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IMMUNE REPERTOIRE DIVERSITY IN ALLOGENEIC STEM CELL TRANSPLANTATION AND ITS IMPLICATIONS FOR INFECTIONS AND THE GRAFT VERSUS LEUKEMIA EFFECT

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Immune Repertoire Diversity in Allogeneic Stem Cell Transplantation and its Implications for Infections and the Graft Versus Leukemia Effect

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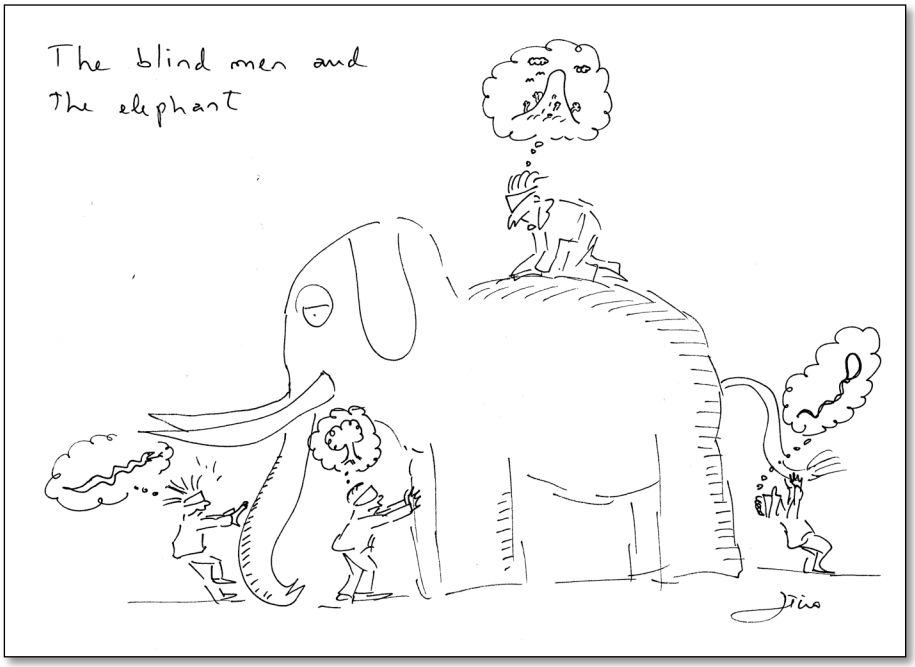
To my fantastic family

The essence of science



By my first scientific mentor Decio Eizirik, 1992

ABSTRACT

The beneficial graft versus leukemia effect (GVL) and its detrimental counterparts, graft versus host disease (GVHD) and susceptibility to infections, are all coupled to a multitude of events during the immune reconstitution (IR) after hematopoietic stem cell transplantation (HSCT). The general aim of this thesis has been to learn more about the IR in HSCT with a particular focus on the impact of infections, natural killer (NK) cell mediated GVL effects and the possibility to apply GVL effects in adoptive cell therapy.

In **paper I**, we identified factors interfering with the IR, thereby making the patients susceptible to late lethal infections. We found that cytomegalovirus (CMV) was an independent risk factor for death in late infections. NK cells are important for controlling CMV infections and patients lacking NK cells suffer from cyclic herpes virus reactivations.[1-3] NK cells are also known to mediate GVL-effects and have been coupled to reduced relapse rates after HSCT. The results of **paper I** thus prompted us to study NK cell-mediated GVL effects and the interaction between CMV and NK cell repertoire dynamics. In **paper II** we examined NK cell-mediated alloreactivity in 105 patients with myeloid malignancies undergoing human leukocyte antigen (*HLA*)-identical sibling transplantation. A longitudinal analysis revealed maintained NK cell tolerance at all time-points during the IR. In agreement with these experimental data, no clinically evident GVL effect was observed based on stratification of missing ligands to killer cell immunoglobulin-like receptors (*KIRs*) in the recipients. In **paper III** we determined the size of the alloreactive subset and graded the ability of different donors to deliver GVL effects in *HLA* -mismatched transplantation. The educated subsets expressing KIRs in presence of a corresponding *HLA* -receptor ligand varied between 12-68% (mean 33%) resulting in 0-62% (mean 8%) alloreactive NK cells depending on recipient *HLA* -ligands. This algorithm served as a template for studies conducted in **paper IV,** where we further dissected the role of pre-transplant NK cell repertoires in the donor and post-transplant repertoires developing after 9-12 months. Unsupervised hierarchical clustering was used to group donors and recipients based on their NK cell receptor repertoires. The result showed that donors with naïve receptor repertoires had less relapse and recipients with a tendency to reset their repertoires towards naivety had less relapse and better overall survival.

In summary this thesis shed new light on the relationships between early and late infections and the recovery of the immune system after HSCT, linking specific NK cell repertoires to protection from relapse and increased overall survival. This knowledge may be useful for the development of new strategies utilizing NK cells in cellular therapies against hematological malignancies.

LIST OF SCIENTIFIC PAPERS

I. **Risk Factors for Fatal Infectious Complications Developing Late after Allogeneic Stem Cell Transplantation**
ANDREAS BJÖRKLUND, Johan Aschan, Myriam Labopin, Mats Remberger, Olle Ringden, Jacek Winiarski and Per Ljungman
Bone Marrow Transplantation, 2007, 40: 1055-1062

II. **NK Cells Expressing Inhibitory KIR for Non–Self-Ligands Remain Tolerant in *HLA* -Matched Sibling Stem Cell Transplantation**
ANDREAS T BJÖRKLUND, Marie Schaffer, Cyril Fauriat, Olle Ringdén, Mats Remberger, Christina Hammarstedt, A. John Barrett, Per Ljungman, Hans-Gustaf Ljunggren and Karl-Johan Malmberg
Blood, 2010, 115: 2686-2694

III. **Estimation of the Size of the Alloreactive NK Cell Repertoire: Studies in Individuals Homozygous for the Group A KIR Haplotype**
Cyril Fauriat, Sandra Andersson, ANDREAS T BJÖRKLUND, Mattias Carlsten, Marie Schaffer, Niklas K. Björkström, Bettina C. Baumann, Jakob Michaélsson, Hans- Gustaf Ljunggren, and Karl-Johan Malmberg
The Journal of Immunology, 2008, 181: 6010–6019

IV. **Integrative Profiling of Natural Killer Cell Repertoires Reveal a Role for Less Differentiated NK cells in Protection from Leukemia Relapse**
ANDREAS T BJÖRKLUND, Trevor Clancy, Jodie Goodridge, Vivien Beziat, Marie Schaffer, Eivind Hovig, Hans-Gustaf Ljunggren, Per Ljungman, Karl-Johan Malmberg
Manuscript

CONTENTS

LIST OF ABBREVIATIONS

ADCC	Antibody-dependent cell-mediated cytotoxicity
APCs	Antigen presenting cells
ATG	Anti-thymocyte globulin
BAFF	B cell-activating factor
CenB	Centromeric haplotype B
CMV	Cytomegalovirus
DC	Dendritic cell
DNAM-1	DNAX Accessory Molecule-1
EBV	Epstein-Barr virus
GVL	Graft versus leukemia effect
HSCT	Allogeneic Hematopoietic Stem Cell Transplantation
IFN	Interferon
IL	Interleukin
ILC	Innate lymphoid cells
IR	Immune reconstitution
KIR	Killer cell immunoglobulin-like receptors
MDSC	Myeloid derived suppressor cells
MHC	Major histocompatibility complex
PTLD	Post-transplant lymphoproliferative disease
TCD	T cell-depleted
TCR	T cell-replete
TGF	Tumor growth factor
TLR	Toll like receptor

1 BACKGROUND

1.1 ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

Implementation of hematopoietic stem cell transplantation (HSCT) during the 80´s, as a therapeutic option for patients with hematological malignancy and other disorders, was the fruit of decades of preclinical and clinical research with some of the most important milestones mentioned below. The continuous refinement of this effective but high-risk procedure has been constantly ongoing ever since.

i. The pioneer animal studies by Medawar, Lorentz, first in irradiated mice[4-6], dogs[7] and monkeys[8] followed by bone marrow infusion and the first patients with leukemia receiving bone marrow after irradiation and accidental irradiation exposure.[9]

ii. The development of transplant immunology. Principles for graft rejection in transplantation delineated by Medawar[10]; the discovery of the major histocompatibility complex (MHC) system in mice by Snell and Gorer[11, 12], the development of serological typing[13] and recent advances within molecular typing.

iii. Identification of transplant-specific complications such as graft versus host disease (GVHD) and morbidity caused by opportunistic infections.[14]

iv. Systematic improvements of conditioning regimens leading to less toxicity, better engraftment and reduction of GVHD and the use of post HSCT immune suppressive therapy.[15, 16]

v. Supportive care with prophylactic and pre-emptive treatment strategies against infections and development of new anti-viral and anti-fungal drugs.

vi. The use of alternative donors haploidentical or cord blood transplantation.

HSCT offers the possibility to cure patients with hematological malignancies incurable by conventional chemotherapy. The principal therapeutic effect is mediated by the immunological process termed graft versus leukemia effect (GVL). The GVL relies on both T and NK cells[17, 18] and is only present in the allogeneic setting. Despite considerable refinement of the transplantation procedures over the years[19], HSCT is still a high-risk treatment associated with life-threatening complications. Several of the complications are coupled to a mismatch between the donor's and the recipient's transplant antigens. This may trigger acute or chronic graft versus

host disease (GVHD), lead to graft rejection or to incomplete immune reconstitution (IR), which in turn predispose for opportunistic infections.

1.2 IMMUNE RECONSTITUTION

1.2.1 General considerations

Conditioning regimens are given to the recipient to hamper T cell mediated rejection, mediate successful engraftment of the stem cell graft and, and in malignancies, to suppress remaining disease. Cytotoxic chemotherapy, irradiation, and anti-T cell antibodies in different combinations all acts to create "immunological space". The choice of regimen depends on the patient's condition, age, and underlying disease.

The reconstitution of the engrafting hematopoietic system can be divided into three different phases:

i) The first (or aplastic) phase, with deficiencies in all immune cells, has a duration of 1-3 weeks.
ii) The second (or acute GVHD) phase lasts for approximately 3 months and is dominated by immature NK cells, granulocytes, monocytes and low numbers of T cells and B cells and a risk for developing acute GVHD.
iii) The third (or late) phase is characterized by B cell deficiency with low levels of IgG_2, IgG_4 and IgA and functional defects of the T cell subsets and a risk for chronic GVHD. The T cell response to alloantigens is usually impaired for up to two years after HSCT. The duration of the late phase is determined by multiple factors including donor or recipient age, source of stem cells, occurrence of GVHD and residual thymic activity.[20, 21]

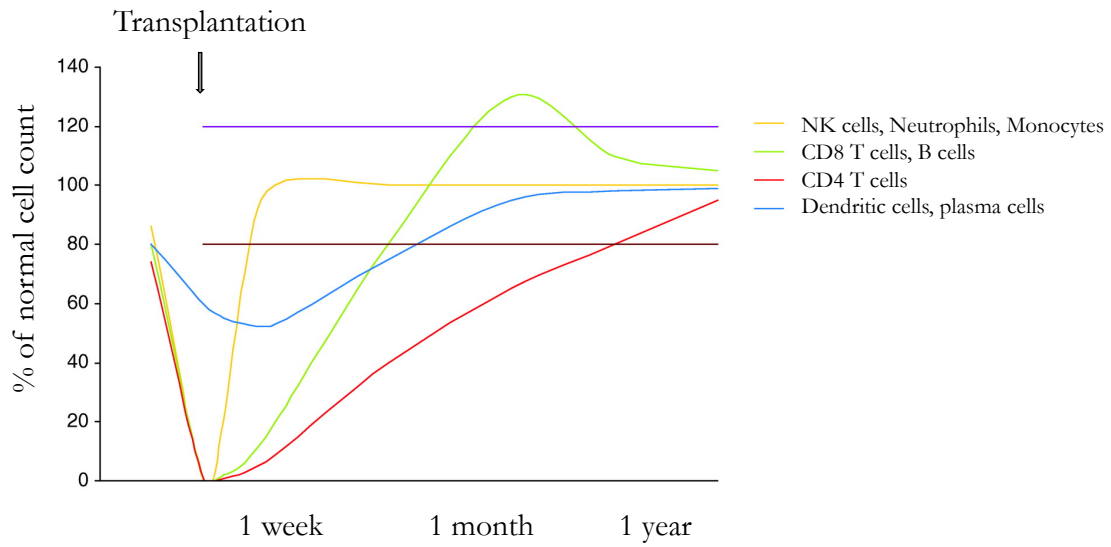


Figure 1. Cellular immune reconstitution after HSCT.

Ref: Adopted from Storek J.Exp.Op.Bi. 20

Reconstitution of different immune cell numbers is depicted in figure 1. Even when the number of cells in each immune subset is normalized, the immune system lacks full function for a long period of time, resulting in increased risk for infections.[22-25] A number of factors reduce the response to antigens. Most T cells present during the first year after transplantation are generated from T cells transferred with the graft after which they expand to fill the empty T cell compartment. High levels of inflammatory cytokines early post-transplant[26, 27]and low T cell numbers favor a fast homeostatic expansion of a limited number of specificities[28-30], lacking the full diversity of a normal T cell repertoire. Homing receptors directing the cells to different lymphoid compartments is downregulated impairing antigen presentation of newly introduced/reintroduced antigens in the host. This is one reason behind the long-standing reduction of B cell responses.[31-34] The functional restoration has also been shown to be further impaired in older recipients.[35, 36]

Infectious spectrum and immune defects after SCT

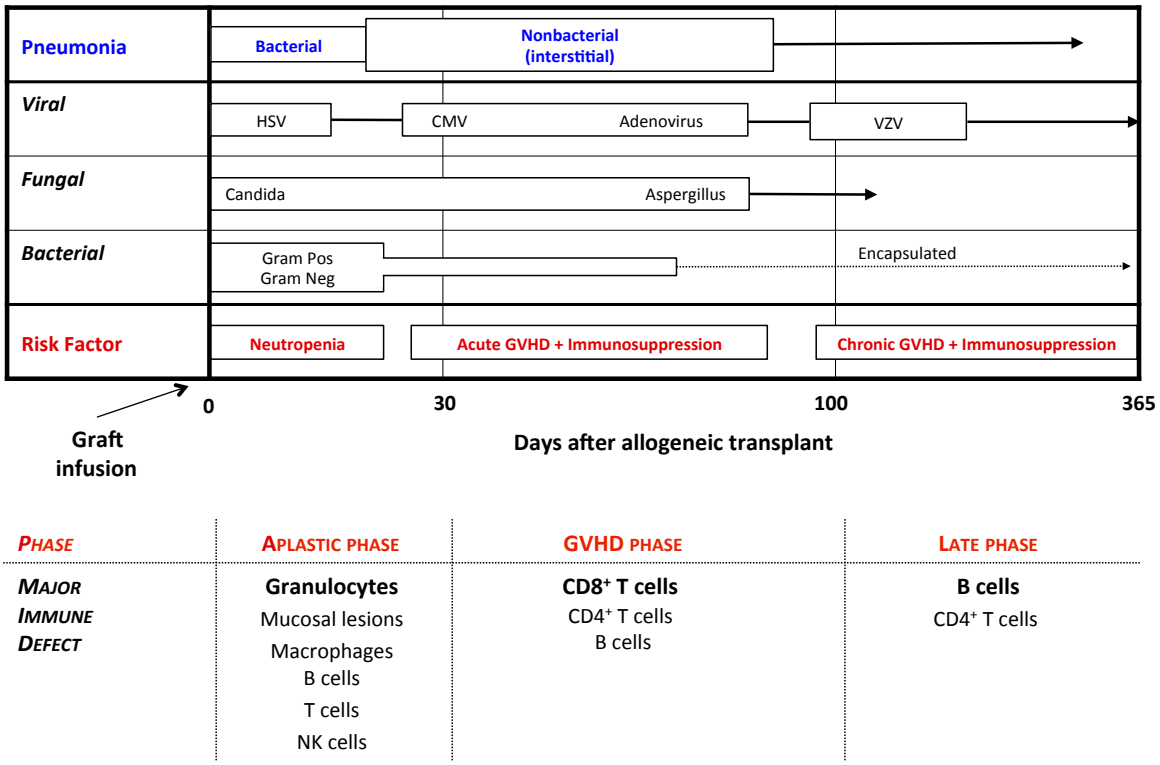


Figure 2. Major immune defects and spectrum of infections post HSCT.

Ref; Extrapolated from figures published in Transplant Infections by Bowden, R.A., Ljungman P, Snydman D.R., 2012.

1.3 INFECTIONS

The immune status of the different phases dictates the spectrum of infections that appears after HSCT (Figure 2). Bacterial infections are common during the aplastic phase. Opportunistic viral infections usually develop during the second phase. Mold infections remain an important problem and are most common during the second phase. Candida infections are today uncommon if prophylaxis is used. For patients with delayed IR and GVHD, the risks for opportunistic infection are prolonged for several months (or years) especially if continuous immune suppressive therapy is needed. The increasing rates of resistant bacteria will pose important management challenges for the future.

Bacterial infections are most commonly seen during aplasia. In a recent large study with HSCT for mixed diseases, 21% developed blood stream infections detectable by culture.[37] Gram-positive bacterial infections (mainly alpha hemolytic streptococci and coagulase-negative staphylococci) were dominating in this cohort, in which quinolone prophylaxis was used, resulting in a mortality from these infections of 3.3%.

Common viral infections during the acute GVHD-phase are cytomegalovirus (CMV) and Epstein-Barr virus (EBV) that uncontrolled can cause severe disease with considerable mortality. However, CMV monitoring with use of preemptive antiviral therapy has been very successful as discussed later to prevent development of end-organ disease. EBV infections are common and may develop into post-transplant lymphoproliferative disease (PTLD) that is a life threatening condition with high mortality. Unrelated or mismatched donor, use of anti-thymocyte globulin (ATG), and splenectomy are some of the risk factors.[38, 39] A preemptive strategy including early treatment with rituximab and, if possible, reduction of immunosuppression, may improve outcome.[40, 41] Adenovirus infections are rare but serious infections more often seen in pediatric patients and can contribute to considerable mortality. Monitoring and early anti-viral treatment proposed as a possible management strategy especially in children undergoing HSCT from alternative donors.[42, 43] BK virus may cause hemorrhagic cystitis and community acquired respiratory viruses such as respiratory syncytial-, influenza-, and parainfluenza viruses, can also cause considerable morbidity and to some extent also mortality.[44]

Aspergillosis and other mold infections remain a major cause of infectious death after HSCT.[45, 46] These infections develop either during the initial neutropenic phase, if the patient was infected before transplantation, or during the acute GVHD-phase. The linkage to ongoing GVHD is strong.[47] Reported incidences are 0-20% partly depending on geographical localization of the transplant center and the mortality rate is around 50%.[46] The prognosis has improved with the introduction of new antifungal drugs used both for prophylaxis in high-risk patients and therapy.[46, 48, 49]

Varicella-zoster virus can reactivate any time, but especially during the first year after SCT and in patients with chronic GVHD needing long-term immunosuppression, and may cause disseminated visceral infection with risk for fatal outcome.[50, 51] Therefore long-term acyclovir prophylaxis at least for one year is today given routinely.[52] The risk for *Pneumocystis jirovecii* is increased for at least 6 months and prophylaxis is indicated. Patients with chronic GVHD are also at increased risk for serious infections caused by pneumococci and *H. influenzae*. Vaccination with conjugated vaccines is the main prophylactic measure and is recommended by international guidelines.[53, 54]

In **paper I** we examine how late developing infections contribute to the transplant related mortality and correlate this to a number of risk factors.

1.4 GRAFT VERSUS HOST DISEASE AND GRAFT VERSUS LEUKEMIA EFFECT

Graft versus host disease (GVHD) is a major problem, lethal in approximately 15% of transplant recipients.[55] Acute GVHD usually develops within the first 100 days, but can also occur later, especially after reduced conditioning and donor lymphocyte infusions. Chronic GVHD usually appears later than 100 days after HSCT. These conditions have classically been described as having two different underlying pathogenic mechanisms. Acute GVHD is dependent on a T_H1 and/or T_H17 immune response with hyperinflammation causing a skin rash (81%), liver dysfunction (50%), and/or gut dysfunction (54%).[56] Chronic GVHD is linked to a T_H2-like response profile causing a slower, scleroderma-like disease affecting oral and ocular mucosal surfaces, the skin, muscles, lungs, liver and gut. However, the immunological mechanisms have in recent studies been shown to be more complex than previously anticipated.[57-61]

The clinical severity of acute GVHD is determined by the extent of involvement of the three main target organs. Overall grades are I (mild), II (moderate), III (severe), and IV (very severe). Severe GVHD has a poor prognosis, with 25% long-term survival (5 years) for grade III disease and 5% for grade IV.[62] A recent update of chronic GVHD clinical grading system includes only three grades (mild, moderate, severe).[63]

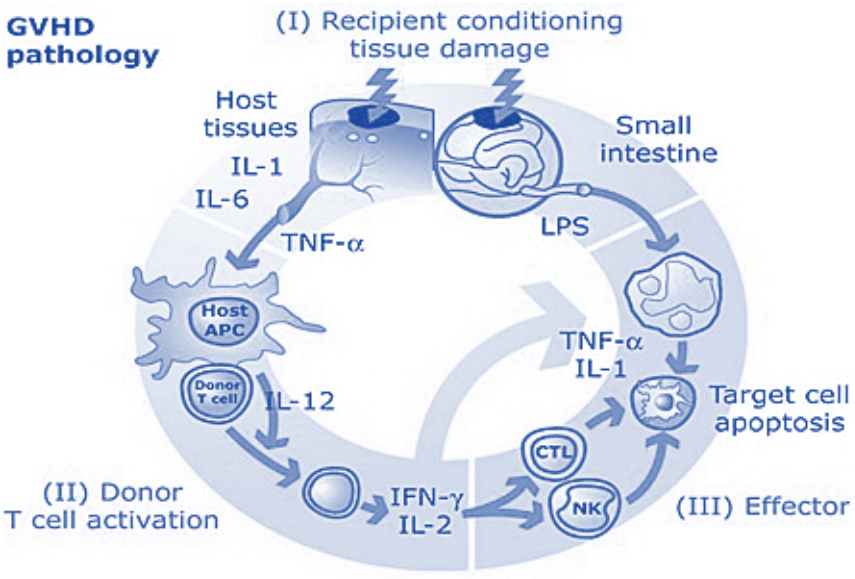


Figure 3

Three phases of GVHD.

Ref; Adopted from. Reddy P. and Ferrara J.L.M. Mouse models of graft versus host disease (Feb, 2009)

1.4.1 Acute GVHD

Acute GVHD is the major cause of mortality and morbidity before day 100 after transplantation.[64, 65] The process has been divided into three phases. The *initial phase* in which the GVHD is triggered by tissue damage from the conditioning regimen or by infection that activates host antigen presenting cells (APCs) and innate immune cells. During the *afferent phase,* these cells activate and promote proliferation of alloantigen-specific T cells that during the *efferent phase* lead to cytokine production such as interleukin (IL)-1 and tumor necrosis factor (TNF)-α initiating tissue necrosis.[66]

1.4.2 Chronic GVHD

The pathophysiology of chronic GVHD is poorly understood, but several facts are known.[57, 66, 67] i) Damage of the thymus mediated by the conditioning regimen or by preceding acute GVHD can impair the negative selection of auto/allo-reactive CD4+ T cells. ii) T_H2 cytokines are released including IL-4, IL-5 and IL-11. This in turn stimulates production of the fibrogenic cytokines IL-2, IL-10 and Tumor growth factor (TGF)-β1, activating macrophages producing Platelet derived growth factor (PDGF). iii) These cytokines stimulate proliferation and activation of tissue fibroblasts. iv) Regulatory T cells have been shown to be low in numbers. v) Dysregulation of B cells with high levels of B cell-activating factor (BAFF) and production of auto-reactive antibodies.[57]

1.4.3 Recent Advances in GVHD Biology

The microbiome and mycobiome of the gut can activate an innate immune response and to trigger acute GVHD.[68] Pattern-recognition receptors (PRRs), such as toll-like receptors (TLR) and nod-like receptors (NLR), are sensors that can activate the innate cells. Bacterial lipopolysaccharide (LPS) and bacterial DNA can, via TLR-4 and TLR-9, trigger the innate immunity and cause a "cytokine storm".[69] Damage-associated molecular patterns (DAMPs) are released after tissue damage and trigger APCs.[70, 71] Studies have shown that intestinal and skin GVHD can be diminished in mice by gut decontamination.[72-74] The bacterial flora can also protect against GVHD[75] and gut decontamination was not shown to improve overall survival.[76] In a recent study, we presented results showing that certain activated innate lymphocyte subsets with homing potential to the gut may mediate protection against acute GVHD.[77] If this is affected by intestinal bacterial and fungal flora remains to be examined.

The presentation of minor histocompatibility antigens by MHC class I molecules on recipient hematopoietic APCs is important, and donor APCs can augment this response. However it was shown that there are only few residual regular APCs. Instead, parenchymal tissue cells can acquire APC functions and promote marked expansion of alloreactive donor T cell populations in the gastrointestinal tract.[78] B cells also seem to interact in the development of both acute and chronic GVHD. Deletion of B cells before, but not after, conditioning reduced the risk for acute GVHD and B-cell dysregulation and CD20 deletion can be efficient in chronic GVHD.[79,80] GVHD is a mainly a result of naïve T cell responses. Central and effector memory T cells do not appear to induce GVHD, but can mediate GVL responses.[81] T_H1 cells and pro-inflammatory molecules such as IL-1, IL-6, IL-12, tumor necrosis factor (TNF) and nitric oxide are important factors in the induction of GVHD. T_H2-type cytokines, such as IL-4, can reduce acute GVHD.[57] T_H17 cells, which are characterized by the production of IL-17A, IL-17F, IL-21 and IL-22, have been suggested to have a direct role in GVHD pathobiology. In patients with acute GVHD, IL-17-producing cells can be found in gut but not in the skin.[58, 82, 83]

1.4.4 Graft versus leukemia effect

The main therapeutic effect mediated by HSCT against malignancies is thought to be the GVL effect. GVL is tightly coupled to immunological allo-reactions causing GVHD where patients transplanted for leukemia that develop GVHD have reduced relapse rates and an increased overall survival. The GVL relies on both T and NK cells[17, 18] and is mainly seen in allogeneic settings. T cell depletion from the graft eliminates GVHD, but at the expense of an increased leukemia relapse rate.[18, 84] The major GVL effectors are cytotoxic T cells that recognize allogeneic histocompatibility antigens presenting tumor-specific peptides and unique tumor antigens. In addition, NK cells and NKT cells can directly recognize MHC class I molecules and stress-induced peptides and mount anti-tumor responses. The magnitude of the GVL effect and thereby the efficacy of HSCT varies between diseases and seem to be greater against myeloid malignancies than lymphoid. The development of a strong GVL effect is linked to a successful immune reconstitution and tapering of the immune suppression, therefore the GVL effect will have more time to develop in slowly than in rapidly progressing diseases where the leukemic cells may cause relapse before the GVL is established.[85, 86]

Even though GVHD and GVL are tightly linked, it has been shown that NK cells can mediate GVL without GVHD.[87] Furthermore, IFN-γ can promote separation of GVHD from GVL by promoting apoptosis and suppressing proliferation of alloreactive T cells, by increasing

Programmed cell death protein 1 (PD-1) expression, leading to elimination of these cells in the affected organ (Figure 3).[88]

1.5 CYTOMEGALOVIRUS

Herpesviruses are DNA viruses that have coevolved with the human species over millions of years and exist in many vertebrates. The family *Herpesviridae* consists of three subfamilies of viruses (alpha, beta, and gamma), where CMV belongs to the beta subfamily. Herpesviruses particles consist of the core, the capsid, the tegument, and the envelope (Figure 4). During the acute phase of infection, CMV has been shown to infect multiple cell types; endothelial cells, epithelial cells, smooth muscle cells, fibroblasts, neuronal cells, hepatocytes, trophoblasts, monocytes/macrophages, and dendritic cells (DCs).[89] The virus can thereafter establish latency/persistence in endothelial cells, cells of the myeloid lineage and $CD34^+$ cells.[90-94]

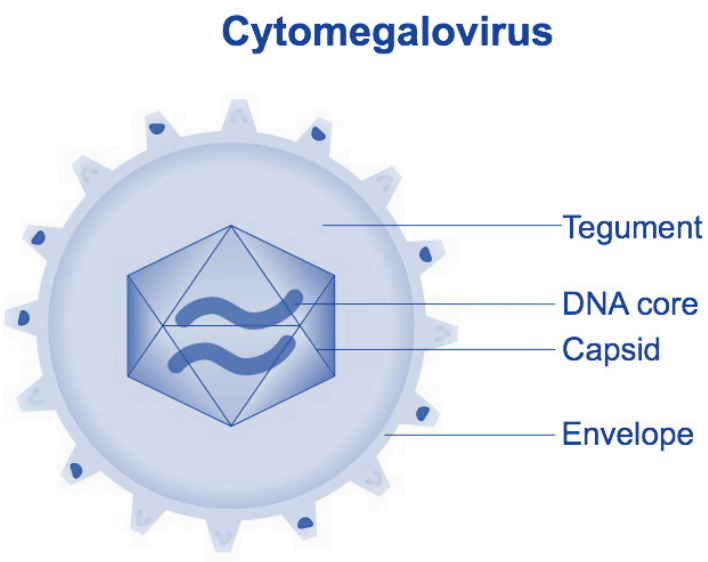


Figure 4. Cytomegalovirus. Schematic presentation.

Innate, cellular and humoral immune responses are all involved in defeating and controlling CMV. T cells are considered most important in controlling latent/persistent infection explaining why CMV infections mostly develop during the first three month after HSCT. Both CD4+ and CD8+ T cells are crucial and mount broad responses against multiple CMV epitopes and approximately 10% of all memory T cells have specificity against CMV.[95] CD4+ cells provide B-cell stimulation and production of CMV specific antibodies. These antibodies are protective, but cannot clear infected cells. Antibodies to different CMV antigens have been described, but few are effectively neutralizing the CMV. Some antibodies with neutralizing properties have, however, been identified with specificities against glycoprotein B, H/LUL-128-131 and the N–M complex. A pentameric complex consisting of gH/gL/UL128-131 is explored for vaccination and neutralizes infection of epithelial and endothelial cells *in vivo*.[96]

CMV is a strong inducer of type 1 immune responses where IL-2 and IFN-γ are dominating the cytokine profile, but it has been described that CMV also trigger type 2 cytokines such as IL-10 [97] producing a mixed response. The induction of an innate immune response is strong, rapid and does not require transcriptionally active viral particles.[98] This may in part be due to the fact that the envelope glycoproteins B and H may interact with integrin heterodimers, toll-like receptors, and entry receptors, leading to early signaling and transcriptional events in infected cells and activating innate immune responses before the outset of viral replication.[98]

The tegument contains most of the viral proteins important for entry, excretion and for triggering immune escape mechanisms.[99-101] CMV has developed numerous mechanisms to avoid recognition by DCs, T and NK cells. In DCs, the infection causes an increase of the co-stimulatory molecules CD40, CD80 and CD86, a down-regulation of both MHC class I and II. After that, surviving DCs have been described to mature rapidly, up-regulate Fas-ligand and TRAIL and thereby acquire capacity to kill peripheral blood mononuclear cells (PBMC) or T cell lines.[102] CMV can also hamper the IL-12 and IL-2 –production by dendritic cells diminishing further stimulation.[103] The monocyte lineage also plays a central role during the latent phase, where the virus can reside until differentiation to macrophages or DCs occurs. This can trigger reactivation of CMV.[92, 104]

T cells can be avoided by the proteins US2, US3, US10 and US11 that increase degradation of *HLA* class I; US6 block TAP and UL83 inhibit the proteasome hindering antigen processing and presenting.[101] To avoid NK cell elimination due to low HLA class I density, the UL18 can mimic HLA-class I and bind inhibitory LILR-1 receptors. The UL40-protein can mimic class I leader peptides and thereby maintain HLA -E expression. The latter promotes inhibition through signaling via inhibitory CD94/NKG2A receptors expressed by NK cells.[105, 106] Another protein, UL16, UL 142 and the virus-encoded micro RNA, miR-U L112, selectively retains stress-induced proteins ULBP1-4 and MICA/B inside the cell and thereby prevent recognition by the activating receptor NKG2D.[107, 108]

Interaction between NK cells and DCs in CMV infection

A crosstalk between NK cells and DCs was described; NK cells can kill immature DCs or promote their maturation and mature DCs can in turn stimulate NK cell cytotoxicity and proliferation. The activating receptors NKp30 and DNAX Accessory Molecule-1 (DNAM-1) is important in this interaction and stimulation leads to increased production of IL-12, IL-15, IL-18, and IFN-α/β.[109-112] NK cells were shown to regulate CMV infection through interactions with autologous APCs. NK cells respond through stimulation of activating receptors NKp46 and DNAM-1 via ligands expressed by infected monocytoid DCs.[113] This early response is followed by a virus-mediated down-regulation of the DNAM-1 ligands CD155 and CD112, which dampens NK cell reactivity and leads to viral escape.

Approximately 50-90% of the European population (74% in Nordic countries) are CMV-seropositive.[114] The infection is transferred via body fluids and is often asymptomatic during the primary infection but can present as mononucleosis, kissing disease [115], with symptoms such as lymph cervical lymphadenopathy, splenomegaly, continuous fever, myalgia and rash.[116] During the latent phase a persistent production of viral particles can occur in endothelial cells allowing a continuous stimulation and modulation of the host immune system.[92] This continuous immune stimulation is thought to contribute to the increase of CMV-specific T cells detected in elderly and to contribute to an immune risk profile leading to increased susceptibility to infections.[117, 118] Interestingly, the NK cell immune compartment is also affected by age, but a correlation to CMV seropositivity has not been possible to show.[119, 120]

1.5.1 CMV in HSCT

For the purpose of this thesis, the two options of primary CMV infection (usually transferred from the donor) in a CMV seronegative recipient and reactivation of a persistent/latent virus in a CMV seropositive patient will be jointly described as CMV infection or episodes of CMV replication. CMV can cause severe disease in the immunocompromised host making CMV one of the most important opportunistic pathogens to control after HSCT.[121-123] During active replication, the virus can be found in most tissues of the body. It can cause pneumonia, hepatitis, gastroenteritis, retinitis and encephalitis, and the disease can develop both early and late after the transplantation procedure.[124, 125]

CMV-replication occurs most frequently during the acute GVHD-phase after HSCT and has been correlated to a reduced function of CMV-specific CD8+ and CD4+ T cells[126] and reduced

signal transducer and activator of transcription 5 phosphorylation (STAT5) levels has been shown in response to IL-2 or IL-7 in patients with more CMV reactivations.[127]

The CMV serological status of donor and recipient was in multiple studies shown to be an important factor for transplant outcome of HSCT. Early diagnosis and modern antiviral treatment have reduced CMV-induced morbidity and mortality[128-134], but CMV seropositive recipients still have a poorer outcome than CMV seronegative patients.[113, 131, 132] The best outcome is seen when using a CMV seronegative donor to a CMV seronegative patient. This combination reduces the morbidity in CMV infection but does also reduce the risk for severe bacterial and fungal infections.[135] However, the effect of donor serostatus in CMV seropositive patients remains a controversial issue. A recent large study encompassing 49542 HSCT patients from the European Group for Blood and Marrow Transplantation (EBMT) was recently published.[136] In this study it was confirmed that using a CMV negative donor to a negative recipient had better overall survival (OS) compared with CMV positive donor to negative recipient. Furthermore, having a CMV positive donor is beneficial in a CMV seropositive recipient, but only if myeloablative conditioning is used. These effects were also only seen in HSCT recipients with unrelated donors and were absent with sibling donors.

1.5.2 CMV and GVL

Besides the risk of developing hazardous CMV-disease, CMV infection has been coupled to reduction in relapse of leukemia. As early as 1984 there was a report from Lönnqvist et al. reported in a small study that CMV infection could lead to reduced relapse of leukemia.[137] Elmaagacli et al. have published similar results from AML patients undergoing myeloablative allogeneic SCT[138] and Ito et al.[139] also found a decreased relapse risk in patients with CML. Green et al. documented a reduction in relapse risk in a population of mixed diseases both at day 100 and at 1 year after transplantation.[140] The mechanism behind this effect is not yet clarified. The effect seems to be due to CMV replication by itself because there was no effect by CMV serological status. Instead, Green et al., found an opposite effect of recipient CMV seropositivity, with an increased relapse risk early after transplantation for acute leukemia and lymphoma. The effect was seen by CMV replication occurring early after SCT since there was no effect when a landmark analysis starting at 50 days after SCT was performed.[140] These studies have also failed to find an increased overall survival despite a reduced risk for relapse since the non-relapse mortality was increased in patients with documented CMV replication resulting in no net benefit. One possible explanation is interplay between CMV and NK cells that could result in increased GVL. This possibility is examined in **paper IV.**

1.5.3 CMV treatment strategies

Controlling CMV infection leads to an improved survival. Through modern strategies with CMV monitoring and pre-emptive antiviral therapy, the risk for CMV-disease have decreased from 20 – 30% to less than 5% in many studies[124, 125, 141]

The decision of preemptive therapy is usually based on either pp65-antigenemia or quantitative PCR measuring viral load. The latter is the most commonly used method today.[142] Many centers have developed their own assays, creating a variability that makes comparisons between laboratories difficult. The level for initiation of antiviral therapy has been debated, but published data suggest that preemptive therapy can safely be initiated from 100 copies/ml to 10.000 copies/ml, depending on the patient group studied.[143, 144] Ganciclovir i.v., the p.o. prodrug valganciclovir, and foscarnet have all been effective as first line therapy.[145-147] However, CMV remains a problem especially in patients experiencing repeated or prolonged CMV replication episodes. An important clinical parameter during treatment is that a slow decrease in viral load was shown to be a risk factor for later development of CMV disease.[141] These patients have an increased risk for toxicity from the existing antiviral drugs and an increased risk for their CMV becoming resistant. Several groups have worked with CMV-specific T cell therapy for several years and finally two randomized controlled trials of this strategy were performed in the UK.[148-151] The results of these studies will be presented later this year. Prophylaxis regimens are used in some centers, but the toxicity is high with presently licensed antiviral drugs. Ganciclovir can for example inhibit lymphocyte proliferation[152] and may cause lymphopenia and neutropenia. The use of foscarnet is limited by nefrotoxicity and electrolytic disturbances.[153] New drugs with less toxicity such as letermovir, brincidofovir and maribavir are under development.[154-156] An unresolved question is, however, if the described beneficial effects of CMV replication on relapse will be affected if a very effective prophylactic regimen against CMV is applied. The development of an adequate CMV control and anti-leukemic effects seen after reactivations may be hampered.

1.6 NK CELL BIOLOGY

The field of innate immunity has expanded vastly during the last years, giving rise to a completely new nomenclature to describe its cellular components. Innate lymphoid cells (ILCs) have several common characteristics. They are developed from common lymphoid progenitors, lack recombined antigen receptors, myeloid and dendritic cell phenotypical markers.[157, 158] They all have the ability to respond to APC cytokine stimulation, but NK cells are so far the only ILC-type that can respond with cytotoxic degranulation. Organ specific NK cells have recently been found and in line with this finding[159] most of the newly discovered ILCs in humans have been found in the mucosal immune system where they are thought to scan this environment and possess regulatory functions[77, 159] They have been compared to T helper cells since they have specific cytokine production profiles.[160] They are divided into three groups based on their capacity to mount a T_H1-, T_H2- or T_H17 (IL-17 and IL-22)- type cytokine response and with specific requirement for transcription factors during ontogeny. In this new nomenclature NK cells belong to the ILC group 1 (Figure 5).

NK cells were discovered by Kiessling, in parallel with Herberman in 1975[161-164] and were defined by their natural ability to kill tumor cells without prior sensitization. Nearly four decades of research has broadened the view of the role played by NK cells, which are now considered to be key cellular components of the innate immune system acting at the interface between innate and adaptive immunity. NK cells produce IFN-γ in response to exogenous cytokine stimulation, display immunoregulatory activity by perforin-dependent killing of activated immune cells, and mediate immune surveillance of virus- and tumor transformed cells[165, 166] through IFN-γ secretion, perforin and FAS-ligand-dependent target cell killing.[167, 168] NK cell functions can complement T cell function by detecting targets having low or no expression of MHC class I molecules at the cell surface. This is called missing self-recognition and is determined by a family of inhibitory receptors called killer cell immunoglobulin-like receptors (KIRs) that specifically bind to different groups of MHC-I and by the dimeric receptor CD94/NKG2A, which binds to HLA –E.[169] In recent years, it has become clear that NK cell function is regulated by the net sum of signals from a vast array of inhibitory and activating receptors.[170] Several of the activating receptors bind to stress- or virus-induced molecules on infected or transformed cells. Hence, NK cell recognition is triggered by loss or alteration of HLA class I expression (missing self) in combination with increased expression of stress-associated ligands for activating receptors (induced self).[171-173]

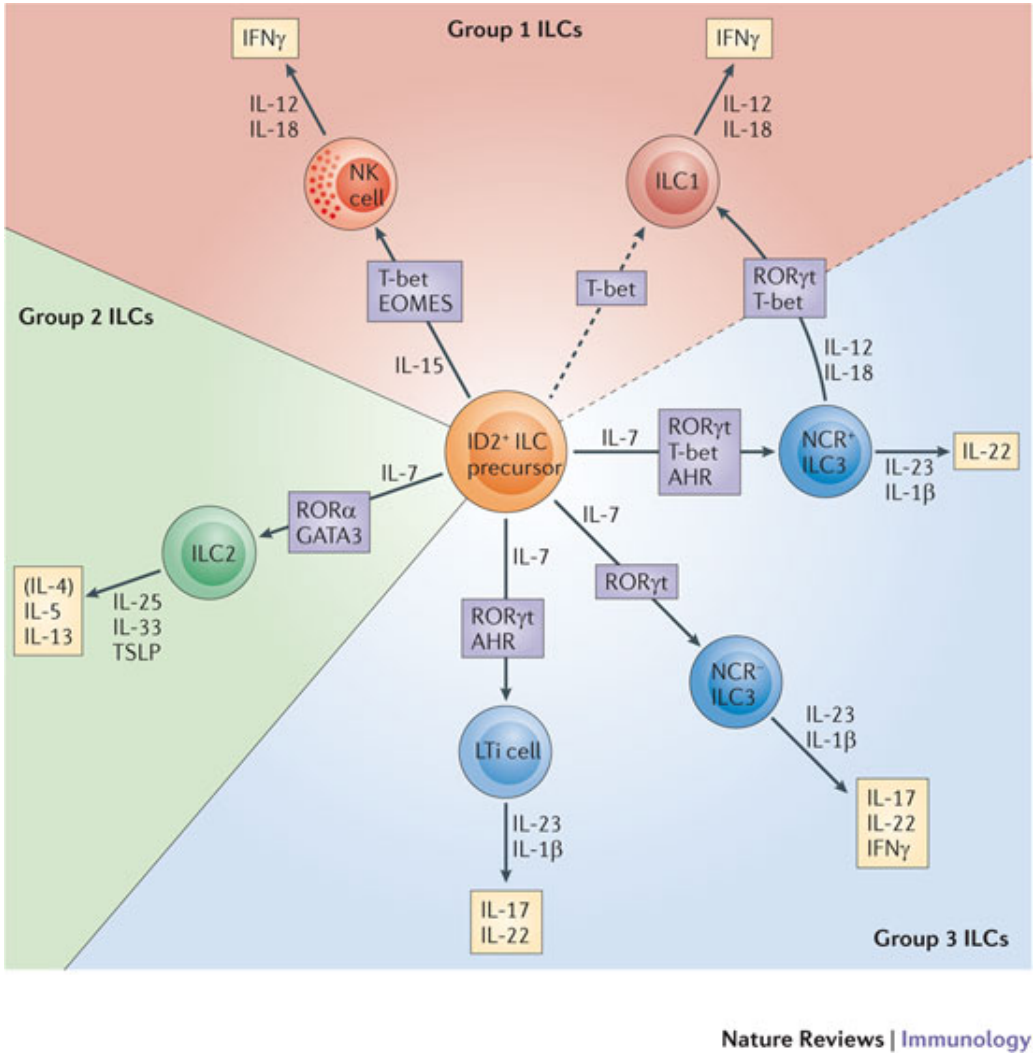


Figure 5. New groups of innate cells and their developmental pathways.

Ref: Spitz et al. (2013), Innate lymphoid cells - A proposal for uniform nomenclature, *Nature Reviews Immunology* **13**, 145

NK cells develop in the bone marrow from early lymphoid precursors to mature NK cells and can then develop further in lymph nodes, thymus, liver and spleen.[174] Work by Caligiuri and colleagues showed that the early NK cell development in humans, from $CD34^{+}$ hematopoietic precursor cells (HPCs) through discrete steps into $CD56^{bright}$ NK cells, occurs in secondary lymphoid tissues.[175-177] Freud et al have proposed following model: Lymphoid progenitor cells that express CD34, CD45RA and integrin β cells are called stage 1 cells. Stage 2 is defined by acquisition of CD117 that is followed by CD161 and CD127 during stage 3.[177] IL-15 has been shown to be central for NK cells differentiation from stage 2 to 4 (Figure 6).[178, 179] Stage 3 cells are fully committed and do not differentiate into T cells or dendritic cells. From stage 4, NK cells are $CD56^{bright}$ $CD94/NKG2A^{+}$ and are readily detected in peripheral blood. During further differentiation these cells down-regulate CD56 to become $CD56^{dim}$. A special distinction can be made between $CD56^{dim}$ and $CD56^{bright}$ subsets where $CD56^{dim}$ cells express CD16 and have cytotoxic potential[180] and $CD56^{bright}$ NK cells is $CD16^{-}$, express IFN-γ and have immunoregulatory function[181]. In recent years, it has become clear that $CD56^{dim}$ NK cells also

produce a range of cytokines and chemokines, in particular following stimulation with cellular targets.[182] The CD56^{bright} NK cells can be attracted to inflammatory sites and are the dominating NK cell subset in lymph nodes.[183] We and others have recently shown that CD56^{dim} NK cells continue to differentiate.[184-186] During this process they lose expression of NKG2A, sequentially acquire inhibitory *KIRs* and CD57, change their expression patterns of homing molecules including CD62L and display a gradual decline in proliferative capacity.[175, 177, 185]

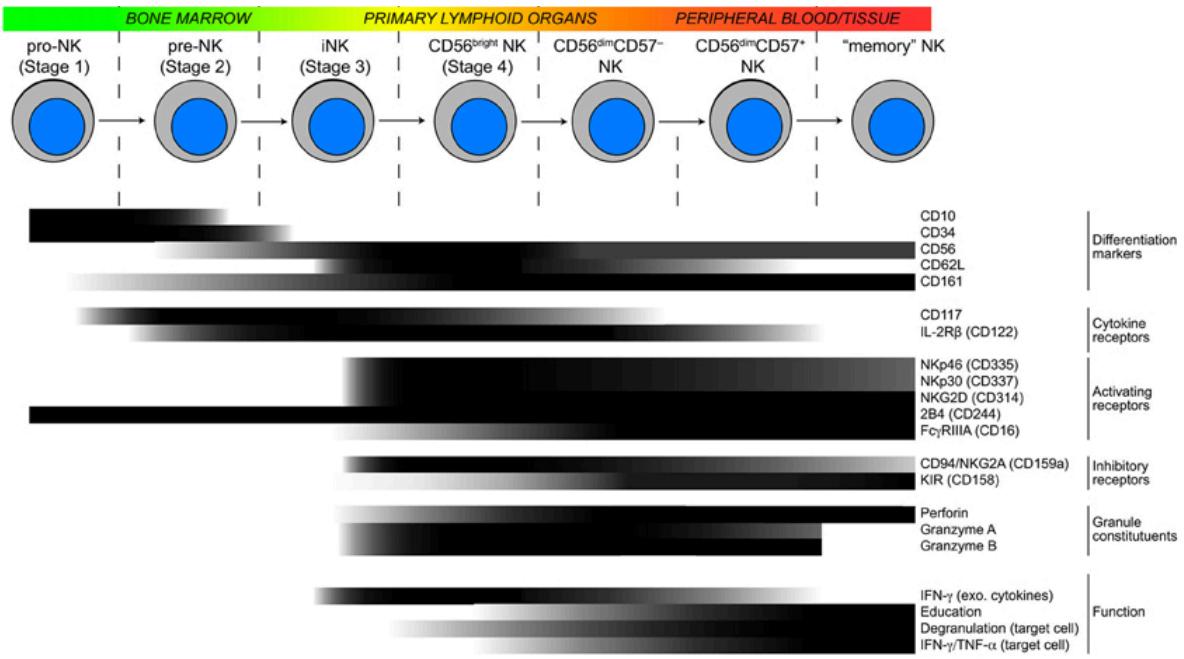


Figure 6. Overview; NK cell development and important markers at different stages.

Ref: Cichocki et al. (2013) Epigenetic regulation of NK cell differentiation and effector functions. Front. Immunol. 4:55.

1.6.1 NK cell education

For long it has been assumed that all mature NK cells are fully functional and ready to kill targets with aberrant expression of HLA class I. Based on characterization of large number of NK clones, it was proposed that every NK cell must express at least one self-specific inhibitory receptor to maintain self-tolerance.[187, 188] However, more recent investigations of unperturbed polyclonal NK cells have found subpopulations of hyporesponsive NK cells lacking inhibitory receptors specific for self-MHC class I molecules (self-KIRs or self-Ly49s in human and mice, respectively.[189, 190] Thus, instead of a selection process that delete potentially auto reactive subsets, NK cells have been shown to undergo an educational process where only NK cells expressing self-KIRs or NKG2A are endowed with functional competence.[189, 191] This is a prerequisite to maintain NK cell tolerance. During education, CD56^{dim} NK cells are functionally fine-tuned by

interactions between cell surface receptors, including inhibitory and activating KIRs and NKG2A with their cognate HLA class I ligands.[191-194] Given the stochastic expression of KIRs at the cell surface, this functional calibration is a prerequisite to maintain NK cell tolerance.[195] Several principles for the educational process have been suggested; The arming or licensing-model[196-198] proposing that NK cells are initially inert and get "licensed to kill" through ligation of inhibitory receptors by ligands expressed on other cells (trans-presentation) or by themselves (cis-interaction-model).[199, 200] The disarming-model propose that NK cells are fully functional or "armed" from the beginning but become hypofunctional when they fail to get inhibitory input from neighboring cells, potentially due to overstimulation.[201] More recently, education was suggested to be more of a dynamic process than an on/off phenomenon. This dynamic functional tuning is termed the rheostat model and does not exclude any of the previously suggested principles.[202-205] The rheostat model is particularly useful in the context of NK cell adaptation to different milieus in different organs and immune responses without mediating too strong response or creating autoreactivity.[206]

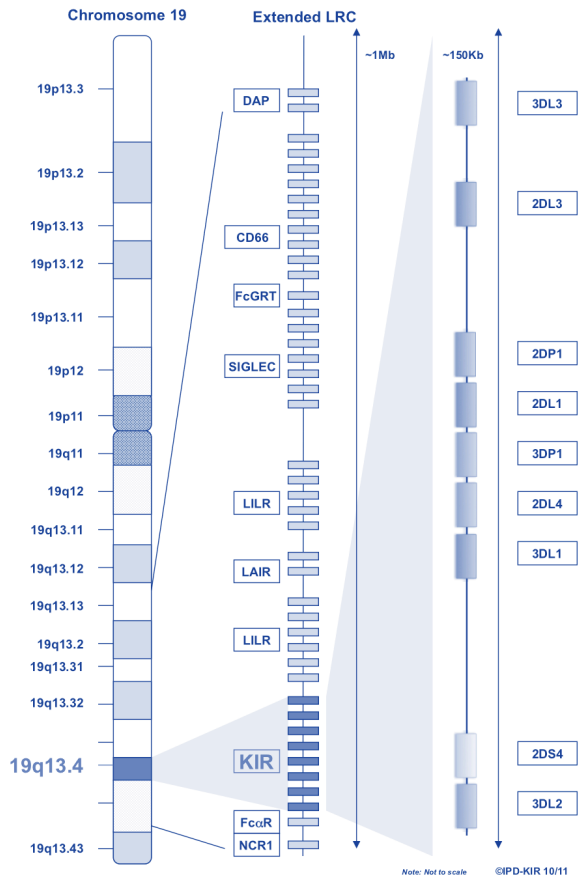
It is tempting to draw the conclusion that hyporesponsive NK cells do not exert any biological function, but this has been questioned in recent studies. Transfer of uneducated cells to immunodeficient mice where shown to provide a better protection against CMV than transfer of the educated fraction.[207] Furthermore, it has been shown that high doses of interleukin-12 (IL-12), IL-18[191, 208] or infection with listeria[209] can reverse the hypo-responsiveness of uneducated NK cells. In a therapeutic setting, uneducated NK cells were shown to be more advantageous in the context of antibody-based targeting of neuroblastoma cells.[210] As we shall discuss in relation to **paper II** in this thesis, uneducated cells may under certain conditions become activated during the IR after HSCT.

1.6.2 NK cell repertoire skewing and memory

Until recently, NK cells were believed to be short-lived innate cells without any involvement in the immunological memory to encountered antigens. This dogma has been overthrown in recent years when it has been shown that viral infections may shape the NK cell repertoire in a way that provide protection against new viral challenges[211-213] This has been taken as a proof for that NK cells also possess the capability to mount memory responses. There are many examples of this repertoire skewing. NK cells expressing the activating receptor CD94/NKG2C have been shown to proliferate in response to CMV-infection.[214, 215] These NKG2C$^+$ cells also expand in response to hepatitis C virus and hantavirus but only in CMV-seropositive individuals.[216, 217] CD94/NKG2C binds to HLA-E and the binding is influenced by the peptide bound to the grove.[105, 169] It is therefore possible that the inhibitory receptor NKG2A and activating receptor NKG2C can distinguish between HLA-E expressing different leader peptides.[218] Several recent

epidemiological studies have shown that the risk for CMV-reactivation is reduced if the donor possesses more activating KIRs[219-222] suggesting that certain NK receptor profiles are associated with a better immunity to infection.

There is evidence for NK cells involvement in the immune response against various infections. Their role in viral infections is the most established but there are also reports of NK cell activity being important against infections with other microorganisms, including *Legionella pneumophila*[223], *Mycobacterium tuberculosis*[224], *Borrelia burgdorferi*[225], *Toxoplasma gondii* [226] and Plasmodium falciparum.[227] As yet, there is no clear evidence for direct, cognate recognition of bacteria, fungi or parasites by NK cells. Bacterial infections were shown to induce the expression of NKG2D ligands and the activation of myeloid cells by Toll-like-receptor ligands results in the production of the proinflammatory cytokines, IL-12 and IL-18, which are potent inducers of interferon-γ (IFN-γ) production in NK cells. A rapid secretion of IFN-γ by NK cells at the site of infection preceding the T cell response will cause an activation of macrophages and dendritic cells, and might be an important component of the immune response in many of these infections.



1.6.3 Killer Cell Immunoglobulin-Like Receptors

KIRs have a central role for the function of NK cells. 15 *KIR* gene loci have been identified (*KIR2DL1, KIR2DL2/L3, KIR2DL4, KIR2DL5A, KIR2DL5B, KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5, KIR3DL1/S1, KIR3DL2, KIR3DL3* and two pseudogenes, *KIR2DP1 and KIR3DP1*). All are encoded within a 100-200 Kb region of the Leukocyte Receptor Complex (LRC) located on chromosome 19 (19q13.4).[228] KIRs are all membrane-bound receptors belonging to immunoglobulin superfamily. The KIRs can be either activating or inhibitory depending on if their intracellular motif is immunoreceptor tyrosine-based inhibitory motif (ITIM) or immunoreceptor tyrosine-based activating motif (ITAM).[229]

Figure 7. KIR gene cluster localization on chromosome 19q13.4

Ref: Robinson J et al.. IPD-the Immuno Polymorphism Database. Nucleic Acids Research (2005), 331:D523-5

KIR genes are narrowly placed and the linkage disequilibrium[230] is high with combinations of *KIRs* being inherited in haplotypes. According to EMBL-EBI Immune Polymorphism Database there are today over 40 haplotypes identified.[231] All haplotypes include 4 framework genes and can be broadly divided into two different groups A and B, where haplotype A only includes inhibitory *KIRs* and the activating *KIR2DS4* and haplotype B is more variable and can include *KIR2DL2, KIR2DL5A/B, KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS5* and *KIR3DS1* genes that are absent in group A.[232, 233] By studying individual gene motifs in the centeromeric (Cen) or telomeric (Tel) end the different haplotypes can be further classified[234] based on their Cen/Tel A/B content (Figure 8).

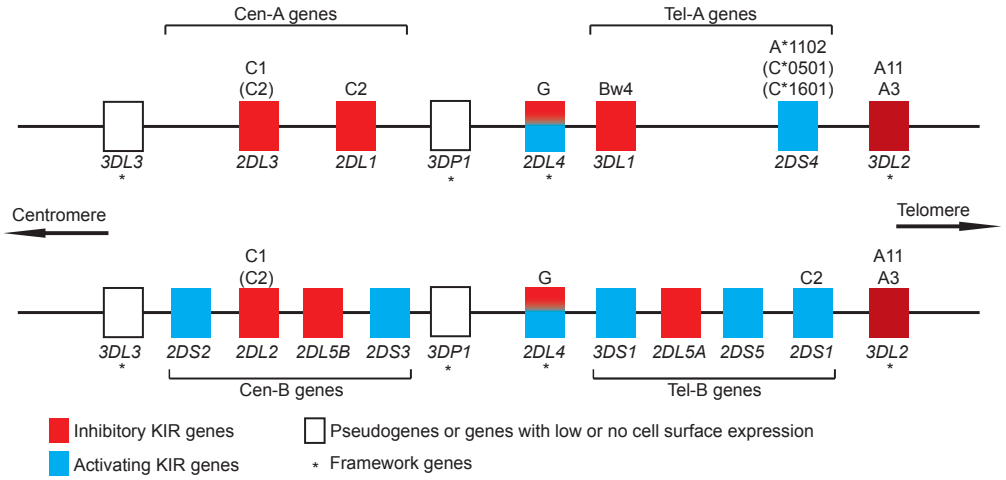


Figure 8. Schematic presentation of A and B haplotypes and definition of the telomeric (Tel) and centromeric (Cen) part.

Ref: Adopted from Malmberg et al. Killer cell immunoglobulin-like receptor workshop. Immunity 2011.

1.6.4 Evidence for NK cell-mediated graft versus leukemia effect in allogeneic stem cell transplantation

Valiante et al. first conceived the concept of NK cell alloreactivity in transplantation based on the stochastic expression of KIR.[187] NK cells have strong alloreactive capabilities that can be triggered by a mismatch between KIRs and their corresponding HLA class I ligands[87, 235-241] or through activating receptors.[242-245]

The first clinical evidence showing that NK alloreactivity had a role in tumor surveillance in humans, came in 2002. Ruggeri et al reported that outcomes from a cohort of AML- and ALL patients, receiving KIR-HLA mismatched, CD34 enriched peripheral stem cell grafts, effectively depleted from T cells.[87] The clinical results were impressive with 0% AML-relapse in the KIR-ligand mismatched group versus 75% in the non-mismatched group. A KIR-ligand mismatched effect was absent in the ALL-group. Furthermore, acute GVHD grade II-IV and rejection was not seen in a single patient in the KIR-ligand mismatched group. The observations were strengthened by the identification of anti-recipient alloreactive clones only in the mismatched setting and by demonstrating the capability of mouse NK cells to eradicate host APC:s and thereby diminishing presentation of alloantigens to T cells and preventing the development of GVHD. In the end of the article, they postulate: "Alloreactive NK cells emerge as a form of cell therapy that might be used in conditioning regimens for host immune suppression and leukemia ablation". These findings triggered a cascade of studies, during more than a decade, engaging many transplant centers around the world to study outcomes based on stratification of KIR HLA genotypes in donors and recipients in different transplantation settings. They have also lead to the definition of a number of distinct framework models to describe NK cell alloreactivity (Figure 9).

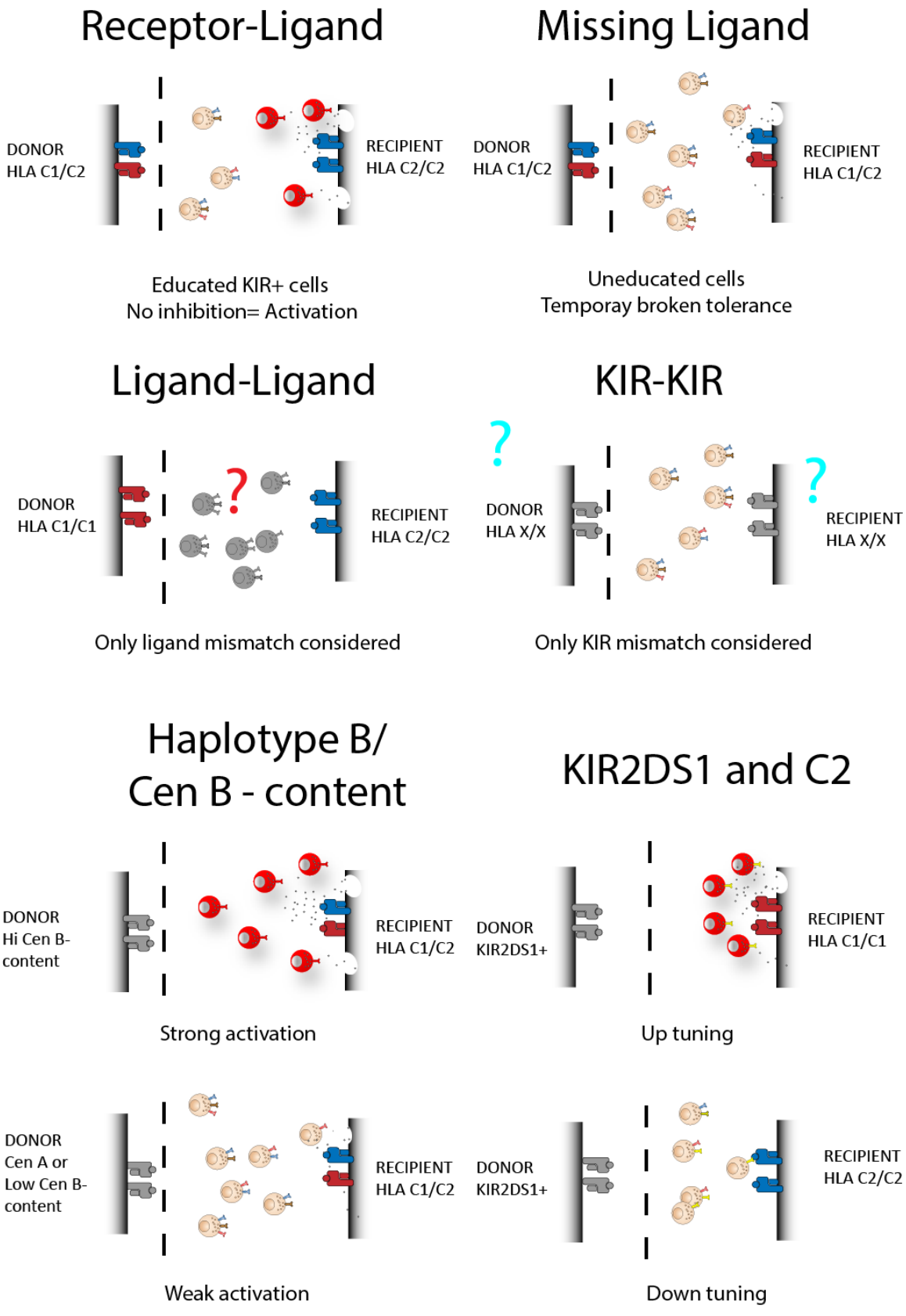


Figure 9. Models of NK cell mediated alloreactivity. As explained in 1.6.4-1.6.5.

TABLE 1

Authors	Marker	Number	OS	aGvHD	Rej	Rel	TCD	Donor	Source	N
Ruggeri et al., 2002	LIG-LIG	AML (57) ALL (35)	B	B	B	B	100%	Haplo	PSC	92
Leung et al. 2004	Receptor-LIG	Ped AML(17) ALL(19)	*na*	*na*	*na*	B	100%	Haplo	PSC	36
Bishara et al., 2004	LIG-LIG	AML (15), ALL (24), CML (13)	D	D	NS	NS	100% (milder)	Haplo	PSC	62
Zaho et al. 2014	Receptor-LIG	CML (97)	NS	NS	*na*	D B (Activ rec)	TCR	Haplo	BM+PSC	97
Symons et al.2010	LIG-LIG, KIR-KIR, Haplo B	AML (25), ALL (7), CML (11)	B	NS (D kir-lig trend)	NS	B	TCR	Haplo	BM	86
Schaffer et al., 2004	Receptor-LIG	AML (57), ALL (43), CML (64)	D (Inf)	NS	NS	NS (Trend less relapse)	ATG	URD	BM/PSC	190
Davies et al., 2002	LIG-LIG	AML (14), ALL, (35), CML (58)	D	NS	NS	NS	34%	URD	BM	175
Giebel et al., 2003	LIG-LIG	AML(22), ALL (38), CML(42)	B	NS	NS	Trend less relapse	ATG	URD	BM	130
Lowe et al. 2003	LIG-LIG	AML (40), ALL (33),	NS (D trend)	NS	NS	NS	100% (mild?)	URD	BM	105
Bornhauser et al., 2004	LIG-LIG	AML/MDS (73), CML (45)	NS	NS	-	D	ATG	URD	BM/PSC	118
Beelen et al., 2005	KIR-KIR	AML/MDS (138), CML (236)	NS	NS	D	B	0% (no ATG)	URD	BM/PSC	374
De Santis et al. 2005	LIG-LIG	AML (17), MDS (17), CML (22),	D	D/ B (If more KIRs)	D	NS	9% (no ATG)	URD	BM/PSC	104
Miller et al. 2007	Missing Ligand	AML (556), MDS (282), CML (1224)	*na*	D (CML) NS (others)	*na*	B	*na*	URD	BM/PSC	2042
Farag et al. 2006	LIG-LIG	AML (419), MDS(252), CML (800)	*na*	NS	*na*	**NS**	TCD/TCR	URD	BM	1571
Hsu et al 2006	LIG-LIG	AML (245), MDS (27), CML(750), ALL (180)	NS	*na*	*na*	B	TCR	URD	BM/PSC	1770
Gagne et al 2009	Receptor- LIG/ KIR-KIR	AML+ALL(138), MDS(23), CML(54)	D (3DL1, 3DS1 in Bw4-)	D (2DS1, 2DL5 mm)		D (3DL1, 3DS1 in Bw4-)	TCR	URD	*na*	264
Cooley et al 2009	Haplo B	AML(488)	B (If B/x don)	D (URD ns MAB high cGVHD)	*na*	**NS**	TCR	URD	BM/PSC (11%)	448
Giebel et al. 2009	KIR-KIR	AML(40) , MDS (4), CML (43). ALL (21)	D (2DS1+don in C2/xRec)-	D (if 2DS1 MM) cGVHD (if 2DS3 MM)	*na*	**NS** (2DS5 D)	ATG	URD/Sib(32%)	BM/PBC	100
Venstrom et al. 2010	3DS1+ copy nr	AML(306), MDS (154), CML (390), ALL(237)	na	B (3DS1+)	*na*	**NS**	TCR/20%TCD	URD	BM (97%)	1087
Cooley et al. 2010	Cen A/ B	AML (1086), ALL(323)	B (Cen/Tel B)	*na*	*na*	B	TCR	URD	BM/PSC	1409
Venstrom et al. 2012	C1/C2, 2DS1	AML (1277)	B	*na*	*na*	B	28% TCD	URD	BM/PSC	1277
Cook et al., 2004	C1/C2, 2DS2	AML (52), CML (49), MDS (11), ALL (54)	D (C1-2DS2pos)	NS	*na*	**NS**	na	Sibl	na	220
Hsu et al., 2005	Missing Ligand	AML (57), MDS (15), CML (61), ALL(45)	B	NS	*na*	B	100%	Sibl	BM	178
Chen et al. 2006	KIR-KIR	AML (40), MDS (5), CML (28), ALL (44)	B	NS	*na*	**NS**	TCR	Sib	BM	131
McQueen 2007	Haplo B	AML (57), CML (28), MDS (21), ALL(21)	Trend for B (HapB/x)	B (Bw4 present)	*na*	B	TCR	Sib	BM/PSC (89%)	202

1.6.5 Dissecting the beneficial effects of KIR-*HLA* mismatch

Delineating the conditions that promote NK-alloreactivity has been more complex than first expected, with many conflicting results (references collected in Table 1). However, after more than a decade of research examining NK cell alloreactivity from multiple angles, some conclusions can be drawn. NK cells ***do*** have strong alloreactive capabilities that can be triggered by a mismatch between *KIRs* and their corresponding *HLA* class I ligands[87, 235-241] or through activating receptors.[242-245]

The clinical effects mediated by KIR-ligand mismatch have been most prominent in the haploidentical HSCT setting, where several other groups, besides Velardi, have shown decreased relapse rate for adult AML (Table 1).[87, 235, 236] There are also reports with positive results from HSCT with unrelated and sibling donors, where most have the common denominator of using effective T cell depletion (TCD) either through CD34 selection or the use of anti-thymocyte globuline (ATG).[237, 238, 240, 241, 243, 246, 247] This is in line with observations that T cells in the graft can interfere with NK cell maturation. In TCD transplantation, NK cells express more KIRs and express less IFN-γ, suggesting a faster maturation and more cytotoxic phenotype.[248] This difference could be mediated by the lack of regulatory T cells, which may inhibit NK cells both through direct inhibition and by a competition of IL-2.[249-252] Another piece of evidence supporting the interference of T regulatory cells with NK cells comes from a recent study by Bachanova et al, where adoptively transferred haploidentical NK cells show a better in vivo expansion and also a better clinical effect after IL-2 diphtheria toxin treatment depleting the host T-regulatory cells.[252] The influence of T cells on NK cell alloreactivity is one of the focuses of **paper II** in the present thesis. Another factor coupled to a better NK cell mediated effect is the high stem cell doses often used in haploidentical transplantation. This notion is supported by experiments in mouse models, where high stem cell doses may overcome rejection of grafted cells[253], something also described in early studies of NK cell tolerance in the context of hybrid resistance.[254] A third factor that may contribute to promote NK cell mediated alloreactivity in haploidentical transplantation and other TCD settings to use no or less immunosuppressive therapy is used post-transplant allowing NK cells to deliver full cytotoxicity.[255]

In recent years, the focus has shifted from KIR-ligand mismatch or missing ligand to studies of the clinical effects of activating KIRs. Two early studies including patients with mixed diagnoses showed that donor-recipient pairs with *KIR* genotypes containing more activating receptors (haplotype B) could affect the outcome.[256, 257] In 2009 Cooley et al presented data from 448 patients with AML showing that patients with at least one group B haplotype have 42% better relapse free survival and 50% improvement in overall survival.[258] In a second study, including

1086 patients with AML and 323 with ALL, the strongest prediction was made by Haplotype B motifs in the centromeric part of the group B haplotype (Cen B) of the *KIR* locus. The relative risk for relapse was 0.34 when comparing homozygosity for the Cen B versus the Cen A KIR gene content. Cooley et al recently dissected effects of the Cen A/B prediction system further and showed that all individual KIR-genes in the haplotype B may influence the outcome as long as the recipient was not C2 homozygous.[259] It was proposed that KIR2DL2 might mediate at least a part of the effect.[259] The cellular mechanism behind this observation remains unclear but it is possible that it relates to the strong educating impact of the KIR2DL2-C1 interaction[260]. Interestingly, seven haplotype B genes were independently linked to better outcome of C1+ patients in the *HLA* mismatched situation. No such effect was seen in the *HLA*-matched situation, which may infer that a mismatch may potentiate these effects, maybe trough a breaking of tolerance.

1.6.6 Downtuning of NK cell responses by KIR2DS1

Among the activating *KIRs*, KIR2DS1 is the only receptor that shows significant functional interactions with a constitutively expressed ligand, HLA -C2.[260] Chewning showed in 2006 that KIR2DS1+ NK cells from C1+ donors mediated alloreactive responses against C2+ targets. In 2009 Giebel published results from a small cohort showing that the use of a KIR2DS1+ donor into C2+ recipients resulted in worse outcome.[261] Moreover, Venstrom et al showed 2012 in a large cohort of 1277 patients transplanted for AML that use of a KIR2DS1+ donor could mediate protection against relapse in C1+ recipients.[245] This is also well in line with *in vitro* results showing that KIR2DS1 tune down NK cell-mediated killing in the presence of C2 homozygous donor cells[262]. The biological function of an activating receptor with weak binding affinity to HLA-C2 remains to be solved. It is not impossible that stronger binding and activation may be mediated by altered ligands caused by pathogens as suggested for other activating receptors.[262, 263] It has also been proposed that activating receptors are needed for education that suggest that KIR2DS1 could have a function even in the absence of C2 ligand.[264]

Although the binding to the proposed cognate ligand HLA-Bw4 is less well documented, the effect of KIR3DS1 has also been examined in several studies.[243, 245, 265, 266] In a study with mixed diagnoses the presence of KIR3DS1 was linked to less relapse in Bw4+ recipients [265] and in larger studies of AML patients it was coupled to a lower risk for acute GVHD II-IV and an increased overall survival.[243, 245] These effects were further potentiated when carrying one or two copies of KIR3DS1 indicating a dose-dependent effect. A direct correlation to copy number

variation of KIR3DS1 was described for controlling HIV-infection[267], suggesting that a higher expression of this activating receptor may influence the functionality of the NK cell repertoire.

Delineating the impact of individual *KIR* genes is complicated because of the strong linkage disequilibrium between the narrowly placed *KIR*-genes.[230] Furthermore, mismatches of HLA-B and -C may trigger T cell alloreactivity, which may be hard to separate from NK cell-mediated GVL. Although it remains an outstanding task to decipher the conditions that foster NK cell alloreactivity in the context of HSCT, the accumulated data permit some conclusions to be drawn:

- NK cells can mediate clinically relevant responses against AML and MDS.
- Responses are most commonly seen when T cells or at least T regulatory cells have been inhibited, probably because NK cells more easily can undergo expansion and mediate their cytotoxic function without inhibition.
- KIR-ligand mismatched donors in don+/rec- direction is preferable in TCD-transplant settings.
- Activating KIR2DS1+ or Cen B+ donors are preferable for C1/X recipients both in matched and mismatched unrelated T cell-replete (TCR) settings.

2 GENERAL AIMS

The general aim of this thesis was to examine new aspects of the immune reconstitution after HSCT by using the latest techniques within multicolor flow cytometry and try to learn more about the role of NK cells in infection and GVL-processes.

More specifically we wanted to gain knowledge about NK biology for the purpose to develop NK cell-based cellular therapies against malignancy.

Since NK cell repertoire formation is influenced by CMV, and CMV is partly controlled by NK cells, we wanted to focus on this interplay after HSCT.

To enable these studies, we established a biobank from patients transplanted at, Karolinska University Hospital, Huddinge. The regional ethics board in Stockholm and at National Institutes of Health, Bethesda, MD, approved all studies. Patients were included between 2005-2011 and were sequentially sampled for serum and peripheral blood. Samples were taken from the donor and at 11 time points after the transplantation in the recipient. Extended *HLA* and *KIR*-genotyping of donors and recipients were performed and patient data was collected prospectively. We also collected lymphocytes from a cohort of healthy donors that were *HLA* and *KIR*-genotyped. These biobanks were used for the studies reported in **paper II-IV**.

3 DISCUSSION

3.1 FACTORS COUPLED TO DELAYED IMMUNE RECONSTITUTION ARE ASSOCIATED WITH LETHAL INFECTION LATE AFTER HSCT

Besides relapse of the underlying disease or GVHD, infections are the most common causes of death in HSCT patients.[268-270] A mixture of viral, bacterial and fungal infections contributes to this increased mortality, indicating that defects in both the innate and adaptive immunity are involved. The early mortality due to infections has been extensively studied. In **paper I**, we therefore concentrated on the contribution of infections developing later than 6 months after HSCT on the transplant related mortality (TRM).

We showed that the spectrum of the lethal infections was similar as described in earlier studies. These results stress the fact that prolonged prophylaxis against varicella, fungal, and bacterial infections is warranted in patients with ongoing chronic GVHD and immune suppression even late after HSCT. These patients should therefore be under careful observation, liberally examined, and early treated when developing signs of infection. It is of course of outermost importance that these patients are vaccinated according to internationally recommended guidelines.[271]

The primary objective of this study was to identify risk factors coupled to late lethal infection and as expected, we found that chronic GVHD and a mismatched/unrelated donor increased the risk for late lethal infection. These factors are known to have a profoundly negative influence on the IR.[272-274] Total body irradiation (TBI) is also known to cause lifelong splenic impairment increasing the risk for encapsulated bacterial infections.[275, 276] We also found that previous acute GVHD grade II-IV, even when not followed by chronic GVHD, was an independent factor increasing the risk seven-fold for late lethal infection. This could in part be due to the direct effects on the IR and cellular function mediated by acute GVHD.[277, 278] The immunological imprint made by treatment with high doses of steroids[279-281] or a need for prolonged treatment with cyclosporine A may also contribute to the increased risk. Further investigation of differences in IR and function of specific lymphocyte subsets may reveal why the risk for late lethal infection is increased in this group of patients.

More surprising was the finding that CMV infection, usually occurring early during the IR after HSCT, could leave an imprint that increases the risk for death in infection years after the transplantation. CMV infection increased the risk five-fold for dying in a lethal infection. As we point out in **paper I,** it is known that acute GVHD increases the risk for CMV infection[50, 282], increases the CMV viral load[141] and the risk for subsequent chronic GVHD.[283-287] The use of unrelated/mismatched donors increases both the risk of CMV infection and acute GVHD. Cytomegalovirus infection has also been associated with an increased risk for chronic GVHD[121, 137, 288], and vice versa.[289] However, when we corrected for all these potential confounding factors in a multivariate analysis, CMV still remained an independent risk factor for late death.

This captured our interest since CMV, the most clinically important virus infection post HSCT, has several known immunomodulatory properties and display heterogeneous reactivation patterns among patients. CMV can suppress T cells, NK cells, macrophages, neutrophils and dendritic cells[290-296], but little is known about the long-term effects of CMV infection on the IR after HSCT. Clinical studies have shown correlations between CMV replication and development of severe bacterial and fungal infection.[47, 285, 297, 298] Here we confirm and extend those findings and show that even an early CMV infection can influence late outcome.

An interesting approach would be to use an early vaccination strategy against CMV, which could improve early CMV immunity and thereby potentially diminishing uncontrolled replication. After decades of research, there are now promising vaccines reaching phase III trials.[299-301] Our findings support the need for continuous efforts to develop such vaccines possibly able to reduce the risk for late lethal infections.

At the time of our first study, a few reports had shown a correlation between CMV infection or donor CMV seropositivity and an increased GVL effect against hematological malignancies; findings that have been verified in several recent studies. Since NK cells play an important role in the control of CMV infection[302, 303] and also are coupled to GVL effects in HSCT, we decided to further study NK cells after HSCT with focus on NK reconstitution, NK-mediated GVL-effects and the interplay between the NK cell compartment and CMV.

3.1.1.1 Conclusions paper I.

The risk to die from late infections is not negligible. Mismatched or unrelated donor, chronic GVHD, previous TBI, acute GVHD or CMV infection increases the risk significantly. We speculate that CMV infection can affect the IR, increasing the risk for developing late infections.

3.2 FUNCTIONAL TUNING OF NK CELLS IN TRANSPLANTATION

One important question addressed in the present thesis is how the alloreactive NK cells behave *in vivo* during the IR after transplantation. By gaining further insights into how NK cells function in the early phase after transplantation, we might be able to delineate why clinical effects are observed in certain transplantation settings but not in others. Beneficial effects have been evident in murine experimental systems, but are harder to reproduce in clinical transplantation. In the haploidentical setting Ruggeri et al found that alloreactive NK cell clones from the patients, expanded *in vitro*, could be detected up to three months after the transplantation. Thereafter these cells disappeared[304], leading to the assumption that tolerance was obtained. In unrelated KIR-ligand mismatched TCR transplantation, educated alloreactive NK cells have been identified up to three years after transplantation as determined by flow cytometry.[305] The continuing education was fully dependent on donor KIR-ligands and followed earlier stipulated principles of education[306], indicating that the cells maintaining the education may be provided with the graft.

The cellular and molecular mechanisms underlying education are still unknown, but it is likely that bone marrow stroma[190] and other donor cells take part in the process.[307-309] A hot candidate for the conductor position could be the dendritic cell. Activated monocytoid DC were recently shown to induce KIR and NKG2A-expression on immature NK cells in a IL12-dependent manner[310]. There is also evidence that education is maintained by *cis*-interactions, within each individual cell, without the need for interactions with stroma or other cellular components.[311, 312] A role for *cis* interactions does not exclude additional tuning by the local milieu.[313] A major

contribution by one or more donor-derived hematopoietic cell types in education seems likely given that NK cells transferred in isolation rapidly adapt to the MHC environment.[314, 315]

3.3 ARE UNEDUCATED CELLS ABLE TO BREAK THEIR TOLERANCE?

In **paper II** we focused on NK cell maturation in HLA-matched sibling transplantation. Since the KIR-locus in chromosome 19 is uncoupled from the HLA -locus on chromosome 6, KIR-mismatches can occur even in the HLA-matched situation (figure 9). There had been earlier reports about beneficial clinical effects of receptor-ligand mismatch in HLA -matched unrelated and TCD HLA-identical sibling transplantation.[240, 241] Stimulated by these findings, we examined the impact of KIR-HLA genetics in our own TCR HLA -identical sibling cohort of 105 patients transplanted for AML and MDS at Karolinska University Hospital between the years 1988-2008.

We could first verify that the NK cell KIR reconstitution in our cohort mimicked that reported in earlier studies.[316-319] The dynamics of $NKG2A^+$ NK cells also followed previously described patterns with high numbers detectable early after transplantation.[185, 320, 321] The KIR expression was gradually acquired over time, resulting in a mature NK cell repertoire, where the frequency of triple KIR expressing cells detectable after six months was similar to those present in the donor.[190, 318, 322-324] Notably, in our cohort, the NK cells had a slight decline in function during the first month but recovered full functionality after 2-3 months. $NKG2A^+$ KIR^- NK cells represented the major responding cell population during the first two months. In contrast to a previous report, NK cells expressing non-educating KIRs remained hyporesponsive at all time points; a finding that recently has been corroborated by others.[208, 305] Given the proposed role for T cells in interfering with NK cell reactivity, we started collaboration with Prof. John Barrett at the NIH, since his group could kindly provide us with patient material from TCD, $CD34^+$ enriched, HLA-matched sibling transplantations. Importantly, NK cell responses in patients undergoing TCD transplantation were similar to those in the TCR setting (**paper II**). Thus, our results suggested that discrepancies in the outcomes between different studies might depend on many other factors than T cell content of the graft.

Our results were in contrast to a study by Yu et al.[325] In their cohort NKG2A did not educate the cells whereas non-self single-KIR expressing cells were highly functional early after HSCT as determined by monitoring IFN-γ and CD107a responses early. This subpopulation of NK cells expressing non-self KIRs gradually became tolerant at later time-points after transplantation, suggesting a window of opportunity for NK cell-mediated alloreactivity by uneducated NK cells. The major differences between the two studies were the following: i) In the cohort examined by

Yu et al. no immune suppression was given after the transplantation while in our TCR setting cyclosporine A was given for three months and also for the first three weeks in the TCD setting. ii) Yu et al. examined a larger group of TCD patients and it is possible that factors increasing cytokine levels, such as GVHD or infections in some patients, could influence the education of NK cells, making the non-self single-*KIR* expressing NK cells prone to overcome the lack of KIR-mediated education. iii) The mode of stimulation and the read-outs were different in the *in vitro* assays used in the two studies. We used K562-target cells while Yu et al. used 721.221-cells, which might have caused slightly different results since 721.221 cells usually elicit less robust responses than K562 cells. iv) We monitored CD107a expression but did not examine IFN-γ production that is known to require a higher degree of stimulation.[182]

In 2011, Foley et al. presented a study that shed further light on differences between TCD and TCR settings.[208] Target cell-induced IFN-γ production was generally lower in the TCD setting. NKG2A was shown to only educate the cells for cytotoxicity and not for production of IFN-γ, something that educating KIRs were capable of. Furthermore, they found discrepancies in the type of response of discrete NK cell subsets to stimulation by different cytokines. IL-12 and IL-18 did promote IFN-γ production but not degranulation, while stimulation with IL-15 could promote both functions. These findings may have implications for whether GVHD or infection can stimulate NK cells to mediate GVL via breaking of the tolerance. Bacterial infections and GVHD may for example primarily cause an increase in IL-12 and viral infections may promote a response where IL-15 production is higher but these mechanisms need further studies before any conclusion could be drawn.

The major differences between keeping the tolerance and breaking it seems to be the T cell depletion and the absence of immune suppressive therapy, factors that also have shown to speed up the NK cell reconstitution, with early expression of more KIRs and less $CD56^{bright}$ [326], which would favor strong cytotoxicity.[326, 327] These differences are important to bear in mind when trying to optimize NK cell-mediated GVL effects in clinical transplantation and/or cellular therapies. T cell depletion seems to be crucial for letting the NK cell work freely and avoidance of IS may also be preferable. Several studies have now shown convincing GVL-effects in the HLA matched settings why even HLA -matched siblings may be considered as donors for adoptive cell therapy, especially if they have CenB *KIR* motif and the cells are pre-stimulated with cytokines that favor breaking of tolerance.

In our cohort of 105 patients transplanted with sibling donor for AML and MDS, there was no correlation between having a missing ligand and OS or RI. The only statistically significant correlation was a 2.8-fold increase in acute GVHD II-IV if the C2 ligand was missing (HR 2.87

(1.29-6.37) p=.01). In addition, there was a trend towards increased TRM (HR 4.01 (0.98-16.40) p=.05) in the same group; a finding we at the time for the analysis interpreted as false positive since patients with combination of missing Bw4 and C2 or C1 fell out in the same way. However, several investigators have now reported a similar pattern with higher acute GVHD in the C1/C1 situation.[328] McQueen et al. propose a reasonable explanation for this. C2-KIR2DL1 has a stronger interaction resulting in stronger inhibition than C1-KIR2DL2/3[257], thereby inhibiting NK cell responses more efficiently and likely also provides less cytokine stimulation to T cells that could augment GVHD. Cook et al. found that C2 homozygous donor/recipient-pairs had a decreased OS.[329] There are also indications from other studies of activating *KIRs* that a "missing C2 effect" could lead to less relapse after transplantation for AML. The effects of donor activating *KIRs* or CenB haplotypes are potentiated in C1+ recipients and Venstrom et al. found increased relapse rate in C2 homozygous recipients having KIR2DS1 bearing donors that could be explained by the previously mentioned down-tuning of NK cell education mediated by KIR2DS1.[245, 259, 262]

3.3.1.1 Conclusions paper II

We could not verify any evident GVL effect of having a missing ligand in our sibling cohort, a finding that in cellular cytotoxicity-assays correlated with intact tolerance of uneducated NK cells during IR. However, reinterpreting our results in light of recent studies give support to the emergence of NK cell-mediated alloreactivity that increases the risk of acute GVHD in patients missing C2.

3.4 DETERMINING THE ALLOREACTIVE POTENTIAL AT THE SUBSET AND POPULATION LEVEL

In **paper III** we examined NK cell repertoires in 31 healthy donors in an attempt to determine their overall potential to deliver alloreactivity in an allogeneic transfer or HSCT setting. To this end, we examined donors homozygous for the group A haplotype to be able to distinguish all *KIRs* phenotypically, without cross reactivity of the commercially available antibodies. As a point of departure, we set out to calculate the size of functionally educated NKG2A- NK cells. However, to do this, we first needed to sort out the prerequisites. The education process is well described for the C1/C2/Bw4[330] interaction but less well examined for the KIR3DL2-A3/11 receptor ligand pair. KIR3DL2 is one of the framework genes included in all KIR-haplotypes and binds to *HLA* A3/A11. This binding has been shown to be dependent on peptides.[331] For example was the EBV-derived peptide, EBNA3A was shown to promote the binding of KIR3DL2 to *HLA* -A3. Self-peptides are thought to be able to play the same role. We studied

KIR3DL2 single positive cells in all *HLA* A3/A11 individuals and found the cells to be hyporesponsive. This suggested that the KIR3DL2 – HLA A3/A11 interaction was not strong enough to provide education. KIR3DL2 may confer education during active a primary EBV-infection. However, based on the experimental data in healthy donors, we concluded that KIR3DL2 should not be included in the algorithm for calculating the alloreactive subset.

The frequency of educated NK cells in this cohort of healthy donors ranged from 12 to 68% (mean 33%) (**paper III**). This vast variability translated into very different potential as allogeneic NK cell donors in *HLA* mismatched settings. As an example, the size of the alloreactive repertoire in a C1/C2 to C2/C2 transplant ranged from 1 to 9 % and in the full cohort between 0-62 % (mean 8%).

Thus, we conclude that genetic algorithms for predicting NK cell alloreactivity needs to be complemented by phenotypic assessment of the alloreactive subset. In a study of adoptively transferred NK cells, in an autologous transplantation setting, against relapsed myeloma, the number of infused alloreactive cells were quantified. However, since the NK cells didn't expand *in vivo*, no conclusions regarding clinical effects could be drawn.[332] This points towards the importance to keep track of the number of alloreactive cells in adoptive cell therapy settings. However, in light of recent studies showing the complexity of KIR-KIR-ligand interactions, phenotypic and functional tests of the donor repertoire may be the best way to reveal the actual alloreactive potential. A recently described method for examining the donor alloreactive repertoire, by co-culturing of donor and recipient or target cells expressing specific KIR-ligands, may be a good way to determine the true alloreactivity capacity of each donor-recipient combination[333]. Larger clinical studies considering the alloreactive subset are warranted to see if this relates to a better outcome before this could become clinical praxis.

3.5 DIVISION OF LABOR BETWEEN NKG2A AND KIRS

It is well established that KIR expression and NKG2A expression are inversely correlated, which has been interpreted as a buffering mechanism to maintain a tolerant and functional repertoire. [187, 334] Extending those findings, we showed that this inverse correlation also exists at the single cell level. Thus, cells expressing more KIRs at the cells surface expressed less NKG2A than those with fewer KIRs (**paper III**). Interestingly NK cells only expressing the non-educating KIR3DL2 also had higher NKG2A than NK cells expressing educating KIRs. Taken together, these data support the notion that functional NK cells always express an inhibitory receptor to self and that the tuning of the cell is dependent on the net strength of the binding that the

receptors can provide.[202] Well in line with these rules are also the findings that activating receptors can down-tune the response in presence of a stimulating ligand to avoid strong auto-reactivity.[262, 263] The inverse correlation of NKG2A and KIR at the single cell level may have consequences extending beyond the buffering of the NK cell repertoire. It was recently described that the HLA-E molecule present other peptides than the HLA-leader-sequences and that CD94/NKG2 receptors can discriminate these HLA-E-peptide complexes.[335, 336] Furthermore, Kuldeep et al. suggest that NKG2A and KIRs may have complementary functions as NKG2A can provide inhibition when HLA-levels are low and KIRs can sense and discriminate changes in environments with denser HLA-levels.[335] The change in receptor profiles from NKG2A- to KIR-dominant during NK cell differentiation may thus lead to division of labor between naïve and more mature NK cells. How these findings should be interpreted in the context of HSCT is not yet clear but one may speculate that a blockade of the CD94/NKG2A or KIR receptor system by monoclonal antibodies would be very beneficial by triggering cytotoxicity against tumor cells with low HLA-expression.

3.5.1.1 Conclusions paper III

The number of alloreactive NK cells may vary considerably between donors 0-62% (mean 8%) alloreactive NK cells depending on recipient HLA -ligands. This important to consider when studying NK cell mediated GVL effects in the context of HSCT.

3.6 INTEGRATIVE PROFILING OF MULTIPLE PHENOTYPIC PARAMETERS AND ITS IMPACT ON OUTCOME IN HSCT

NK cell populations are extremely diversified, both between populations and individuals, because of the large variation in *KIR*-alleles, *KIR* gene copy number giving rise to a high number of haplotypes.[337] This diversity is reflected in phenotypic differences and variations in the NK cell repertoire between individuals. Environmental factors, for example latent viruses, have been shown to further skew these repertoires causing differences in maturation status and by memory like responses.[216, 338, 339]

We hypothesized that the constitution of the NK cell repertoire in the donor could affect the outcome after HSCT and at the same time we wanted to examine how common clinical events in the recipient after the transplantation could interfere with repertoire development. We defined NK cell repertoires based on five phenotypic characteristics coupled to NK-repertoire maturity and memory and used these data to cluster all individual profiles with statistically similar repertoires. The correlation of these groups with clinical outcomes where thereafter examined.

The parameters were selected based on the differentiation model described by Björkström et al, where immature $CD56^{bright}$, $NKG2A^+$ NK cells differentiate to become $CD56^{dim}$ NK cells and thereafter lose NKG2A, start expressing KIRs and CD57.[185] To cover the key steps of this process we included the overall frequencies of $CD56^{bright}$ NK cells, the frequency of $NKG2A^+$ NK cells, the frequency of CD57+ NK cells as the three first parameters. As a fourth parameter, we established the size of the educated repertoire based on the algorithm described in **paper III**. Furthermore, NK cells that expand in the response to CMV have been shown to express educated KIRs together with the activating receptor NKG2C [216, 302, 340], which was included as the fifth and final parameter. This approach was applied on one cohort of 106 peripheral stem cell donors to study the impact on outcome mediated by the donor repertoire and 65 donor-recipient pairs to be able to study the impact of dynamic changes in the repertoire after transplantation.

3.7 CORRELATION BETWEEN DONOR PHENOTYPE AND CLINICAL OUTCOME

When analyzing the donor cohort, three major cluster groups with different receptor expression became apparent. The clinical outcome was different in one of the groups. Somewhat surprisingly we found that the second cluster consisting of donors characterized by having more naïve NK repertoires had significantly less relapse (**paper IV**). This finding was opposite to what we had anticipated based on the biological data. More mature repertoires, dominated by NK cells with a higher differentiation status and thereby more potent in mediating ADCC and natural cytotoxicity, might be expected to provide better anti-leukemic activity. The strong impact on the NK repertoires mediated by CMV and the triggering of memory like responses has also been suggested to protect against relapse[341, 342], especially since CMV reactivation in several studies has been shown to mediate protection against relapse.[138, 140, 343] Important to note is that the protective effects of CMV reactivation are only visible in large cohorts and seem to be more pronounced in patients having myeloablative conditioning and not in their cohort undergoing reduced conditioning.[343]

This raises the question about which NK subset that best mediate the GVL effect? NKG2C+ NK cells expressing self-KIRs, expanded in vitro, have a potent function but are incapable of killing HLA-matched AML-blasts (Liu et al., manuscript in preparation). If these educated, differentiated and maximally responsive NK cells are shown not to mediate the most beneficial clinical effects, alternative explanations have to be considered. It has been shown in several

studies that uneducated NK cells can provide effector functions. Furthermore, antibody-dependent cell-mediated cytotoxicity (ADCC)-stimulation via CD16, also providing very strong stimulation *in vitro*, can override weak education.[344, 345] *In vivo* transfer studies in mice have demonstrated that the KIR-negative, uneducated NK cells mediate a stronger protection against CMV infection than the educated KIR^+ cells.[207] The same authors propose that uneducated NK cells also could be responsible for mediating GVL effects in transplantation. Our results suggest that the more immature fraction of the NK cells may be more important than previously thought in terms of promoting GVL-effects. In support of this notion, Foley et al. showed that NKG2A provides strong educating stimuli for cytotoxicity indicating that this "immature" subset may provide GVL and that stimulation through educating self KIRs are needed to acquire IFN-γ production.

Another factor that could affect the terminally differentiated cells more than the naïve cells is induction of senescence or exhaustion. Exhaustion has been well described in the T cell compartment, where CMV has been shown to contribute to increased senescence[346], but this is less well established for NK cells. Since NK cells are rapidly renewing, compared to T cells, one could speculate that this is less important, but exhaustion markers as; cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), PD-1 and T cell immunoglobulin- and mucin-domain-containing molecule-3 (Tim-3) has been described to be expressed also on NK cells and shown to block function in presence of tumor.[347-349] Provided that a tumor or an inflammatory environment more easily turns terminally differentiated NK cells into exhaustion, one could speculate that there might be an advantage to have stem cell donors with more naïve repertoires which are more resistant to these effects.

3.8 CORRELATION BETWEEN CHANGES IN RECIPIENT PHENOTYPE AND CLINICAL OUTCOME

Since CMV can promote NK cell differentiation, towards more mature repertoires, we examined the impact of CMV infection on the NK cell pool in our cohort. Extending earlier studies,[341, 350] we found that the increase in NKG2C and educated KIRs correlated with the number of replication episodes the patients had experienced. Thus, a less controlled and prolonged infection seems to provide a stronger stimulus for NK differentiation.

When applying unsupervised hierarchical clustering on the recipient repertoires at 9-12 months after HSCT, the strong impact of CMV became apparent. 38 % of the recipients had a more mature repertoire characterized by hi NKG2C, educated KIRs, and CD57 combined with low

frequencies of NKG2A and CD56^{bright} NK cells. Recipients belonging to this cluster had received more CMV treatment and had more complicated/prolonged CMV episodes. Importantly, the statistical examination of the outcomes of the recipients belonging to this cluster corroborated the findings in the donor cohort. Hence, recipients with more naïve repertoires at 9-12 months had a less relapse and increased overall survival. The non-naïve group had a nine-fold higher risk to die after 9 months; and a six-fold increased risk for developing a relapse later than 9 months after HSCT compared to the larger group having more naïve repertoires. The causes of death were a combination of relapse (n=5) and late infections caused by aspergillus (n=2).

To analyze the dynamic changes in the repertoires, we clustered donor-recipient delta values determined by subtracting the values of the individual parameters in the recipient with those in the donors. Again the patients switching to more naïve repertoires had a better outcome. The group consisting of 36 (of 65) patients that lowered their number of CD57+ NK cells had 6 times lower relapse incidence and 5 times lower OS.

Since we are studying changes to the NK cell repertoire induced by clinical events after the transplantation, and thereafter make correlations to later outcome, there is a risk for bias. A main concern for the analysis in the recipient cohort is to what extent these results are influenced by other factors. But notably, there was no statistical difference with regards to the length of time on immune suppressive therapy, acute GVHD, chronic GVHD or disease risk index (DRI)[351] between the compared groups. Corroborating the clustering algorithm, the need for CMV treatment was strongly associated with the groups developing a more mature repertoire.

3.8.1.1 Conclusions paper IV

In **paper I** we found that CMV reactivation causes an increased risk for late lethal infections uncoupled from GVHD and donor status. In **paper IV** we verify that the patients having viremia above the threshold for treatment skew their NK cell repertoire towards a more mature phenotype, something that is further enhanced by having multiple reactivations. The patients having larger positive skewing against terminally differentiated repertoire, judged by changes after 9-12 months, had a significantly decreased survival, mainly caused by relapse. The fact that the two only cases of late deaths occurred in the same group may suggest that the immune defect caused by CMV reactivation may at least in part be coupled to skewing of NK cell repertoire. Whether this is mediated by a direct effect dependent on the NK cell repertoire *per se* or if the skewed repertoire is a marker of a global defect in the full immune network towards worse control of infection and tumor cells remain to be examined. For example NK cells have recently been suggested to be important as rheostats controlling the T cell response to viral infections.[352]

In a mouse model it was shown that NK cells delete CD4+ T cells, thereby hindering lethally strong CD8+ T cell responses against murine-CMV (MCMV) and promoting the persistence of MCMV. This mechanism points toward the importance of a having a well-functioning NK cell compartment, in order to be able to maintain adequate immunity at least against viruses and highlights the interplay between chronic CMV-infection and the immune network.

Of note, in this smaller cohort, we could not detect any impact of CMV reactivation on the incidence of relapse, when analysed in isolation. The use of peripheral stem cell grafts may have contributed to this outcome.[343]

4 PROSPECTS OF USING NK CELLS IN CANCER THERAPY

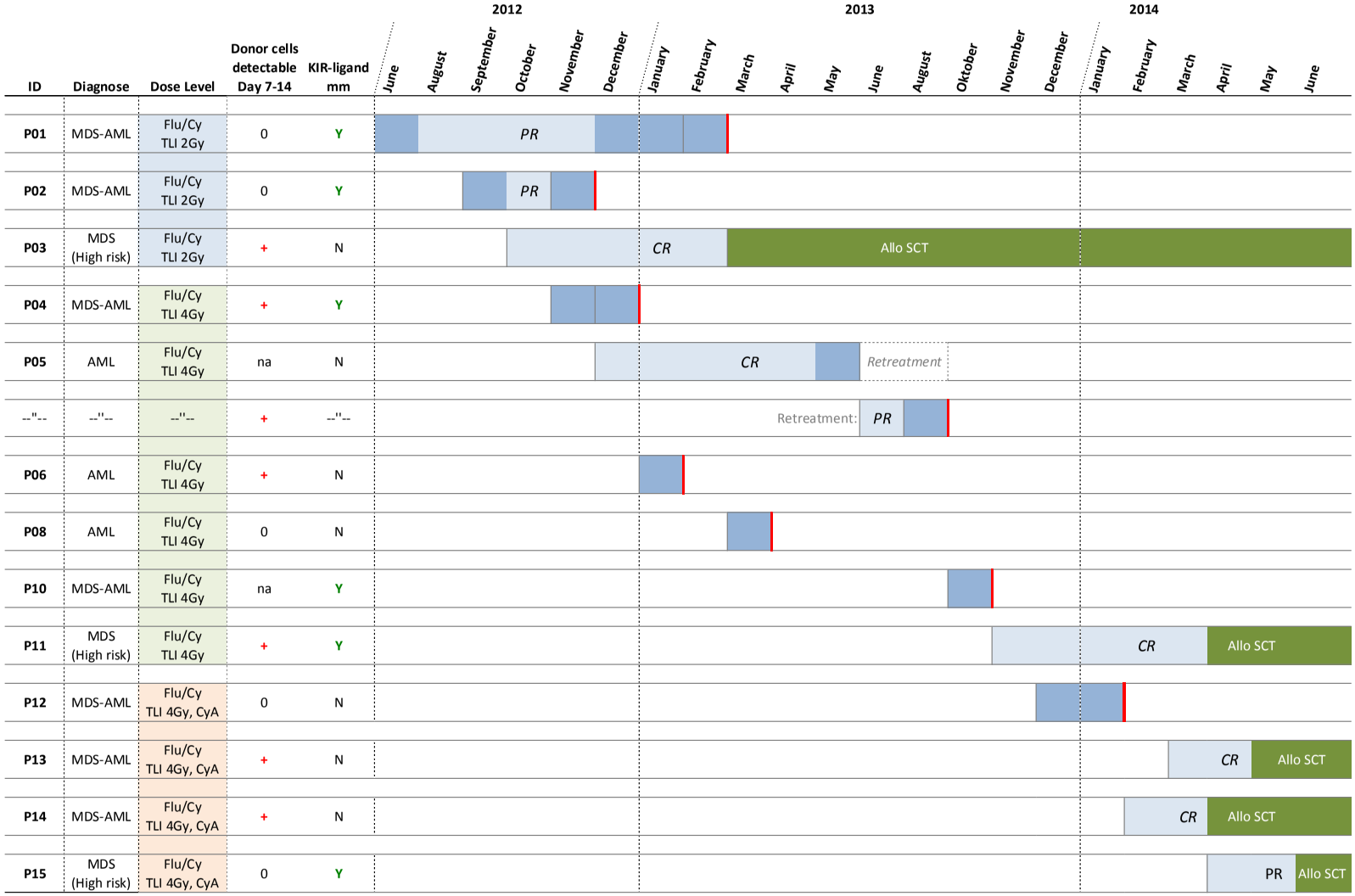
In 2005 Miller et al published a study where adoptive transfer of haploidentical NK cells were used against refractory AML.[353] In this study they used conditioning with cyclophosphamide and fludarabine (Flu/Cy) followed by IL-2 stimulation *in vivo*, a conditioning similar to the one used for adoptive T cell therapy.[354] Adoptive transfer was possible without major complications and NK cells could sometimes expand and persist in the donor for a couple of weeks. Furthermore this resulted in a more efficient leukemia clearance.[353] Five of 19 patients responded with complete remission and later a CR rate of 21% was shown.[252] However, to date, the responses after a single treatment with NK cells have not been durable and the duration of the response has varied between 2-18 months. To be curative, the treatment needs to be combined with stem cell transplantation. In more recent work from Miller et al the standard conditioning regimen has been combined with myeloablative TBI and stem cell support given after the NK-transfer. This resulted in much better expansion rates and in better clinical responses. (Miller personal communication) In their most recent publication the Flu/Cy regimen was combined with T regulatory cell depletion that resulted in a complete response rate of 52% at day 35, something that stress the fact that the inhibition of regulatory cells is crucial.

4.1 ONGOING CLINICAL EXPLORATION

Inspired by early trials of adoptive NK cell therapy [353] we launched our own study in 2012. The study was designed as a Phase I/II clinical trial with 3 + 3 + 3 patients receiving escalating doses of total lymphoid irradiation (TLI) 2 Gy vs 4 Gy and in third dose level receiving cyclosporine A. The choice of conditioning was based on the previous experiences made by Rosenberg and Miller[353] but using TLI instead of total body irradiation (TBI) to be able to treat the patient without stem cell support since it is less myelotoxic. TLI is non-myeloablative and does not markedly influence myeloid diseases.[355-357] In addition, we use lower doses of fludarabine (25mg/m2 for four days) and cyclophosphamide (25 mg/kg for two days) than in previous studies. This lower dose regimen was selected to allow inclusion of patients with myelodysplastic syndrome (MDS), who tend to have few healthy stem cells to regenerate after myelotoxic treatment, thereby hopefully avoiding prolonged aplasia. Fludarabine and cyclophosphamide are two drugs that have high ability to lymphodeplete the recipient thereby allowing space for transferred lymphocytes to expand, eliminating rejection and regulatory cells that could hamper the cytotoxicity of transferred NK cells. Donors were chosen among relatives. Haploidentical

Time line NK cell therapy study

TABLE 2



donors mismatched for KIR-ligands, with a high number of alloreactive cells and activating KIRs were chosen when available. To date thirteen patients with either refractory AML or high risk MDS not eligible for standard therapy regimen or suitable for allogeneic stem cell transplantation have been included so far. The current results are presented in table 2. Eight of the 13 patients had reduced tumor burden or complete remission 1 month after the treatment. Six patients stabilized their disease enough to be accepted for HSCT and five had suitable donors and proceeded to HSCT. The duration of the response after HSCT is too early to evaluate.

The criterion of expansion of NK cells to 100 cells/μl at day 14 was not reached. Instead donor NK cells were detected by quantitative PCR, termed microchimerism. A positive microchimerim was correlated to a complete response with 4/6 patients with detectable donor NK cells at day 7-14 achieved CR while 5/5 patients without detectable donor cells did not achieve CR indicating that NK cells expanding above the threshold for detection by PCR may be sufficient to mediate an effect.

Another question is raised by the fact that responses were seen even in the KIR-ligand matched situation. One explanation could be that the conditioning is effective by itself even though both fludarabine and cyclophosphamide are drugs that have been proven effective against lymphoid malignancies and are here used primarily for their immune suppressive effects. Besides their cytotoxic properties effects, fludarabine inhibits STAT-1 signaling [358] and cyclophosphamide can beside its lymphodepletion properties inhibit T-regulatory cells. [359-361] TLI is also mainly affecting the lymphoid compartment leaving the myeloid compartment untouched[357, 362, 363] In several MDS and MDS-AML patients a rapid disease-progression was halted by the NK cell therapy and the course of the disease was stabilized for several months. Although direct killing of MDS blasts by infused NK cells may be one contributing mechanisms of the current therapy[364], other effects may also be considered including a general immunomodulatory effect affecting the T cell compartment and perhaps also myeloid cells including DC and myeloid derived suppressor cells (MDSC).[365-368] It is possible that the treatment in parallel with targeting rapid progressive tumor cells also target pathogenic immune cells and thereby changing the tumor microenvironment.

Preliminary conclusions: Our protocol had tolerable toxicity in high-risk MDS- and AML-patients. NK cells did not expand to the predetermined level but were detected by PCR day 7-14. The majority of clinical responses were seen in the group with positive microchimerism.

5 CONCLUSIONS

1) Infections are important contributors to late death and the risk factors are unrelated donor, GVHD, TBI and CMV reactivation.

2) CMV infection is a statistically independent risk factor for late death indicating that early CMV viremia may skew the immune network leading to a prolonged or persistent immune defect.

3) CMV infection *per se* does not provide protection against relapse in our cohort of HSCT with peripheral stem cell donors after transplantation.

4) CMV infection after HSCT alters the NK-repertoire to a more differentiated state and the magnitude of this response correlates with the severity of the CMV reactivation pattern

5) Phenotypic differences in the donor NK cell repertoire can lead to differences in outcome after HSCT

6) Transplantation with donors having a more naïve NK cell repertoire, defined with unsupervised hierarchical clustering, may provide protection against relapse of AML and MDS.

7) A more naïve donor NK cell repertoire confer a decreased relapse risk but increases the risk for dying by infection early after transplantation.

8) NK cell tolerance is maintained early and late after HLA-matched HSCT in both TCD and TCR settings.

9) NKG2A is a strong educating stimulus dominating the NKR repertoire early after transplantation.

10) KIR3DL2 does not provide functional education of NK cells in healthy donors.

6 FUTURE OUTLOOK:

In this post Hugo-project era, the scientific community has come to the conclusion that genes are not everything… The focus has instead been directed against proteomics and epigenetics, since the study of gene products, rather than the genes, was shown to describe biological processes more accurately. We have chosen to study phenotypically defined NK cell repertoires with multicolor flow cytometry; a technique that soon has reached its maximum number of parameters possible to analyze on one single cell. The most recent development in single cell analysis platforms, including the mass cytometry CyTOF[369], in combination with softwares for automated analysis such as SPADE[370] and ACCENSE[371] hold promise to embrace and visualize the complexity of the almost endless number of subclones that exists within the immune cell compartment. These technological advances provide a framework for more comprehensive approaches to study the immune system. We may now be able to refine the present knowledge to better understand the full complexity of interactions and communication between immune cells. It is now time to bring these techniques into the clinic.

Exploiting such technologies may also allow us to determine the "immune profile" of each patient, which will make it easier to study and identify functional immune deficiencies in regards to tumor development, autoimmunity and other diseases coupled to inflammation. With this information it may be possible to explore new methods for intervention and correcttion of the defects with immunomodulatory techniques such as; immunomodulatory drugs, cytokines such as IFN, IL-2/15 or by targeted cell therapies or a full transplantation.

A similar development is ongoing in the emerging field of tumor profiling. This knowledge in combination with immune profiling could make it possible to find ways to circumvent tumor immune escape mechanisms. We have to learn how to hit the cancer cell from the right angle and how to combine several strategies to eradicate or control the most resistant tumors. Understanding tumor heterogeneity may aid in defining ideal targets, both for new small molecular compounds, and for immune therapy. It is also of uttermost importance to examine in which sequence therapies are delivered. For example the tumor mass may have to be reduced to small numbers before immunomodulation is used and short acting single doses of short acting cells (as in current adoptive transfer settings) have to be used at the right "window of opportunity" to potentiate and maximize the effects.

New knowledge about tumor heterogeneity and about which stem cells that drives the tumor will make it possible to direct the effector cells, both NK cells and T cells, towards the most central targets by using for example common or bispecific (BIKEs) monoclonal antibodies or

permanently transduced chimeric antigen-receptors (CARs). To maximize these effects the knowledge about how the target tumor can be sensitized to killing by up-regulate ligands for activating ligands or setting them in a state where the cells become prone to self-destruction by apoptosis.

To reach these goals we need to relate the above gained knowledge to environmental factors, such as infections and ageing, that shape and intervene with the immune network both on the phenotypic, epigenetic and genetic level.

The knowledge how to select the right donor for adoptive cellular therapy and transplantation have to be developed in combination with methods to expand the cells to sufficient numbers without exhaustion keeping the selective killing properties without risk for severe side effects in the patient.

Strong connections between clinical doctors and specialized pre-clinical researchers will therefore be instrumental to catalyze the translation of modern immunology into the clinic. A key element is to build biobanks and running prospective sampling of large patient cohorts including immune profiling along with conventional and new treatments.

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8 REFERENCES

1. Biron CA, Byron KS, Sullivan JL. Severe herpesvirus infections in an adolescent without natural killer cells. *N Engl J Med* 1989; **320**(26)**:** 1731-1735. e-pub ahead of print 1989/06/29; doi: 10.1056/NEJM198906293202605

2. Etzioni A, Eidenschenk C, Katz R, Beck R, Casanova JL, Pollack S. Fatal varicella associated with selective natural killer cell deficiency. *J Pediatr* 2005; **146**(3)**:** 423-425. doi: 10.1016/j.jpeds.2004.11.022

3. Eidenschenk C, Dunne J, Jouanguy E, Fourlinnie C, Gineau L, Bacq D *et al.* A novel primary immunodeficiency with specific natural-killer cell deficiency maps to the centromeric region of chromosome 8. *Am J Hum Genet* 2006; **78**(4)**:** 721-727. doi: 10.1086/503269

4. Lorenz E, Uphoff D, Reid TR, Shelton E. Modification of irradiation injury in mice and guinea pigs by bone marrow injections. *Journal of the National Cancer Institute* 1951; **12**(1)**:** 197-201.

5. Main JM, Prehn RT. Successful skin homografts after the administration of high dosage X radiation and homologous bone marrow. *Journal of the National Cancer Institute* 1955; **15**(4)**:** 1023-1029.

6. Barnes DW, Corp MJ, Loutit JF, Neal FE. Treatment of murine leukaemia with X rays and homologous bone marrow; preliminary communication. *British medical journal* 1956; **2**(4993)**:** 626-627.

7. Thomas ED, Lochte HL, Jr., Lu WC, Ferrebee JW. Intravenous infusion of bone marrow in patients receiving radiation and chemotherapy. *N Engl J Med* 1957; **257**(11)**:** 491-496. doi: 10.1056/NEJM195709122571102

8. van Bekkum DW, de Vries MJ, van der Waay D. Lesions characteristic of secondary disease in germfree heterologous radiation chimeras. *Journal of the National Cancer Institute* 1967; **38**(2)**:** 223-231.

9. Mathe G, Amiel JL, Schwarzenberg L, Cattan A, Schneider M. Adoptive immunotherapy of acute leukemia: experimental and clinical results. *Cancer Res* 1965; **25**(9)**:** 1525-1531.

10. Medawar PB. A second study of the behaviour and fate of skin homografts in rabbits: A Report to the War Wounds Committee of the Medical Research Council. *Journal of anatomy* 1945; **79**(Pt 4)**:** 157-176 154.

11. Snell GD, Russell E, Fekete E, Smith P. Resistance of various inbred strains of mice to tumor homoiotransplants, and its relation to the H-2 allele which each carries. *Journal of the National Cancer Institute* 1953; **14**(3)**:** 485-491.

12. Amos DB, Gorer PA, Mikulska ZB. An analysis of an antigenic system in the mouse (the H-2 system). *Proceedings of the Royal Society of London. Series B, Containing papers of a Biological character. Royal Society* 1955; **144**(916)**:** 369-380.

13. Mann DL, Rogentine GN, Jr., Fahey JL, Nathenson SG. Molecular heterogeneity of human lymphoid (HL-A) alloantigens. *Science* 1969; **163**(3874)**:** 1460-1462.

14. Billingham RE. Reactions of grafts against their hosts. *Science* 1959; **130**(3381)**:** 947-953.

15. Uphoff DE. Alteration of homograft reaction by A-methopterin in lethally irradiated mice treated with homologous marrow. *Proceedings of the Society for Experimental Biology and Medicine. Society for Experimental Biology and Medicine* 1958; **99**(3)**:** 651-653.

16. Santos GW. Busulfan (Bu) and cyclophosphamide (Cy) for marrow transplantation. *Bone Marrow Transplant* 1989; **4 Suppl 1:** 236-239.

17. Kolb HJ. Graft-versus-leukemia effects of transplantation and donor lymphocytes. *Blood* 2008; **112**(12)**:** 4371-4383. e-pub ahead of print 2008/11/26; doi: 112/12/4371 [pii]

10.1182/blood-2008-03-077974

18. Apperley JF, Mauro FR, Goldman JM, Gregory W, Arthur CK, Hows J *et al.* Bone marrow transplantation for chronic myeloid leukaemia in first chronic phase: importance of a graft-versus-leukaemia effect. *Br J Haematol* 1988; **69**(2)**:** 239-245.

19. Remberger M, Ackefors M, Berglund S, Blennow O, Dahllof G, Dlugosz A *et al.* Improved survival after allogeneic hematopoietic stem cell transplantation in recent years. A single-center study. *Biol Blood Marrow Transplant* 2011; **17**(11)**:** 1688-1697. e-pub ahead of print 2011/05/31; doi: 10.1016/j.bbmt.2011.05.001

20. Storek J. Immunological reconstitution after hematopoietic cell transplantation - its relation to the contents of the graft. *Expert opinion on biological therapy* 2008; **8**(5)**:** 583-597. doi: 10.1517/14712598.8.5.583

21. Bosch M, Khan FM, Storek J. Immune reconstitution after hematopoietic cell transplantation. *Current opinion in hematology* 2012; **19**(4)**:** 324-335. doi: 10.1097/MOH.0b013e328353bc7d

22. Storek J, Dawson MA, Storer B, Stevens-Ayers T, Maloney DG, Marr KA *et al.* Immune reconstitution after allogeneic marrow transplantation compared with blood stem cell transplantation. *Blood* 2001; **97**(11)**:** 3380-3389.

23. Storek J, Gooley T, Witherspoon RP, Sullivan KM, Storb R. Infectious morbidity in long-term survivors of allogeneic marrow transplantation is associated with low CD4 T cell counts. *American journal of hematology* 1997; **54**(2)**:** 131-138.

24. Reusser P, Riddell SR, Meyers JD, Greenberg PD. Cytotoxic T-lymphocyte response to cytomegalovirus after human allogeneic bone marrow transplantation: pattern of recovery and correlation with cytomegalovirus infection and disease. *Blood* 1991; **78**(5)**:** 1373-1380.

25. Lucas KG, Small TN, Heller G, Dupont B, O'Reilly RJ. The development of cellular immunity to Epstein-Barr virus after allogeneic bone marrow transplantation. *Blood* 1996; **87**(6)**:** 2594-2603.

26. Chklovskaia E, Nowbakht P, Nissen C, Gratwohl A, Bargetzi M, Wodnar-Filipowicz A. Reconstitution of dendritic and natural killer-cell subsets after allogeneic stem cell transplantation: effects of endogenous flt3 ligand. *Blood* 2004; **103**(10)**:** 3860-3868. doi: 10.1182/blood-2003-04-1200

27. Antin JH, Ferrara JL. Cytokine dysregulation and acute graft-versus-host disease. *Blood* 1992; **80**(12)**:** 2964-2968.

28. Roux E, Helg C, Chapuis B, Jeannet M, Roosnek E. T-cell repertoire complexity after allogeneic bone marrow transplantation. *Hum Immunol* 1996; **48**(1-2)**:** 135-138.

29. Gorochov G, Debre P, Leblond V, Sadat-Sowti B, Sigaux F, Autran B. Oligoclonal expansion of CD8+ CD57+ T cells with restricted T-cell receptor beta chain variability after bone marrow transplantation. *Blood* 1994; **83**(2)**:** 587-595.

30. Hentschke P, Omazic B, Mattsson J, Nasman-Bjork I, Lundkvist I, Gigliotti D *et al.* T-cell receptor Vbeta repertoire after myeloablative and reduced intensity conditioning allogeneic haematopoietic stem cell transplantation. *Scandinavian journal of immunology* 2005; **61**(3)**:** 285-294. doi: 10.1111/j.1365-3083.2005.01564.x

31. Omazic B, Hentschke P, Nasman-Bjork I, Mattsson J, Oxelius VA, Ringden O *et al.* Reconstitution of the Ig heavy chain CDR3 repertoire after allogeneic haematopoietic stem cell transplantation with myeloablative or reduced-intensity conditioning regimens. *Scandinavian journal of immunology* 2005; **61**(1)**:** 72-81. doi: 10.1111/j.0300-9475.2005.01528.x

32. Storek J, Witherspoon RP, Luthy D, Storb R. Low IgG production by mononuclear cells from marrow transplant survivors and from normal neonates is due to a defect of B cells. *Bone Marrow Transplant* 1995; **15**(5)**:** 679-684.

33. Glas AM, van Montfort EH, Storek J, Green EG, Drissen RP, Bechtold VJ *et al.* B-cell-autonomous somatic mutation deficit following bone marrow transplant. *Blood* 2000; **96**(3)**:** 1064-1069.

34. Suzuki I, Milner EC, Glas AM, Hufnagle WO, Rao SP, Pfister L *et al.* Immunoglobulin heavy chain variable region gene usage in bone marrow transplant recipients: lack of somatic mutation indicates a maturational arrest. *Blood* 1996; **87**(5)**:** 1873-1880.

35. Mackall CL, Fleisher TA, Brown MR, Andrich MP, Chen CC, Feuerstein IM *et al.* Age, thymopoiesis, and CD4+ T-lymphocyte regeneration after intensive chemotherapy. *N Engl J Med* 1995; **332**(3)**:** 143-149. doi: 10.1056/NEJM199501193320303

36. Small TN, Papadopoulos EB, Boulad F, Black P, Castro-Malaspina H, Childs BH *et al.* Comparison of immune reconstitution after unrelated and related T-cell-depleted bone marrow transplantation: effect of patient age and donor leukocyte infusions. *Blood* 1999; **93**(2)**:** 467-480.

37. Blennow O, Ljungman P, Sparrelid E, Mattsson J, Remberger M. Incidence, risk factors, and outcome of bloodstream infections during the pre-engraftment phase in 521 allogeneic hematopoietic stem cell transplantations. *Transplant infectious disease : an official journal of the Transplantation Society* 2014; **16**(1)**:** 106-114. doi: 10.1111/tid.12175

38. Sundin M, Le Blanc K, Ringden O, Barkholt L, Omazic B, Lergin C *et al.* The role of HLA mismatch, splenectomy and recipient Epstein-Barr virus seronegativity as risk factors in post-transplant lymphoproliferative disorder following allogeneic hematopoietic stem cell transplantation. *Haematologica* 2006; **91**(8)**:** 1059-1067.

39. Uhlin M, Wikell H, Sundin M, Blennow O, Maeurer M, Ringden O *et al.* Risk factors for Epstein-Barr virus-related post-transplant lymphoproliferative disease after allogeneic hematopoietic stem cell transplantation. *Haematologica* 2014; **99**(2)**:** 346-352. doi: 10.3324/haematol.2013.087338

40. Rasche L, Kapp M, Einsele H, Mielke S. EBV-induced post transplant lymphoproliferative disorders: a persisting challenge in allogeneic hematopoetic SCT. *Bone Marrow Transplant* 2014; **49**(2)**:** 163-167. doi: 10.1038/bmt.2013.96

41. Styczynski J, Gil L, Tridello G, Ljungman P, Donnelly JP, van der Velden W *et al.* Response to rituximab-based therapy and risk factor analysis in Epstein Barr Virus-related lymphoproliferative disorder after hematopoietic stem cell transplant in children and adults: a study from the Infectious Diseases Working Party of the European Group for Blood and Marrow Transplantation. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2013; **57**(6)**:** 794-802. doi: 10.1093/cid/cit391

42. Matthes-Martin S, Feuchtinger T, Shaw PJ, Engelhard D, Hirsch HH, Cordonnier C *et al.* European guidelines for diagnosis and treatment of adenovirus infection in leukemia and stem cell transplantation: summary of ECIL-4 (2011). *Transplant infectious disease : an official journal of the Transplantation Society* 2012; **14**(6)**:** 555-563. doi: 10.1111/tid.12022

43. Lion T, Baumgartinger R, Watzinger F, Matthes-Martin S, Suda M, Preuner S *et al.* Molecular monitoring of adenovirus in peripheral blood after allogeneic bone marrow transplantation permits early diagnosis of disseminated disease. *Blood* 2003; **102**(3)**:** 1114-1120. doi: 10.1182/blood-2002-07-2152

44. Hirsch HH, Martino R, Ward KN, Boeckh M, Einsele H, Ljungman P. Fourth European Conference on Infections in Leukaemia (ECIL-4): guidelines for diagnosis and treatment of human respiratory syncytial virus, parainfluenza virus, metapneumovirus, rhinovirus, and coronavirus. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2013; **56**(2)**:** 258-266. doi: 10.1093/cid/cis844

45. Marr KA, Carter RA, Crippa F, Wald A, Corey L. Epidemiology and outcome of mould infections in hematopoietic stem cell transplant recipients. *CLIN INFECT DIS* 2002; **34**(7)**:** 909-917. doi: 10.1086/339202

46. Blennow O, Remberger M, Klingspor L, Omazic B, Fransson K, Ljungman P *et al.* Randomized PCR-based therapy and risk factors for invasive fungal infection following reduced-intensity conditioning and hematopoietic SCT. *Bone Marrow Transplant* 2010; **45**(12)**:** 1710-1718. doi: 10.1038/bmt.2010.38

47. Marr KA, Carter RA, Boeckh M, Martin P, Corey L. Invasive aspergillosis in allogeneic stem cell transplant recipients: changes in epidemiology and risk factors. *Blood* 2002; **100**(13)**:** 4358-4366. doi: 10.1182/blood-2002-05-1496

48. Ullmann AJ, Lipton JH, Vesole DH, Chandrasekar P, Langston A, Tarantolo SR *et al.* Posaconazole or fluconazole for prophylaxis in severe graft-versus-host disease. *N Engl J Med* 2007; **356**(4)**:** 335-347. doi: 10.1056/NEJMoa061098

49. Herbrecht R, Denning DW, Patterson TF, Bennett JE, Greene RE, Oestmann JW *et al.* Voriconazole versus amphotericin B for primary therapy of invasive aspergillosis. *N Engl J Med* 2002; **347**(6)**:** 408-415. doi: 10.1056/NEJMoa020191

50. Boeckh M, Kim HW, Flowers ME, Meyers JD, Bowden RA. Long-term acyclovir for prevention of varicella zoster virus disease after allogeneic hematopoietic cell transplantation--a randomized double-blind placebo-controlled study. *Blood* 2006; **107**(5)**:** 1800-1805. doi: 10.1182/blood-2005-09-3624

51. Erard V, Guthrie KA, Varley C, Heugel J, Wald A, Flowers ME *et al.* One-year acyclovir prophylaxis for preventing varicella-zoster virus disease after hematopoietic cell transplantation: no evidence of rebound varicella-zoster virus disease after drug discontinuation. *Blood* 2007; **110**(8)**:** 3071-3077. doi: 10.1182/blood-2007-03-077644

52. Tomblyn M, Chiller T, Einsele H, Gress R, Sepkowitz K, Storek J *et al.* Guidelines for preventing infectious complications among hematopoietic cell transplantation recipients: a global perspective. *Biol Blood Marrow Transplant* 2009; **15**(10)**:** 1143-1238. doi: 10.1016/j.bbmt.2009.06.019

53. Ljungman P, Cordonnier C, Einsele H, Englund J, Machado CM, Storek J *et al.* Vaccination of hematopoietic cell transplant recipients. *Bone Marrow Transplant* 2009; **44**(8)**:** 521-526. doi: 10.1038/bmt.2009.263

54. Rubin LG, Levin MJ, Ljungman P, Davies EG, Avery R, Tomblyn M *et al.* 2013 IDSA clinical practice guideline for vaccination of the immunocompromised host. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2014; **58**(3)**:** 309-318. doi: 10.1093/cid/cit816

55. Pasquini MC, Wang Z, Horowitz MM, Gale RP. 2010 report from the Center for International Blood and Marrow Transplant Research (CIBMTR): current uses and outcomes of hematopoietic cell transplants for blood and bone marrow disorders. *Clinical transplants* 2010**:** 87-105.

56. Martin PJ, Schoch G, Fisher L, Byers V, Anasetti C, Appelbaum FR *et al.* A retrospective analysis of therapy for acute graft-versus-host disease: initial treatment. *Blood* 1990; **76**(8)**:** 1464-1472. doi: papers3://publication/uuid/8E7C6025-903D-470B-8DD7-A93EF8F995C9

57. Blazar BR, Murphy WJ, Abedi M. Advances in graft-versus-host disease biology and therapy. *Nat Rev Immunol* 2012; **12**(6)**:** 443-458. doi: 10.1038/nri3212

58. Broady R, Yu J, Chow V, Tantiworawit A, Kang C, Berg K *et al.* Cutaneous GVHD is associated with the expansion of tissue-localized Th1 and not Th17 cells. *Blood* 2010; **116**(25)**:** 5748-5751. doi: 10.1182/blood-2010-07-295436

59. Imanguli MM, Swaim WD, League SC, Gress RE, Pavletic SZ, Hakim FT. Increased T-bet+ cytotoxic effectors and type I interferon-mediated processes in chronic graft-versus-host disease of the oral mucosa. *Blood* 2009; **113**(15)**:** 3620-3630. doi: 10.1182/blood-2008-07-168351

60. Murphy WJ, Welniak LA, Taub DD, Wiltrout RH, Taylor PA, Vallera DA *et al.* Differential effects of the absence of interferon-gamma and IL-4 in acute graft-versus-host disease after allogeneic bone marrow transplantation in mice. *J Clin Invest* 1998; **102**(9)**:** 1742-1748. doi: 10.1172/JCI3906

61. Nikolic B, Lee S, Bronson RT, Grusby MJ, Sykes M. Th1 and Th2 mediate acute graft-versus-host disease, each with distinct end-organ targets. *J Clin Invest* 2000; **105**(9)**:** 1289-1298. doi: 10.1172/JCI7894

62. Cahn JY, Klein JP, Lee SJ, Milpied N, Blaise D, Antin JH *et al.* Prospective evaluation of 2 acute graft-versus-host (GVHD) grading systems: a joint Societe Francaise de Greffe de Moelle et Therapie Cellulaire (SFGM-TC), Dana Farber Cancer Institute (DFCI), and International Bone Marrow Transplant Registry (IBMTR) prospective study. *Blood* 2005; **106**(4)**:** 1495-1500. doi: 10.1182/blood-2004-11-4557

63. Filipovich AH, Weisdorf D, Pavletic S, Socie G, Wingard JR, Lee SJ *et al.* National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. *Biol Blood Marrow Transplant* 2005; **11**(12)**:** 945-956. doi: 10.1016/j.bbmt.2005.09.004

64. Flowers MED, Inamoto Y, Carpenter PA, Lee SJ, Kiem H-P, Petersdorf EW *et al.* Comparative analysis of risk factors for acute graft-versus-host disease and for chronic graft-versus-host disease according to National Institutes of Health consensus criteria. *Blood* 2011; **117**(11)**:** 3214-3219. doi: papers3://publication/doi/10.1182/blood-2010-08-302109

65. Billingham RE. The biology of graft-versus-host reactions. *Harvey lectures* 1966; **62:** 21-78.

66. Ferrara JL, Levine JE, Reddy P, Holler E. Graft-versus-host disease. *Lancet* 2009; **373**(9674)**:** 1550-1561. doi: 10.1016/S0140-6736(09)60237-3

67. Socie G, Ritz J. Current issues in chronic graft-versus-host disease. *Blood* 2014; **124**(3)**:** 374-384. doi: 10.1182/blood-2014-01-514752

68. van der Velden WJ, Netea MG, de Haan AF, Huls GA, Donnelly JP, Blijlevens NM. Role of the mycobiome in human acute graft-versus-host disease. *Biol Blood Marrow Transplant* 2013; **19**(2)**:** 329-332. doi: 10.1016/j.bbmt.2012.11.008

69. Hill GR, Ferrara JL. The primacy of the gastrointestinal tract as a target organ of acute graft-versus-host disease: rationale for the use of cytokine shields in allogeneic bone marrow transplantation. *Blood* 2000; **95**(9)**:** 2754-2759.

70. Wilhelm K, Ganesan J, Muller T, Durr C, Grimm M, Beilhack A *et al.* Graft-versus-host disease is enhanced by extracellular ATP activating P2X7R. *Nat Med* 2010; **16**(12)**:** 1434-1438. doi: 10.1038/nm.2242

71. Lee KH, Park SS, Kim I, Kim JH, Ra EK, Yoon SS *et al.* P2X7 receptor polymorphism and clinical outcomes in HLA-matched sibling allogeneic hematopoietic stem cell transplantation. *Haematologica* 2007; **92**(5)**:** 651-657.

72. van Bekkum DW, Knaan S. Role of bacterial microflora in development of intestinal lesions from graft-versus-host reaction. *Journal of the National Cancer Institute* 1977; **58**(3)**:** 787-790.

73. Lampert IA, Moore RH, Huby R, Cohen J. Observations on the role of endotoxin in graft-versus-host disease. *Progress in clinical and biological research* 1988; **272:** 351-359.

74. van Bekkum DW, Roodenburg J, Heidt PJ, van der Waaij D. Mitigation of secondary disease of allogeneic mouse radiation chimeras by modification of the intestinal microflora. *Journal of the National Cancer Institute* 1974; **52**(2)**:** 401-404.

75. Jenq RR, Ubeda C, Taur Y, Menezes CC, Khanin R, Dudakov JA *et al.* Regulation of intestinal inflammation by microbiota following allogeneic bone marrow transplantation. *J Exp Med* 2012; **209**(5)**:** 903-911. doi: 10.1084/jem.20112408

76. Potter V, Moore J. Randomised trials of Graft versus Host Disease prophylaxis in haemopoietic stem cell transplantation. *Reviews on recent clinical trials* 2008; **3**(2)**:** 130-138.

77. Munneke JM, Björklund AT, Mjösberg JM, Garming-Legert K, Bernink JH, Blom B *et al.* Activated innate lymphoid cells are associated with a reduced susceptibility to graft

versus host disease. *Blood* 2014; **124**(5)**:** 812-821. doi: papers3://publication/doi/10.1182/blood-2013-11-536888

78. Koyama M, Kuns RD, Olver SD, Raffelt NC, Wilson YA, Don AL *et al.* Recipient nonhematopoietic antigen-presenting cells are sufficient to induce lethal acute graft-versus-host disease. *Nat Med* 2012; **18**(1)**:** 135-142. doi: 10.1038/nm.2597

79. Wang X, Li H, Matte-Martone C, Cui W, Li N, Tan HS *et al.* Mechanisms of antigen presentation to T cells in murine graft-versus-host disease: cross-presentation and the appearance of cross-presentation. *Blood* 2011; **118**(24)**:** 6426-6437. doi: 10.1182/blood-2011-06-358747

80. Schultz KR, Paquet J, Bader S, HayGlass KT. Requirement for B cells in T cell priming to minor histocompatibility antigens and development of graft-versus-host disease. *Bone Marrow Transplant* 1995; **16**(2)**:** 289-295.

81. Zheng H, Matte-Martone C, Li H, Anderson BE, Venketesan S, Sheng Tan H *et al.* Effector memory CD4+ T cells mediate graft-versus-leukemia without inducing graft-versus-host disease. *Blood* 2008; **111**(4)**:** 2476-2484. doi: 10.1182/blood-2007-08-109678

82. Shlomchik WD, Couzens MS, Tang CB, McNiff J, Robert ME, Liu J *et al.* Prevention of graft versus host disease by inactivation of host antigen-presenting cells. *Science* 1999; **285**(5426)**:** 412-415.

83. Matte CC, Liu J, Cormier J, Anderson BE, Athanasiadis I, Jain D *et al.* Donor APCs are required for maximal GVHD but not for GVL. *Nat Med* 2004; **10**(9)**:** 987-992. doi: 10.1038/nm1089

84. Horowitz MM, Gale RP, Sondel PM, Goldman JM, Kersey J, Kolb HJ *et al.* Graft-versus-leukemia reactions after bone marrow transplantation. *Blood* 1990; **75**(3)**:** 555-562. doi: papers3://publication/uuid/9B7EEBF7-413E-423D-B906-CE0E42732589

85. Zhang P, Chen BJ, Chao NJ. Prevention of GVHD without losing GVL effect: windows of opportunity. *Immunologic research* 2011; **49**(1-3)**:** 49-55. doi: 10.1007/s12026-010-8193-7

86. Horowitz MM, Gale RP, Sondel PM, Goldman JM, Kersey J, Kolb HJ *et al.* Graft-versus-leukemia reactions after bone marrow transplantation. *Blood* 1990; **75**(3)**:** 555-562.

87. Ruggeri L, Capanni M, Urbani E, Perruccio K, Shlomchik WD, Tosti A *et al.* Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. *Science* 2002; **295**(5562)**:** 2097-2100.

88. Wang H, Yang YG. The complex and central role of interferon-gamma in graft-versus-host disease and graft-versus-tumor activity. *Immunol Rev* 2014; **258**(1)**:** 30-44. doi: 10.1111/imr.12151

89. Sinzger C, Digel M, Jahn G. Cytomegalovirus cell tropism. *Curr Top Microbiol Immunol* 2008; **325:** 63-83.

90. Cheung AK, Gottlieb DJ, Plachter B, Pepperl-Klindworth S, Avdic S, Cunningham AL *et al.* The role of the human cytomegalovirus UL111A gene in down-regulating CD4+ T-cell recognition of latently infected cells: implications for virus elimination during latency. *Blood* 2009; **114**(19)**:** 4128-4137. doi: 10.1182/blood-2008-12-197111

91. Reeves MB, Sinclair JH. Analysis of latent viral gene expression in natural and experimental latency models of human cytomegalovirus and its correlation with histone modifications at a latent promoter. *The Journal of general virology* 2010; **91**(Pt 3)**:** 599-604. doi: 10.1099/vir.0.015602-0

92. Jarvis MA, Nelson JA. Human cytomegalovirus persistence and latency in endothelial cells and macrophages. *Current opinion in microbiology* 2002; **5**(4)**:** 403-407.

93. McGeoch DJ, Dolan A, Ralph AC. Toward a comprehensive phylogeny for mammalian and avian herpesviruses. *Journal of virology* 2000; **74**(22)**:** 10401-10406. doi: papers3://publication/uuid/E733591B-C10C-49F7-A4E3-C93BB6A9D1B9

94. Mocarski ES, Jr. Immune escape and exploitation strategies of cytomegaloviruses: impact on and imitation of the major histocompatibility system. *Cell Microbiol* 2004; **6**(8)**:** 707-717. doi: 10.1111/j.1462-5822.2004.00425.x

95. Sylwester AW, Mitchell BL, Edgar JB, Taormina C, Pelte C, Ruchti F *et al.* Broadly targeted human cytomegalovirus-specific CD4+ and CD8+ T cells dominate the memory compartments of exposed subjects. *J Exp Med* 2005; **202**(5)**:** 673-685. doi: 10.1084/jem.20050882

96. Dasari V, Smith C, Khanna R. Recent advances in designing an effective vaccine to prevent cytomegalovirus-associated clinical diseases. *Expert Rev Vaccines* 2013; **12**(6)**:** 661-676. doi: 10.1586/erv.13.46

97. Essa S, Pacsa A, Raghupathy R, Said T, Nampoory MR, Johny KV *et al.* Low levels of Th1-type cytokines and increased levels of Th2-type cytokines in kidney transplant recipients with active cytomegalovirus infection. *Transplantation proceedings* 2009; **41**(5)**:** 1643-1647. doi: 10.1016/j.transproceed.2008.10.098

98. Rossini G, Cerboni C, Santoni A, Landini MP, Landolfo S, Gatti D *et al.* Interplay between human cytomegalovirus and intrinsic/innate host responses: a complex bidirectional relationship. *Mediators of inflammation* 2012; **2012:** 607276. doi: 10.1155/2012/607276

99. Varnum SM, Streblow DN, Monroe ME, Smith P, Auberry KJ, Pasa-Tolic L *et al.* Identification of proteins in human cytomegalovirus (HCMV) particles: the HCMV proteome. *Journal of virology* 2004; **78**(20)**:** 10960-10966. doi: 10.1128/JVI.78.20.10960-10966.2004

100. Kalejta RF. Tegument proteins of human cytomegalovirus. *Microbiology and molecular biology reviews : MMBR* 2008; **72**(2)**:** 249-265, table of contents. doi: 10.1128/MMBR.00040-07

101. Loewendorf A, Benedict CA. Modulation of host innate and adaptive immune defenses by cytomegalovirus: timing is everything. *Journal of internal medicine* 2010; **267**(5)**:** 483-501. doi: papers3://publication/doi/10.1111/j.1365-2796.2010.02220.x

102. Raftery MJ, Schwab M, Eibert SM, Samstag Y, Walczak H, Schonrich G. Targeting the function of mature dendritic cells by human cytomegalovirus: a multilayered viral defense strategy. *Immunity* 2001; **15**(6)**:** 997-1009.

103. Andrews DM, Andoniou CE, Granucci F, Ricciardi-Castagnoli P, Degli-Esposti MA. Infection of dendritic cells by murine cytomegalovirus induces functional paralysis. *Nature Immunology* 2001; **2**(11)**:** 1077-1084. doi: 10.1038/ni724

104. Hargett D, Shenk TE. Experimental human cytomegalovirus latency in CD14+ monocytes. *Proc Natl Acad Sci U S A* 2010; **107**(46)**:** 20039-20044. doi: 10.1073/pnas.1014509107

105. Tomasec P, Braud VM, Rickards C, Powell MB, McSharry BP, Gadola S *et al.* Surface expression of HLA-E, an inhibitor of natural killer cells, enhanced by human cytomegalovirus gpUL40. *Science (New York, N.Y* 2000; **287**(5455)**:** 1031.

106. Ulbrecht M, Martinozzi S, Grzeschik M, Hengel H, Ellwart JW, Pla M *et al.* Cutting edge: the human cytomegalovirus UL40 gene product contains a ligand for HLA-E and prevents NK cell-mediated lysis. *J Immunol* 2000; **164**(10)**:** 5019-5022.

107. Stern-Ginossar N, Elefant N, Zimmermann A, Wolf DG, Saleh N, Biton M *et al.* Host immune system gene targeting by a viral miRNA. *Science (New York, N.Y* 2007; **317**(5836)**:** 376-381. doi: 10.1126/science.1140956

108. Lodoen MB, Lanier LL. Viral modulation of NK cell immunity. *Nat Rev Microbiol* 2005; **3**(1)**:** 59-69.

109. Moretta L, Ferlazzo G, Bottino C, Vitale M, Pende D, Mingari MC *et al.* Effector and regulatory events during natural killer-dendritic cell interactions. *Immunol Rev* 2006; **214:** 219-228. doi: 10.1111/j.1600-065X.2006.00450.x

110. Fernandez NC, Lozier A, Flament C, Ricciardi-Castagnoli P, Bellet D, Suter M *et al.* Dendritic cells directly trigger NK cell functions: cross-talk relevant in innate anti-tumor immune responses in vivo. *Nat Med* 1999; **5**(4)**:** 405-411. doi: 10.1038/7403

111. Gerosa F, Baldani-Guerra B, Nisii C, Marchesini V, Carra G, Trinchieri G. Reciprocal activating interaction between natural killer cells and dendritic cells. *J Exp Med* 2002; **195**(3)**:** 327-333.

112. Ferlazzo G, Tsang ML, Moretta L, Melioli G, Steinman RM, Munz C. Human dendritic cells activate resting natural killer (NK) cells and are recognized via the NKp30 receptor by activated NK cells. *J Exp Med* 2002; **195**(3)**:** 343-351.

113. Magri G, Muntasell A, Romo N, Saez-Borderias A, Pende D, Geraghty DE *et al.* NKp46 and DNAM-1 NK-cell receptors drive the response to human cytomegalovirus-infected myeloid dendritic cells overcoming viral immune evasion strategies. *Blood* 2011; **117**(3)**:** 848-856. doi: 10.1182/blood-2010-08-301374

114. Ljungman P, Brand R. Factors influencing cytomegalovirus seropositivity in stem cell transplant patients and donors. *Haematologica* 2007; **92**(8)**:** 1139-1142. doi: papers3://publication/uuid/CE4B42AA-F8B5-4C86-B399-F62B39322219

115. Hendrie CA, Brewer G. Kissing as an evolutionary adaptation to protect against Human Cytomegalovirus-like teratogenesis. *Medical hypotheses* 2010; **74**(2)**:** 222-224. doi: 10.1016/j.mehy.2009.09.033

116. Landolfo S, Gariglio M, Gribaudo G, Lembo D. The human cytomegalovirus. *Pharmacology & therapeutics* 2003; **98**(3)**:** 269-297.

117. Olsson J, Wikby A, Johansson B, Löfgren S, Nilsson BO, Ferguson FG. Age-related change in peripheral blood T-lymphocyte subpopulations and cytomegalovirus infection in the very old: the Swedish longitudinal OCTO immune study. *Mech. Ageing Dev.* 2000; **121**(1-3)**:** 187-201. doi: papers3://publication/uuid/1651C5FF-EE9F-4B9E-BE2E-C0AEDCDBCD87

118. Pawelec G, Derhovanessian E. Role of CMV in immune senescence. *Virus research* 2011; **157**(2)**:** 175-179. doi: papers3://publication/doi/10.1016/j.virusres.2010.09.010

119. Solana R, Campos C, Pera A, Tarazona R. Shaping of NK cell subsets by aging. *Current opinion in immunology* 2014; **29C:** 56-61. doi: papers3://publication/doi/10.1016/j.coi.2014.04.002

120. Bengner M, Beziat V, Ernerudh J, Nilsson BO, Lofgren S, Wikby A *et al.* Independent skewing of the T cell and NK cell compartments associated with cytomegalovirus infection suggests division of labor between innate and adaptive immunity. *Age (Dordr)* 2014; **36**(2)**:** 571-582. e-pub ahead of print 2013/09/26; doi: 10.1007/s11357-013-9587-y

121. Ljungman P, Aschan J, Lewensohn-Fuchs I, Carlens S, Larsson K, Lonnqvist B *et al.* Results of different strategies for reducing cytomegalovirus-associated mortality in allogeneic stem cell transplant recipients. *Transplantation* 1998; **66**(10)**:** 1330-1334.

122. Meyers J, Flournoy N, Thomas E. Nonbacterial pneumonia after allogeneic marrow transplantation: review of ten years' experience. *Rev Infect Dis* 1982; **4:** 1119-1131.

123. Zaia JA, Forman SJ. Cytomegalovirus infection in the bone marrow transplant recipient. *Infectious Disease Clinics of North America* 1995; **9**(4)**:** 879-900.

124. Boeckh M, Nichols WG, Papanicolaou G, Rubin R, Wingard JR, Zaia J. Cytomegalovirus in hematopoietic stem cell transplant recipients: Current status, known challenges, and future strategies. *Biol Blood Marrow Transplant* 2003; **9**(9)**:** 543-558.

125. Boeckh M, Ljungman P. How we treat cytomegalovirus in hematopoietic cell transplant recipients. *Blood* 2009; **113**(23)**:** 5711-5719. doi: 10.1182/blood-2008-10-143560

126. Ozdemir E, St John LS, Gillespie G, Rowland-Jones S, Champlin RE, Molldrem JJ *et al.* Cytomegalovirus reactivation following allogeneic stem cell transplantation is associated with the presence of dysfunctional antigen-specific CD8+ T cells. *Blood* 2002; **100**(10)**:** 3690-3697. doi: 10.1182/blood-2002-05-1387

127. Perez-Bercoff L, Vudattu NK, Byrareddy SN, Mattsson J, Maeurer M, Ljungman P. Reduced IL-7 responsiveness defined by signal transducer and activator of transcription 5 phosphorylation in T cells may be a marker for increased risk of developing cytomegalovirus disease in patients after hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2014; **20**(1)**:** 128-132. doi: 10.1016/j.bbmt.2013.10.006

128. Bacigalupo A, Tedone E, Sanna MA, Moro F, Van LM, Grazi G *et al.* CMV infections following allogeneic BMT: risk factors, early treatment and correlation with transplant related mortality. *Haematologica* 1992; **77**(6)**:** 507-513.

129. Meyers JD, Flournoy N, Thomas ED. Risk factors for cytomegalovirus infection after human marrow transplantation. *Journal of Infectious Diseases* 1986; **153**(3)**:** 478-488.

130. Takenaka K, Gondo H, Tanimoto K, Nagafuji K, Fujisaki T, Mizuno S *et al.* Increased incidence of cytomegalovirus (CMV) infection and CMV- associated disease after allogeneic bone marrow transplantation from unrelated donors. The Fukuoka Bone Marrow Transplantation Group. *Bone Marrow Transplant* 1997; **19**(3)**:** 241-248.;

131. Craddock C, Szydlo RM, Dazzi F, Olavarria E, Cwynarski K, Yong A *et al.* Cytomegalovirus seropositivity adversely influences outcome after T- depleted unrelated donor transplant in patients with chronic myeloid leukaemia: the case for tailored graft-versus-host disease prophylaxis. *British journal of haematology* 2001; **112**(1)**:** 228-236.;

132. Broers AE, van Der Holt R, van Esser JW, Gratama JW, Henzen-Logmans S, Kuenen-Boumeester V *et al.* Increased transplant-related morbidity and mortality in CMV-seropositive patients despite highly effective prevention of CMV disease after allogeneic T-cell-depleted stem cell transplantation. *Blood* 2000; **95**(7)**:** 2240-2245.

133. Ljungman P, Hakki M, Boeckh M. Cytomegalovirus in hematopoietic stem cell transplant recipients. *Hematol Oncol Clin North Am* 2011; **25**(1)**:** 151-169. e-pub ahead of print 2011/01/18; doi: S0889-8588(10)00166-8 [pii]

10.1016/j.hoc.2010.11.011

134. Schmidt-Hieber M, Labopin M, Beelen D, Volin L, Ehninger G, Finke J *et al.* CMV serostatus has still an important prognostic impact in de novo acute leukemia patients after allogeneic stem cell transplantation: a report from the acute leukemia working party of EBMT. *Blood* 2013. e-pub ahead of print 2013/09/17; doi: 10.1182/blood-2013-05-499830

135. Nichols WG, Corey L, Gooley T, Davis C, Boeckh M. High risk of death due to bacterial and fungal infection among cytomegalovirus (CMV)-seronegative recipients of stem cell

transplants from seropositive donors: evidence for indirect effects of primary CMV infection. *J Infect Dis* 2002; **185**(3)**:** 273-282.

136. Ljungman P, Brand R, Hoek J, de la Camara R, Cordonnier C, Einsele H *et al.* Donor cytomegalovirus status influences the outcome of allogeneic stem cell transplant: a study by the European group for blood and marrow transplantation. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2014; **59**(4)**:** 473-481. doi: papers3://publication/doi/10.1093/cid/ciu364

137. Lonnqvist B, Ringden O, Wahren B, Gahrton G, Lundgren G. Cytomegalovirus infection associated with and preceding chronic graft-versus-host disease. *Transplantation* 1984; **38**(5)**:** 465-468.

138. Elmaagacli AH, Steckel NK, Koldehoff M, Hegerfeldt Y, Trenschel R, Ditschkowski M *et al.* Early human cytomegalovirus replication after transplantation is associated with a decreased relapse risk: evidence for a putative virus-versus-leukemia effect in acute myeloid leukemia patients. *Blood* 2011; **118**(5)**:** 1402-1412. doi: 10.1182/blood-2010-08-304121

139. Ito S, Pophali P, Co W, Koklanaris EK, Superata J, Fahle GA *et al.* CMV reactivation is associated with a lower incidence of relapse after allo-SCT for CML. *Bone Marrow Transplant* 2013; **48**(10)**:** 1313-1316. doi: 10.1038/bmt.2013.49

140. Green ML, Leisenring WM, Xie H, Walter RB, Mielcarek M, Sandmaier BM *et al.* CMV reactivation after allogeneic HCT and relapse risk: evidence for early protection in acute myeloid leukemia. *Blood* 2013; **122**(7)**:** 1316-1324. doi: 10.1182/blood-2013-02-487074

141. Ljungman P, Perez-Bercoff L, Jonsson J, Avetisyan G, Sparrelid E, Aschan J *et al.* Risk factors for the development of cytomegalovirus disease after allogeneic stem cell transplantation. *Haematologica* 2006; **91**(1)**:** 78-83.

142. Baldanti F, Lilleri D, Gerna G. Monitoring human cytomegalovirus infection in transplant recipients. *Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology* 2008; **41**(3)**:** 237-241. doi: 10.1016/j.jcv.2007.12.001

143. Lilleri D, Gerna G, Furione M, Bernardo ME, Giorgiani G, Telli S *et al.* Use of a DNAemia cut-off for monitoring human cytomegalovirus infection reduces the number of pre-emptively treated children and young adults receiving haematopoietic stem cell transplantation as compared to qualitative pp65-antigenemia. *Blood* 2007.

144. Milano F, Pergam SA, Xie H, Leisenring WM, Gutman JA, Riffkin I *et al.* Intensive strategy to prevent CMV disease in seropositive umbilical cord blood transplant recipients. *Blood* 2011; **118**(20)**:** 5689-5696. e-pub ahead of print 2011/09/23; doi: 10.1182/blood-2011-06-361618

145. Reusser P, Einsele H, Lee J, Volin L, Rovira M, Engelhard D *et al.* Randomized multicenter trial of foscarnet versus ganciclovir for preemptive therapy of cytomegalovirus infection after allogeneic stem cell transplantation. *Blood* 2002; **99**(4)**:** 1159-1164.

146. Goodrich JM, Mori M, Gleaves CA, Du Mond C, Cays M, Ebeling DF *et al.* Early treatment with ganciclovir to prevent cytomegalovirus disease after allogeneic bone marrow transplantation. *N Engl J Med* 1991; **325**(23)**:** 1601-1607.

147. Volin L, Barkholt L, Nihtinen A, J.Aschan J, Uotinen H, Hägglund H *et al.* An open-label randomised study of oral valganciclovir versus intravenous ganciclovir for pre-emptive therapy of cytomegalovirus infection after allogeneic stem cell transplantation. *Bone Marrow Transplantation* 2008; **42**(Suppl.1)**:** S47.

148. Walter EA, Greenberg PD, Gilbert MJ, Finch RJ, Watanabe KS, Thomas ED *et al.* Reconstitution of cellular immunity against cytomegalovirus in recipients of allogeneic bone marrow by transfer of T-cell clones from the donor. *N Engl J Med* 1995; **333**(16)**:** 1038-1044. doi: 10.1056/NEJM199510193331603

149. Einsele H, Roosnek E, Rufer N, Sinzger C, Riegler S, Loffler J *et al.* Infusion of cytomegalovirus (CMV)-specific T cells for the treatment of CMV infection not responding to antiviral chemotherapy. *Blood* 2002; **99**(11)**:** 3916-3922.

150. Leen AM, Bollard CM, Mendizabal AM, Shpall EJ, Szabolcs P, Antin JH *et al.* Multicenter study of banked third-party virus-specific T cells to treat severe viral infections after hematopoietic stem cell transplantation. *Blood* 2013; **121**(26)**:** 5113-5123. doi: 10.1182/blood-2013-02-486324

151. Uhlin M, Gertow J, Uzunel M, Okas M, Berglund S, Watz E *et al.* Rapid salvage treatment with virus-specific T cells for therapy-resistant disease. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2012; **55**(8)**:** 1064-1073. doi: 10.1093/cid/cis625

152. Battiwalla M, Wu Y, Bajwa RP, Radovic M, Almyroudis NG, Segal BH *et al.* Ganciclovir inhibits lymphocyte proliferation by impairing DNA synthesis. *Biol Blood Marrow Transplant* 2007; **13**(7)**:** 765-770. doi: 10.1016/j.bbmt.2007.03.009

153. Ljungman P, Larsson K, Kumlien G, Aschan J, Barkholt L, Gustafsson-Jernberg A *et al.* Leukocyte depleted, unscreened blood products give a low risk for CMV infection and disease in CMV seronegative allogeneic stem cell transplant recipients with seronegative stem cell donors. *Scand J Infect Dis* 2002; **34**(5)**:** 347-350.

154. Marschall M, Stamminger T, Urban A, Wildum S, Ruebsamen-Schaeff H, Zimmermann H *et al.* In vitro evaluation of the activities of the novel anticytomegalovirus compound AIC246 (letermovir) against herpesviruses and other human pathogenic viruses. *Antimicrobial agents and chemotherapy* 2012; **56**(2)**:** 1135-1137. doi: 10.1128/AAC.05908-11

155. Marty FM, Winston DJ, Rowley SD, Vance E, Papanicolaou GA, Mullane KM *et al.* CMX001 to prevent cytomegalovirus disease in hematopoietic-cell transplantation. *N Engl J Med* 2013; **369**(13)**:** 1227-1236. doi: 10.1056/NEJMoa1303688

156. Chemaly RF, Ullmann AJ, Stoelben S, Richard MP, Bornhauser M, Groth C *et al.* Letermovir for cytomegalovirus prophylaxis in hematopoietic-cell transplantation. *N Engl J Med* 2014; **370**(19)**:** 1781-1789. doi: 10.1056/NEJMoa1309533

157. Hazenberg MD, Spits H. Human innate lymphoid cells. *Blood* 2014; **124**(5)**:** 700-709. doi: 10.1182/blood-2013-11-427781

158. Luetke-Eversloh M, Killig M, Romagnani C. Signatures of human NK cell development and terminal differentiation. *Frontiers in immunology* 2013; **4:** 499. doi: 10.3389/fimmu.2013.00499

159. Shi FD, Ljunggren HG, La Cava A, Van Kaer L. Organ-specific features of natural killer cells. *Nat Rev Immunol* 2011; **11**(10)**:** 658-671. doi: 10.1038/nri3065

160. Cella M, Miller H, Song C. Beyond NK cells: the expanding universe of innate lymphoid cells. *Frontiers in immunology* 2014; **5:** 282. doi: 10.3389/fimmu.2014.00282

161. Kiessling R, Klein E, Pross H, Wigzell H. "Natural" killer cells in the mouse. II. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Characteristics of the killer cell. *European journal of immunology* 1975; **5**(2)**:** 117-121. doi: papers3://publication/doi/10.1002/eji.1830050209

162. Kiessling R, Klein E, Wigzell H. "Natural" killer cells in the mouse. I. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Specificity and distribution according to genotype. *European journal of immunology* 1975; **5**(2)**:** 112-117. doi: papers3://publication/doi/10.1002/eji.1830050208

163. Herberman RB, Nunn ME, Holden HT, Lavrin DH. Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic and allogeneic tumors. II. Characterization of effector cells. *Int J Cancer* 1975; **16**(2)**:** 230-239.

164. Herberman RB, Nunn ME, Lavrin DH. Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic acid allogeneic tumors. I. Distribution of reactivity and specificity. *Int J Cancer* 1975; **16**(2)**:** 216-229.

165. Biron C, Nguyen K, Pien G, Cousens L, Salazar-Mather T. NATURAL KILLER CELLS IN ANTIVIRAL DEFENSE: Function and Regulation by Innate Cytokines. *Annu. Rev. Immunol.* 1999; **17**(1)**:** 189-220. doi: doi:10.1146/annurev.immunol.17.1.189

166. Wu J, Lanier L. Natural killer cells and cancer. *Advances in cancer research* 2003; **90:** 127-156.

167. Lodoen MB, Lanier LL. Natural killer cells as an initial defense against pathogens. *Curr Opin Immunol* 2006; **18**(4)**:** 391-398. doi: 10.1016/j.coi.2006.05.002

168. Vesely MD, Kershaw MH, Schreiber RD, Smyth MJ. Natural innate and adaptive immunity to cancer. *Annu Rev Immunol* 2011; **29:** 235-271. doi: 10.1146/annurev-immunol-031210-101324

169. Braud VM, Allan DS, O'Callaghan CA, Soderstrom K, D'Andrea A, Ogg GS *et al.* HLA-E binds to natural killer cell receptors CD94/NKG2A, B and C. *Nature* 1998; **391**(6669)**:** 795-799.

170. Lanier LL. NK cell recognition. *Annu Rev Immunol* 2005; **23:** 225-274.

171. Diefenbach A, Raulet DH. The innate immune response to tumors and its role in the induction of T-cell immunity. *Immunol Rev* 2002; **188:** 9-21.

172. Karre K, Ljunggren HG, Piontek G, Kiessling R. Selective rejection of H-2-deficient lymphoma variants suggests alternative immune defence strategy. *Nature* 1986; **319**(6055)**:** 675-678.

173. Ljunggren HG, Karre K. In search of the 'missing self': MHC molecules and NK cell recognition. *Immunol Today* 1990; **11**(7)**:** 237-244.

174. Huntington ND, Legrand N, Alves NL, Jaron B, Weijer K, Plet A *et al.* IL-15 trans-presentation promotes human NK cell development and differentiation in vivo. *J Exp Med* 2009; **206**(1)**:** 25-34. e-pub ahead of print 2008/12/24; doi: jem.20082013 [pii]

10.1084/jem.20082013

175. Freud AG, Yokohama A, Becknell B, Lee MT, Mao HC, Ferketich AK *et al.* Evidence for discrete stages of human natural killer cell differentiation in vivo. *J Exp Med* 2006; **203**(4)**:** 1033-1043. doi: 10.1084/jem.20052507

176. Freud AG, Becknell B, Roychowdhury S, Mao HC, Ferketich AK, Nuovo GJ *et al.* A human CD34(+) subset resides in lymph nodes and differentiates into CD56bright natural killer cells. *Immunity* 2005; **22**(3)**:** 295-304. doi: 10.1016/j.immuni.2005.01.013

177. Freud AG, Caligiuri MA. Human natural killer cell development. *Immunol Rev* 2006; **214:** 56-72.

178. Buckley RH. Molecular defects in human severe combined immunodeficiency and approaches to immune reconstitution. *Annu Rev Immunol* 2004; **22:** 625-655. doi: 10.1146/annurev.immunol.22.012703.104614

179. Gilmour KC, Fujii H, Cranston T, Davies EG, Kinnon C, Gaspar HB. Defective expression of the interleukin-2/interleukin-15 receptor beta subunit leads to a natural killer cell-deficient form of severe combined immunodeficiency. *Blood* 2001; **98**(3)**:** 877-879.

180. Cooper MA, Fehniger TA, Caligiuri MA. The biology of human natural killer-cell subsets. *Trends in immunology* 2001; **22**(11)**:** 633-640.

181. Cooper MA, Fehniger TA, Turner SC, Chen KS, Ghaheri BA, Ghayur T *et al.* Human natural killer cells: a unique innate immunoregulatory role for the CD56(bright) subset. *Blood* 2001; **97**(10)**:** 3146-3151.

182. Fauriat C, Long EO, Ljunggren HG, Bryceson YT. Regulation of human NK-cell cytokine and chemokine production by target cell recognition. *Blood* 2010; **115**(11)**:** 2167-2176. doi: 10.1182/blood-2009-08-238469

183. Freud AG, Yu J, Caligiuri MA. Human natural killer cell development in secondary lymphoid tissues. *Semin Immunol* 2014; **26**(2)**:** 132-137. doi: 10.1016/j.smim.2014.02.008

184. Juelke K, Killig M, Luetke-Eversloh M, Parente E, Gruen J, Morandi B *et al.* CD62L expression identifies a unique subset of polyfunctional CD56dim NK cells. *Blood* 2010; **116**(8)**:** 1299-1307. e-pub ahead of print 2010/05/28; doi: blood-2009-11-253286 [pii]

10.1182/blood-2009-11-253286

185. Bjorkstrom NK, Riese P, Heuts F, Andersson S, Fauriat C, Ivarsson MA *et al.* Expression patterns of NKG2A, KIR, and CD57 define a process of CD56dim NK-cell differentiation uncoupled from NK-cell education. *Blood* 2010; **116**(19)**:** 3853-3864. doi: 10.1182/blood-2010-04-281675

186. Lopez-Verges S, Milush JM, Pandey S, York VA, Arakawa-Hoyt J, Pircher H *et al.* CD57 defines a functionally distinct population of mature NK cells in the human CD56dimCD16+ NK-cell subset. *Blood* 2010; **116**(19)**:** 3865-3874. doi: 10.1182/blood-2010-04-282301

187. Valiante NM, Uhrberg M, Shilling HG, Lienert-Weidenbach K, Arnett KL, D'Andrea A *et al.* Functionally and structurally distinct NK cell receptor repertoires in the peripheral blood of two human donors. *Immunity* 1997; **7**(6)**:** 739-751.

188. Ciccone E, Pende D, Vitale M, Nanni L, Di Donato C, Bottino C *et al.* Self class I molecules protect normal cells from lysis mediated by autologous natural killer cells. *Eur J Immunol* 1994; **24**(4)**:** 1003-1006. doi: 10.1002/eji.1830240434

189. Anfossi N, Andre P, Guia S, Falk C, Roetynck S, Stewart C *et al.* Human NK Cell Education by Inhibitory Receptors for MHC Class I. *Immunity* 2006; **25**(2)**:** 331-342.

190. Cooley S, Xiao F, Pitt M, Gleason M, McCullar V, Bergemann TL *et al.* A subpopulation of human peripheral blood NK cells that lacks inhibitory receptors for self-MHC is developmentally immature. *Blood* 2007; **110**(2)**:** 578-586.

191. Kim S, Poursine-Laurent J, Truscott S, Lybarger L, Song Y, Yang L *et al.* Licensing of natural killer cells by host major histocompatibility complex class I molecules. *Nature* 2005; **436**(7051)**:** 709-713. doi: 10.1038/nature03847

192. Anfossi N, Andre P, Guia S, Falk CS, Roetynck S, Stewart CA *et al.* Human NK cell education by inhibitory receptors for MHC class I. *Immunity* 2006; **25**(2)**:** 331-342.

193. Fauriat C, Ivarsson MA, Ljunggren HG, Malmberg KJ, Michaelsson J. Education of human natural killer cells by activating killer cell immunoglobulin-like receptors. *Blood*; **115**(6)**:** 1166-1174. e-pub ahead of print 2009/11/12; doi: blood-2009-09-245746 [pii]

10.1182/blood-2009-09-245746

194. Brodin P, Karre K, Hoglund P. NK cell education: not an on-off switch but a tunable rheostat. *Trends Immunol* 2009; **30**(4)**:** 143-149. e-pub ahead of print 2009/03/14; doi: S1471-4906(09)00039-8 [pii]

10.1016/j.it.2009.01.006

195. Andersson S, Malmberg JA, Malmberg KJ. Tolerant and diverse natural killer cell repertoires in the absence of selection. *Exp Cell Res* 2010; **316**(8)**:** 1309-1315. e-pub ahead of print 2010/03/09; doi: S0014-4827(10)00092-3 [pii]

10.1016/j.yexcr.2010.02.030

196. Kim S, Poursine-Laurent J, Truscott SM, Lybarger L, Song YJ, Yang L *et al.* Licensing of natural killer cells by host major histocompatibility complex class I molecules. *Nature* 2005; **436**(7051)**:** 709-713. doi: 10.1038/nature03847

197. Peterson ME, Long EO. Inhibitory receptor signaling via tyrosine phosphorylation of the adaptor Crk. *Immunity* 2008; **29**(4)**:** 578-588. doi: 10.1016/j.immuni.2008.07.014

198. Yokoyama WM, Kim S. Licensing of natural killer cells by self-major histocompatibility complex class I. *Immunol Rev* 2006; **214:** 143-154. doi: 10.1111/j.1600-065X.2006.00458.x

199. Doucey MA, Scarpellino L, Zimmer J, Guillaume P, Luescher IF, Bron C *et al.* Cis association of Ly49A with MHC class I restricts natural killer cell inhibition. *Nat Immunol* 2004; **5**(3)**:** 328-336. doi: 10.1038/ni1043

200. Andersson KE, Williams GS, Davis DM, Hoglund P. Quantifying the reduction in accessibility of the inhibitory NK cell receptor Ly49A caused by binding MHC class I proteins in cis. *Eur J Immunol* 2007; **37**(2)**:** 516-527. doi: 10.1002/eji.200636693

201. Raulet DH, Vance RE. Self-tolerance of natural killer cells. *Nat Rev Immunol* 2006; **6**(7)**:** 520-531. doi: 10.1038/nri1863

202. Brodin P, Lakshmikanth T, Johansson S, Karre K, Hoglund P. The strength of inhibitory input during education quantitatively tunes the functional responsiveness of individual natural killer cells. *Blood* 2008. e-pub ahead of print 2008/11/01; doi: blood-2008-05-156836 [pii]

10.1182/blood-2008-05-156836

203. Hoglund P, Brodin P. Current perspectives of natural killer cell education by MHC class I molecules. *Nat Rev Immunol* 2010. e-pub ahead of print 2010/09/08; doi: nri2835 [pii]

10.1038/nri2835

204. Johansson S, Johansson M, Rosmaraki E, Vahlne G, Mehr R, Salmon-Divon M *et al.* Natural killer cell education in mice with single or multiple major histocompatibility complex class I molecules. *J Exp Med* 2005; **201**(7)**:** 1145-1155. doi: 10.1084/jem.20050167

205. Joncker NT, Fernandez NC, Treiner E, Vivier E, Raulet DH. NK cell responsiveness is tuned commensurate with the number of inhibitory receptors for self-MHC class I: the rheostat model. *J Immunol* 2009; **182**(8)**:** 4572-4580. doi: 10.4049/jimmunol.0803900

206. Pradeu T, Jaeger S, Vivier E. The speed of change: towards a discontinuity theory of immunity? *Nat Rev Immunol* 2013; **13**(10)**:** 764-769. doi: 10.1038/nri3521

207. Orr MT, Murphy WJ, Lanier LL. 'Unlicensed' natural killer cells dominate the response to cytomegalovirus infection. *Nature immunology* 2010; **11**(4)**:** 321-327. doi: papers3://publication/doi/10.1038/ni.1849

208. Foley B, Cooley S, Verneris MR, Curtsinger J, Luo X, Waller EK *et al.* NK cell education after allogeneic transplantation: dissociation between recovery of cytokine-producing and cytotoxic functions. *Blood* 2011; **118**(10)**:** 2784-2792. doi: papers3://publication/doi/10.1182/blood-2011-04-347070

209. Fernandez NC, Treiner E, Vance RE, Jamieson AM, Lemieux S, Raulet DH. A subset of natural killer cells achieves self-tolerance without expressing inhibitory receptors specific for self-MHC molecules. *Blood* 2005; **105**(11)**:** 4416-4423. doi: 10.1182/blood-2004-08-3156

210. Tarek N, Le Luduec JB, Gallagher MM, Zheng J, Venstrom JM, Chamberlain E *et al.* Unlicensed NK cells target neuroblastoma following anti-GD2 antibody treatment. *J Clin Invest* 2012; **122**(9)**:** 3260-3270. doi: 10.1172/JCI62749

211. Sun JC, Beilke JN, Lanier LL. Adaptive immune features of natural killer cells. *Nature* 2009; **457**(7229)**:** 557-561. doi: 10.1038/nature07665

212. Cooper MA, Elliott JM, Keyel PA, Yang L, Carrero JA, Yokoyama WM. Cytokine-induced memory-like natural killer cells. *Proc Natl Acad Sci U S A* 2009; **106**(6)**:** 1915-1919. doi: 10.1073/pnas.0813192106

213. O'Leary JG, Goodarzi M, Drayton DL, von Andrian UH. T cell- and B cell-independent adaptive immunity mediated by natural killer cells. *Nat Immunol* 2006; **7**(5)**:** 507-516. doi: 10.1038/ni1332

214. Braud V, Allan D, O'Callaghan C, Soderstrom K, D'Andrea A, Ogg G *et al.* HLA-E binds to natural killer cell receptors CD94/NKG2A, B and C. *Nature* 1998; **391**(6669)**:** 795-799.

215. Guma M, Budt M, Saez A, Brckalo T, Hengel H, Angulo A *et al.* Expansion of CD94/NKG2C+ NK cells in response to human cytomegalovirus-infected fibroblasts. *Blood* 2006; **107**(9)**:** 3624-3631. doi: 10.1182/blood-2005-09-3682

216. Beziat V, Dalgard O, Asselah T, Halfon P, Bedossa P, Boudifa A *et al.* CMV drives clonal expansion of NKG2C+ NK cells expressing self-specific KIRs in chronic hepatitis patients. *Eur J Immunol* 2012; **42**(2)**:** 447-457. e-pub ahead of print 2011/11/23; doi: 10.1002/eji.201141826

217. Bjorkstrom NK, Lindgren T, Stoltz M, Fauriat C, Braun M, Evander M *et al.* Rapid expansion and long-term persistence of elevated NK cell numbers in humans infected with hantavirus. *J Exp Med* 2011; **208**(1)**:** 13-21. e-pub ahead of print 2010/12/22; doi: jem.20100762 [pii]

10.1084/jem.20100762

218. Kaiser BK, Pizarro JC, Kerns J, Strong RK. Structural basis for NKG2A/CD94 recognition of HLA-E. *Proc Natl Acad Sci USA* 2008; **105**(18)**:** 6696-6701. doi: 10.1073/pnas.0802736105

219. Cook M, Briggs D, Craddock C, Mahendra P, Moss P. Donor KIR genotype has a major influence on the rate of cytomegalovirus reactivation following T-cell replete stem cell transplantation *Blood* 2006; **107**(3)**:** 1230-1232.

220. Chen C, Busson M, Rocha V, Appert M-L, Lepage V, Dulphy N *et al.* Activating KIR genes are associated with CMV reactivation and survival after non-T-cell depleted HLA-identical sibling bone marrow transplantation for malignant disorders. *Bone Marrow Transplant* 2006; **38**(6)**:** 437-444. doi: 10.1038/sj.bmt.1705468

221. Zaia JA, Sun JY, Gallez-Hawkins GM, Thao L, Oki A, Lacey SF *et al.* The effect of single and combined activating killer immunoglobulin-like receptor genotypes on cytomegalovirus infection and immunity after hematopoietic cell transplantation. *Biol Blood Marrow Transplant* 2009; **15**(3)**:** 315-325. doi: 10.1016/j.bbmt.2008.11.030

222. Stern M, Elsässer H, Hönger G, Steiger J, Schaub S, Hess C. The number of activating KIR genes inversely correlates with the rate of CMV infection/reactivation in kidney transplant recipients. *Am J Transplant* 2008; **8**(6)**:** 1312-1317. doi: 10.1111/j.1600-6143.2008.02242.x

223. Spörri R, Joller N, Albers U, Hilbi H, Oxenius A. MyD88-dependent IFN-gamma production by NK cells is key for control of Legionella pneumophila infection. *J Immunol* 2006; **176**(10)**:** 6162-6171.

224. Feng CG, Kaviratne M, Rothfuchs AG, Cheever A, Hieny S, Young HA *et al.* NK cell-derived IFN-gamma differentially regulates innate resistance and neutrophil response in T cell-deficient hosts infected with Mycobacterium tuberculosis. *J Immunol* 2006; **177**(10)**:** 7086-7093.

225. Brown CR, Reiner SL. Activation of natural killer cells in arthritis-susceptible but not arthritis-resistant mouse strains following Borrelia burgdorferi infection. *Infect Immun* 1998; **66**(11)**:** 5208-5214.

226. Sher A, Oswald IP, Hieny S, Gazzinelli RT. Toxoplasma gondii induces a T-independent IFN-gamma response in natural killer cells that requires both adherent accessory cells and tumor necrosis factor-alpha. *J Immunol* 1993; **150**(9)**:** 3982-3989.

227. Hansen DS, D'Ombrain MC, Schofield L. The role of leukocytes bearing Natural Killer Complex receptors and Killer Immunoglobulin-like Receptors in the immunology of malaria. *Current Opinion in Immunology* 2007; **19**(4)**:** 416-423. doi: 10.1016/j.coi.2007.07.011

228. Trowsdale J. Genetic and functional relationships between MHC and NK receptor genes. *Immunity* 2001; **15**(3)**:** 363-374.

229. Trowsdale J, Barten R, Haude A, Stewart CA, Beck S, Wilson MJ. The genomic context of natural killer receptor extended gene families. *Immunol Rev* 2001; **181:** 20-38.

230. Gourraud P-A, Meenagh A, Cambon-Thomsen A, Middleton D. Linkage disequilibrium organization of the human KIR superlocus: implications for KIR data analyses. *Immunogenetics* 2010; **62**(11-12)**:** 729-740. doi: papers3://publication/doi/10.1007/s00251-010-0478-4

231. Vilches C, Parham P. KIR: diverse, rapidly evolving receptors of innate and adaptive immunity. *Annu Rev Immunol* 2002; **20:** 217-251.

232. Marsh SG, Parham P, Dupont B, Geraghty DE, Trowsdale J, Middleton D *et al.* Killer-cell immunoglobulin-like receptor (KIR) nomenclature report, 2002. *Tissue Antigens* 2003; **62**(1)**:** 79-86.

233. Martin AM, Kulski JK, Gaudieri S, Witt CS, Freitas EM, Trowsdale J *et al.* Comparative genomic analysis, diversity and evolution of two KIR haplotypes A and B. *Gene* 2004; **335:** 121-131. doi: 10.1016/j.gene.2004.03.018

234. Pyo CW, Guethlein LA, Vu Q, Wang R, Abi-Rached L, Norman PJ *et al.* Different patterns of evolution in the centromeric and telomeric regions of group A and B haplotypes of the human killer cell Ig-like receptor locus. *PLoS One* 2010; **5**(12)**:** e15115. doi: 10.1371/journal.pone.0015115

235. Leung W, Iyengar R, Turner V, Lang P, Bader P, Conn P *et al.* Determinants of antileukemia effects of allogeneic NK cells. *J Immunol* 2004; **172**(1)**:** 644-650.

236. Symons HJ, Leffell MS, Rossiter ND, Zahurak M, Jones RJ, Fuchs EJ. Improved survival with inhibitory killer immunoglobulin receptor (KIR) gene mismatches and KIR haplotype B donors after nonmyeloablative, HLA-haploidentical bone marrow transplantation. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation* 2010; **16**(4)**:** 533-542. doi: papers3://publication/doi/10.1016/j.bbmt.2009.11.022

237. Schaffer M, Malmberg KJ, Ringden O, Ljunggren HG, Remberger M. Increased infection-related mortality in KIR-ligand-mismatched unrelated allogeneic hematopoietic stem-cell transplantation. *Transplantation* 2004; **78**(7)**:** 1081-1085.

238. Giebel S, Locatelli F, Lamparelli T, Velardi A, Davies S, Frumento G *et al.* Survival advantage with KIR ligand incompatibility in hematopoietic stem cell transplantation from unrelated donors. *Blood* 2003; **102**(3)**:** 814-819.

239. Beelen DW, Ottinger HD, Ferencik S, Elmaagacli AH, Peceny R, Trenschel R *et al.* Genotypic inhibitory killer immunoglobulin-like receptor ligand incompatibility enhances the long-term antileukemic effect of unmodified allogeneic hematopoietic stem cell transplantation in patients with myeloid leukemias. *Blood* 2005; **105**(6)**:** 2594-2600.

240. Miller JS, Cooley S, Parham P, Farag SS, Verneris MR, McQueen KL *et al.* Missing KIR ligands are associated with less relapse and increased graft-versus-host disease (GVHD) following unrelated donor allogeneic HCT. *Blood* 2007; **109**(11)**:** 5058-5061.

241. Hsu KC, Keever-Taylor CA, Wilton A, Pinto C, Heller G, Arkun K *et al.* Improved outcome in HLA-identical sibling hematopoietic stem-cell transplantation for acute myelogenous leukemia predicted by KIR and HLA genotypes. *Blood* 2005; **105**(12)**:** 4878-4884. doi: 10.1182/blood-2004-12-4825

242. Zhao XY, Chang YJ, Xu LP, Zhang XH, Liu KY, Li D *et al.* HLA and KIR genotyping correlates with relapse after T-cell-replete haploidentical transplantation in chronic myeloid leukaemia patients. *British Journal of Cancer* 2014. doi: papers3://publication/doi/10.1038/bjc.2014.423

243. Venstrom JM, Gooley TA, Spellman S, Pring J, Malkki M, Dupont B *et al.* Donor activating KIR3DS1 is associated with decreased acute GVHD in unrelated allogeneic hematopoietic stem cell transplantation. *Blood* 2010; **115**(15)**:** 3162-3165. doi: 10.1182/blood-2009-08-236943

244. Cooley S, Weisdorf DJ, Guethlein LA, Klein JP, Wang T, Le CT *et al.* Donor selection for natural killer cell receptor genes leads to superior survival after unrelated transplantation for acute myelogenous leukemia. *Blood* 2010; **116**(14)**:** 2411-2419. doi: papers3://publication/doi/10.1182/blood-2010-05-283051

245. Venstrom JM, Pittari G, Gooley TA, Chewning JH, Spellman S, Haagenson M *et al.* HLA-C-dependent prevention of leukemia relapse by donor activating KIR2DS1. *N Engl J Med* 2012; **367**(9)**:** 805-816. e-pub ahead of print 2012/08/31; doi: 10.1056/NEJMoa1200503

246. Hsu KC, Gooley T, Malkki M, Pinto-Agnello C, Dupont B, Bignon J-D *et al.* KIR ligands and prediction of relapse after unrelated donor hematopoietic cell transplantation for hematologic malignancy. *Biology of Blood and Marrow Transplantation* 2006; **12**(8)**:** 828-836. doi: papers3://publication/doi/10.1016/j.bbmt.2006.04.008

247. Clausen J, Wolf D, Petzer AL, Gunsilius E, Schumacher P, Kircher B *et al.* Impact of natural killer cell dose and donor killer-cell immunoglobulin-like receptor (KIR) genotype on outcome following human leucocyte antigen-identical haematopoietic stem cell transplantation. *Clinical & Experimental Immunology* 2007; **148**(3)**:** 520-528. doi: papers3://publication/doi/10.1111/j.1365-2249.2007.03360.x

248. Cooley S, McCullar V, Wangen R, Bergemann TL, Spellman S, Weisdorf DJ *et al.* KIR reconstitution is altered by T cells in the graft and correlates with clinical outcomes after unrelated donor transplantation. *Blood* 2005; **106**(13)**:** 4370-4376.

249. Ghiringhelli F, Ménard C, Terme M, Flament C, Taieb J, Chaput N *et al.* CD4+CD25+ regulatory T cells inhibit natural killer cell functions in a transforming growth factor-beta-

dependent manner. *Journal of Experimental Medicine* 2005; **202**(8)**:** 1075-1085. doi: papers3://publication/doi/10.1084/jem.20051511

250. Gasteiger G, Hemmers S, Firth MA, Le Floc'h A, Huse M, Sun JC *et al.* IL-2-dependent tuning of NK cell sensitivity for target cells is controlled by regulatory T cells. *The Journal of experimental medicine* 2013; **210**(6)**:** 1167-1178. doi: papers3://publication/doi/10.1084/jem.20122462

251. Lee S-H, Fragoso MF, Biron CA. Cutting edge: a novel mechanism bridging innate and adaptive immunity: IL-12 induction of CD25 to form high-affinity IL-2 receptors on NK cells. *J. Immunol.* 2012; **189**(6)**:** 2712-2716. doi: papers3://publication/doi/10.4049/jimmunol.1201528

252. Bachanova V, Cooley S, Defor TE, Verneris MR, Zhang B, McKenna DH *et al.* Clearance of acute myeloid leukemia by haploidentical natural killer cells is improved using IL-2 diphtheria toxin fusion protein. *Blood* 2014; **123**(25)**:** 3855-3863. doi: papers3://publication/doi/10.1182/blood-2013-10-532531

253. Bachar-Lustig E, Rachamim N, Li HW, Lan F, Reisner Y. Megadose of T cell-depleted bone marrow overcomes MHC barriers in sublethally irradiated mice. *Nature medicine* 1995; **1**(12)**:** 1268-1273. doi: papers3://publication/uuid/29A71680-4652-4D58-93B8-E83A1CFACE4E

254. Bennett M. Biology and genetics of hybrid resistance. *Adv Immunol* 1987; **41:** 333-445. e-pub ahead of print 1987/01/01;

255. Davies SM, Ruggieri L, DeFor T, Wagner JE, Weisdorf DJ, Miller JS *et al.* Evaluation of KIR ligand incompatibility in mismatched unrelated donor hematopoietic transplants. Killer immunoglobulin-like receptor. *Blood* 2002; **100**(10)**:** 3825-3827. doi: papers3://publication/doi/10.1182/blood-2002-04-1197

256. Kroger N, Binder T, Zabelina T, Wolschke C, Schieder H, Renges H *et al.* Low number of donor activating killer immunoglobulin-like receptors (KIR) genes but not KIR-ligand mismatch prevents relapse and improves disease-free survival in leukemia patients after in vivo T-cell depleted unrelated stem cell transplantation. *Transplantation* 2006; **82**(8)**:** 1024-1030. doi: papers3://publication/doi/10.1097/01.tp.0000235859.24513.43

257. McQueen KL, Dorighi KM, Guethlein LA, Wong R, Sanjanwala B, Parham P. Donor-recipient combinations of group A and B KIR haplotypes and HLA class I ligand affect the outcome of HLA-matched, sibling donor hematopoietic cell transplantation. *Hum Immunol* 2007; **68**(5)**:** 309-323.

258. Cooley S, Trachtenberg E, Bergemann TL, Saeteurn K, Klein J, Le CT *et al.* Donors with group B KIR haplotypes improve relapse-free survival after unrelated hematopoietic cell transplantation for acute myelogenous leukemia. *Blood* 2009; **113**(3)**:** 726-732. e-pub ahead of print 2008/10/24; doi: blood-2008-07-171926 [pii]

10.1182/blood-2008-07-171926

259. Cooley S, Weisdorf DJ, Guethlein LA, Klein JP, Wang T, Marsh SGE *et al.* Donor killer cell Ig-like receptor B haplotypes, recipient HLA-C1, and HLA-C mismatch enhance the clinical benefit of unrelated transplantation for acute myelogenous leukemia. *J. Immunol.* 2014; **192**(10)**:** 4592-4600. doi: papers3://publication/doi/10.4049/jimmunol.1302517

260. Moesta AK, Graef T, Abi-Rached L, Older Aguilar AM, Guethlein LA, Parham P. Humans differ from other hominids in lacking an activating NK cell receptor that recognizes the C1 epitope of MHC class I. *J Immunol* 2010; **185**(7)**:** 4233-4237. doi: 10.4049/jimmunol.1001951

261. Giebel S, Nowak I, Dziaczkowska J, Czerw T, Wojnar J, Krawczyk-Kulis M *et al.* Activating killer immunoglobulin-like receptor incompatibilities enhance graft-versus-host disease and affect survival after allogeneic hematopoietic stem cell transplantation. *European journal of haematology* 2009; **83**(4)**:** 343-356. doi: papers3://publication/doi/10.1111/j.1600-0609.2009.01280.x

262. Fauriat C, Ivarsson MA, Ljunggren HG, Malmberg KJ, Michaelsson J. Education of human natural killer cells by activating killer cell immunoglobulin-like receptors. *Blood* 2010; **115**(6)**:** 1166-1174. doi: 10.1182/blood-2009-09-245746

263. Ivarsson MA, Michaëlsson J, Fauriat C. Activating killer cell Ig-like receptors in health and disease. *Frontiers in immunology* 2014; **5:** 184. doi: papers3://publication/doi/10.3389/fimmu.2014.00184

264. Guia S, Jaeger BN, Piatek S, Mailfert S, Trombik T, Fenis A *et al.* Confinement of activating receptors at the plasma membrane controls natural killer cell tolerance. *Science signaling* 2011; **4**(167)**:** ra21. doi: papers3://publication/doi/10.1126/scisignal.2001608

265. Gagne K, Busson M, Bignon J-D, BalEre-Appert M-L, Loiseau P, Dormoy A *et al.* Donor KIR3DL1/3DS1 gene and recipient Bw4 KIR ligand as prognostic markers for outcome in unrelated hematopoietic stem cell transplantation. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation* 2009; **15**(11)**:** 1366-1375. doi: papers3://publication/doi/10.1016/j.bbmt.2009.06.015

266. Gagne K, Busson M, Balere-Appert ML, Absi L, Jollet I, Bignon JD *et al.* Relevance of KIR gene matching in unrelated hematopoietic stem cell transplantations. *Tissue Antigens*

2007; **69 Suppl 1:** 118-122. doi: papers3://publication/doi/10.1111/j.1399-0039.2006.76210.x

267. Pelak K, Need AC, Fellay J, Shianna KV, Feng S, Urban TJ *et al.* Copy number variation of KIR genes influences HIV-1 control. *PLoS biology* 2011; **9**(11)**:** e1001208. doi: papers3://publication/doi/10.1371/journal.pbio.1001208

268. Gratwohl A, Brand R, Frassoni F, Rocha V, Niederwieser D, Reusser P *et al.* Cause of death after allogeneic haematopoietic stem cell transplantation (HSCT) in early leukaemias: an EBMT analysis of lethal infectious complications and changes over calendar time. *Bone marrow transplantation* 2005; **36**(9)**:** 757-769. doi: papers3://publication/doi/10.1038/sj.bmt.1705140

269. Ochs L, Shu XO, Miller J, Enright H, Wagner J, Filipovich A *et al.* Late infections after allogeneic bone marrow transplantations: comparison of incidence in related and unrelated donor transplant recipients. *Blood* 1995; **86**(10)**:** 3979-3986. doi: papers3://publication/uuid/12FD5E6E-2D8E-44F9-8EE7-962078165E73

270. Socié G, Stone JV, Wingard JR, Weisdorf D, Henslee-Downey PJ, Bredeson C *et al.* Long-term survival and late deaths after allogeneic bone marrow transplantation. Late Effects Working Committee of the International Bone Marrow Transplant Registry. *New England Journal of Medicine* 1999; **341**(1)**:** 14-21. doi: papers3://publication/doi/10.1056/NEJM199907013410103

271. Ljungman P, Cordonnier C, Einsele H, Englund J, Machado CM, Storek J *et al.* Vaccination of hematopoietic cell transplant recipients. *Bone marrow transplantation* 2009; **44**(8)**:** 521-526. doi: papers3://publication/doi/10.1038/bmt.2009.263

272. Maury S, Mary JY, Rabian C, Schwarzinger M, Toubert A, Scieux C *et al.* Prolonged immune deficiency following allogeneic stem cell transplantation: risk factors and complications in adult patients. *Br J Haematol* 2001; **115**(3)**:** 630-641.

273. Abrahamsen IW, Somme S, Heldal D, Egeland T, Kvale D, Tjonnfjord GE. Immune reconstitution after allogeneic stem cell transplantation: the impact of stem cell source and graft-versus-host disease. *Haematologica* 2005; **90**(1)**:** 86-93.

274. Kalhs P, Panzer S, Kletter K, Minar E, Stain-Kos M, Walter R *et al.* Functional asplenia after bone marrow transplantation. A late complication related to extensive chronic graft-versus-host disease. *Ann Intern Med* 1988; **109**(6)**:** 461-464.

275. Kulkarni S, Powles R, Treleaven J, Riley U, Singhal S, Horton C *et al.* Chronic graft versus host disease is associated with long-term risk for pneumococcal infections in recipients of bone marrow transplants. *Blood* 2000; **95**(12)**:** 3683-3686.

276. Knecht H, Jost R, Gmur J, Burger J, Fehr J. Functional hyposplenia after allogeneic bone marrow transplantation is detected by epinephrine stimulation test and splenic ultrasonography. *European journal of haematology* 1988; **41**(4)**:** 382-387.

277. Zimmerli W, Zarth A, Gratwohl A, Speck B. Neutrophil function and pyogenic infections in bone marrow transplant recipients. *Blood* 1991; **77**(2)**:** 393-399. doi: papers3://publication/uuid/EA080575-F8F7-43A3-93E9-7CF39FEBC085

278. Lin Y, Hu X, Cheng H, Pang Y, Wang L, Zou L *et al.* Graft-Versus-Host Disease Causes Broad Suppression of Hematopoietic Primitive Cells and Blocks Megakaryocyte Differentiation in a Murine Model. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation* 2014. doi: papers3://publication/doi/10.1016/j.bbmt.2014.05.009

279. Sionov RV, Cohen O, Kfir S, Zilberman Y, Yefenof E. Role of mitochondrial glucocorticoid receptor in glucocorticoid-induced apoptosis. *Journal of Experimental Medicine* 2006; **203**(1)**:** 189-201. doi: papers3://publication/doi/10.1084/jem.20050433

280. Zen M, Canova M, Campana C, Bettio S, Nalotto L, Rampudda M *et al.* The kaleidoscope of glucorticoid effects on immune system. *Autoimmun Rev* 2011; **10**(6)**:** 305-310. doi: papers3://publication/doi/10.1016/j.autrev.2010.11.009

281. Claman HN. Corticosteroids and lymphoid cells. *N Engl J Med* 1972; **287**(8)**:** 388-397.

282. Miller W, Flynn P, McCullough J, Balfour HH, Jr., Goldman A, Haake R *et al.* Cytomegalovirus infection after bone marrow transplantation: an association with acute graft-v-host disease. *Blood* 1986; **67**(4)**:** 1162-1167.

283. Cornelissen JJ, Carston M, Kollman C, King R, Dekker AW, Löwenberg B *et al.* Unrelated marrow transplantation for adult patients with poor-risk acute lymphoblastic leukemia: strong graft-versus-leukemia effect and risk factors determining outcome. *Blood* 2001; **97**(6)**:** 1572-1577. doi: papers3://publication/uuid/D86A8BDC-A51F-4506-BFA4-26500E3A5935

284. Broers AE, van Der Holt R, van Esser JW, Gratama JW, Henzen-Logmans S, Kuenen-Boumeester V *et al.* Increased transplant-related morbidity and mortality in CMV-seropositive patients despite highly effective prevention of CMV disease after allogeneic

T-cell-depleted stem cell transplantation. *Blood* 2000; **95**(7)**:** 2240-2245. doi: papers3://publication/uuid/DC2C6A98-B43D-48F8-9543-92AD48C564CB

285. Ringden O, Paulin T, Lonnqvist B, Nilsson B. An analysis of factors predisposing to chronic graft-versus-host disease. *Experimental hematology* 1985; **13**(10)**:** 1062-1067.

286. Carlens S, Ringden O, Remberger M, Lonnqvist B, Hagglund H, Klaesson S *et al.* Risk factors for chronic graft-versus-host disease after bone marrow transplantation: a retrospective single centre analysis. *Bone Marrow Transplant* 1998; **22**(8)**:** 755-761.

287. Larsson K, Aschan J, Remberger M, Ringden O, Winiarski J, Ljungman P. Reduced risk for extensive chronic graft-versus-host disease in patients receiving transplants with human leukocyte antigen-identical sibling donors given polymerase chain reaction-based preemptive therapy against cytomegalovirus. *Transplantation* 2004; **77**(4)**:** 526-531. doi: papers3://publication/doi/10.1097/01.TP.0000109778.39235.F4

288. Soderberg C, Larsson S, Rozell BL, Sumitran-Karuppan S, Ljungman P, Moller E. Cytomegalovirus-induced CD13-specific autoimmunity--a possible cause of chronic graft-vs-host disease. *Transplantation* 1996; **61**(4)**:** 600-609.

289. Boeckh M, Leisenring W, Riddell SR, Bowden RA, Huang ML, Myerson D *et al.* Late cytomegalovirus disease and mortality in recipients of allogeneic hematopoietic stem cell transplants: importance of viral load and T-cell immunity. *Blood* 2003; **101**(2)**:** 407-414.

290. Lehner PJ, Wilkinson GW. Cytomegalovirus: from evasion to suppression? *Nat Immunol* 2001; **2**(11)**:** 993-994.

291. Boeckh M, Nichols WG. Immunosuppressive effects of beta-herpesviruses. *Herpes : the journal of the IHMF* 2003; **10**(1)**:** 12-16.

292. Laursen AL, Mogensen SC, Andersen HM, Andersen PL, Ellermann-Eriksen S. The impact of CMV on the respiratory burst of macrophages in response to Pneumocystis carinii. *Clinical and experimental immunology* 2001; **123**(2)**:** 239-246.

293. Ho M. Immunology of cytomegalovirus: immunosuppressive effects during infections. *Birth defects original article series* 1984; **20**(1)**:** 131-147.

294. Giebel S, Maccario R, Lilleri D, Zecca M, Avanzini MA, Marconi M *et al.* The immunosuppressive effect of human cytomegalovirus infection in recipients of allogeneic

hematopoietic stem cell transplantation. *Bone marrow transplantation* 2005; **36**(6)**:** 503-509. doi: papers3://publication/doi/10.1038/sj.bmt.1705094

295. Jackson SE, Mason GM, Wills MR. Human cytomegalovirus immunity and immune evasion. *Virus research* 2011; **157**(2)**:** 151-160. doi: papers3://publication/doi/10.1016/j.virusres.2010.10.031

296. Avetisyan G, Aschan J, Hägglund H, Ringden O, Ljungman P. Evaluation of intervention strategy based on CMV-specific immune responses after allogeneic SCT. *Bone marrow transplantation* 2007; **40**(9)**:** 865-869. doi: papers3://publication/doi/10.1038/sj.bmt.1705825

297. Kutza AS, Muhl E, Hackstein H, Kirchner H, Bein G. High incidence of active cytomegalovirus infection among septic patients. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 1998; **26**(5)**:** 1076-1082.

298. Mikulska M, Raiola AM, Bruno B, Furfaro E, Van Lint MT, Bregante S *et al.* Risk factors for invasive aspergillosis and related mortality in recipients of allogeneic SCT from alternative donors: an analysis of 306 patients. *Bone marrow transplantation* 2009; **44**(6)**:** 361-370. doi: papers3://publication/doi/10.1038/bmt.2009.39

299. Krause PR, Bialek SR, Boppana SB, Griffiths PD, Laughlin CA, Ljungman P *et al.* Priorities for CMV vaccine development. *Vaccine* 2013; **32**(1)**:** 4-10. doi: papers3://publication/doi/10.1016/j.vaccine.2013.09.042

300. Dasari V, Smith C, Khanna R. Recent advances in designing an effective vaccine to prevent cytomegalovirus-associated clinical diseases. *Expert Rev Vaccines* 2013; **12**(6)**:** 661-676. doi: papers3://publication/doi/10.1586/erv.13.46

301. Rieder F, Steininger C. Cytomegalovirus vaccine: phase II clinical trial results. *Clin. Microbiol. Infect.* 2014; **20 Suppl 5**(s5)**:** 95-102. doi: papers3://publication/doi/10.1111/1469-0691.12449

302. Guma M, Angulo A, Vilches C, Gomez-Lozano N, Malats N, Lopez-Botet M. Imprint of human cytomegalovirus infection on the NK cell receptor repertoire. *Blood* 2004; **104**(12)**:** 3664-3671. doi: 10.1182/blood-2004-05-2058

303. Hanley P, Bollard C. Controlling Cytomegalovirus: Helping the Immune System Take the Lead. *Viruses* 2014; **6**(6)**:** 2242-2258. doi: papers3://publication/doi/10.3390/v6062242

304. Ruggeri L, Capanni M, Casucci M, Volpi I, Tosti A, Perruccio K *et al.* Role of natural killer cell alloreactivity in HLA-mismatched hematopoietic stem cell transplantation. *Blood* 1999; **94**(1)**:** 333-339. doi: papers3://publication/uuid/FDAA482F-93D2-4600-8D42-7B635050A47A

305. Haas P, Loiseau P, Tamouza R, Cayuela J-M, Moins-Teisserenc H, Busson M *et al.* NK-cell education is shaped by donor HLA genotype after unrelated allogeneic hematopoietic stem cell transplantation. *Blood* 2011; **117**(3)**:** 1021-1029. doi: papers3://publication/doi/10.1182/blood-2010-02-269381

306. Höglund P, Brodin P. Current perspectives of natural killer cell education by MHC class I molecules. *Nature Publishing Group* 2010; **10**(10)**:** 724-734. doi: papers3://publication/doi/10.1038/nri2835

307. Zeis M, Uharek L, Glass B, Gaska T, Steinmann J, Gassmann W *et al.* Allogeneic NK cells as potent antileukemic effector cells after allogeneic bone marrow transplantation in mice. *Transplantation* 1995; **59**(12)**:** 1734-1736. doi: papers3://publication/uuid/71B88745-3746-462E-8D54-EF999310F737

308. JP OK, Kociban DL, Machen LL, Buffo MJ. Evidence for a possible role of Asialo-GM1-positive cells in the graft-versus-leukemia repression of a murine type-C retroviral leukemia. *Bone Marrow Transplant* 1995; **16**(3)**:** 451-456. e-pub ahead of print 1995/09/01;

309. Koh CY, Ortaldo JR, Blazar BR, Bennett M, Murphy WJ. NK-cell purging of leukemia: superior antitumor effects of NK cells H2 allogeneic to the tumor and augmentation with inhibitory receptor blockade. *Blood* 2003; **102**(12)**:** 4067-4075.

310. Curran SA, Romano E, Kennedy MG, Hsu KC, Young JW. Phenotypic and Functional Activation of Hyporesponsive, KIRnegNKG2Aneg Human NK Cell Precursors Requires IL-12p70 Provided by Poly(I:C) Matured Monocyte-derived Dendritic Cells. *Cancer immunology research* 2014. doi: papers3://publication/doi/10.1158/2326-6066.CIR-14-0054-T

311. Chalifour A, Scarpellino L, Back J, Brodin P, Devevre E, Gros F *et al.* A Role for cis Interaction between the Inhibitory Ly49A receptor and MHC class I for natural killer cell education. *Immunity* 2009; **30**(3)**:** 337-347. e-pub ahead of print 2009/03/03; doi: S1074-7613(09)00098-3 [pii]

10.1016/j.immuni.2008.12.019

312. Scarpellino L, Oeschger F, Guillaume P, Coudert JD, Levy F, Leclercq G *et al.* Interactions of Ly49 family receptors with MHC class I ligands in trans and cis. *J Immunol* 2007; **178**(3)**:** 1277-1284.

313. Johansson MH, Bieberich C, Jay G, Karre K, Hoglund P. Natural killer cell tolerance in mice with mosaic expression of major histocompatibility complex class I transgene. *Journal of Experimental Medicine* 1997; **186**(3)**:** 353-364. doi: papers3://publication/uuid/8851BB0C-B405-428D-90E3-CBFE5F864701

314. Joncker NT, Raulet DH. Regulation of NK cell responsiveness to achieve self-tolerance and maximal responses to diseased target cells. *Immunol Rev* 2008; **224:** 85-97. e-pub ahead of print 2008/09/02; doi: IMR658 [pii]

10.1111/j.1600-065X.2008.00658.x

315. Elliott JM, Wahle JA, Yokoyama WM. MHC class I-deficient natural killer cells acquire a licensed phenotype after transfer into an MHC class I-sufficient environment. *J Exp Med* 2010; **207**(10)**:** 2073-2079. e-pub ahead of print 2010/09/08; doi: jem.20100986 [pii]

10.1084/jem.20100986

316. Savani BN, Mielke S, Adams S, Uribe M, Rezvani K, Yong ASM *et al.* Rapid natural killer cell recovery determines outcome after T-cell-depleted HLA-identical stem cell transplantation in patients with myeloid leukemias but not with acute lymphoblastic leukemia. *Leukemia* 2007; **21**(10)**:** 2145-2152. doi: 10.1038/sj.leu.2404892

317. Triplett BM, Horwitz EM, Iyengar R, Turner V, Holladay MS, Gan K *et al.* Effects of activating NK cell receptor expression and NK cell reconstitution on the outcomes of unrelated donor hematopoietic cell transplantation for hematologic malignancies. *Leukemia* 2009; **23**(7)**:** 1278-1287. doi: 10.1038/leu.2009.21

318. Shilling HG, McQueen KL, Cheng NW, Shizuru JA, Negrin RS, Parham P. Reconstitution of NK cell receptor repertoire following HLA-matched hematopoietic cell transplantation. *Blood* 2003; **101**(9)**:** 3730-3740. doi: 10.1182/blood-2002-08-2568

319. Shilling H. Reconstitution of NK cell receptor repertoire following HLA-matched hematopoietic cell transplantation. *Blood* 2003; **101**(9)**:** 3730-3740. doi: 10.1182/blood-2002-08-2568

320. Yawata M, Yawata N, Draghi M, Partheniou F, Little AM, Parham P. MHC class I-specific inhibitory receptors and their ligands structure diverse human NK cell repertoires towards a balance of missing-self response. *Blood* 2008.

321. Andersson S, Fauriat C, Malmberg JA, Ljunggren HG, Malmberg KJ. KIR acquisition probabilities are independent of self-HLA class I ligands and increase with cellular KIR

expression. *Blood* 2009. e-pub ahead of print 2009/03/24; doi: blood-2008-10-184549 [pii]

10.1182/blood-2008-10-184549

322. Dulphy N, Haas P, Busson M, Belhadj S, Peffault de Latour R, Robin M *et al.* An unusual CD56(bright) CD16(low) NK cell subset dominates the early posttransplant period following HLA-matched hematopoietic stem cell transplantation. *J Immunol* 2008; **181**(3)**:** 2227-2237. e-pub ahead of print 2008/07/22; doi: 181/3/2227 [pii]

323. Nguyen S, Dhedin N, Vernant JP, Kuentz M, Al Jijakli A, Rouas-Freiss N *et al.* NK-cell reconstitution after haploidentical hematopoietic stem-cell transplantations: immaturity of NK cells and inhibitory effect of NKG2A override GvL effect. *Blood* 2005; **105**(10)**:** 4135-4142. e-pub ahead of print 2005/02/03; doi: 2004-10-4113 [pii]

10.1182/blood-2004-10-4113

324. Vago L, Forno B, Sormani MP, Crocchiolo R, Zino E, Di Terlizzi S *et al.* Temporal, quantitative, and functional characteristics of single-KIR-positive alloreactive natural killer cell recovery account for impaired graft-versus-leukemia activity after haploidentical hematopoietic stem cell transplantation. *Blood* 2008; **112**(8)**:** 3488-3499. e-pub ahead of print 2008/07/23; doi: blood-2007-07-103325 [pii]

10.1182/blood-2007-07-103325

325. Yu J, Venstrom JM, Liu XR, Pring J, Hasan RS, O'Reilly RJ *et al.* Breaking tolerance to self, circulating natural killer cells expressing inhibitory KIR for non-self HLA exhibit effector function after T cell-depleted allogeneic hematopoietic cell transplantation. *Blood* 2009; **113**(16)**:** 3875-3884. e-pub ahead of print 2009/01/31; doi: blood-2008-09-177055 [pii]

10.1182/blood-2008-09-177055

326. Ruggeri L, Aversa F, Martelli MF, Velardi A. Allogeneic hematopoietic transplantation and natural killer cell recognition of missing self. *Immunological reviews* 2006; **214**(1)**:** 202-218. doi: papers3://publication/doi/10.1111/j.1600-065X.2006.00455.x

327. Salagianni M, Lekka E, Moustaki A, Iliopoulou EG, Baxevanis CN, Papamichail M *et al.* NK cell adoptive transfer combined with Ontak-mediated regulatory T cell elimination induces effective adaptive antitumor immune responses. *J. Immunol.* 2011; **186**(6)**:** 3327-3335. doi: papers3://publication/doi/10.4049/jimmunol.1000652

328. Ludajic K, Balavarca Y, Bickeböller H, Rosenmayr A, Fae I, Fischer GF *et al.* KIR genes and KIR ligands affect occurrence of acute GVHD after unrelated, 12/12 HLA

matched, hematopoietic stem cell transplantation. *Bone marrow transplantation* 2009; **44**(2)**:** 97-103. doi: papers3://publication/doi/10.1038/bmt.2008.432

329. Cook MA, Milligan DW, Fegan CD, Darbyshire PJ, Mahendra P, Craddock CF *et al.* The impact of donor KIR and patient HLA-C genotypes on outcome following HLA-identical sibling hematopoietic stem cell transplantation for myeloid leukemia. *Blood* 2004; **103**(4)**:** 1521-1526. doi: papers3://publication/doi/10.1182/blood-2003-02-0438

330. Yu J, Heller G, Chewning J, Kim S, Yokoyama WM, Hsu KC. Hierarchy of the human natural killer cell response is determined by class and quantity of inhibitory receptors for self-HLA-B and HLA-C ligands. *J Immunol* 2007; **179**(9)**:** 5977-5989.

331. Hansasuta P, Dong T, Thananchai H, Weekes M, Willberg C, Aldemir H *et al.* Recognition of HLA-A3 and HLA-A11 by KIR3DL2 is peptide-specific. *Eur J Immunol* 2004; **34**(6)**:** 1673-1679.

332. Shi J, Tricot G, Szmania S, Rosen N, Garg TK, Malaviarachchi PA *et al.* Infusion of haplo-identical killer immunoglobulin-like receptor ligand mismatched NK cells for relapsed myeloma in the setting of autologous stem cell transplantation. *Br J Haematol* 2008; **143**(5)**:** 641-653. doi: 10.1111/j.1365-2141.2008.07340.x

333. De Santis D, Foley B, Witt CS, Christiansen FT. The detection of NK cell alloreactivity by flow cytometric CD107a assay. *Methods Mol Biol* 2012; **882:** 477-489. doi: 10.1007/978-1-61779-842-9_27

334. Shilling HG, Young N, Guethlein LA, Cheng NW, Gardiner CM, Tyan D *et al.* Genetic control of human NK cell repertoire. *J Immunol* 2002; **169**(1)**:** 239-247.

335. Cheent KS, Jamil KM, Cassidy S, Liu M, Mbiribindi B, Mulder A *et al.* Synergistic inhibition of natural killer cells by the nonsignaling molecule CD94. *Proceedings of the National Academy of Sciences of the United States of America* 2013; **110**(42)**:** 16981-16986. doi: papers3://publication/doi/10.1073/pnas.1304366110

336. Lampen MH, Hassan C, Sluijter M, Geluk A, Dijkman K, Tjon JM *et al.* Alternative peptide repertoire of HLA-E reveals a binding motif that is strikingly similar to HLA-A2. *Molecular Immunology* 2013; **53**(1-2)**:** 126-131. doi: papers3://publication/doi/10.1016/j.molimm.2012.07.009

337. Li H, Pascal V, Martin MP, Carrington M, Anderson SK. Genetic control of variegated KIR gene expression: polymorphisms of the bi-directional KIR3DL1 promoter are

associated with distinct frequencies of gene expression. *PLoS Genet* 2008; **4**(11)**:** e1000254. e-pub ahead of print 2008/11/15; doi: 10.1371/journal.pgen.1000254

338. Beziat V, Liu LL, Malmberg JA, Ivarsson MA, Sohlberg E, Bjorklund AT *et al.* NK cell responses to cytomegalovirus infection lead to stable imprints in the human KIR repertoire and involve activating KIRs. *Blood* 2013; **121**(14)**:** 2678-2688. doi: 10.1182/blood-2012-10-459545

339. Beziat V, Hervier B, Achour A, Boutolleau D, Marfain-Koka A, Vieillard V. Human NKG2A overrides NKG2C effector functions to prevent autoreactivity of NK cells. *Blood* 2011; **117**(16)**:** 4394-4396. e-pub ahead of print 2011/04/23; doi: 10.1182/blood-2010-11-319194

340. Lopez-Verges S, Milush JM, Schwartz BS, Pando MJ, Jarjoura J, York VA *et al.* Expansion of a unique CD57+NKG2Chi natural killer cell subset during acute human cytomegalovirus infection. *Proc Natl Acad Sci U S A* 2011. e-pub ahead of print 2011/08/10; doi: 1110900108 [pii]

10.1073/pnas.1110900108

341. Foley B, Cooley S, Verneris MR, Pitt M, Curtsinger J, Luo X *et al.* Cytomegalovirus reactivation after allogeneic transplantation promotes a lasting increase in educated NKG2C+ natural killer cells with potent function. *Blood* 2012; **119**(11)**:** 2665-2674. e-pub ahead of print 2011/12/20; doi: 10.1182/blood-2011-10-386995

342. Knorr DA, Bachanova V, Verneris MR, Miller JS. Clinical utility of natural killer cells in cancer therapy and transplantation. *Semin Immunol* 2014; **26**(2)**:** 161-172. doi: 10.1016/j.smim.2014.02.002

343. Manjappa S, Bhamidipati PK, Stokerl-Goldstein KE, DiPersio JF, Uy GL, Westervelt P *et al.* Protective effect of cytomegalovirus reactivation on relapse after allogeneic hematopoietic cell transplantation in acute myeloid leukemia patients is influenced by conditioning regimen. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation* 2014; **20**(1)**:** 46-52. doi: papers3://publication/doi/10.1016/j.bbmt.2013.10.003

344. Du J, Lopez Verges S, Pitcher BN, Johnson J, Jung S-H, Zhou L *et al.* CALGB 150905 (Alliance): Rituximab Broadens the Antilymphoma Response by Activating Unlicensed NK Cells. *Cancer immunology research* 2014. doi: papers3://publication/doi/10.1158/2326-6066.CIR-13-0158

345. Tarek N, Le Luduec J-B, Gallagher MM, Zheng J, Venstrom JM, Chamberlain E *et al.* Unlicensed NK cells target neuroblastoma following anti-GD2 antibody treatment. *J. Clin. Invest.* 2012; **122**(9)**:** 3260-3270. doi: papers3://publication/doi/10.1172/JCI62749

346. Akbar AN, Henson SM. Are senescence and exhaustion intertwined or unrelated processes that compromise immunity? *Nature Publishing Group* 2011; **11**(4)**:** 289-295. doi: papers3://publication/doi/10.1038/nri2959

347. Gill S, Vasey AE, De Souza A, Baker J, Smith AT, Kohrt HE *et al.* Rapid development of exhaustion and down-regulation of eomesodermin limit the antitumor activity of adoptively transferred murine natural killer cells. *Blood* 2012; **119**(24)**:** 5758-5768. doi: papers3://publication/doi/10.1182/blood-2012-03-415364

348. Stojanovic A, Fiegler N, Brunner-Weinzierl M, Cerwenka A. CTLA-4 is expressed by activated mouse NK cells and inhibits NK Cell IFN-γ production in response to mature dendritic cells. *J. Immunol.* 2014; **192**(9)**:** 4184-4191. doi: papers3://publication/doi/10.4049/jimmunol.1302091

349. Benson DM, Bakan CE, Mishra A, Hofmeister CC, Efebera Y, Becknell B *et al.* The PD-1/PD-L1 axis modulates the natural killer cell versus multiple myeloma effect: a therapeutic target for CT-011, a novel monoclonal anti-PD-1 antibody. *Blood* 2010; **116**(13)**:** 2286-2294. doi: papers3://publication/doi/10.1182/blood-2010-02-271874

350. Foley B, Cooley S, Verneris MR, Curtsinger J, Luo X, Waller EK *et al.* Human cytomegalovirus (CMV)-induced memory-like NKG2C(+) NK cells are transplantable and expand in vivo in response to recipient CMV antigen. *J Immunol* 2012; **189**(10)**:** 5082-5088. doi: 10.4049/jimmunol.1201964

351. Armand P, Gibson CJ, Cutler C, Ho VT, Koreth J, Alyea EP *et al.* A disease risk index for patients undergoing allogeneic stem cell transplantation. *Blood* 2012; **120**(4)**:** 905-913. doi: papers3://publication/doi/10.1182/blood-2012-03-418202

352. Waggoner SN, Cornberg M, Selin LK, Welsh RM. Natural killer cells act as rheostats modulating antiviral T cells. *Nature* 2012; **481**(7381)**:** 394-398. e-pub ahead of print 2011/11/22; doi: 10.1038/nature10624

353. Miller JS, Soignier Y, Panoskaltsis-Mortari A, McNearney SA, Yun GH, Fautsch SK *et al.* Successful adoptive transfer and in vivo expansion of human haploidentical NK cells in patients with cancer. *Blood* 2005; **105**(8)**:** 3051-3057.

354. Dudley ME, Wunderlich JR, Robbins PF, Yang JC, Hwu P, Schwartzentruber DJ *et al.* Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. *Science* 2002; **298**(5594)**:** 850-854. doi: 10.1126/science.1076514

355. Kohrt HE, Turnbull BB, Heydari K, Shizuru JA, Laport GG, Miklos DB *et al. TLI and ATG conditioning with low risk of graft-versus-host disease retains antitumor reactions after allogeneic hematopoietic cell transplantation from related and unrelated donors*, vol. 114. 2009.

356. Benjamin J, Chhabra S, Kohrt HE, Lavori P, Laport GG, Arai S *et al.* Total Lymphoid Irradiation–Antithymocyte Globulin Conditioning and Allogeneic Transplantation for Patients with Myelodysplastic Syndromes and Myeloproliferative Neoplasms. *Biology of Blood and Marrow Transplantation* 2014; **20**(6)**:** 837-843. doi: http://dx.doi.org/10.1016/j.bbmt.2014.02.023

357. Lowsky R, Takahashi T, Liu YP, Dejbakhsh-Jones S, Grumet FC, Shizuru JA *et al.* Protective conditioning for acute graft-versus-host disease. *N Engl J Med* 2005; **353**(13)**:** 1321-1331. doi: 10.1056/NEJMoa050642

358. Frank DA, Mahajan S, Ritz J. Fludarabine-induced immunosuppression is associated with inhibition of STAT1 signaling. *Nat Med* 1999; **5**(4)**:** 444-447. doi: 10.1038/7445

359. Lutsiak ME, Semnani RT, De Pascalis R, Kashmiri SV, Schlom J, Sabzevari H. Inhibition of CD4(+)25+ T regulatory cell function implicated in enhanced immune response by low-dose cyclophosphamide. *Blood* 2005; **105**(7)**:** 2862-2868. doi: 10.1182/blood-2004-06-2410

360. Ghiringhelli F, Larmonier N, Schmitt E, Parcellier A, Cathelin D, Garrido C *et al.* CD4+CD25+ regulatory T cells suppress tumor immunity but are sensitive to cyclophosphamide which allows immunotherapy of established tumors to be curative. *Eur J Immunol* 2004; **34**(2)**:** 336-344. doi: 10.1002/eji.200324181

361. Beyer M, Kochanek M, Darabi K, Popov A, Jensen M, Endl E *et al.* Reduced frequencies and suppressive function of CD4+CD25hi regulatory T cells in patients with chronic lymphocytic leukemia after therapy with fludarabine. *Blood* 2005; **106**(6)**:** 2018-2025. doi: 10.1182/blood-2005-02-0642

362. Benjamin J, Chhabra S, Kohrt HE, Lavori P, Laport GG, Arai S *et al.* Total lymphoid irradiation-antithymocyte globulin conditioning and allogeneic transplantation for patients with myelodysplastic syndromes and myeloproliferative neoplasms. *Biol Blood Marrow Transplant* 2014; **20**(6)**:** 837-843. doi: 10.1016/j.bbmt.2014.02.023

363. Kohrt HE, Turnbull BB, Heydari K, Shizuru JA, Laport GG, Miklos DB *et al.* TLI and ATG conditioning with low risk of graft-versus-host disease retains antitumor reactions after allogeneic hematopoietic cell transplantation from related and unrelated donors. *Blood* 2009; **114**(5)**:** 1099-1109. doi: 10.1182/blood-2009-03-211441

364. Carlsten M, Baumann BC, Simonsson M, Jadersten M, Forsblom AM, Hammarstedt C *et al.* Reduced DNAM-1 expression on bone marrow NK cells associated with impaired killing of CD34(+) blasts in myelodysplastic syndrome. *Leukemia* 2010. e-pub ahead of print 2010/07/09; doi: leu2010149 [pii]

10.1038/leu.2010.149

365. Alfinito F, Sica M, Luciano L, Pepa RD, Palladino C, Ferrara I *et al.* Immune dysregulation and dyserythropoiesis in the myelodysplastic syndromes. *Br J Haematol* 2010; **148**(1)**:** 90-98. doi: 10.1111/j.1365-2141.2009.07921.x

366. Chen X, Eksioglu EA, Zhou J, Zhang L, Djeu J, Fortenbery N *et al.* Induction of myelodysplasia by myeloid-derived suppressor cells. *J. Clin. Invest.* 2013; **123**(11)**:** 4595-4611. doi: papers3://publication/doi/10.1172/JCI67580

367. Saft L, Björklund E, Berg E, Hellström-Lindberg E, Porwit A. Bone marrow dendritic cells are reduced in patients with high-risk myelodysplastic syndromes. *Leukemia Research* 2013; **37**(3)**:** 266-273. doi: http://dx.doi.org/10.1016/j.leukres.2012.10.010

368. Zeng W, Miyazato A, Chen G, Kajigaya S, Young NS, Maciejewski JP. Interferon-gamma-induced gene expression in CD34 cells: identification of pathologic cytokine-specific signature profiles. *Blood* 2006; **107**(1)**:** 167-175. doi: papers3://publication/doi/10.1182/blood-2005-05-1884

369. Bendall SC, Nolan GP, Roederer M, Chattopadhyay PK. A deep profiler's guide to cytometry. *Trends Immunol* 2012; **33**(7)**:** 323-332. doi: 10.1016/j.it.2012.02.010

370. Qiu P, Simonds EF, Bendall SC, Gibbs Jr KD, Bruggner RV, Linderman MD *et al.* Extracting a cellular hierarchy from high-dimensional cytometry data with SPADE. *Nat Biotech* 2011; **29**(10)**:** 886-891. doi: 10.1038/nbt.1991

http://www.nature.com/nbt/journal/v29/n10/abs/nbt.1991.html - supplementary-information

371. Shekhar K, Brodin P, Davis MM, Chakraborty AK. Automatic Classification of Cellular Expression by Nonlinear Stochastic Embedding (ACCENSE). *Proc Natl Acad Sci U S A* 2014; **111**(1)**:** 202-207. doi: 10.1073/pnas.1321405111