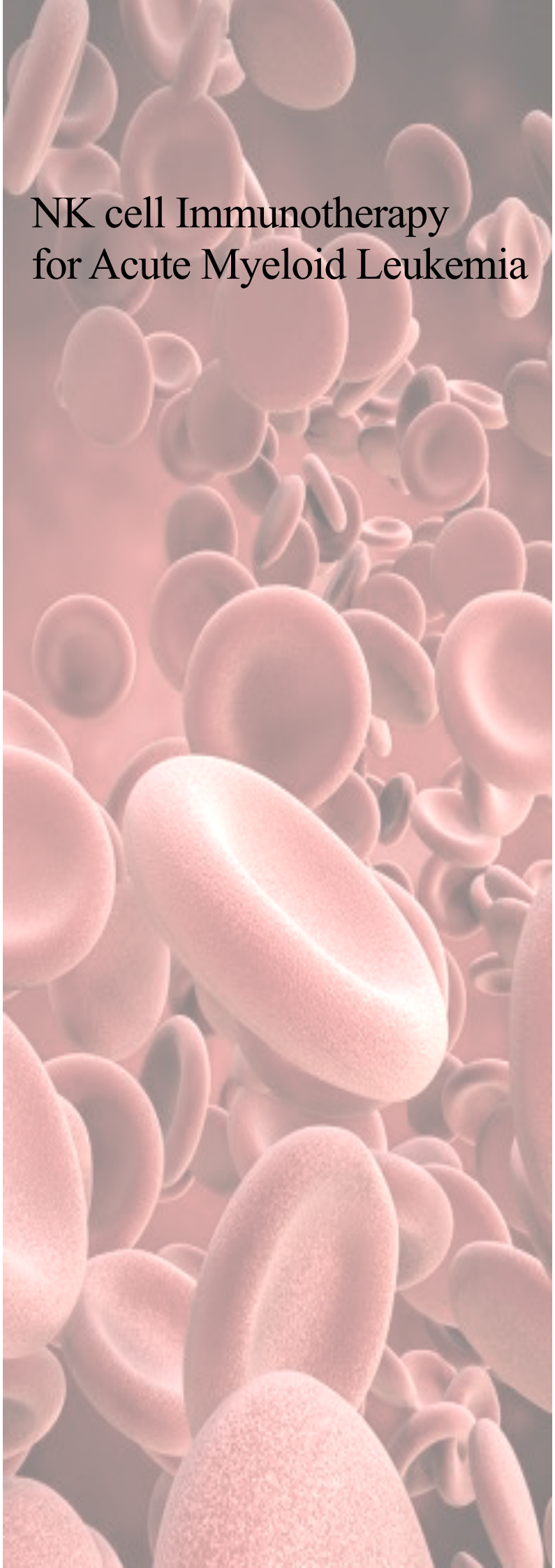

NK cell Immunotherapy for Acute Myeloid Leukemia

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4th of July 2019

Summary

Acute myeloid leukemia (AML) is an aggressive hematological disease, characterized by an increasing incidence by age and high mortality. The standard treatment of AML is chemotherapy, possibly followed by a hematopoietic stem cell transplantation (HSCT) for patients with a high risk of recurrence. The high relapse rate is a major problem in AML. This is mainly due to residual AML cells that remain in the body after chemotherapy. Therefore, it is necessary to develop a therapy that also attacks these residual AML cells and thus improves the overall survival in AML patients. Immunotherapy has been of great interest in the past years for therapeutic strategies in various cancers. Recent advances with chimeric antigen receptor (CAR) T-cells for other hematological malignancies have generated interest for this method in AML patients, but also early results for natural killer (NK) cell therapy are promising for the treatment of AML. NK cells are cells of the innate immunity, although they also have been described to play a role in adaptive immunity. NK cells are in possession of strong cytolytic responses and have the ability to release mediators, such as cytokines and chemokines that mediate inflammatory responses. They control cancer cells by interacting with them or enhancing the function of other (immune) cells in the tumor environment. Many studies have been performed about priming allogeneic NK cells to activate them and with the idea of administering these activated cells to AML patients and induce anti-tumor responses in this way. Even clinical trials have already shown promising results for this strategy. In this review, the possibilities of NK cells in immunotherapeutic strategies against AML blasts will be discussed.

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Introduction

Acute myeloid leukemia

Acute myeloid leukemia (AML), also known as acute myelogenous leukemia, is a heterogeneous disease, defined by clonal expansion of myeloid progenitor cells in the bone marrow and peripheral blood (Saultz JN and Garzon R, 2016). Annually, 30.000 new patients are diagnosed with AML in Europe, primarily affecting older individuals at a median age of 66 years. AML accounts for approximately 80% of all acute leukemia in adults. The 5-year survival of adults is around 30% in Europe, with particularly dismal outcomes in the elderly (Visser O et al., 2012). Risk factors for developing AML are environmental factors and simply bad luck. Most mutations in AML are somatic and not familial (Steensma DP et al., 2015). Therefore, genetic predisposition does not play a role in most cases. The most common symptoms of AML are B-symptoms like fatigue, fever, and weight loss, but also anemia-related symptoms, like shortness of breath and symptoms due to thrombocytopenia, like excessive bruising and nosebleeds (Davis AS et al., 2014).

Pathogenesis of AML

Normal hematopoiesis starts with the multipotential hematopoietic stem cell (HSC). This stem cell is capable of self-renewal and it can differentiate into all mature cells of the hematopoietic system (Pang WW et al., 2017). The myeloid progenitor cells in the bone marrow, that form out of the HSCs, are able to differentiate in several cell types, among which erythrocytes, thrombocytes, and monocytes (Figure 1). Since the myeloid progenitor cells are affected in AML, the symptoms fitting AML are thus due to the replacement of normal blood cells with leukemic cells.

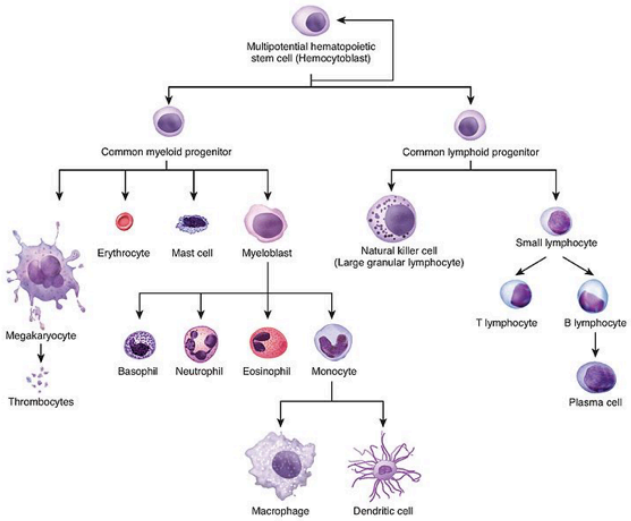

Figure 1: A schematic overview of normal hematopoiesis. The HSC divides into two progenitor lines; the common myeloid progenitor and common lymphoid progenitor. Out of these progenitor cells, all hematological cells can be formed.

The evolution of AML is a multistep process, in which clonal hematopoiesis of indeterminate potential (CHIP) plays an important role. CHIP is defined by somatic mutations in leukemia-associated driver genes resulting in the expansion of a genetically identical clone of marrow and blood cells (Steensma DP, 2018). More than 80% of the observed mutations in The Cancer Genome Atlas dataset were in 19 genes, heretofore associated with leukemia or lymphoma, including ASXL1, TP53, BCORL1, GNAS and SF3B1, as well as DNMT3A, TET2 and JAK2 (Steensma DP et al., 2015). The most common genes identified as mutated were the hematologic malignancy-associated DNMT3A, ASXL1, and TET2. Individuals with clonal mutations have an increased risk of a hematological malignancy diagnosis and death compared to age-matched individuals without mutations (Steensma DP et al., 2015). Most of the mutations found in AML genomes are random events that occur in the HSCs. The mutations keep existing as the clone expands and, in many cases, one or two cooperation mutations are necessary to generate the malignant founding clone. Cells from the malignant clone can acquire additional mutations and therefore develop subclones that can contribute to the progression of the disease and/or relapse (Welch JS et al., 2012).

In addition, leukemic stem cells (LSCs) and leukemia-initiating cells (LICs), a subpopulation of AML cells, that have long-term repopulating potential, are located in the bone marrow microenvironment. These LSCs are a main problem in AML because they are drug-resistant. Chemotherapeutic drugs that target fast-dividing cells are unlikely to affect the stem cell population because LSCs maintain a quiescent cell cycle status. They also may own natural mechanisms of survival, as they are more primitive developed than tumor cells and the LSCs are biologically similar to healthy cells since they have fewer oncogenic lesions (Siveen KS et al., 2017). Relapse from AML is a common problem and mainly due to those remnant LSCs in the bone marrow (Shafat MS et al., 2017).

Treatment of AML

The treatment of patients with AML depends in the first place on genetics. This dictates the risk category and therefore the therapeutic intervention. The genetic profile of the patient is useful to decide whether a bone marrow transplant should be performed or whether novel therapies are considered, such as inhibitors against e.g. IDH or FLT3 (DiNardo CD and Cortes JE, 2016). Thereby, the treatment is also dependent on the health state and age of the patient. Older patients are often not capable of dealing with high-intensity chemotherapy and are generally too weak for hematopoietic stem cell therapy (HSCT) (Acheampong DO et al., 2018). The treatment for AML is divided into two phases; induction and consolidation, which includes stem cell transplantation. Induction focusses on eliminating the bulk of the tumor and hopefully also as much as possible of the LSCs to achieve complete remission (CR), while the aim of consolidation is to eradicate the residual leukemia cells, including LSCs that persist after induction (Ferrara F et al., 2013). Even though chemotherapy seems to be a good first treatment option, AML stem cells can develop resistance to chemotherapy and thus respond less likely than expected. Cancer cells can be recognized by the immune system via immunosurveillance. This consequently eliminates the tumor cells. However, tumor cells are able to escape immunosurveillance by expressing inhibiting molecules or secretion of immunosuppressive cytokines (Beatty GL and Gladney WL, 2015). Immunotherapy aims at enhancing the immunosurveillance and thereby killing the cancer cells.

Cellular microenvironment in AML

The bone marrow microenvironment plays an important role in AML. The bone marrow is soft tissue, located in the cavities of bones. The bone marrow stromal cells (BMSCs) are responsible for the formation of the microenvironment for hematopoiesis, but they also give rise to other cells like adipose tissue, bone cartilage, and myofibers. AML blasts are capable of affecting and manipulating BMSCs in the bone marrow microenvironment. This way, the AML blasts create their own microenvironment that supports their survival and proliferation (Shafat MS et al., 2017). The leukemic cells formed in AML express leukemia-associated antigens (LAAs). Some of these LAAs, like proteinase 3 (PR3) and Wilm's tumor antigen 1 (WT1), can cause a cytotoxic T cell response (Van Driessche A et al., 2005). This, in contrast to other molecules expressed on the membrane of AML cells, which contribute to escaping immunosurveillance. Important markers for this principle are cluster of differentiation 47 (CD47) and programmed death-ligand 1 (PD-L1) (Jaiswal S et al., 2009; Chen X et al., 2008).

Aim of this review

Immunotherapy is a highly interesting principle for the treatment of various cancers. More and more mechanisms of the immune system and its role in immunosurveillance in cancer become clear and thereby, specific molecules that play a role in immunosurveillance can be targeted. There also seem to be several targets for immunotherapy in AML. In most cases of AML, chemotherapy does not achieve the satisfactory effect that is desired. Thereby, chemotherapy goes together with high cytotoxicity and high risk of relapse after the patients achieved remission. Thence, it would be interesting to use immunotherapy in AML patients, to achieve CR and lower relapse rates. This review will focus on the current therapy for AML and the possibilities of immunotherapy. Since the strategies of immunotherapy diverge a lot and NK cell therapy has renewed interest in AML therapy, only the possibilities of using NK cells in immunotherapy for AML will be discussed in this review.

Current therapy for AML

Chemotherapy

Chemotherapy is a form of cancer treatment, that consists of one or a combination of several anti-cancer drugs. It can be given to patients with curative intent, but it can also be given with the aim to prolong life and reduce symptoms. Latter is used in palliative care. For most cancers, including AML, chemotherapy is the standard treatment option, because it is readily available and affordable (Acheampong DO et al., 2018). Chemotherapeutic drugs act on the division of cells, especially fast-dividing cells. The agents prevent mitosis of cells by damaging the DNA and inhibiting cellular mechanisms of the cell (Malhotra V et al., 2003). And since cancerous cells undergo uncontrolled growth together with other malignant properties, called the 'hallmarks of cancer', chemotherapy is effective on these cells (Hanahan D and Weinberg RA, 2011). Nonetheless, chemotherapy also has quite a few disadvantages.

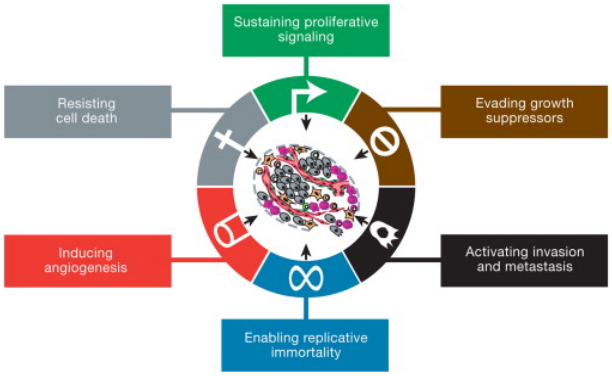

Figure 2: Overview of the 'Hallmarks of Cancer'. These are abilities of cancerous cells, that help to survive and escape the host's immune system. (Hanahan D and Weinberg RA, 2011)

First, chemotherapy is not a curative treatment for AML in most cases. Figure 3 shows the overall survival (OS) and the disease-free survival (DFS) of patients younger than 60 years (Figure 3 A, B) and older than 60 years (Figure 3 C, D), according to a study of Mrózek et al. (Mrózek K et al. 2012). The patients were subcategorized in favorable, intermediate I, intermediate II and adverse genetic groups. The OS and DFS of younger patients are longer compared to older patients. And besides, this figure also demonstrates the importance of genetics concerning the treatment, since the adverse genetic group shows much poorer outcomes after chemotherapy.

Secondly, the side effects of chemotherapy are a major problem in the treatment of cancer. The chemotherapeutic agents do not affect the fast-dividing cancerous cells but also other fast-dividing healthy tissues in the human body, like cells from the bone marrow, epithelial lining of the intestinal and oral mucosae and hair follicles (Acheampong DO et al., 2018). Therefore, these cells are likely to be affected by chemotherapy, which was targeted to affect the cancer cells.

Thereby, a lot of tumors contain heterogeneity and possible resistant cells. Although, combination chemotherapy provides maximum cell kill and offers a broader range of coverage of resistant cells and might prevent or at least slow down the development of new drug-resistant cell lines (Malhotra V et al., 2003). It is likely to think the more chemotherapeutic agents are used, the higher the cytotoxicity of the treatment.

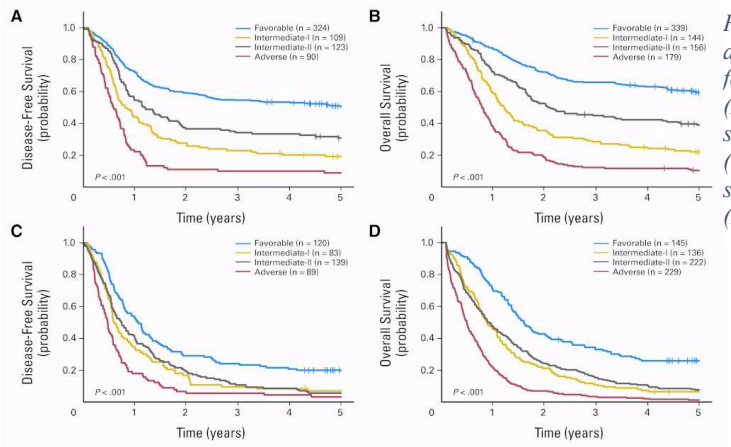

Figure 3: The outcome of patients with AML after first-line treatment classified into the four European LeukemiaNet genetic groups. (A) Disease-free survival and (B) overall survival of patients younger than 60 years. (C) Disease-free survival and (D) overall survival of patients older than 60 years (Mrózek K et al. 2012).

Furthermore, relapse commonly occurs in AML patients. It is known LSCs can remain in the bone marrow after treatment of AML and it was suggested that a lot of LSCs are quiescent, which results in their ability to escape the killing effect of cytotoxic chemotherapeutic agents, which target rapidly dividing cells (Jordan CT et al., 2006; Saito Y et al., 2010). Nevertheless, a more recent study by Farge et al. demonstrated contrasting results. In patient-derived xenografts, treated with cytarabine (AraC), the AraC-resistant pre-existing and persisting cells showed high levels of reactive oxygen species, increased mitochondrial mass, and retained active polarized mitochondria consistent with a high oxidative phosphorylation (OXPHOS) status. The high OXPHOS but not low OXPHOS human AML cell lines were resistant to chemotherapy in vivo. This study thus demonstrates that essential mitochondrial functions of the AML cells also contribute to AraC resistance to in the treatment of AML (Farge T et al., 2017).

As treatment for AML, adults get administered with cytarabine and an anthracycline. The standard combination is the '7+3 regimen', with a 7-day continuous infusion of cytarabine (100 or 200 mg/m^2 per day) on days 1 to 7 and daunorubicin (60 mg/m^2 per day) on days 1 to 3 (Dombret H et al., 2015). Although, the intensity of the treatment depends on the age and health state of the patient. Younger patients often undergo more intensive chemotherapy, sometimes followed by a hematopoietic stem cell transplantation, if they are at high risk of relapse (Acheampong DO et al., 2018).

Hematopoietic Stem Cell Transplantation

With a hematopoietic stem cell transplantation (HSCT), multipotent hematopoietic stem cells (HSCs), usually derived from bone marrow, peripheral blood or umbilical cord, are transplanted. The stem cells can be from the patient itself (autologous), from a donor (allogeneic) or from an identical twin (synergic) for the induction of tissue regeneration and immunological lysis of pathogen or malignant cells. For avoiding host-versus-graft rejections, tissue typing of human leukocyte antigens (HLA) for tissue and organ transplant as well as the use of immunosuppressant drugs is recommended (Mahla RS, 2016). Besides risking rejection of the graft, infection is also a major complication after HSCT (Park B et al., 2015). In AML, allogeneic HSCT provides a high rate of curability, but is also associated with transplant-related morbidity and mortality (TRM). The risk for TRM increases with the use of an unmatched HLA donor. Autologous HSCT in AML patients enables the intensification of chemotherapy, is associated with a lower TRM, and the transplant graft is highly available. However, these benefits may be confuted by a higher rate of relapse, because the graft-versus-leukemia effect is not as much as with an allogeneic HSCT (Takami A, 2018).
Therefore, allogeneic HSCT is preferred over autologous HSCT. But to receive an allogeneic transplant, patients must be relatively healthier and thus often younger to be good candidates, because of the risk of serious complications. The most severe complication after allogeneic HSCT is graft-versus-host disease (GVHD), which is the leading cause of morbidity and mortality. GVHD could be described as an unwanted, exaggerated normal inflammatory mechanism, in which lymphocytes of the donor recognize the antigens of the host as foreign, which induces inflammation, and thus damages the patient's own body tissues (Ferrara JLM et al., 2009).
In addition, mutations of TET2 and DNMT3A, already known to be associated with a poor prognosis in AML patients, also lead to a shorter OS after allogeneic HSCT in patients with myelodysplastic syndrome (MDS) (Bejar R et al., 2014). It is likely to think that these mutations would have a similar effect on the OS of AML patients after allogeneic HSCT.

In conclusion, the treatments that are available for AML nowadays do not have a satisfactory effect. Every year, doctors diagnose around 19520 people in the United States with AML, of which 10670 deaths occur on a yearly basis. The remission rate is around 67%, but patients older than 60 years do not typically respond to the treatment and only achieve a remission rate of approximately 50%. Thereby, people who achieve remission do not always stay in remission. For many, AML can return over time (Nall R and Chun C, 2018). This, together with the fact that the treatments nowadays are highly cytotoxic, and the patients are at risk of side effects and severe complications, demonstrates the need of developing better and less toxic treatments for AML.

Immunotherapy as alternative option for AML

Cancer and the immune system

The immune system has been described to play a role in cancer. Some immune responses can promote the initiation or progression of cancer, while other immune responses can protect the host from developing a tumor (Schreiber RD et al., 2011). The innate and adaptive immune system both participate in this tumor surveillance. Progression of AML and certain other cancers is only possible if they are able to escape the immunosurveillance mechanisms of the immune system (Marcus A et al., 2014). Cancer cells can escape immunosurveillance through several approaches. This process is known as 'cancer immunoediting'. The clones can evolve and express specific proteins (e.g. PD-L1) to avoid immune-mediated elimination by leukocytes that have anti-tumor properties. Or they recruit immunosuppressive leukocytes which create a microenvironment that disturbs the anti-tumor response (Figure 4) (Beatty GL and Gladney WL, 2015). The identification of inhibiting molecules, such as PD-L1, expressed by tumor cells has led to the revival of immune therapy research.

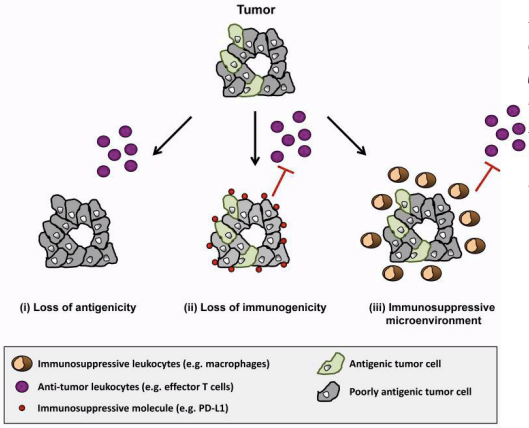

Figure 4: Immune escape mechanisms in cancer. (i) Loss of antigenicity may be achieved through the asset of defects in antigen processing and presentation. (ii) Loss of immunogenicity can be reduced by the expression of immunosuppressive molecules (e.g. PD-L1) or secretion of suppressive cytokines (e.g. IL-10, TGF- β. (iii) Tumors can also establish an immunosuppressive microenvironment by recruiting immunosuppressive leukocytes, such as macrophages (Beatty GL and Gladney WL, 2015).

Methods in immunotherapy

Cancer immunotherapy can be divided into passive and active immunotherapy (Table 1) (Zhang H et al., 2018). With passive immunotherapy, the drugs are used to enhance the existing anti-tumor response, while active immunotherapy focusses on stimulating the host's immune system to destroy the tumor cells (Kakimi K et al., 2017).

Table 1: Cancer immunotherapy divided into active and passive immunotherapy. Passive immunotherapy consists immunomodulating antibodies and adoptive cell therapy. Active immunotherapy can be sub-divided in specific and non-specific therapy.

Passive immunotherapy		Active immunotherapy	
Immunomodulating antibodies	**Adoptive immunotherapy**	**Specific**	**Non-specific**
Immune-checkpoint inhibitors	Tumor-infiltrating lymphocytes	Vaccines	Immune adjuvants
Immune co-stimulatory antibodies	TCR-gene modifies lymphocytes		Cytokines
	Chimeric Antigen Receptors (CARs)		

At present, there are four promising methods, which will be discussed briefly. To start with monoclonal antibodies (mAbs). These are modified proteins that are aimed to target specific parts of deregulated signal transduction pathways in cancer or to interfere with immunological processes. A lot of mAbs are already approved for the treatment of both solid and hematological malignancies, and also new clinical trials with mAbs are ongoing (Henricks LM et al., 2015).

Cancer vaccines are a form of active immunotherapy and have the aim to stimulate or restore the ability of the immune system to fight cancer. The vaccines can be classified into preventive vaccines and therapeutic vaccines. Preventive vaccines, such as the vaccines against hepatitis B virus (HBV) and human papillomavirus (HPV), limit the chance of developing the cancer, that is caused by the virus

(Speiser DE et al., 2014). Therapeutic vaccines directly target the immune system and then enhance the system's attack on tumor cells (Shore ND et al., 2015).
For hematological malignancies as well as solid cancers, adoptive cell transfer (ACT) of tumor-associated antigen-specific T cells is a very attractive form of immunotherapy (Restifo NP et al., 2012). Tumor-infiltrating lymphocytes (TILs) were used in ACT studies, for having promising effects in metastatic melanoma patients, but later this approach has been limited by the difficulty in expanding viable TILs and only showing their effector functions. To overcome this problem, CAR- and T cell receptor engineered T cells have been developed by genetic modifications and their promising effects have been observed in various studies for several cancers (Zhang H et al., 2018).
Last but not least, the immune checkpoint blockage. Immune checkpoints are molecules that regulate the intensity of the T cell response and are critical for avoiding autoimmunity. Only at the same time, they also limit the power and duration of the beneficial anti-tumor response. Molecules that are crucial in the checkpoint regulation involve CTLA-4, PD-1, T cell immunoglobulin and mucin domain containing protein 3 (Tim-3) and lymphocyte activation gene 3 (LAG-3) on the T cell surface (Makkouk A et al., 2015). In 2011, the first immune checkpoint therapy was approved for use in metastatic melanoma and since then, many more have been approved for the treatment of other cancers. At present, more than 100 trials are ongoing to study the effect of immune checkpoint blockers in various cancer types (Zhang H et al., 2018).

Immunotherapy in AML
Also, many studies about immunotherapeutic options are ongoing for AML. But until now, since 2000, the only immunotherapeutic option that is approved for clinical use in the United States is gemtuzumab ozogamicin (GO), a humanized anti-CD33 antibody conjugated to calicheamicin. However, this treatment was withdrawn from the market in 2010, because of high rates of mortality and hepatic veno-occlusive disease (Y Liu 2019). In 2017, the treatment was re-approved in a different schedule for the treatment of $CD33^+$ AML patients (Barron J et al., 2018). But anti-CD33 is not the only antibody of interest in AML. Other studies focus for example on CD45, CD30, and CD44 too. But also, the other immunotherapeutic methods, such as cancer vaccines, immune checkpoint inhibitors, and adoptive cell therapy are being studied for the treatment of AML (Liu Y et al., 2019). CAR T cells seem to be a very promising treatment option (S Hoffman 2019). But even though two studies showed initially blast reduction after using CAR T cells, eventually all the patients relapsed or even showed disease progression (Ritchie DS et al., 2013; Wang QS et al., 2015). Other immune cells that have shown hopeful results for a possible treatment are NK cells. Their therapeutic potential will be further discussed below.

NK cell therapy

NK cells
The immune system is divided into two sub-systems; the innate immune system and the adaptive immune system, which respectively roughly represent the non-specific first response and the antigen-specific response of the body. NK cells play an important role in innate immunity as well as in adaptive immunity, in which its function is more recently discovered and described (Paust S et al., 2011). They are in possession of strong cytolytic responses against tumor or virus-infected cells and they have the ability to release cytokines and chemokines that mediate inflammatory responses to induce hematopoiesis and modulate the adaptive immune responses (Moretta L et al., 2005).

Role of NK cells in cancer
Innate immune cells can control cancer cells by directly interacting with them or by enhancing the function of other (immune) cells in the tumor environment (Marcus A et al., 2014). NK cells are able to recognize and kill malignant cells without prior sensitization. NK cells express $CD3^-CD56^+$ and are divided into two leading subtypes: $CD16^+CD56^{dim}$ and $CD16^-CD56^{bright}$. The latter subtype includes the less mature, cytokine producing NK cells and resides predominantly in lymphoid tissue. $CD16^+CD56^{dim}$ NK cells are the mature, cytotoxic cells, which represent 90% of the NK cells in circulation (Hansrivijit P et al., 2019). NK cells express inhibitory and activating receptors on their surface to mediate their function. Cytotoxicity of NK cells is triggered when the activating signals overcome the inhibitory

signals (Morvan MG and Lanier LL, 2016). The most important inhibitory signals act via killer immunoglobulin-like receptors (KIRs), which recognize major histocompatibility complex (MHC) class I antigens on autologous cells (Hansrivijit P et al., 2019). Activating signals are mediated through C-type lectin receptors and natural cytotoxicity receptors (Moretta A et al., 2000).
NK cells are capable of directly killing tumor cells through several pathways. One of them is direct tumor cell lysis by secreting perforin and granzymes (Van den Broek MF et al., 1995). But the NK cells can also eliminate tumor cells through death-receptor ligands such as FasL (Lee RK et al., 1996) or by the expression of CD16 which leads to antibody dependent cellular cytotoxicity (ADCC) (Brehm C et al., 2014).

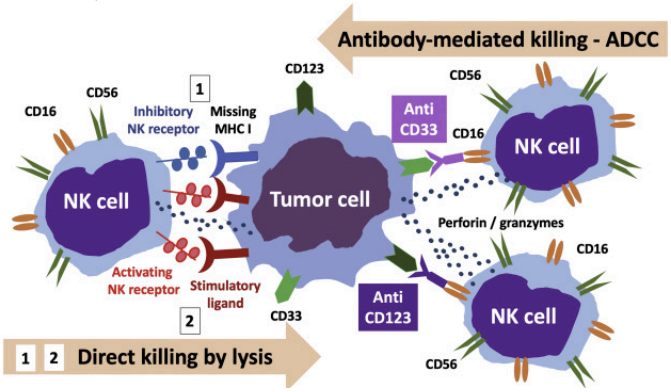

Figure 5: NK cytotoxicity against leukemic cells. NK cells can kill tumor cells by direct activation or by an antibody-mediated mechanism. Direct activation occurs when the inhibitory NK cell receptor fails to recognize self-antigen on MHC I (1) or when the activating NK receptors bind stimulatory ligands (2). ADCC of NK cells occurs when tumor antigens bind to their antibodies. These antibodies bind to CD16 on NK cells, which results in the release of cytotoxic enzymes (Hansrivijit P et al., 2019).

However, NK cells from AML patients seem to have an abnormal phenotype, decreased function and decreased cytokine production (Costello RT et al., 2002; Stingaris K et al., 2014). Thereby, autologous NK cells fail to recognize and kill AML blasts due to their dysfunction. This suggests that therapy with NK cells should be done with allogeneic NK cells to be effective. Nonetheless, the findings of Costello and Stingaris are in contrast with a study of Yang et al. in 2018. They investigated the characteristics of NK cells in the leukemic microenvironment and their effect on T cells. They used an MLL-AF9 induced AML mouse model for this study. And they discovered that in the early and middle stages of AML, an increase of NK cells occurs in the AML spleen. This splenic microenvironment promotes NK cell activation and cytotoxicity molecules and cytokines were upregulated in the activated NK cells. Additionally, the NK cells from the AML microenvironment regulated T cell function, not only by controlling their activation but also their differentiation (Yang F et al., 2018).
These findings are not the only ones that suggest that NK cells play an important role in T cell activation. It was already described before that NK cells enhance T cell polarization via IFN-ɣ secretion (Morandi B et al., 2006).
Studies about the role of NK cells in acute lymphoblastic leukemia (ALL), another form of acute leukemia, also showed that NK cells promote anti-tumor activity (Boieri M et al., 2017; Jin F et al., 2016). All these findings demonstrate that NK cells play an important role in the tumor environment and that they are an interesting target for immunotherapeutic strategies.

NK immunotherapy in AML
The NK cell function in AML has already been studied for a long time and many ideas about using NK cells in immunotherapy have been acquired. Brune et al. discovered in 1996 that monocytes inhibit the anti-leukemic abilities of NK cells. However, they also found out that histamine reverses this inhibitory signal of monocytes and thereby, imitates the NK cell-activating cytokines to induce killing of AML blasts (Brune M et al., 1996). In 2016, this effect of histamine combined with interleukin 2 (IL-2) is studied as maintenance treatment in AML patients, who already received chemotherapy. Their data suggest that this therapy supports the reconstitution of a deficient NK cell fraction (Cuapio A et al., 2016).

NK cells can be activated by cytokines or by specific interactions. Research conducted by Wang et al. (2015) included that NK cells play a significant role in immunosurveillance at the early stage of AML through CD226/CD155 interactions. They depleted NK cells and discovered that the cells were necessary for the elimination of leukemia cells in their AML mouse model. In addition, their results

show that the CD266/CD155 interaction primarily mediated the contact between NK cells and leukemic cells and thus contributes to the anti-tumor effects of NK cells in AML. Other interactions such as the NK activating receptor NKG2D with its ligand RAE also seemed to play a role in the immunosurveillance. Another finding in their study was the upregulation of a subtype of MHC-I, which might contribute to the development of resistance of the leukemic cells to NK cell killing (Wang Y et al., 2015).
Baessler and coworkers have studied another interaction between NK cells and AML blasts, which is CD137-CD137L. They identified CD137L on the AML cells as an inhibitor of the anti-leukemic response of NK cells. Signaling via CD137L into the AML blasts stimulates the release of IL-10 and tumor necrosis factor (TNF). IL-10 is known to mediate immunosuppression. TNF is a molecule with potent immunomodulatory functions, but that depends on the cellular context and cell type. Blocking CD137 in cocultures of CD137L-expressing AML blasts with allogeneic NK cells increased the granule mobilization, cytotoxicity and IFN-ɣ production of the NK cells (Baessler T et al. 2010).

An important cytokine for NK homeostasis is IL-15. Sczepanski and coworkers cultured autologous NK cells from AML patients with IL-15 and they found out that IL-15 caused upregulation of the expression of activating receptors (e.g. NKG2C and NKG2D) on the NK cells (Figure 6). Together, the cytotoxic activity of NK cells against AML blasts was increased after stimulation with IL-15 (Szczepanski MJ et al., 2010). The pre-activation of NK cells with IL-15 also seems to be effective in other hematological malignancies, such as T-ALL (M Boieri et al., 2017). In 2011, a clinical trial for haploidentical donor NK cell infusion with IL-15 in adult AML patients has been performed. But unfortunately, the results of this trial were not presented (ClinicalTrials.gov, Identifier: NCT01385423).

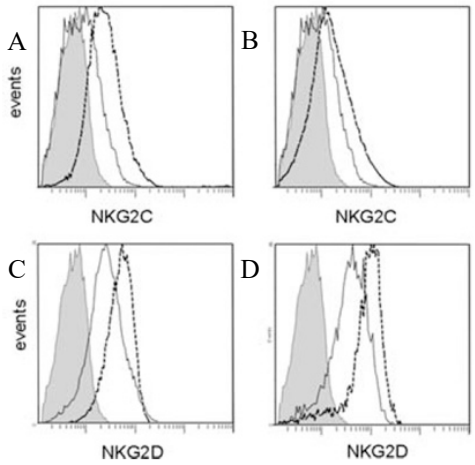

Figure 6: Upregulation of expression of NK activating receptors after 72h culture with IL-15 in NK subsets in AML patients. (A) and (C) present the upregulation of NKD2C and NKG2D respectively for the CD16^{-}CD56^{bright} subtype. (B) and (D) present the upregulation of the receptors for the CD16^{+}CD56^{dim} subtype. The grey histogram indicates isotype control, black line indicates day 0 values and the dotted line indicates expression levels after 72h culture with IL-15 (Szczepanski MJ et al., 2010).

Not only pre-activation with IL-15 causes more effective NK cells. Cytokine-induced memory-like NK cells can also differentiate and exhibit enhanced responses to cytokine or activating receptor stimulation after pre-activation with the combination of IL-12, IL-15, and IL-18 (Romee R et al., 2016). Their study even showed in the context of a phase 1 clinical trial, that the adoptively transferred NK cells proliferated and expanded in AML patients, while they demonstrated strong responses against leukemia targets. Eventually, four complete remissions were observed out of 9 evaluable patients.

However, cytokines and receptor interactions are not the only methods for “priming” NK cells. They can also be primed with leukemia cell lysate. Recently, Fehniger et al. performed a phase 1 clinical trial, in which they used CTV-1 leukemia cell line lysate CNDO-109 for priming HLA-haploidentical donor NK cells. These NK cells were administered to AML patients who achieved remission after chemotherapy but who were at high risk of recurrence. This therapy led to prolonged remission in three of the twelve patients, of which 2 remained relapse-free (Fehniger TA et al., 2018). NK cell therapy can also be used after allogeneic HSCT, in patients with high risk of relapse. Promising improvements in relapse rate, immune reconstitution, and viral control have been demonstrated after allogeneic HSCT by using donor NK cells in a phase 1 clinical trial performed by Ciurea et al. (Figure 7). They expanded the NK cells ex-vivo with membrane-bound IL-21 (mbIL21) and showed that it was safe to infuse high-doses of ex-vivo mbIL21 expanded donor NK cells (Ciurea SO et al., 2017).

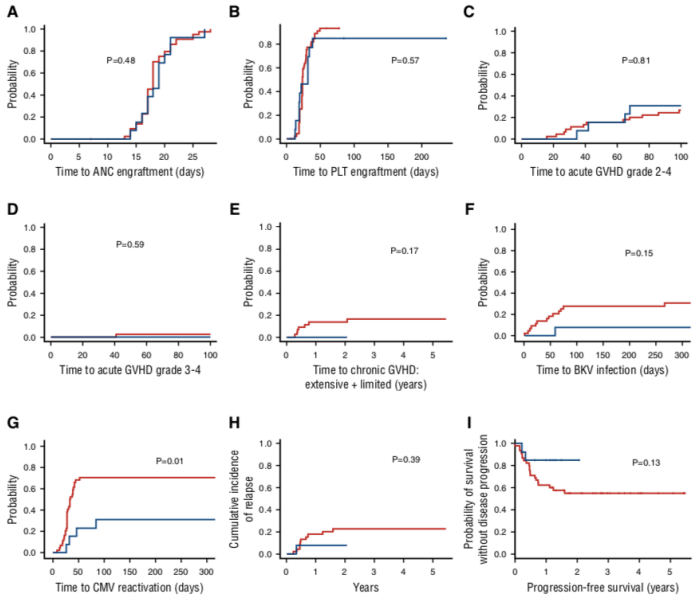

Figure 7: Comparison of clinical outcomes between patients treated with NK cells (blue line) and without NK cells (red line). (A) Time to neutrophil engraftment. (B) Time to platelet engraftment. (C) Cumulative incidence of grade 2-4 acute GVHD (aGVHD). (D) Cumulative incidence of grade 3-4 aGVHD. (E) Cumulative incidence of chronic GVHD limited 1 extensive. (F) Incidence of BK polyomavirus (BKV) cystitis. (G) Incidence of cytomegalovirus (CMV) reactivation. (H) Cumulative incidence of relapse. (I) Probability of progression-free survival (Ciurea SO et al., 2017).

Conclusion

AML is a complex disease, which mainly develops due to somatic mutations and environmental factors. The standard treatment nowadays does not achieve the results that are desired and that is also why the overall survival of patients with AML remains too low, especially in the elderly. In the past years, immunotherapy has been of great importance in research about new therapeutic strategies for cancer. Those strategies diverge from cancer vaccines to adoptive cell therapies. For AML, a lot of research has been performed about different immunotherapeutic strategies, but for now, only gemtuzumab ozogamicin (GO) is approved for clinical use in the United States (Barron J et al., 2018).
An interesting field of immunotherapy is adoptive cell therapy. CAR T cells are an example of this, but also NK cells seem to have great opportunities. These cells play a significant role in the response against tumor cells and leukemic blasts. Yang et al. discovered in an AML mouse model that NK cells get activated in the spleen and they also stimulate proliferation and differentiation of T cells (Yang F et al., 2018). However, this study was performed in mice and there are no results yet that confirm this idea about NK cells in AML patients. In studies from Costello et al. and Stingaris et al. is even demonstrated that NK cells in AML patients have evolved into an abnormal phenotype and thus decreased in functional activity (Costello RT et al., 2002; Stingaris K et al., 2014).

Adoptive cell therapy is a form of passive immunotherapy and thus focusses on enhancing the existing anti-tumor response. Active immunotherapy aims at stimulating the host's immune system to destroy the tumor cells, but since NK cells in AML patients seem to have an abnormal phenotype, forms of active immunotherapy with autologous NK cells might seem less relevant to study. However, Szczepanski et al. showed promising results about the upregulation of expression of NK cell activating receptors, such as NKG2D and NKD2C, after culturing autologous NK cells with the cytokine IL-15. The NK activating receptor NKG2D has been described later to play an important role in immunosurveillance against AML blasts (Wang Y et al., 2015). Other interactions between the NK cells and AML blasts that are of interest for their immunotherapeutic potential are CD226/CD155 and CD137/CD137L.
Allogeneic NK cells can be pre-activated in several ways; via stimulation of NK cell activating receptors, via cytokines or via leukemia cell line lysate. Specific interactions, such as the aforementioned CD226/CD155, contribute to the activation of NK cells and their anti-tumor response against leukemic cells. Cytokines are another pathway to activate NK cells. IL-15 alone, or in combination with IL-12 and IL-18, seems to have a stimulating effect on NK cells in AML and other hematological malignancies, like T-ALL (Szczepanski MJ et al., 2010; Boieri M et al., 2017). Recently, a phase 1

clinical trial is performed, in which they investigated haploidentical allogeneic NK cells primed with leukemic cell lysate, to prevent relapse in patients, who already achieved remission (Fehniger TA et al., 2018).

Consequently, NK cell therapy on its own is not capable yet of achieving remission in AML patients, but it seems to be a very promising therapy to prevent relapse in patients, who underwent chemotherapy or an allogeneic HSCT, as demonstrated by Ciurea et al. Thereby, their study also demonstrated that the infusion of high doses of NK cells is a safe application and NK cells can be relatively easily derived from peripheral blood of donors (Ciurea SO et al., 2017). Furthermore, there are several methods discovered to pre-activate the NK cells before eventually administering them to patients. It would be interesting to study the effects of some of these priming methods in patients, as they are now only tested ex-vivo or in AML mouse models.

In summary, immunotherapies for AML have already achieved some successes in clinical trials and other studies in this field of interest have demonstrated that NK cell therapy can be a potential therapy for AML to reduce relapse rates and increase overall survival. NK cell therapy seems to have promising capabilities, but more research and clinical trials are necessary to determine the therapeutic value of this strategy.

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