

From the Department of Laboratory Medicine  
Karolinska Institutet, Stockholm, Sweden

**GRAFT-VERSUS-HOST DISEASE AND  
TREATMENT WITH MESENCHYMAL STROMAL CELLS**

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**Karolinska  
Institutet**

Stockholm 2016

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Published by Karolinska Institutet.

Printed by E-PRINT AB 2016

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Front picture: Photograph of mesenchymal stromal cells in culture by Jessica Alm.

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ISBN 978-91-7676-263-9

# Graft-versus-host disease and treatment with mesenchymal stromal cells

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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*Till Bisse*

*Samarbetet mellan människan och naturen har alltid varit väldigt sofistikerat. Från antikens greker som använde giftig sjölök, fylld av hjärtglykosider, som ett hjärtstimulerande medel, till örtgumman som tipsade forskaren William Withering om att den vackra blomman digitalis hjälper mot ödem; från äldre tiders svenskar som helt modigt drack liljekonvaljsnaps mot hjärtproblem och hela vägen fram till den moderna medicinen digoxin (som används mot paroxysmal supraventrikulär takykardi) – människor har alltid varit instinktiva farmakognoser!*

Elin Unnes, författare till boken “Herbariet – växter till mat, magi och medicin”



# ABSTRACT

Graft-versus-host disease of both the acute (aGvHD) and chronic (cGvHD) variety remains a major cause of mortality and morbidity after allogeneic hematopoietic stem cell transplantation (HSCT). During the last 15 years, mesenchymal stromal cells (MSC) have been explored as a promising new treatment for aGvHD, but there are many questions to be answered in this young field.

The aim of this thesis is to expand our understanding of MSC treatment and GvHD with a specific focus on safety, factors affecting the outcome of MSC therapy and the possibility of treating also cGvHD with MSC.

In **paper I** we performed a long-term follow up study of the first patients treated with MSC, and reported on their outcome. We demonstrated a high frequency of infections and recommend the use of prophylactic drugs and close surveillance of patients during and following MSC treatment. Regarding factors affecting the outcome, we reported an association between low passage MSC and better clinical outcome, indicating that MSC lose some of their potency with extensive culturing. In **paper II**, we analysed autopsy reports and tissue samples from patients treated with MSC and could demonstrate that MSC do not appear to engraft in the patients. The risk of malignant transformation of donated MSC should therefore be very low.

In **paper III** we demonstrated a correlation between vitamin D deficiency prior to HSCT and an increased incidence of cGvHD, indicating vitamin D deficiency as a possible risk factor for cGvHD.

**Paper IV** reports on a clinical trial of MSC therapy in refractory cGvHD. Eleven patients were included; of whom nine received up to six repeated infusions of MSC and could be evaluated for response. Of these nine, six patients responded to MSC therapy with durable improvement in cGvHD symptoms and could significantly reduce systemic immunosuppression.

To summarize, this thesis provides new data regarding the safety of MSC therapy and suggests that the use of MSC is relatively safe, provided that necessary precautions are taken regarding infectious complications. With this information at hand, we could move forward to expanding the use of MSC in conditions with less dire expectations than refractory aGvHD, such as cGvHD. The clinical study of MSC therapy in cGvHD is one of the largest reported worldwide and suggests that repeated infusions of MSC could be a valuable treatment option for these patients.

# POPULÄRVETENSKAPLIG SAMMANFATTNING

I benmärgen finns de stamceller som bildar våra blodceller, såväl röda blodkroppar som de vita blodkroppar som utgör vårt immunsystem. Vid benmärgstransplantation byter man därför ut ett sjukt immunsystem, vid exempelvis leukemi, mot ett friskt från en donator. Men när inte immunsystemet matchar kroppens egna celler perfekt kan man få en avstötningsreaktion som kallas transplantat-kontra-värd reaktion, eller GvH av den engelska förkortningen. Denna kan komma snabbt efter transplantationen och ge en häftig, akut reaktion med blåsor i huden, diarré och leverpåverkan som kan vara dödlig trots behandling. Den kan också komma senare, i en kronisk form som kan visa sig upp till ett par år efter transplantationen och mer likna reumatiska sjukdomar med ledvärk, torra slemhinnor och hudutslag.

I benmärgen finns också en annan typ av stamceller som bildar ben, fett och bindväv och skapar en anpassad livsmiljö för blodstamcellerna. Dessa så kallade mesenkymala celler interagerar även direkt med immunförsvarets celler och verkar ofta som en dämpande kraft för att lugna ner immunsystemet och motverka skada när immuncellerna är alltför aggressiva. Därför har man sedan början av 2000-talet provat att behandla akut GvH med donerade mesenkymala celler. De lämpar sig också väl för behandling, eftersom de kan mångfaldigas i cellodling och inte behöver matchas mellan givare och mottagare. Behandlingen har visat sig ha viss effekt, men väckte också en del oro för biverkningar som ökade infektioner eller att de donerade cellerna skulle bilda tumörer i kroppen. Mycket återstår också att förstå kring behandlingen, som vilka faktorer hos patienten, donatorn eller hanteringen av cellerna som kan påverka effekten.

I den här avhandlingen studeras säkerheten hos behandling med mesenkymala celler, och vilka faktorer som kan påverka eller förutsäga effekten av behandlingen. Vi har också studerat effekten av D-vitaminbrist innan transplantationen, framför allt vad gäller uppkomsten av kronisk GvH. Slutligen har vi studerat effekten av behandling med mesenkymala celler vid kronisk GvH.

**I det första arbetet** genomförde vi en återblickande långtidsuppföljning på alla patienter som fått mesenkymala celler på Karolinska Universitetssjukhuset Huddinge från 2002 (då den första behandlingen i världen gjordes) till 2007. Det var 31 patienter, varav de flesta (23) hade fått mesenkymala celler som behandling för akut GvH. Övriga 8 patienter hade fått dem som behandling för blödning i urinblåsan, vilket också är en allvarlig komplikation efter benmärgstransplantation.

Det visade sig att risken att dö av infektioner var hög även lång tid efter tillfrisknande från den akuta sjukdomen. Vi försökte spegla behandlingen i provrörsexperiment där patientens immunceller fick reagera på donatorns mesenkymala celler. Resultatet av experimenten kunde dock inte förutsäga huruvida en patient skulle svara på behandlingen i verkligheten. Däremot verkar celler som genomgått färre odlingscykler ha bättre effekt än de som odlats



längre. Patienterna som fått celler som odlats i högst två cykler svarade bättre på behandlingen och överlevde i större utsträckning än övriga.

I **det andra arbetet** gick vi igenom obduktioner som gjorts på 18 patienter som avlidit efter att ha genomgått behandling med mesenkymala celler. Hos ingen av dem fanns tecken till tumörer eller vävnad som bildats av de donerade cellerna. Vävnadsprover från 15 av patienterna analyserades närmare med DNA-teknik, vilket visade mycket små mängder av kvarvarande donerade celler i patientens kropp. Ju kortare tid som gått från att cellerna givits tills att vävnadsprovet togs, desto större var chansen att några celler skulle kunna påträffas. Slutsatsen blev att de donerade mesenkymala cellerna endast verkar överleva en kort tid i kroppen och att behandlingen därmed verkar säker ur det hänseendet.

I **det tredje arbetet** fokuserade vi på D-vitaminbrist, och om det kan påverka det nybildade immunförsvaret efter benmärgstransplantation på ett negativt sätt. D-vitamin har en funktion i regleringen av immunsvaret, och brist på D-vitamin misstänks kunna öka risken för autoimmuna sjukdomar som reumatoid artrit, MS och typ 1 diabetes. Vi kunde utnyttja blodprover som tagits innan transplantationen och sparats frysta för att i efterhand ta reda på vilka patienter som haft D-vitaminbrist före transplantationen, och sedan utifrån journalen läsa ut hur det gått för dem. Vi kunde då se att de patienter som haft låga D-vitaminnivåer före transplantationen hade en högre risk att utveckla kronisk GvH. Utifrån denna studie kan vi dock inte dra några slutsatser om huruvida det är D-vitaminbristen som orsakar den ökade risken och om det i så fall skulle löna sig att ge extra D-vitamin inför transplantationen, utan man skulle behöva gå vidare och undersöka det med hjälp av en kontrollgrupp.

**Det fjärde arbetet** är en behandlingsstudie där vi testat att ge upprepade behandlingar av mesenkymala stamceller för att behandla patienter med svår kronisk GvH. Elva patienter har påbörjat behandlingen, två fick avbryta i förtid och vi kan därför inte utvärdera om deras behandling haft någon effekt. Av de nio som fullföljt minst ett halvårs behandling har sex stycken svarat med lindring av symtomen och kunnat minska ned på övrig immunhämmande behandling. De har behandlats i ungefär nio månader och förbättringen verkar hålla i även efter att vi avslutat behandlingen, flera patienter är nu mer än ett år efter sista behandlingen med mesenkymala celler och är fortfarande klart förbättrade. Två patienter har kunnat upphöra med alla immunhämmande läkemedel.

Slutsatserna i avhandlingen är att behandling med mesenkymala celler verkar vara relativt säkert, men att vi bör ge förebyggande behandling mot svampinfektioner och ha noga uppsikt på andra infektioner även lång tid efter att patienterna läkt ut sin GvH, något som nu är rutin på kliniken. Mesenkymala celler verkar också lovande som behandling av kronisk GvH, men vi behöver gå vidare med fler studier för att förstå varför vissa patienter inte svarar på behandlingen och om vi kan hitta sätt att göra behandlingen effektivare för fler patienter.

Trots 15 års kliniska studier har hittills ingen genomfört en övertygande, kontrollerad studie som visar effekt av mesenkymala celler. Det behöver inte betyda att det inte finns någon effekt, men området är svårt att studera. Kliniska studier på GvH har flera inneboende

svårigheter, det är ett sällsynt tillstånd med svårt sjuka patienter där man ibland måste fatta snabba beslut om behandling. Behandlingen med mesenkymala celler har även den sina svårigheter, med variationer mellan olika donatorer, oklarheter i vilken hantering av cellerna som är bäst och avsaknaden av test som visar vilka celler som har bäst effekt. Sammantaget tror jag att vi har en större chans att kunna visa effekt av cellbehandling vid andra sjukdomar än GvH, till exempel inflammatoriska tarmsjukdomar eller reumatologiska sjukdomar, och det bedrivs också mycket studier på dessa sjukdomar. Vi borde samarbeta mer mellan forskningsgrupper och lära oss av deras resultat. Ett exempel är behovet av ett test som förutsäger cellernas effekt, om sådana studier kunde bedrivas parallellt med stora kliniska studier i flera sjukdomsgrupper kunde resultaten sedan användas för att förbättra cellprodukten och därmed resultaten.

## LIST OF SCIENTIFIC PAPERS

- I. **von Bahr L**, Sundberg B, Lönnies L, Sander B, Karbach H, Hägglund H, Ljungman P, Gustafsson B, Karlsson H, Le Blanc K\*, Ringdén O\*. Long-term complications, immunologic effects, and role of passage for outcome in mesenchymal stromal cell therapy. *Biol Blood Marrow Transplant*. 2012 Apr;18(4):557–64.  
\* equal contribution
- II. **von Bahr L**, Batsis I, Moll G, Hägg M, Szakos A, Sundberg B, Uzunel M, Ringden O, Le Blanc K. Analysis of tissues following mesenchymal stromal cell therapy in humans indicates limited long-term engraftment and no ectopic tissue formation. *Stem Cells*. 2012 Jul;30(7):1575–8.
- III. **von Bahr L**, Blennow O, Alm J, Björklund A, Malmberg K-J, Mougiakakos D, Le Blanc A, Oefner PJ, Labopin M, Ljungman P, Le Blanc K. Increased incidence of chronic GvHD and CMV disease in patients with vitamin D deficiency before allogeneic stem cell transplantation. *Bone Marrow Transplant*. 2015 Sep;50(9):1217–23.
- IV. **von Bahr L**, Heldring N, Afram G, Modin C, Garming-Legert K, Ljungman P, Davies LC, Le Blanc K. Treatment of Chronic GvHD with Mesenchymal Stromal Cells Induces Durable Responses; a Phase II Study. Manuscript.

## OTHER RELEVANT PUBLICATIONS

- I. Moll G, Jitschin R, **von Bahr L**, Rasmusson-Duprez I, Sundberg B, Lönnies L, Elgue G, Nilsson-Ekdahl K, Mougiakakos D, Lambris JD, Ringdén O, Le Blanc K, Nilsson B. Mesenchymal stromal cells engage complement and complement receptor bearing innate effector cells to modulate immune responses. *PLoS ONE*. 2011;6(7):e21703.
- II. Moll G, Rasmusson-Duprez I, **von Bahr L**, Connolly-Andersen A-M, Elgue G, Funke L, Hamad OA, Lönnies H, Magnusson PU, Sanchez J, Teramura Y, Nilsson-Ekdahl K, Ringdén O, Korsgren O, Nilsson B, Le Blanc K. Are therapeutic human mesenchymal stromal cells compatible with human blood? *Stem Cells*. 2012 Jul;30(7):1565–74.
- III. Jitschin R, Mougiakakos D, **von Bahr L**, Völkl S, Moll G, Ringden O, Kiessling R, Linder S, Le Blanc K. Alterations in the cellular immune compartment of patients treated with third-party mesenchymal stromal cells following allogeneic hematopoietic stem cell transplantation. *Stem Cells*. 2013 Aug;31(8):1715–25.
- IV. Moll G, Hult A, **von Bahr L**, Alm JJ, Heldring N, Hamad OA, Stenbeck-Funke L, Larsson S, Teramura Y, Roelofs H, Nilsson B, Fibbe WE, Olsson ML, Le Blanc K. Do ABO blood group antigens hamper the therapeutic efficacy of mesenchymal stromal cells? *PLoS ONE*. 2014;9(1):e85040.
- V. Moll G, Alm JJ, Davies LC, **von Bahr L**, Heldring N, Stenbeck-Funke L, Hamad OA, Hinsch R, Ignatowicz L, Locke M, Lönnies H, Lambris JD, Teramura Y, Nilsson-Ekdahl K, Nilsson B, Le Blanc K. Do cryopreserved mesenchymal stromal cells display impaired immunomodulatory and therapeutic properties? *Stem Cells*. 2014 Sep;32(9):2430–42.
- VI. Garming-Legert K, Tour G, Sugars R, **von Bahr L**, Davies LC, Le Blanc K. Enhanced oral healing following local mesenchymal stromal cell therapy. *Oral Oncol*. 2015 Dec;51(12):e97-99.

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## LIST OF ABBREVIATIONS

ATG	Anti-thymocyte globulin
BAFF	B-cell activating factor
CLL	Chronic lymphocytic leukaemia
CMV	Cytomegalovirus
CXCL	C-X-C motif ligand
EBV	Epstein-Barr virus
ECP	Extracorporeal photopheresis
FCS	Foetal calf serum
G-CSF	Granulocyte colony stimulating factor
GvHD	Graft-versus-host disease
<i>aGvHD</i>	<i>Acute graft-versus-host disease</i>
<i>cGvHD</i>	<i>Chronic graft-versus-host disease</i>
GvT	Graft-versus-tumour effect
HC	Haemorrhagic cystitis
HLA	Human leukocyte antigen
HSC	Hematopoietic stem cells
HSCT	Allogeneic hematopoietic stem cell transplantation
IBMIR	Instant blood mediated inflammatory reaction
IDO	Indoleamine 2,3-dioxygenase
IG	Immunoglobulin
IL	Interleukin
ILC	Innate lymphoid cell
INF	Interferon
LPS	Lipopolysaccharide
MHC	Major histocompatibility antigen
miRNA	Micro-RNA
MLR	Mixed lymphocyte reaction
MSC	Mesenchymal stromal cell
NIH	National Institute of Health

NK-cells	Natural killer cells
PBSC	Peripheral blood stem cells
PBMC	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
PDL	Programmed death-1 ligand
PDGFR	Platelet derived growth factor receptor
PGE2	Prostaglandin E2
RIC	Reduced intensity conditioning
TFH	T follicular helper cell
TGF- $\beta$	Transforming growth factor beta
Th	T helper cell
TLR	Toll-like receptor
TNF- $\alpha$	Tumour necrosis factor alpha
Treg	Regulatory T-cell
VZV	Varicella zoster virus



# 1 INTRODUCTION

Imagine a garden. There are flowers, bushes and trees, but beneath the plants, vital for the growth, is the soil. If the soil is thin and dry, the plants will not be able to prosper. In a rich soil, full of nutrients, the plants will thrive. This is a picture of the haematopoietic niche of the bone marrow, where the haematopoietic stem cells (HSC) thrive in a supporting environment.

The milieu in the bone marrow is the stroma (from Greek, meaning "layer, bed, bed covering"); vasculature, bone, fat and connective tissue. This microenvironment is crucial for the regulation of HSC differentiation and self-renewal, as mutations affecting the stromal cells of the bone marrow can seriously hamper haematopoiesis (1). Mesenchymal stromal cells (MSC) in the bone marrow, the source of bone, cartilage, fat and connective tissue cells, also have direct effects on many of the differentiated immune cells, orchestrating the inflammatory reaction.

Following HSC transplantation, the HSCs enter a bone marrow that has been damaged by conditioning cytototherapy and radiation. The few new stem cells, about 5% of the stem cell mass of a healthy bone marrow, must expand to fill the niche and give rise to progenitor cells that can differentiate into blood and immune cells. This happens under the pressure of inflammatory signalling from damaged tissues. When the regulation of the developing immune system goes awry it reacts against the host tissue, creating graft-versus-host disease (GvHD).

Here we question: If the bone marrow stromal cells can interact with the immune system and dampen immune responses, maybe infusion of donated MSC could be effective in treating GvHD? This has been tried during the last decade with some success. But infusion of third-party cells with stem cell capacity raises safety issues, such as the risk of tumour formation.

The aim of this thesis is to expand our understanding of GvHD and MSC treatment. It focuses on the safety and potential efficacy of the treatment for chronic GvHD, as well as factors that could predict or improve patient outcome. The study also explores the possible association between vitamin D deficiency and the development of chronic GvHD.

## 2 HEMATOPOIETIC STEM CELL TRANSPLANTATION

### 2.1 HISTORY

The first initiative to explore transplantation of HSCs came in the wake of the atomic bomb, when animal experiments demonstrated the possibility of rescuing mice exposed to lethal doses of radiation by transferring bone marrow from a healthy animal (2). The first human transplantation ensued shortly after (3), but the initial enthusiasm rapidly declined due to dismal results in the early years (4). With the discovery of the human leukocyte antigen (HLA)-system (5) and the possibility to choose a “suitable” donor, in combination with better understanding of the importance of pre-treatment of the patient, hematopoietic stem cell transplantation (HSCT) in patients with severe haematological malignancies grew exponentially during the 1970s (6). In 2013, the total number of performed transplantations worldwide reached 1 million, the majority of which were for haematological malignancies (7).

In the early stages, all transplantations performed were allogeneic, transferring bone marrow from another (healthy) person to the patient. The development of autologous transplantation quickly followed (8). In this case the patient’s own hematopoietic cells are cryopreserved whilst the patient undergoes high dose radiation and/or chemotherapy. This treatment effectively targets the malignancy at the expense of bone marrow toxicity, which can then be rescued by re-infusion of the preserved hematopoietic cells. Autologous transplantation is safer with regards to immunological complications as the problem of immunological compatibility is avoided. However, there is a risk of contaminating the graft with malignant cells when the malignancy is present in the haematological compartment. In addition, a major advantage of allogeneic transplantation is the immunological clearance of malignant cells achieved by the graft-versus-tumour (GvT) effect, explored in chapter 2. Autologous transplantation will not be discussed further in this thesis and the abbreviation HSCT will be used referring to allogeneic transplantations herein.

### 2.2 HLA TYPING

HLAs are the human equivalents of major histocompatibility complex (MHC) molecules (reviewed in 5). MHC class I correspond to HLA-A, B and C and present peptides produced inside the cell, either endogenous or viral, for recognition by CD8<sup>+</sup> cytotoxic T-cells. MHC class II correspond to HLA-DR, DP and DQ and are present on specialized antigen-presenting cells, such as dendritic cells, where they present peptides from phagocytized foreign cells, such as bacteria, and are recognized by CD4<sup>+</sup> T-helper cells.

HLA antigens are coded for on chromosome 6. A person carries two HLA haplotypes, one inherited from the mother and one from the father, therefore the chance of a sibling carrying

the same two haplotypes, to be HLA-identical, is 25%. A donor with one identical HLA haplotype (for example in the case of a parent donating HSCs to a child, or vice versa) is called haploidentical. When searching for an unrelated donor, HLA-matching on at least 8 HLA antigens (2 each for HLA-A, B, C and DR) is sought after. Typing in our institution is performed for 12 HLA antigens.

This is an extremely simplified explanation of compatibility, complicated by the existence of minor histocompatibility antigens inherited outside of the HLA gene complex (9). This means that HLA-identical siblings are still not entirely immunologically compatible like genetically identical (syngeneic) twins are.

### **2.3 TRANSPLANTATION PROCEDURE**

The transplantation procedure involves:

- i) A conditioning regimen with chemotherapy and/or radiation that weakens the patient's own immune system to pave the road for the transplanted cells. This also serves to eradicate as many as possible of remaining malignant cells.
- ii) Infusion of donated HSCs
- iii) GvHD prophylaxis
- iv) Supportive care including prophylaxis for infectious diseases

The conditioning regimen varies with the underlying disease and the clinical status of the patient. High dose myeloablative regimens, frequently involving whole-body irradiation, were initially ubiquitous and are effective in eradicating both the host immune system and malignant cells. This results in low rates of graft rejection and relapse, but at a cost of high cellular toxicity (10). In the late 1990s, reduced-intensity conditioning (RIC) regimens were introduced (11), making it possible to transplant older patients with more co-morbidities.

HSCs can be obtained directly from the bone marrow by iliac aspiration, from apheresis of peripheral blood after mobilization with granulocyte colony stimulating factor (G-CSF) or from umbilical cord blood. Peripheral blood stem cells (PBSC) are the most commonly used, with the advantage of faster engraftment, reducing the risk of neutropenic infections, but with increased risk of cGvHD compared to bone marrow (12). Umbilical cord blood expands the available donor pool due to less HLA restriction, but is associated with slower engraftment and increased risk of opportunistic infections (reviewed in 13), therefore making it an option only when another suitable donor cannot be found.

Regardless of stem cell source, the cell graft is made up of a mixture of HSCs, progenitor cells and mature mononuclear cells, though the proportions vary somewhat. The cell graft is infused into a central venous catheter and the HSCs home from the circulation to the bone marrow (14).

GvHD prophylaxis is necessary in all allogeneic settings, but as it counteracts the beneficial GvT effect the regimen is adjusted both by the risk of GvHD and the risk of relapse. The routine at Karolinska University Hospital generally follows the recommendations of the European Group for Blood and Marrow Transplantation (EBMT) and the European Leukaemia Network (ELN) (15). The basis of prophylaxis is a calcineurin inhibitor directed against interleukin (IL)-2 mediated T-cell activation. This treatment strategy is continued for a minimum of 3 months after HSCT, longer when the donor was unrelated, and up to a year in non-malignant disease. It is combined with a short course of methotrexate early post-transplant. *In vivo* T-cell depletion with anti-thymocyte globulin (ATG) is added in unrelated donor transplants or non-malignant disease.

## **2.4 IMMUNE RECONSTITUTION AND INFECTIONS**

During the initial neutropenic phase, patients are deeply immunosuppressed and mucosal barriers damaged due to conditioning therapies, leading to a high risk of bacterial infections (16) as well as invasive candida infections (17). Antibiotic and antifungal prophylaxis during the neutropenic phase is routine at our centre.

Circulating neutrophil and monocyte levels return to normal within a few weeks post-transplant, though neutrophil functions, such as chemotaxis and phagocytosis, can be impaired especially in the setting of GvHD (18). Macrophages are more resistant to chemotherapy and initially tissue macrophages of host origin can be found, but they are gradually exchanged with donor macrophages (19). Natural killer (NK) cells are restored to normal levels within the first month post-transplant (20). With a relatively restored innate immune system and intact mucosal barriers, the risk of bacterial infections drops significantly after the first month.

In contrast, the T- and B-cells of the adaptive immune system take longer to recover. An early expansion of mature T-cells in the graft gives rise to a limited repertoire during the first year post-HSCT, followed by thymus-dependent development of naïve T-cells (21). This process can be delayed or inhibited by factors affecting the thymus function, most notably older age and GvHD (22). B-cells are first undetectable in peripheral blood, starting to increase during the second month post-HSCT, but maintaining an immature phenotype with limited immunoglobulin (Ig)G production for up to two years post-transplant (23).

This prolonged immunodeficiency, augmented by GvHD and GvHD treatments, leaves the patients at high risk of viral infections. Re-activation of latent viruses, including cytomegalovirus (CMV) (24), Epstein-Barr virus (EBV) (25) and varicella zoster (VZV) (26) are major threats and routine monitoring of CMV, as well as EBV in risk patients, is performed. Valaciclovir-prophylaxis to prevent shingles is administered for the first year post-HSCT. Toxoplasma can also re-activate, causing severe infections, and prophylaxis is needed if the patient is sero-positive (27). Acute respiratory viral infections are common both during and after the neutropenic phase and can lead to life threatening lower respiratory tract

infections (28). *Pneumocystis carinii*-associated pneumonia is rare due to routine prophylaxis, but associated with significant mortality and morbidity (29). Invasive fungal infections are a risk primarily in the setting of GvHD, where prophylaxis is recommended (30). A summary of immune reconstitution, infection risk and recommended prophylaxis is presented in **Figure 1**.

Time period	Neutropenic phase (0 to 10-30 days)	Early post-engraftment (< 100 days)	Mid post-engraftment (<1 year)	Late post-engraftment (> 1 year)
<b>Immune deficiencies</b>	Neutropenia			
	B-cell			
	T-cell			If GvHD
<b>Infections</b>	Bacteremia			
	Respiratory viruses			
		CMV, VZV, EBV		If GvHD
	Invasive fungi		If GvHD	
		<i>Pneumocystis carinii</i>		If GvHD
<b>Recommended prophylaxis</b>	Antibiotic			
	Valaciclovir			If GvHD
	Antifungal		If GvHD	
		<i>Pneumocystis carinii</i>		If GvHD

**Figure 1.** Schematic overview of the time-line of immune deficiencies, infections and recommended prophylaxis after HSCT.

### 3 GRAFT-VERSUS-HOST DISEASE AND GRAFT-VERSUS-TUMOUR EFFECT

#### 3.1 ACUTE GRAFT-VERSUS-HOST DISEASE

The main effector cell population in aGvHD are cytotoxic T-cells from the graft. Depletion of mature T-cells from the graft significantly reduces the risk of aGvHD (31). The immunological response of these T-cells is, in essence, completely normal as the cells react to the foreign environment, a process further triggered by danger signals from tissues damaged by conditioning therapy or infections (32). Damage to the gut mucosa causes translocation of intestinal bacteria over the mucosal barrier (33), and bacterial toxins, such as lipopolysaccharide (LPS), further increase the “cytokine storm” that propagates aGvHD (34).

The major organs affected by aGvHD are the skin, gut and liver. Most commonly, patients first present with a rash and at initiation of therapy approximately 81% of patients have skin involvement, 54% gut dysfunction and 50% liver dysfunction (35). The diagnosis is mainly clinical, but histopathological evaluation is sometimes needed to distinguish from other disorders such as CMV colitis (36). The liver is rarely biopsied due to the risk of bleeding complications. The severity of aGvHD is usually graded according to the revised Glucksberg criteria (37) (**Table 1**), where each organ is staged 0-4 and then an overall grade of I-IV is derived based on the combination of organ stages. The overall grade correlates to survival, with approximately 25% long-term survivors in patients with grade III and less than 5% survivors for grade IV (38).

As aGvHD usually develops during the first 100 days after HSCT (35), patients are generally still on calcineurin-inhibitor based prophylaxis. First-line treatment for aGvHD is methylprednisolone (15), but durable, complete remissions are only achieved in about 35% of patients treated with steroids alone (39). If the patient does not respond to prednisolone, defined as no response after 7 days or clear progression after 5 days (15), there is no standard second-line treatment option and the general recommendation is that patients should, if possible, be treated in clinical trials (15).

**Table 1.** Revised Seattle Glucksberg scoring system for aGvHD

<b>Stage</b>	<b>Skin</b>	<b>Liver</b> (bilirubin)	<b>GI</b> (diarrhoea volume)
<b>0</b>	No rash	<2 mg/dL	< 500 mL/day
<b>1</b>	Rash <25% of BSA	2-3 mg/dL	500-999 mL/day or persistent nausea*
<b>2</b>	Rash 25-50% of BSA	3.1-6 mg/dL	1000-1500 mL/day
<b>3</b>	Rash >50% of BSA	6.1-15 mg/dL	>1500 mL/day
<b>4</b>	Generalized erythema and bullae	>15 mg/dL	Severe abdominal pain or ileus
<b>Grade</b>	<b>Skin</b>	<b>Liver</b>	<b>GI</b>
<b>I</b>	Stage 1-2	0	0
<b>II</b>	Stage 3 or:	Stage 1 or:	Stage 1
<b>III</b>	-	Stage 2-3 or:	Stage 2-4
<b>IV</b>	Stage 4 or:	Stage 4	-

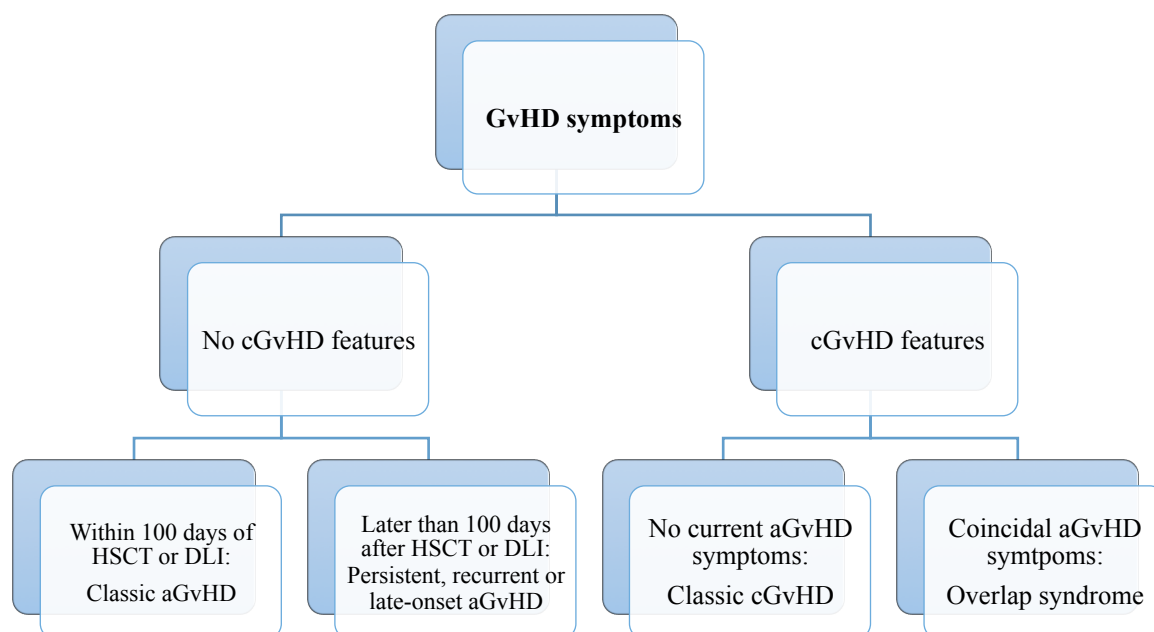
Adapted from Przepiora et al, Bone Marrow Transplant 1995. \* Persistent nausea with histological evidence of aGvHD in the stomach or duodenum.

## 3.2 CHRONIC GRAFT-VERSUS-HOST DISEASE

### 3.2.1 Diagnosis and staging

Traditionally, all GvHD appearing >100 days after HSCT was termed chronic (cGvHD). However, this fails to reflect the different pathophysiological mechanisms, as well as distinct clinical features, in the acute and chronic forms. aGvHD can be seen more than 100 days after HSCT, especially in reduced intensity transplants, late tapering of immunosuppressive drugs or following donor lymphocyte infusions. To clear this distinction and ameliorate studies in the field, a National Institute of Health (NIH) conference was held in 2004 to establish consensus criteria for diagnosis and staging of cGvHD (40) and response criteria for conducting clinical studies in cGvHD (41). This was later followed by a second conference in 2014, with some revision of the initial criteria (42, 43).

Under this definition, GvHD is divided into aGvHD or cGvHD based on the clinical characteristics. Patients demonstrating symptoms associated with aGvHD (erythema, vomiting, diarrhoea, cholestatic liver disease) without meeting the criteria of cGvHD are diagnosed as aGvHD, regardless of time from HSCT. Patients with classical cGvHD symptoms (sclerosis of skin and mucosa, bronchiolitis obliterans, fasciitis, joint contractures) are diagnosed as cGvHD, with a further sub-division of classic chronic (in the absence of aGvHD features) or overlap syndrome (if also at least one symptom associated with aGvHD is present). See **Figure 2**.



**Figure 2.** Schematic of differential diagnosis of acute and chronic GvHD. HSCT: haematopoietic stem cell transplantation DLI: donor lymphocyte infusion. Adapted from Jagasia et al, BBMT 2015.



After diagnosis of cGvHD, the stage is defined as mild, moderate or severe according to the degree of organ involvement and severity of symptoms (40,42).

### **3.2.2 Treatment**

Corticosteroids, with or without the addition of calcineurin inhibitors, constitute the basis of cGvHD treatment (15). Unfortunately, only approximately half of the patients achieve long-term remission on this first-line treatment (44) and as in aGvHD, there is no established second-line treatment (15, 45). Prolonged treatment with corticosteroids carries significant risks including type II diabetes, osteoporosis, muscle atrophy, hypertension, psychological disturbances and infections.

The most well documented second-line treatment is extracorporeal photopheresis (ECP), with reported response rates of approximately 60% (46). It is the only second-line treatment in cGvHD that has been evaluated in a randomized controlled study (48), but this study failed to reach its primary endpoint. A 2010 survey of EBMT centres reported that the most commonly used second-line therapies were ECP (53%), mycophenolate mofetil (36%), rituximab (12%), calcineurin inhibitors (12%), mammalian target of rapamycin (mTOR) inhibitors (9%), corticosteroids (8%) and tyrosine kinase inhibitors (6%) (48). More recent additions include low-dose IL-2 (49) and bortezomib (50).

## **3.3 PATHOPHYSIOLOGY OF CHRONIC GVHD**

The pathophysiology of cGvHD is complex and not fully understood. It involves both the adaptive and the innate immune system and it is possible that different pathophysiological mechanisms dominate the picture in different clinical manifestations of the disease. Some of the main pathophysiological mechanisms discussed below are summarised in a schematic overview in **Figure 3**.

### **3.3.1 T-cells**

Alloreactive T-cells from the graft as well as decreased thymic function are involved in the initiation of cGvHD. The classical paradigm stated that cGvHD, as opposed to acute, was a T-helper (Th)2-driven disease (51). This has been challenged by recent studies showing an active role of Th1, as well as Th17 cells, in at least sclerotic skin cGvHD (52), with IL-17A produced by CD8<sup>+</sup> cytotoxic T-cells as well as CD4<sup>+</sup> Th17 cells seemingly central to the development of sclerodermatous disease (53). CD4<sup>+</sup> lymphopenia with a skewing towards less regulatory T cells (Tregs), in relation to conventional T-cells, is a common feature of cGvHD (54); with clinical studies aiming at expanding Tregs with low-dose IL-2 therapy demonstrating encouraging early results (55).

Another T-cell subset that has recently come into focus in cGvHD research is T follicular helper cells (TFH). TFH cells interact with B-cells in germinal centres of lymphatic tissue and promote B-cell activation and Ig production (56). Aberrant TFH activation could impair

the positive selection of B-cell clones and has been suggested as a pathophysiological mechanism in several autoimmune diseases, where increased levels of circulating activated TFH cells have been found to correlate with disease activity (reviewed in 57). Irregular TFH functionality with increased frequencies of activated TFH cells have also been reported in cGvHD (58).

### **3.3.2 B-cells**

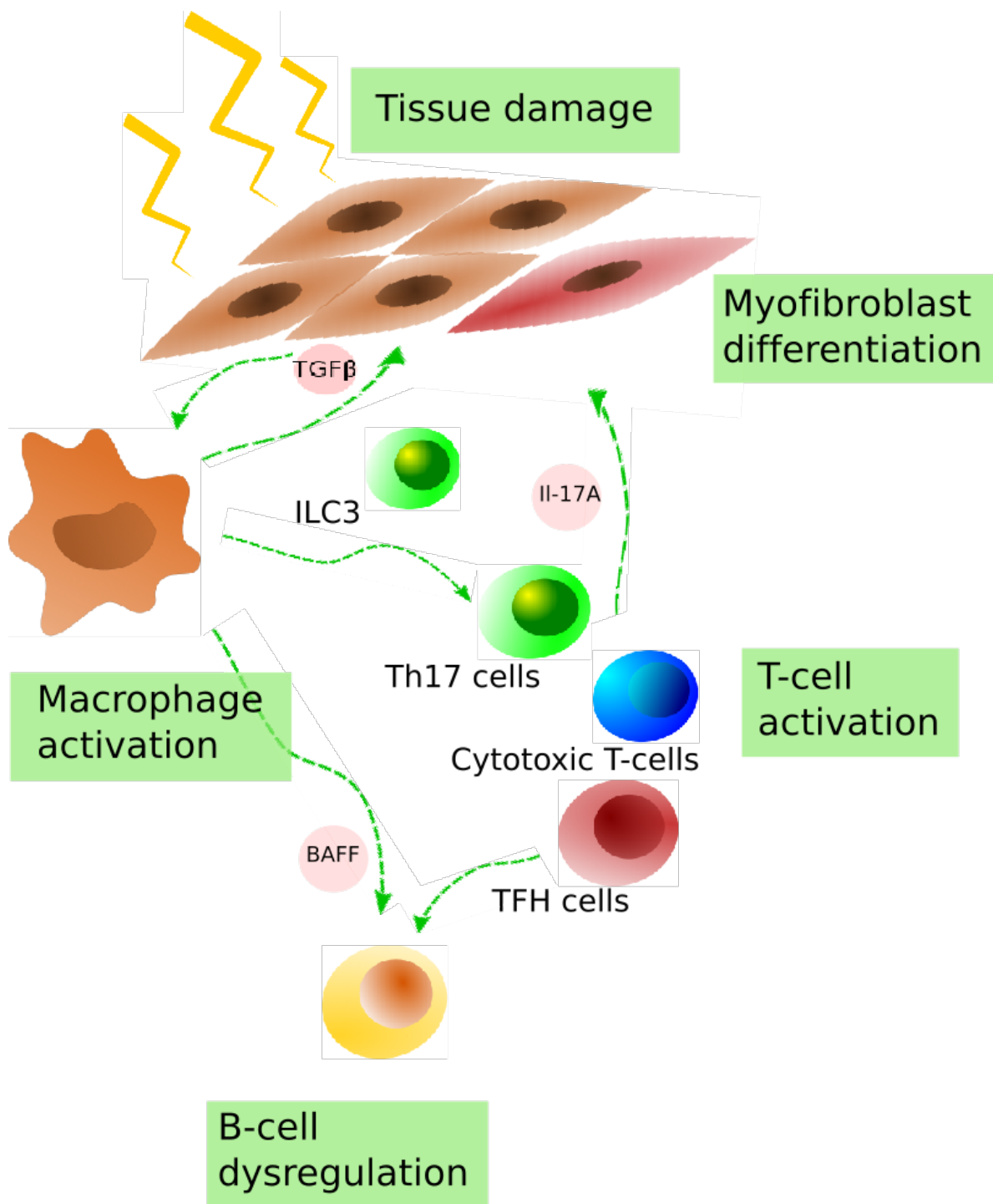
B-cell dysregulation seems to be at the heart of cGvHD development (reviewed in 59), with slow B-cell regeneration (60) and high levels of circulating B-cell activating factor (BAFF) detected (61). This elevated BAFF/B-cell ratio impairs the normal negative selection of alloreactive B-cells (62). However, the mechanisms behind this dysregulation and role of B-cells in the pathophysiology of cGvHD remain largely unknown and areas of intense investigation.

Auto-antibody production is a common feature in cGvHD (63, 64). Anti-platelet derived growth factor receptor (PDGFR)-antibodies have been suggested to exert a direct effect in promoting tissue fibrosis (65), but the functional relevance of these antibodies is unclear (63, 66). Rather, the role of B-cells as antigen-presenting cells and their production of pro- or anti-inflammatory cytokines have been the primary focus to date (reviewed in 67).

Allen et al. reported that B-cells derived from cGvHD patients are highly activated and resistant to apoptosis (68). Specific subsets, including transitional CD21<sup>-</sup> B-cells are increased in cGvHD (69, 70), whilst CD27<sup>+</sup> memory B-cells (69) and regulatory B-cells (71) are reduced. It is important to note however, that these data can be somewhat contradictory, with other reports indicating higher levels of CD27<sup>+</sup> cells (72). CD21<sup>-</sup> B-cells have also been demonstrated to be anergic (73) and thus might not be relevant to the disease pathophysiology.

### **3.3.3 Fibrosis and the innate immune system**

Central to the cGvHD pathology is the development of fibrosis, similar to several other chronic inflammatory diseases such as systemic sclerosis, primary biliary cirrhosis or idiopathic pulmonary fibrosis. Fibrosis is characterised by differentiation of myofibroblasts that lay down excessive amounts of extracellular matrix (reviewed in 74). The mechanisms driving and regulating myofibroblast differentiation are poorly understood, but macrophages appear to play a complex, orchestrating role with both pro- and anti-fibrotic properties (75, 76). Macrophages display several phenotypes, depending on the surrounding environment, and different phenotypes exhibit different features in the development of fibrosis (reviewed in 77). Transforming growth factor (TGF)  $\beta$ , secreted by macrophages, is a major driving force in the development of fibrosis (75, 78). But macrophages are also instrumental in the resolution of fibrosis (79) and can under certain circumstances inhibit inflammation-driven fibrosis (76).



**Figure 3. Schematic overview of some pathophysiological mechanisms in cGvHD.**

Tissue damage, from aGvHD, pathogens or other trauma, initiates danger signals that trigger activation of the innate immune system. Macrophages and T follicular helper (TFH) cells drive B-cell dysregulation. Activated Th17 cells and cytotoxic T-cells react towards allo-antigens. IL-17A from T-cells and innate lymphoid cells (ILC) as well as TGF β from macrophages induce myofibroblast differentiation and fibrosis development.

Innate lymphoid cells (ILC) are cells of lymphoid origin but lacking antigen-specific receptors and therefore part of the innate immune system (80). They are rare in the circulation, but enriched in tissues near epithelial borders where they interact closely with tissue-resident macrophages and are involved in chronic inflammation and fibrosis (reviewed in 81). ILC are divided into three subtypes ILC1, ILC2 and ILC3, mirroring the T helper subtypes Th1, Th2 and Th17 (80). The ILC2 subtype is implicated in pulmonary fibrosis (82, 83), whilst ILC3 is an important source of IL-17A, which as discussed above appears to be central to the development of sclerodermatous cGvHD (53). In psoriasis patients, increased proportions of ILC3 have been isolated from active skin lesions (84).

Monocytes, macrophages and dendritic cells are also major producers of BAFF (85, 86) and dysregulation of BAFF production in monocytes has been demonstrated in patients with Sjögren's syndrome (87). This might be a further driving mechanism in the B cell pathology discussed above. B-cells and BAFF can also have direct effects on fibroblasts, promoting fibrosis in systemic sclerosis (88).

### **3.4 RISK FACTORS FOR GVHD**

Many known risk factors for GvHD can be classified as either relating to immune mismatch between the donor and the patient or to tissue damage and impaired restorative capacity in the patient, which can be understood in the context of disease pathophysiology as described above.

Belonging to the first category are HLA-mismatch between donor and patient (89), allo-immunization of the donor by transfusion or pregnancy (90) and female donor to male recipient (90,91). The composition of the donor cell graft also impacts the outcome, with a higher risk of GvHD in PBSC transplants compared to bone marrow grafts (92). In umbilical cord blood cell transplantations instead, some degree of HLA-mismatch can be allowed without increasing the risk of GvHD (93), though the HLA-matching remains important (94). This can, to some extent, be explained by the proportions of mature, alloreactive T-cells in the grafts, as a higher total nucleated cell dose is associated with higher risk of cGvHD (95).

In the second category we find older patient age (90) and higher intensity conditioning (mainly total body irradiation) (96). For cGvHD, a major risk factor is previous aGvHD (97), which falls within this category both through direct damage to target tissues such as skin and through destruction of the thymus, leading to impaired T-cell reconstitution (98).

Many studies reporting on risk factors predate the NIH diagnostic criteria for cGvHD and thus the distinction between acute and chronic forms is not clear. A large study from 2011, comprising nearly 3000 patients, aimed to compare the effect of various risk factors on aGvHD and NIH-defined cGvHD (99). PBSC and older patient age were found to be only associated with cGvHD, whilst total body irradiation was only linked to aGvHD. In addition, many risk factors were found to be common to both forms of GvHD but the statistical

measures of cGvHD risk were not affected by adjusting for prior aGvHD, indicating independent mechanisms.

### **3.5 VITAMIN D**

A newly suggested risk factor for cGvHD is vitamin D deficiency (100). Cholecalciferol, or vitamin D, is a fat-soluble vitamin obtained partly from ingestion of certain foods, but mainly by the transformation of 7-dehydrocholesterol in the skin when exposed to sufficient levels of sunlight. As many HSCT patients have been hospitalized for long periods of time, in addition to suffering from mucosal damage and limited nutrition, it is not surprising that the incidence of vitamin D deficiency is high within this population (101).

Cholecalciferol needs to be activated by the enzyme 1- $\alpha$ -hydroxylase. This was previously believed to take place only in the kidneys, but 1- $\alpha$ -hydroxylase has later been found to be active in many parts of the body, including dendritic cells and macrophages (102). Active vitamin D may play a vital role in regulating the immune system, including inhibition of alloreactive T-cells (103). Several observational studies have reported on associations between vitamin D deficiency and autoimmune diseases, including multiple sclerosis (104) and type 1 diabetes (105). Deficiency of vitamin D has also been implicated as a risk factor for rejection of solid organ transplants (106).

In 2012, Glotbecker et al. reported on 53 adult patients demonstrating a significant association between low vitamin D levels prior to HSCT and increased incidence of cGvHD (107). This finding could however not be confirmed by a later study comprising 123 paediatric patients (108), but the latter study reported a very low overall incidence of cGvHD which could explain the lack of correlation. More studies are needed to explore the question of whether vitamin D deficiency is a risk factor for the development of cGvHD.

### **3.6 GRAFT-VERSUS-TUMOUR EFFECT**

The other side of the GvHD coin is the immunological anti-tumour effect known as graft-versus-tumour (GvT) or graft-versus-leukaemia (GvL). This was suggested in early animal experiments (109) and was later confirmed as clinically relevant in human HSCT (110,111). Patients with aGvHD or cGvHD displayed lower relapse rates than unaffected patients.

However, Horowitz et al. (111) further indicated that the GvT effect, to some extent, was mediated by allogeneic T-cells independent of the GvHD. Patients receiving T-cell depleted grafts, with or without GvHD, displayed higher relapse rates than in non-T-cell depleted transplants without GvHD. Furthermore, patients receiving a graft from an identical twin had more than twice the relapse risk of other patients without GvHD.

Later studies have confirmed an association between cGvHD and lower relapse rates (112), whilst the impact of aGvHD seems to be dependent also upon the conditioning regimen (113, 114). A reduced intensity conditioning does not confer a significant anti-tumour effect in itself but must rely more heavily on the GvT than in the myeloablative setting. Regardless, it appears to be possible to achieve lesser GvHD rates with T-cell depleted grafts (115) or *in vivo* T-cell depletion using ATG (116) without affecting the leukaemia-free survival, at least in unrelated transplants.

On a biological level it is evident that T-cells driving the GvT and GvH response might respond to the same mechanisms but have different antigen specificities. In GvT the T-cells react towards hematopoietic cells and possibly malignancy-specific antigens, whilst in GvH epithelial and other tissue-antigens are in focus. Different techniques for isolating malignancy-specific T-cells for adoptive transfer (117) or vaccination against malignancy-associated epitopes (118) are currently being explored.

## 4 MESENCHYMAL STROMAL CELLS

### 4.1 BACKGROUND

In 1968, Friedenstein et al. published a characterisation of cells found in the bone marrow that could form ectopic bone tissue (119). These cells, originally named mesenchymal stem cells, constitute about 0.001 to 0.01% of the bone marrow mononuclear cells and can differentiate into adipose tissue, bone and cartilage (120). Cells with the same phenotypic properties have later been derived from other tissues, notably umbilical cord blood (121), adipose tissue (122) and skin (123).

### 4.2 DEFINITION

Today we appreciate that mesenchymal stem cells represents a heterogeneous composition, with no single, definitive marker identified to date. In order to better define this cell population, criteria regarding phenotype and function were imposed by The International Society for Cellular Therapy in 2006 (124). We therefore define this cell population by their adherence to plastic, spindle-like morphology and their tri-lineage plasticity; the ability to differentiate into bone, cartilage and fat. In addition, the cells have to be at least 95% positive for surface markers CD73, CD90 and CD105, whilst negative for haematopoietic markers CD34, CD45, CD11b, CD14, CD19 and CD79 $\alpha$ , as well as HLA-DR (124).

The same association proposed that the name multipotent mesenchymal stromal cells (MSC) was to be preferred over mesenchymal stem cells (125), to account for the fact that the definition includes many cell populations, several of which do not fulfil the self-renewal and multi-lineage potential of true stem cells. Through this thesis MSC will refer to mesenchymal stromal cells as defined above.

As these cells are so rare *in vivo*, knowledge of their functions comes from studies using MSC that have been expanded *in vitro*, a process that most certainly affects their functionality. What immunological functions endogenous MSC actually exert *in vivo* still remains to be established. In this chapter, I will focus on the immunomodulatory properties exerted by expanded bone marrow MSC *in vitro* and in animal models.

### 4.3 LICENSING AND POLARIZATION

The interactions between MSC and the immune system are highly complex and dependent on environmental triggers (reviewed in 126). In the absence of pro-inflammatory stimuli, MSC support the survival of T-cells (127), as well as B-cells (128), and rescue T-cells from activation-induced cell death (127). When stimulated with interferon- $\gamma$  (IFN- $\gamma$ ) MSC display potent anti-inflammatory properties (129), a process known as ‘licensing’ (126). This

licensing appears dependent on the concentration and duration of IFN- $\gamma$  stimulation (130) and is also affected by several other cytokines such as tumour necrosis factor-  $\alpha$  (TNF- $\alpha$ ) (131). However, in the presence of bacterial LPS, MSC can react through activation of toll-like receptor (TLR) 4 with a production of pro-inflammatory cytokines such as IL-6 and IL-8 and stimulate T-cell activation (132). This discovery has led to the postulation that MSC can be polarized into two distinct phenotypes, the pro-inflammatory MSC1 and the anti-inflammatory MSC2 (132).

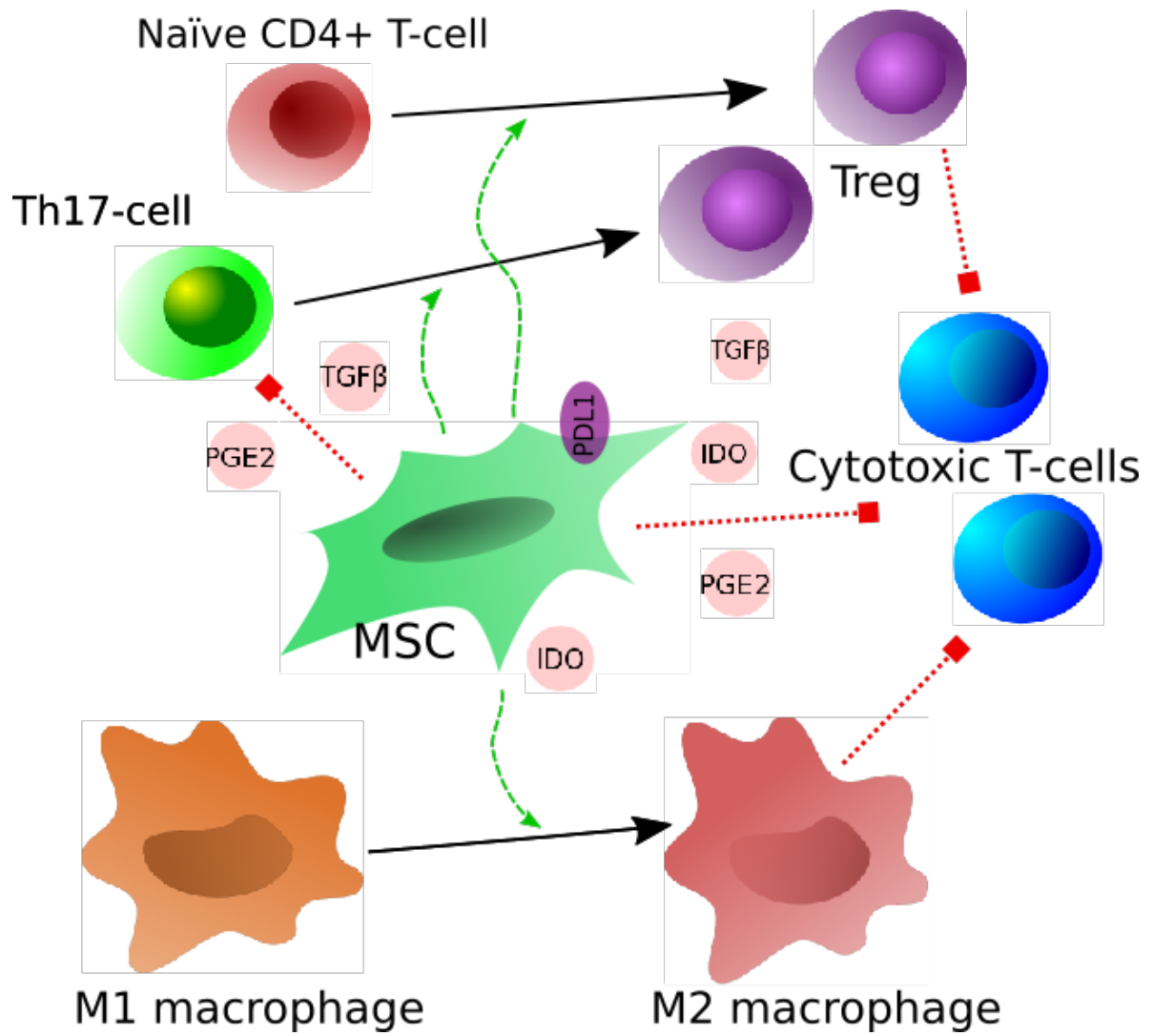
#### **4.4 MECHANISMS OF IMMUNOMODULATION**

There are several, possibly mutually redundant, mechanisms for MSC to exert an immunosuppressive effect. When co-cultured with activated T-cells in mixed lymphocyte reactions (MLR), they suppress T-cell proliferation (133). This suppression could be achieved in transwell (133) and several soluble factors have been identified as effectors, including TGF- $\beta$  (133), prostaglandin E2 (PGE2) (134) and indoleamine 2,3-dioxygenase (IDO) (135). However, more effective T-cell suppression could be achieved when cell contact was allowed (133). In addition to the numerous immunomodulatory soluble factors MSC secrete, they also produce chemoattractants (131), promoting lymphocyte migration and adhesion molecules (136), thereby allowing them to tightly bind lymphocytes. This potentially allows the soluble factors to work more efficiently, as well as providing an opportunity for membrane-bound factors, such as programmed death ligand 1 and 2 (PDL 1/2), to interact with specific lymphocyte receptors, PD-1 in this case (137).

Besides directly suppressing cytotoxic T-cells, licensed MSC can induce CD4<sup>+</sup> T-cells, including Th17 cells (138), into immunosuppressive regulatory T-cells, Tregs (139). MSC-induced Treg induction is dependent on monocytes being present in the co-culture and skewing of these monocytes by MSC into anti-inflammatory M2 macrophages (140). Tregs and M2 macrophages are both potent anti-inflammatory mediators. In a mouse sepsis model, injected MSC accumulated in the lungs together with monocytes and macrophages. These macrophages were induced to produce large amounts of IL-10, which then mediated the protective effect against sepsis-related organ failure observed in the MSC-treated mice (141). These findings indicate the ability of MSC to both directly immunosuppress as well as indirectly modulating anti-inflammatory immune cells to coordinate the suppressive effects.

In addition to their ability to immunomodulate through soluble factor secretion, MSCs are reported to modulate their environment through the production of micro-RNAs (miRNA) (142). These short, non-coding, single-stranded RNA molecules regulate the expression of target genes by binding to target sequences on mRNA. miRNAs can be transported from one cell to another in exosomes or microvesicles (143). The potential of MSC derived miRNAs have been demonstrated in a mouse model of hypoxic injury, where effects were ameliorated by transferring of MSC exosomes (144). miRNA-containing exosomes from human MSCs have also been reported to modulate macrophage activation (145)





**Figure 4. Immunomodulatory mechanisms of MSC.** Direct inhibitory effects on cytotoxic T-cells and Th17-cells via soluble factors, including IDO, PGE2 and  $TGF\beta$ , and membrane-bound PDL-1. Indirect effects via reprogramming of monocytes and M1 macrophages into anti-inflammatory M2 macrophages, and naïve T-cells and Th17-cells into Tregs.

## 4.5 MSC AND FIBROSIS

*In vivo*, circulating or tissue-resident cells of mesenchymal lineage have been shown to differentiate into pro-fibrotic myofibroblasts (rev in 74). TGF- $\beta$ , one of the main soluble factors secreted by MSC (133), is also a major inducer of myofibroblast differentiation and extracellular matrix production (146). Intravenous administration of MSC on the other hand has repeatedly been demonstrated to inhibit or ameliorate fibrosis in animal models (147-150).

This apparent paradox can to some extent be explained by the anti-inflammatory effects of MSC discussed above, as chronic inflammation is a major driving mechanism in the development of fibrosis. MSC and monocyte/macrophage interactions are probably a central feature of the inhibition of fibrosis as this usually is accompanied by less infiltration of macrophages (148, 150), and depletion of macrophages early in the inflammatory process is associated with less fibrosis development in tissue damage models (151).

Another factor is the multi-functionality of TGF- $\beta$ . TGF- $\beta$  is secreted as an inactive pro-peptide complex that after secretion can be bound to extracellular matrix components (reviewed in 152). This makes the concentration of free, active TGF- $\beta$  dependent on several factors separate from the production. There are also three isoforms of TGF- $\beta$ , where TGF- $\beta$ 1 and 2 have the most prominent fibrotic properties while TGF- $\beta$ 3 has been suggested to be anti-fibrotic (153). A mouse model of wound healing indicated that locally injected MSC reduced fibrosis through shifting the ratio between TGF- $\beta$ 1 and TGF- $\beta$ 3 (154).

## 5 MSC THERAPY

### 5.1 BASIC PRINCIPLES

MSC have several properties that make them interesting for cell-based therapies. They express low levels of HLA class I and have been described as immune privileged, in that they do not elicit an immune response when transplanted across HLA barriers (155). Though this concept has been challenged (156), it remains true that MSC from unmatched, third-party donors can be transplanted without immunological reactions (157). MSC can be expanded *in vitro* (158) and cryopreserved (159), which allows for prepared doses of third-party MSC to be stored for future use.

MSC have been investigated for cell therapy in several areas, including regenerative medicine (160), to support haematological recovery after HSCT (161) and as an immunosuppressive treatment for auto- or allo-immune reactions. Different modes of administration have also been investigated, including systemic infusion, local injection (162) and seeding on scaffolds to be implanted (163). In this chapter I will focus on systemic use of MSC as an immunosuppressive treatment.

### 5.2 MSC PRODUCTS

MSC for clinical use can be obtained from various tissues, most commonly bone marrow, adipose tissue (164) or perinatal tissues such as umbilical cord or foetal tissues (165). In experimental models, other tissues such as skin (166) and synovial membranes (167) are also being explored. The origin of the cells most likely greatly affects their immunomodulatory properties (168).

Isolation of MSC from aspirated bone marrow relies on the plastic adherence of the MSC population, with some protocols plating whole unprocessed bone marrow for expansion (169). More commonly, the mononuclear fraction is first separated using density-gradient separation. The mononuclear cells, a mixture of haematopoietic stem and progenitor cells, lymphoid cells, monocytes, endothelial progenitor cells and MSC are then plated in flasks, where the MSCs adhere to the plastic and alter their morphology to a characteristic, spindle-like shape. The medium needs to contain growth factors to allow for cellular proliferation and this was initially achieved by adding foetal calf serum (FCS). FCS is problematic in several ways, as it displays great batch-to-batch variation and carries a risk of unknown infectious agents. In later years, FCS has often been replaced by human serum or platelet-rich plasma.

There is evidence that different protocols for isolation and expansion of MSC can greatly affect the properties of the cells (170-172). Add to this variability the fact that MSC from different donors can differ in secretory and immunomodulatory capabilities (173) and we

realize that there is currently no such thing as a uniform MSC product to be compared over different studies.

### 5.3 ACUTE GVHD

The first case of MSC treatment reported was in a 9-year-old child with severe aGvHD, reported by Le Blanc et al. (174). This initial patient was followed in 2008 by a multicentre report on 55 patients with steroid-refractory aGvHD (175), showing complete responses in 55% and partial response in 16%, with no immediate side-effects to MSC administration.

These encouraging results spurred several further studies, including an industry-sponsored randomized, placebo-controlled phase III trial that was presented as an abstract at the 2010 American Society for Blood and Marrow Transplantation Tandem Meeting (176), but to date has not been published in full. The results of the phase III trial were discouraging, failing to meet the primary endpoint of increased durable complete responses for MSC compared to placebo. However, there are many reasons to be careful in interpreting this single trial as evidence that MSC are ineffective in treating aGvHD (177). A subgroup analysis demonstrated that patients with gut and/or liver involvement responded significantly better with MSC than placebo, though the overall effect was diminished by the patients with only skin involvement, where no difference in response was seen. It is also noteworthy to state that the MSC product used (Prochymal®) was highly expanded, up to 10 000 doses produced from few donors, whilst cells used in academic trials usually only produce 5-10 doses per donor. Our data indicate that extensive passaging could have a negative effect on MSC clinical efficiency (**paper I**).

Recent reviews of the literature (178, 179) concluded that there is support for an effect of MSC on aGvHD, but stresses the need for a randomized phase III-study. There are currently 13 active studies registered on clinicaltrials.gov with MSC therapy for the indication aGvHD, of which 7 are recruiting patients, as well as a European multicentre randomized, double-blinded phase III-study led by the HOVON group (**Table 2**).

Commercial MSC products have been approved in Japan for treatment of aGvHD and in Canada and New Zealand for steroid-refractory aGvHD in children. The response rate in children is usually reported to be higher than that observed in adults (180, 181), though there is no randomized study on a paediatric population.

**Table 2: Studies registered on *clinicaltrials.gov* on MSC treatment of aGvHD**

Phase	Control group	Status*	Sponsor	NCT identifier <sup>§</sup>
I/II	No	Completed	Andalusian Initiative for Advanced Therapies	NCT01222039
II	No	Recruiting	Nanfang Hospital of Southern Medical University	NCT01765634
I/II	No	Completed	Grupo Espanol de transplantes hematopoyeticos y terapia celular	NCT01956903
III	Yes (1)	Completed	Osiris Therapeutics	NCT00366145
II	No	Recruiting	University Hospital of Liege	NCT00603330
I/II	No	Not yet recruiting	Andalusian Initiative for Advanced Therapies	NCT02687646
II	Yes (3)	Recruiting	Royal Perth Hospital	NCT01589549
I/II	No	Recruiting	National Heart, Lung and Blood Institute	NCT02379442
II/III	Yes (3)	Recruiting	Nanfang Hospital of Southern Medical University	NCT02241018
I/II	No	Recruiting	A.O. Ospedale Papa Giovanni XXIII	NCT02032446
I/II	No	Completed	UMC Utrecht	NCT00827398
I/II	No	Ongoing, not recruiting	Affiliated Hospital to Academy of Military Medical Sciences	NCT01754454
III	No	Recruiting	Mesoblast International Sàrl	NCT02336230

Search 2016-04-04 on “graft-versus-host OR gvhd / mesenchymal OR MSC”. Studies that are terminated or of unknown status are excluded, as are studies on cGvHD, of MSC as GvHD prophylaxis and local administration of MSC. \* Study status as indicated on *clinicaltrials.gov*.

<sup>§</sup> Unique study identification number on *clinicaltrials.gov*. Control groups: 1 = randomized, placebo-controlled, double-blind. 2= randomized, placebo-controlled, single-blind (patient). 3= randomized, open label, placebo or alternate treatment.

## 5.4 CHRONIC GVHD

Compared to aGvHD, data on MSC treatment in cGvHD is sparse. The only studies including more than 10 patients come from Sun Yat-Sen University, Guangzhou, China, that have published several reports on MSC treatment on up to 38 patients and reporting response rates between 74% and 87% (182-185). However, it is not clear from the publications to what extent the patients are overlapping between the studies, so it is impossible to know the total number of patients treated. Common between the publications was that repeated infusions of MSC were administered (median 2-3) and that the responses were observed late (median time to best response 233 days in (183), between 3 and 6 months in (184)).

Besides this group, there are only four published reports of MSC treatment in cGvHD. First, Ringdén et al. (186) included one cGvHD patient in their initial report on MSC treatment in GvHD, with a transient response to a single MSC infusion. In 2010, Zhou et al. (187) reported successful treatment of four patients with sclerodermatous cGvHD with repeated infusions (1 per week, total 4-8 infusions) of MSC. This was followed by mixed results in a study by Pérez-Simon et al. (188), reporting 8 patients with refractory cGvHD receiving 1-3 doses of MSC with 4 patients responding (of which 2 were transient responses). Finally Hermann et al. (189) treated 7 cGvHD patients, with a median of 8 infusions of MSC, achieving 4 responses.

## 5.5 OTHER INFLAMMATORY DISEASES

Following the positive reports in aGvHD, MSC therapy has been applied to a variety of other inflammatory diseases. A large number of phase I/II studies have been published in inflammatory bowel disease (190) and multiple sclerosis (191), with a smaller but substantial number in inflammatory arthritis (192) and systemic autoimmune diseases as systemic lupus erythematosus, systemic sclerosis and Sjögrens syndrome (reviewed in 193). To date, several randomized, placebo-controlled studies have been registered in ClinicalTrials.gov (see **table 3**), but no results have been published so far.

Drawing on the regenerative potential of transplanted MSCs, as well as immunomodulatory properties, MSC have also been explored to ameliorate damage caused by ischemia and reperfusion injury (194). This includes ischemic heart disease where a randomized, double-blinded placebo-controlled study has been published (195) with encouraging results, but underpowered to demonstrate efficacy. Uncontrolled studies have also shown some improvement in liver function in end-stage liver disease (196) and preservation of  $\beta$ -cell function in type 1 diabetes (197).

**Table 3: Studies, with control groups, registered on *clinicaltrials.gov* on MSC treatment of inflammatory diseases**

Indication	Phase	Control group <sup>#</sup>	Status*	Sponsor	NCT identifier <sup>§</sup>
Crohn's disease	II/III	3	Not yet recruiting	Nanfeng Hospital of Southern Medical University, Sun Yat-sen University	NCT02532738
	III	1	Active, not recruiting	Mesoblast International Srl	NCT00482092
	III	1	Active, not recruiting	Mesoblast International Srl	NCT01233960
Ulcerative colitis	I/II	2	Recruiting	Affiliated Hospital to Academy of Military Medical Sciences	NCT02442037
Rheumatoid arthritis	II/III	3	Completed	Royan Institute	NCT01873625
	II	1	Recruiting	Mesoblast International Srl	NCT01851070
	I/II	2	Completed	TiGenix S.A.U	NCT01663116
Systemic lupus erythematosus	II	1	Not yet recruiting	Medical University of South Carolina	NCT02633163
Type I diabetes mellitus	II	1	Recruiting	Uppsala University	NCT02057211
	II	1	Completed	Mesoblast International Srl	NCT00690066
Multiple sclerosis	I/II	1	Completed	Andalusian Initiative for Advanced Therapies	NCT01056471
Neuromyelitis optica	II	3	Recruiting	Tianjin Medical University General Hospital	NCT02249676

Search 2016-04-04 on “autoimmune OR crohns OR inflammatory bowel disease OR ulcerative colitis OR systemic sclerosis OR arthritis OR SLE OR scleroderma OR multiple sclerosis | mesenchymal OR msc”. # Only studies with a randomized control group are included. Studies that are terminated or of unknown status are excluded, as are studies using local administration of MSC. \* Study status as indicated on *clinicaltrials.gov*. § Unique study identification number on *clinicaltrials.gov*. Control groups: 1 = randomized, placebo-controlled, double-blind. 2= randomized, placebo-controlled, single-blind (patient). 3= randomized, open label, placebo or alternate treatment.

## 5.6 SAFETY OF MSC TREATMENT

With a new therapy there are naturally questions regarding safety. MSC were quickly shown not to induce transfusion reactions, acute toxicity or clinically significant pulmonary embolisms (186) in immunocompromised patients. This has also later been confirmed in a meta-analysis including immunocompetent patients (157). However, there remained two main concerns. Firstly the risks associated with engraftment of the transplanted cells giving rise to ectopic tissue or malignant tumours in the recipient (198). Secondly, that the immunosuppressive effect might increase the sensitivity to opportunistic infections or recurrence of the malignant disease in the case of HSCT patients.

Malignant transformation of murine MSC in culture has been widely reported (199), but conflicting reports were published regarding human cells (200, 201). This dispute was settled when the reports on malignant transformation could be shown to be misidentifications of contaminating cancer cell lines in the lab (202, 203). Human MSC thus seem a safer option in this regard than embryonic stem cells or induced pluripotent stem cells (198, 204).

In systemic or local infections, MSC seem to have the capacity to act as a double-edged sword with both positive and negative effects reported. Their activation can be modulated by binding of TLRs (205) and by direct interaction with various bacteria (206), which allows the adaptation of the response in an infected environment. MSC can increase the phagocytic capacity of monocyte/macrophages (207), as well as neutrophils (208), whilst reducing the inflammation-induced organ damage. *In vitro*, MSC even exhibit direct anti-microbial effects mediated through LL-37 (209) and IDO (210). This suggests MSC therapy could be beneficial even in severe infections such as sepsis (211).

Re-activation of viral infections such as CMV, EBV and adenovirus is a major risk in HSCT, especially in the setting of aGvHD. There has been conflicting evidence on whether MSC would impair the virus-specific immune response. Karlsson et al. reported that MSC did not inhibit proliferation of CMV- or EBV-specific T-cells *in vitro* and that CMV-specific cells could be obtained from the peripheral blood of two patients after MSC treatment for aGvHD (212). In contrast, Malcherek et al. demonstrated strong inhibition of CMV- and influenza-specific T-cells by MSC in culture (213). MSC can also be directly infected by CMV (214) and this infection could markedly hamper the immunomodulatory capacities of the MSC (215). In follow-up studies of patient cohorts, MSC treatment has not been associated with higher incidence or mortality in CMV or EBV infections, but with a higher mortality in adenovirus infections (216, 217).

Some studies report an increased incidence of invasive fungal infections (218) or pneumonia-associated death (a large proportion of which was due to fungal pneumonia) (219) in patients treated with MSC. In treatment studies lacking control groups there are difficulties separating the increased risk of infectious complications due to the underlying disease from that of MSC, but in a randomized study on MSC as GvHD prophylaxis there was still a slightly higher risk of infections in the MSC-treated patients, despite them having a lower incidence



of aGvHD than the controls (220). This study by Ning et al. (220) also reported a markedly higher incidence of relapse in the patients with haematological malignancies. Though this relapse rate might explain some of the infections, it is in itself alarming. This phenomenon has not been confirmed in any other studies on co-transplantation of MSC in HSCT (reviewed in 221).

## 6 AIMS

The principal aim of this thesis is to expand our understanding of GvHD and MSC treatment. Within this aim, focus has been placed on answering the following questions:

1. **Safety of MSC treatment.** The early patient cohort at Karolinska University Hospital was the first to undergo MSC treatment. Follow-up studies of the treated patients were performed to evaluate the safety of systemic MSC treatment and long term complications with regards to infections, relapse, ectopic tissue formation and secondary malignancies.
2. **Prediction of response.** Why do some patients respond to MSC treatment whilst others do not? Is this dependent on the individual patient, the physiology of disease, the MSC product or a combination of these factors?
3. **Vitamin D deficiency.** Is there an association in patients undergoing HSCT between vitamin D deficiency and the risk of developing cGvHD or other related complications?
4. **MSC treatment of cGvHD.** Could systemic treatment with MSCs be a potentially safe and effective treatment of refractory cGvHD?

## 7 PATIENTS AND METHODS

### 7.1 PATIENTS

**Paper I** details a long-term follow-up of MSC treated patients. All patients treated with MSC for aGvHD (n = 23) and haemorrhagic cystitis (HC) (n = 8) between 2002 and 2007 at the Karolinska University Hospital were included. The cohort consisted of 24 males and 7 females, with a median age of 53 years (range 1-67). Data was collected from the patient medical records and complications were recorded from the date of first MSC infusion until the last date of data collection in November 2009.

In **paper II** the engraftment of infused MSC and the risk of ectopic tissue formation or malignant transformation was evaluated. Nineteen patients treated with MSCs at Karolinska University Hospital between 2002 and 2010 were included, partially overlapping with the cohort of paper I. The patients received MSCs for GvHD (n = 11), HC (n = 5), haemophagocytic lymphohistiocytosis (n = 2) and for the promotion of engraftment (n = 1). Eighteen of the patients were evaluated by autopsy and samples were analysed for MSC donor DNA within multiple organs from 15 patients.

**Paper III** is a retrospective cohort analysis including 166 consecutive patients (> 12 years of age) undergoing HSCT between 2005 and 2011 at Karolinska University Hospital. In the analysis of cGvHD incidence, patients with graft failure (n=13) or with a survival after HSCT of less than 100 days (n=14) were excluded. All data was taken from patient medical records, and all complications, except infections, were recorded from the date of HSCT to the last date of data collection, in April 2014. Infectious complications were only recorded for the 1<sup>st</sup> year following HSCT.

**Paper IV** forms a clinical study for the treatment of cGvHD with systemic, allogeneic MSC therapy. Patients diagnosed with cGvHD of grade moderate to severe, refractory to or not tolerating 3 months standard treatment of calcineurin inhibitor plus steroids were included. Eleven patients with severe cGvHD were enrolled, 6 female and 5 male, with a median age of 50 (range 20-61). Patients were clinically evaluated up to 1 year after last infusion for response.

All studies were conducted in accordance with the Helsinki convention and approved by the regional ethical committee in Stockholm. Written informed consent was obtained, for paper I and II at time of MSC treatment, for paper III at HSCT and for paper IV at study enrolment.

### 7.2 DEFINITIONS

In **paper I** and **III**, data concerning complications were obtained from medical records.

CMV disease was defined according to Ljungman et al. (222). Invasive fungal disease was defined according to De Pauw (223). Only probable and proven invasive fungal infections were considered in paper III, whilst local fungal infections are reported separately in paper I. Diagnosis of pneumonia required either a combination of new pulmonary infiltrates on chest

X-ray or CT scan with symptoms of respiratory infection such as cough, dyspnoea or fever, excluding idiopathic pulmonary syndrome or autopsy-verified infectious pneumonia. Bacteraemia was defined as the first positive blood culture during a 10 day time period. Repeated positive blood cultures >10 days after the first were considered new episodes. In paper I, bacterial and viral infections were classified as severe (causing hospitalization or organ damage) or mild (other).

Rejection (paper I) or graft failure (paper III) was defined as lack of engraftment, engraftment with recipient cells or later developing full (>95%) recipient chimerism in the absence of relapse of the underlying disease. In paper I, transplant-related mortality was defined as death occurring in the absence of relapse. In paper III, disease-free survival was defined as survival with no evidence of relapse or progression for malignant disease. Overall survival was defined as the time from HSCT to death, regardless of cause.

In **paper III and IV**, cGvHD was diagnosed and scored according to the NIH consensus criteria (40), with both classic cGvHD and overlap syndrome included, but not late onset aGvHD. Only cGvHD of moderate and severe grade were considered in the analysis in paper III. Response evaluation in paper IV followed the NIH consensus recommendations (43).

### **7.3 MSC THERAPY**

MSCs from the bone marrow of healthy donors were harvested and expanded following a procedure developed by the European Group for Blood and Marrow Transplantation Developmental Committee and accredited by the Swedish National Board of Health and Welfare under Swedish law 2008:286 (Cell- och vävnadslagen) (approvals number 952/2009, 6.3.3-8874/2011, 6.1.3-9791/2013 and 6.1.3-16411/2015). Donors provided written, informed consent before the procedure.

Bone marrow was harvested under sterile conditions by aspiration from the iliac crest. Bone marrow mononuclear cells were seeded in cell culture flasks in culture medium supplemented with 10% foetal calf serum. The cells were detached with 0.05% Trypsin-EDTA when they were 90% confluent and replated in new flasks for 1-4 passages. After harvest, the cells were cryopreserved. Release criteria were based on the absence of visible clumps, spindle shape morphology, the absence of contamination by pathogens (bacteria and mycoplasma) and viability >95%. The MSCs expressed CD73, CD90, CD105 and HLA-ABC and were negative for CD14, CD31, CD45 and HLA-DR as assessed by flow cytometry.

In **paper I and II**, the majority of patients received a single infusion of MSC at a dose of  $1-2 \times 10^6$  cells/kg bodyweight. Ten patients in paper I and 9 in paper II received repeated MSC infusions, because of recurring or worsening symptoms after an initial good response, or because of lack of response. In **paper IV**, a dose of  $2 \times 10^6$  MSC/ kg was infused at 4-6 week intervals. A minimum of 6 doses was given; in the case of response to treatment after 6 doses an additional 1-3 doses were infused.

## 7.4 LABORATORY ASSAYS

Two different **T-cell suppression assays** were used in **paper I**, both using Ficoll-separated peripheral blood mononuclear cells (PBMCs) from the patients and MSCs from the same donor given as treatment. The PBMCs were activated using either irradiated PBMCs from five unrelated donors or phytohemagglutinin. Irradiated MSCs were then added in a proportion of 10% MSC to patient PBMCs and the proliferation of patient PBMCs was estimated by tritiated thymidine incorporation. Lower proliferation compared to controls (without MSC) was interpreted as T-cell suppression.

**Polymerase chain reaction (PCR)** was used in **paper II** to identify cells of MSC donor origin in tissue samples. It relies on finding a DNA-sequence that is present in the MSC donor but not in the patient or the HSCT donor. In the first three patients a nested PCR method was used based on differences in the HLA type. This method is not quantitative and can thus only determine whether or not MSC donor DNA was present in the tissue. In the remaining analysed patients, quantitative real-time (qRT) PCR was used, based on single nucleotide polymorphisms (SNPs). The sensitivity in both assays was the same, between  $1/10^5$  and  $1/10^6$  as assessed by serial dilutions.

## 7.5 STATISTICS

**Papers I, II and IV** are mainly descriptive in nature and the statistical methods used are the Mann-Whitney U-test, Student's T-test or Fisher's exact test depending on the data. Probability of survival was estimated using the Kaplan-Meier method and compared using the log-rank test (Mantel-Cox).

In **paper III**, cumulative incidence functions (CIF) were used to estimate GvHD, considering death and relapse to be competing events. Probabilities of DFS and OS were calculated using Kaplan-Meier estimates. Univariate analyses were performed using Gray's test for CIF and the log-rank test for DFS and OS. Associations of patient and graft characteristics with outcomes were evaluated in multivariate analysis, using Cox proportional hazards model for dichotomous variables, or negative binomial regression analysis for outcomes with repeated events or continuous variables. For two-sample comparisons, the Wilcoxon rank-sum test or Fisher's exact test was used.

GraphPad Prism® 6, IBM SPSS version 21 and R 3.0.1 software were used.

## 8 RESULTS AND DISCUSSION

### 8.1 SAFETY OF MSC TREATMENT (PAPER I, II, IV)

The first reported case of MSC treatment in human was in 2002, at Karolinska University Hospital (174). Between 2002 and 2007 30 further patients were treated with MSC at our institution for either aGvHD or HC. At this time not much was known about possible negative long-term effects of the MSC treatment. As we had the largest treated patient cohort in the world, as well as the longest experience, we wanted to summarize the outcome in a follow-up report in **paper I**. In **paper II**, the focus was the risk of ectopic tissue formation or malignant transformation of infused MSC.

To be able to separate the effect of MSC treatment from that of other immunosuppressive treatments, and from the aGvHD itself, it would have been necessary to have a control group. For a control group to be appropriate it needs to be well matched, preferably by randomization, and both groups need to be sufficiently sized (powered) to allow for statistical analysis. As there was no suitable control group available, we chose to report the complications in the MSC-treated cohort descriptively in both absolute numbers and as a function of observation period (incidence per 1000 patient-days).

#### 8.1.1 Infections

We could demonstrate a high incidence of infectious complications, even long after MSC treatment and resolution of aGvHD. Out of 13 patients who recovered from aGvHD following MSC treatment, 7 (54%) later died from infections. Furthermore, the HC patients, who did not receive as much immunosuppression as the aGvHD patients, also displayed a high rate of infections including invasive fungal infections (see **table 4** reproduced from **paper I**). Other reports from our institution have later confirmed an association between MSC treatment and infections (218, 219), whilst a large meta-analysis concluded the opposite (157). In this meta-analysis mainly patients without concurrent immunosuppression were included, which might account for the discrepancy in results. It should be noted that no routine anti-fungal or anti-viral prophylaxis was given during the time period under study. We recommend prophylaxis as well as close surveillance of patients treated with MSC to reduce the risk of infections and this is now routine in our clinic.

In **paper IV**, repeated infusions of MSC were administered over a time period of 6-12 months, in 9 patients with severe cGvHD. All the patients received prophylaxis against *Pneumocystis jirovecii* and were followed regularly with PCR for CMV viremia. We recommended anti-fungal prophylaxis with posaconazol, but because of intolerable side effects some patients received fluconazol or no anti-fungal agent. All patients were monitored closely for infectious complications during the MSC treatment period and for 12 months after the last infusion. No invasive fungal infections or CMV reactivations were recorded during this time, and a total of five events of grade 3 infections occurred.

**Table 4.** Severe infectious complications recorded in paper I

	<i>MSC GvHD n = 23</i>		<i>MSC HC n = 8</i>	
	<i>Total</i>	<i>Incidence /1,000 d</i>	<i>Total</i>	<i>Incidence /1,000 d</i>
Severe bacterial infection	27	2.0	8	2.8
Severe viral infection	5	0.4	3	1.0
Severe fungal infection	10	0.7	3	1.0
EBV-activation	7	0.5	1	0.3
PTLD	2	0.1	0	0
<b>CMV</b>	<i>MSC GvHD n = 19</i>		<i>MSC HC n = 7</i>	
Peak (log <sub>10</sub> ) Mean ± 95% CI	4.1 ± 0.22		3.2 ± 0.37	
CMV disease Total (%)	6 (32%)		0 (0%)	

MSC: mesenchymal stromal cells, GvHD: graft-versus-host disease, HC: hemorrhagic cystitis, EBV: epstein-barr virus, CMV: cytomegalovirus. In the CMV columns, seronegative patients receiving a seronegative graft are excluded. Adapted from von Bahr et al., BBMT 2012.

### 8.1.2 Relapse

With strong immunosuppression in patients with an underlying malignant disease, the risk of abrogating the GvT effect and causing a relapse is present. One early study of co-transplantation of MSC at HSCT demonstrated a higher incidence of leukaemia relapse (220), although later studies have not been able to confirm this finding (reviewed in 221).

In **paper I**, 2 out of 27 patients (7%) with haematological malignancy suffered relapse, one with myeloma and one with acute lymphocytic leukaemia (ALL). In **paper IV**, 2 out of 11 patients had relapse, one with myeloma and one with chronic lymphocytic leukaemia (CLL). As cGvHD is associated with a stronger GvT effect than aGvHD (112) and the treatment as well as follow-up time was longer in **paper IV**, this incidence is worth noting for future studies on cGvHD. On the other hand, both myeloma and CLL are diseases known to have relatively high relapse rates following HSCT and the patient cohort is too small for any conclusive results. In other published reports on MSC trials in cGvHD, a total of 6 cases of relapse have been reported among a total of 40 patients (185, 187-189). It should be noted though that it is not entirely evident from these publications how long a follow-up this reflects and thus no event rate can be calculated. The incidence of relapse in MSC treated patients needs to be followed up in future studies, preferably with larger cohorts and appropriate controls.

### 8.1.3 Engraftment

MSCs have a capability *in vitro* to differentiate into bone, adipose tissue and cartilage (120), and at least a portion of the culture-expanded MSCs infused intravenously for immunomodulatory treatment can be assumed to retain this capacity. Engraftment and proliferation of infused cells could lead to the development of ectopic tissue in the patients and possibly also malignant transformation. To address this question, we investigated autopsies of 18 patients as well as tissue samples from 15 patients who had received MSC. This study was published in **paper II**.

No ectopic tissue and no evidence of malignant transformation of MSC could be found on the 18 autopsies. In 7 patients, low levels of MSC donor DNA could be detected in samples from one or more tissues. One patient was severely ill with septicaemia and massive gastrointestinal bleeding at the time of MSC infusion and later developed disseminated intravascular coagulopathy, before passing away 7 days after receiving MSC. In this patient extensive micro- and macro-embolization was found at autopsy as well as detectable MSC donor DNA in all sampled tissues. We believe that the disturbed haemostasis in this patient explains the wide distribution of MSC in the tissues and that under normal circumstances the engraftment of infused MSC is very low. The detection of MSC donor DNA was negatively correlated with time from infusion to sampling, indicating that remaining cells may be subsequently cleared by phagocytosis.



## 8.2 PREDICTION OF RESPONSE (PAPER I AND IV)

We know that some patients seem to respond well to MSC treatment, whilst others do not (175). This could depend on MSC donor variability, other factors related to the MSC product, patient factors or a combination thereof, including the matching between MSC donor and patient. If we understood more about the factors determining the response, this could be extremely helpful in selecting the best MSC product as well as choosing the patients that will benefit from the treatment.

### 8.2.1 MSC donor and product

In **paper I**, different factors of the MSC donor and MSC product were correlated to clinical outcome, divided into response (complete or partial) or no response. Thirty-one patients received a total of 45 infusions, on the indication of aGvHD (n=23) or HC (n=8) and each infusion was analysed separately for short-term response. No correlation was found between MSC donor age or sex and clinical response. The dose of MSC, varying between 0.65 and 3.0 x10<sup>6</sup> /kg bodyweight, was also not correlated to response.

The number of expansion passages of the MSC did however seem to affect the outcome, indicating a possible negative effect of *in vitro* culturing on the MSC properties. Patients receiving early-passage MSC (harvested after one or two passages) displayed both better short-term response and overall survival than patients receiving MSC of higher passage (three or four). In later studies, we have demonstrated that MSC can trigger the instant blood mediated inflammatory reaction (IBMIR) at intravenous infusion and that this triggering is stronger with higher passage MSC (224). Triggering of IBMIR may cause the MSC to be lysed and cleared from the circulation to a higher degree, potentially explaining the better outcome using low passage cells. Cryopreservation of high passage MSC might aggravate this problem, as later indicated in a small cohort of patients (225). We now aim at delivering low passage MSC for treatment. However, in order to achieve sufficient numbers of cells for therapy, expansion for, on average, three passages is needed. We therefore continue to work on improving the handling of the cells to minimize the negative effects of passaging and cryopreservation.

### 8.2.2 MSC donor – patient matching

MSC can safely be transferred across HLA barriers (155) and the use of HLA-matched MSC was not associated with better clinical response (**paper I**) or engraftment (**paper II**). This does not exclude some possible positive effects of HLA-matching, as the patient cohorts are small, but it is safe to say that no major disadvantage could be demonstrated for patients receiving unmatched cells. As using unmatched cryopreserved cells allows for swift administration of the MSC treatment, this is of great importance especially in the setting of aGvHD, where disease progression is rapid.

In **paper I**, we investigated whether the ability of MSCs from a particular donor to suppress the individual patients' T-cells in an *in vitro* assay could be used to predict a clinical effect of

MSC from that donor in the same patient. This appeared not to be the case, as we found no correlation between *in vitro* suppressive activity and clinical response. Probably the *in vitro* assays used today are too blunt to capture the complex interactions *in vivo* between the MSC, different immune cells and other factors such as the coagulation and complement systems.

### 8.2.3 Patient factors

In **paper I**, we demonstrated a better clinical outcome in children than in adults with aGvHD. This has also been confirmed in later studies (180, 181) but is most likely not unique for MSC treatment. Patients with CMV disease before MSC treatment responded poorly, which is interesting as it has later been found that MSC can be infected with CMV and that this impairs their immunomodulatory properties (215). It could also be argued that the patient's symptoms were more associated with the CMV disease than aGvHD and that the lack of response was caused by a delay in CMV-specific treatment.

In **paper IV** we have investigated potential biomarkers for response in MSC treatment. We have considered both biomarkers that could distinguish responders from non-responders before initiation of treatment and early indicators of clinical response that would enable a faster evaluation than the current 6 months needed for clinical evaluation in cGvHD treatment.

Among the screened possible biomarkers, C-X-C motif ligand (CXCL)-9 and CXCL10 displayed a pattern that could be indicative of a predictive biomarker for response. Both act as chemokines to attract inflammatory cells to a site of tissue damage and are ligands for the receptor CXCR3, expressed on lymphocytes (226, 227). They are secreted from M1 macrophages when stimulated with IFN- $\gamma$  (228) and elevated plasma levels of both CXCL9 and CXCL10 have previously been demonstrated to be associated with active cGvHD (229).

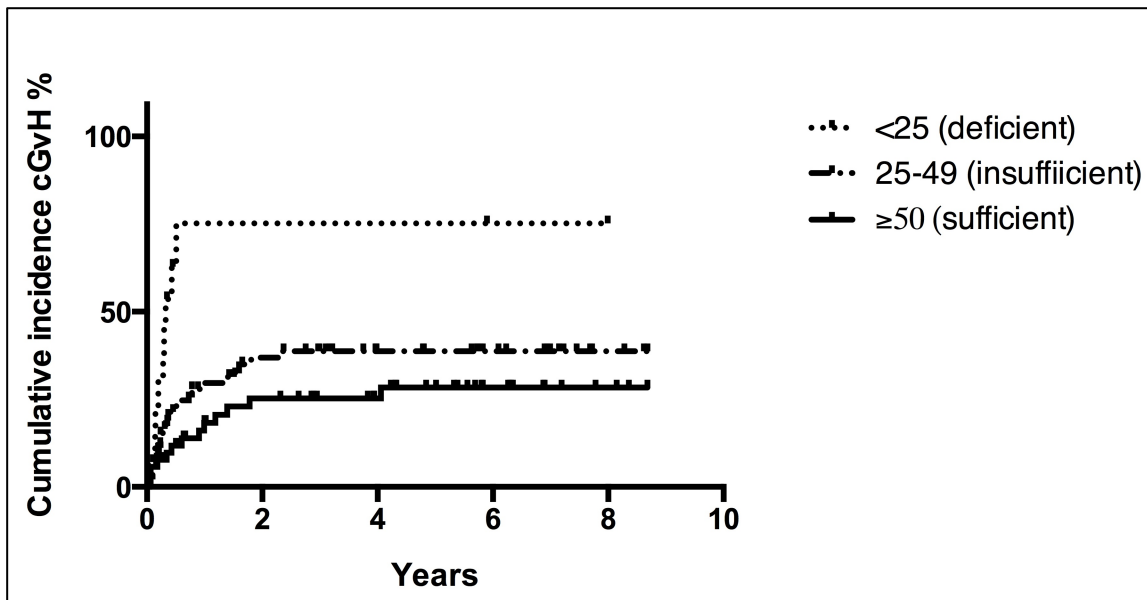
In the evaluated patients, a reduction in plasma CXCL9 and CXCL10 corresponded to clinical response, whilst non-responders increased their plasma levels of both chemokines during the course of the treatment. What makes these chemokines especially promising as biomarkers is that in five of the seven analysed patients this distinction between responders and non-responders was evident after only one MSC infusion, long before clinical response could be evaluated. This did not reach statistical significance among the few patients included, but will be explored further in future patient cohorts.

A problem common to all the MSC studies in this thesis is the small study cohort. Biomarker screening normally requires very large study cohorts, since corrections have to be made to account for type I errors due to multiple comparisons. However, when the aim is to identify biomarkers with a predictive value on the individual patient level, this small study cohort can be made into an advantage. Markers with a low predictive value will not be identified due to the low statistical power in the study and thus the identified candidate markers have a potential of being of real value. Whether these candidate markers do display a true correlation to response will need to be confirmed in further cohorts.

### 8.3 VITAMIN D DEFICIENCY (PAPER III)

Vitamin D is important for the function of the immune system (reviewed in 230) and deficiency has been associated with an increased risk of both autoimmune disease (104, 105, 231) and infection (232). In **paper III**, we performed a retrospective cohort study of 166 patients who had undergone HSCT at Karolinska University Hospital and where serum samples from immediately before HSCT were stored in a bio bank. Vitamin D levels at the time of HSCT were retrospectively measured and correlated to clinical outcome.

We demonstrated a higher risk of cGvHD in patients with low levels of vitamin D, with a threshold at 60 nmol/L and increasing incidence of cGvHD with decreasing vitamin D levels below this threshold (**Figure 5**). This confirmed previously published findings by Glotzbecker et al. (107) of an association between vitamin D deficiency and cGvHD.



**Figure 5. Correlation between vitamin D level and cGvHD incidence (paper III)**

Cumulative incidence of cGvHD, grade moderate-severe, in cohorts stratified by levels of 25-OH-D<sub>3</sub>, in nmol/L, prior to transplantation (N=139). Figure from von Bahr et al., BMT 2015.

In addition, we could see indications of a lower overall survival in patients with vitamin D deficiency and in a multivariate model low vitamin D level at HSCT was a significant risk factor for death. Relapse incidence did not differ between vitamin D deficient and sufficient patients, despite the difference in cGvHD incidence.

Infections during the first year following HSCT were recorded and correlated to vitamin D levels. After correction for multiple comparisons, CMV disease was positively correlated to low vitamin D levels in a multivariate model. Nine cases of CMV disease were recorded,

with all of the affected patients presenting with a vitamin D level before HSCT below the insufficiency level of 50 nmol/L.

As this was a retrospective study, all findings are associations and even though care has been taken to account for confounding factors, it cannot be determined from these data whether vitamin D supplementation would affect the risk. The next step would be to perform a prospective study, including patients before HSCT and randomizing them to either vitamin D supplementation or placebo.

#### **8.4 MSC TREATMENT OF CHRONIC GVHD (PAPER IV)**

We performed a clinical trial of multiple infusions of MSC in patients with cGvHD, refractory to or not tolerating standard therapy of calcineurin inhibitors plus high dose steroids. The primary endpoint was clinical response according to NIH criteria (43) at the end of treatment. Patients should receive a minimum of six infusions to be evaluated for response.

Eleven patients were included, one died of progressive cGvHD after only one infusion and one was taken off the study after three infusions because of a threatening CLL relapse. Of the nine evaluable patients, six were classified as responders and three as non-responders. The patients were followed in the study for 12 months after the last MSC infusion and clinically outside of the study for a median follow-up time of 38 months (12-55) from time of inclusion. The responders continued to show stable or declining symptoms for the entire follow-up period.

This clinical improvement was matched by a reduction in immunosuppression, with two patients completely off systemic immunosuppression and two more free from steroids and tapering calcineurin inhibitors. Patient-reported quality of life also improved in the responders, whilst it decreased in the non-responders. Taken together, these results indicate that repeated infusions of MSC could lead to a significant and durable reduction in cGvHD symptoms in the majority of patients.

The strengths of this study are the rigorous response evaluations, long follow-up time and the combination of physician-observed symptoms with patient-reported measures and biological analyses. Weaknesses are the small treatment group and the lack of a control group and thus no conclusions regarding efficacy of MSC treatment can be made. What we have learned from this study will however be of utmost importance to plan and set up a larger study. Based on the expected response rate we can make a power calculation to estimate the number of patients to include in treatment and control groups in order to be able to show a statistically significant effect.

One problem in such trial design is estimating the chance of spontaneous improvement in the control group. The randomized study of ECP by Flowers et al. (47) reports improvement in 28% of the control group at 12 weeks. Although this study was only focusing on skin GvHD,

this might be a relevant estimate. Using the response rate to MSC in our study (66%) and the response rate in the control group above, a basic power calculation aiming at 80% power indicates that we would need 25 patients in each group. This does not take into account the fact that some patients will likely be excluded during the study, in our study 2/11 (18%) and in the study by Flowers et al. 12%. If we account for a 15% drop out rate, we would need to include approximately 60 patients. Considering the scarcity of cGvHD patients, as further discussed in chapter 9, a study of this size would not be possible to conduct in a single site in Sweden. It would need a multi-centre approach, possibly including centres outside of Sweden as well.

## 9 CONCLUSION AND FUTURE DIRECTIONS

The main conclusions drawn within this thesis are:

- 1) That MSC treatment appears relatively safe, but is probably associated with an increased risk of infections, at least in previously immunosuppressed patients. Prophylactic treatments as well as close surveillance of patients is highly recommended.
- 2) Vitamin D deficiency at time of HSCT is associated with an increased risk of cGvHD, CMV disease and a lower overall survival. Whether these risks can be diminished by vitamin D supplementation should be evaluated in prospective, randomized trials.
- 3) Repeated infusions of MSC could lead to clinically significant, durable improvement in patients with severe refractory cGvHD.

Despite 15 years of clinical research in MSC therapy, no randomized trial has been published demonstrating an immunomodulatory effect by systemic MSC treatment. Using clinical response as the outcome in studies is naturally the most relevant measure, but also logistically challenging and dependent on large, randomized patient cohorts. Focusing the clinical therapeutic studies in the area of GvHD further complicates the situation, as both aGvHD and cGvHD pose major difficulties for clinical studies.

Both forms of GvHD are rare diseases, with a high estimate of incidence being approximately 50 patients with refractory aGvHD and 25 with refractory cGvHD per year in Sweden (based on incidence of aGvHD and cGvHD in **paper III**, responsiveness to steroids as reported by McMillan et al. (39) and Koc et al. (44) and the number of patients undergoing HSCT in Sweden 2015 (personal communication, prof. Per Ljungman)). This could be compared to a prevalence of 20 000 patients with Crohn's disease in Sweden (233).

aGvHD can be difficult to diagnose adequately, at least without good availability of colonoscopy and histopathological examination and is a rapidly progressing disease with high mortality. This forces physicians to often begin treating aggressively without a definite diagnosis, which in many cases excludes patients from being entered into clinical trials. Even more difficult is the randomization of patients to placebo, which can be deemed unethical in acute situations.

In cGvHD the urgency of diagnosis and initiation of treatment is not an issue, but instead the slow progress of the disease poses difficulties for the evaluation of response and also for the ethical questions regarding placebo. Response evaluation, finally, is a major obstacle in chronic but also to some extent in aGvHD, relying solely on clinical observations.

I believe there is a great need for well-executed randomized placebo-controlled studies on MSC in large patient cohorts, where an actual clinical effect could be demonstrated as proof-of-principle. For reasons mentioned above, the conditions best suited for randomized trials of MSC treatment might not be found in the HSCT setting. Instead, I believe that diagnoses, such as Crohn's disease or rheumatoid arthritis, with phase III trials on-going, might be the first where we could see efficacy data in placebo-controlled settings.

This does not mean to say that we should not aim for placebo-controlled studies in GvHD, but we should learn from the experiences made in other diseases and collaborate if possible. In these studies, large effort could be dedicated to finding possible biomarkers for response and preferably laboratory studies on the MSCs used clinically could be performed using a variety of immunological assays to find surrogate markers for clinical response. If an immunological assay could be identified that correlated with clinical efficacy, this would be immensely useful to compare different MSC preparations in the purpose of improving the MSC product for clinical use.





## 10 ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to all the people who have helped and supported me during my work on this thesis.

**Katarina**, thank you so much for believing in me and welcoming me into your world, as a student and a colleague.

A great big thank you to all, past and present, in the Le Blanc group, **Lili, Lena, Berit, Carolina, Maritha, Anton, Cecilia, Rachael, Guido, Regina, Jessica, Nina, Ellen, Greg, Caroline, Srinivasa** and **Erik** for support, interesting discussions and great company. And **Lindsay**, thank you for always being there, for guiding me through the intricacies of laboratory analysis, for accepting to be my co-supervisor and for sharing my passion for gardening!

My patients living with chronic GvHD. Every time I see you I know why I want to pursue this research, and my motivation surges. Without you this thesis would not have been possible.

**Olle Ringdén** and **Mats Remberger**, thank you for being my co-supervisors and for introducing me to GvHD research.

**Per Ljungman**, for being such an amazing expert in the field and always ready to share your knowledge. I look forward to working with you in the clinic soon again.

**Birgitta Sander**, for always welcoming me even though you are always busy, and taking an active interest in my work. I haven't given up on our study on bone marrow FACS, even though it didn't make it to the thesis.

**Karin Garming-Legert**, for all your help with the oral GvHD patients and your enthusiasm for the study.

**EJ Shpall**, thank you for agreeing to be my external opponent and for flying all the way to Sweden to discuss my thesis.

Research nurses **Carina** and **Eva**, and all the amazing nurses in the allo unit, **Kerstin, Anna, Carina** and **Klas**, for your care for the patients and your enthusiasm and support whenever I come asking for help.

My co-authors for all your efforts in improving our joint publications.

All my colleagues at the haematology clinic, especially **Johanna, Andreas** and my clinical supervisor **Stefan**, for helping out and encouraging me through clinic and research.

All researchers at HERM, for interesting questions and discussions at seminars and retreats.

**Jonas Mattson** and the staff at CAST, for your dedication to the patients and for welcoming me at your clinic. I hope that I will stay longer next time!

All my friends, for taking me sailing, on diving trips and to parties. Bokklubben, for all the interesting discussions and for forcing me to read fiction instead of research papers. **Ellen** and **Agri**, for writing company and for an introduction to other research areas than medicine.

**Pappa**, som introducerade mig till forskningen i form av ålar som ständigt smet. Jag fastnade för nyfikenheten, men aktar mig sedan dess för lab-forskning. **Mamma**, som alltid finns där och alltid tror på mig så länge jag inte blir kirurg. **Karin**, min fantastiska lillasyster som jag ständigt försöker imponera på. **Mormor** och **morfar**, som bröt ny mark och gjorde den akademiska världen tillgänglig för oss som kom efter. Min älskade, saknade moster **Bisse** för ditt ständiga stöd, alla dagishämtningar och för ditt osvikliga leende. **Åsa**, **Sara** och alla andra som funnits där genom Bisses sjukdom och bortgång.

**Martin**, tack för att du orkar stötta mig när jag behöver kämpa och stoppar mig när jag behöver vila. **Rasmus**, för glädjen, nyfikenheten och din fascination för makrofager.

## 11 REFERENCES

1. **McCulloch EA, Siminovitch L, Till JE, Russell ES, Bernstein SE.** The cellular basis of the genetically determined hemopoietic defect in anemic mice of genotype Sl-Sld. *Blood*. 1965 Oct;26(4):399–410.
2. **Lorenz E, Uphoff D, Reid TR, Shelton E.** Modification of irradiation injury in mice and guinea pigs by bone marrow injections. *J Natl Cancer Inst*. 1951 Aug;12(1):197–201.
3. **Thomas ED, Lochte HL, Lu WC, Ferrebee JW.** Intravenous Infusion of Bone Marrow in Patients Receiving Radiation and Chemotherapy. *New England Journal of Medicine*. 1957 Sep 12;257(11):491–6.
4. **Bortin MM.** A compendium of reported human bone marrow transplants. *Transplantation*. 1970 Jun;9(6):571–87.
5. **Turner D.** The human leucocyte antigen (HLA) system. *Vox Sang*. 2004 Jul;87 Suppl1:87–90.
6. **Thomas ED, Buckner CD, Banaji M, Clift RA, Fefer A, Flournoy N, et al.** One hundred patients with acute leukemia treated by chemotherapy, total body irradiation, and allogeneic marrow transplantation. *Blood*. 1977 Apr;49(4):511–33.
7. **Gratwohl A, Pasquini MC, Aljurf M, Atsuta Y, Baldomero H, Foeken L, et al.** One million haemopoietic stem-cell transplants: a retrospective observational study. *Lancet Haematol*. 2015 Mar;2(3):e91–100.
8. **Gorin NC, Najman A, Duhamel G.** Autologous bone-marrow transplantation in acute myelocytic leukaemia. *Lancet*. 1977 May 14;1(8020):1050.
9. **Roopenian D, Choi EY, Brown A.** The immunogenomics of minor histocompatibility antigens. *Immunological Reviews*. 2002 Dec 1;190(1):86–94.
10. **Barrett AJ.** Conditioning regimens for allogeneic stem cell transplants. *Curr Opin Hematol*. 2000 Nov;7(6):339–42.
11. **Bacigalupo A.** Hematopoietic stem cell transplants after reduced intensity conditioning regimen (RI-HSCT): report of a workshop of the European group for Blood and Marrow Transplantation (EBMT). *Bone Marrow Transplant*. 2000 Apr;25(8):803–5.
12. **Holtick U, Albrecht M, Chemnitz JM, Theurich S, Skoetz N, Scheid C, et al.** Bone marrow versus peripheral blood allogeneic haematopoietic stem cell transplantation for haematological malignancies in adults. *Cochrane Database Syst Rev*. 2014;4:CD010189.
13. **Danby R, Rocha V.** Improving engraftment and immune reconstitution in umbilical cord blood transplantation. *Front Immunol*. 2014;5:68.
14. **Lapidot T, Dar A, Kollet O.** How do stem cells find their way home? *Blood*. 2005 Sep 15;106(6):1901–10.
15. **Ruutu T, Gratwohl A, de Witte T, Afanasyev B, Apperley J, Bacigalupo A, et al.** Prophylaxis and treatment of GVHD: EBMT–ELN working group recommendations for a standardized practice. *Bone Marrow Transplant*. 2014 Feb;49(2):168–73.
16. **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. *Transpl Infect Dis*. 2014 Feb;16(1):106–14.
17. **Wingard JR.** Fungal infections after bone marrow transplant. *Biol Blood Marrow Transplant*. 1999;5(2):55–68.
18. **Zimmerli W, Zarth A, Gratwohl A, Speck B.** Neutrophil function and pyogenic infections in bone marrow transplant recipients. *Blood*. 1991 Jan 15;77(2):393–9.
19. **Nakata K, Gotoh H, Watanabe J, Uetake T, Komuro I, Yuasa K, et al.** Augmented proliferation of human alveolar macrophages after allogeneic bone marrow transplantation. *Blood*. 1999 Jan 15;93(2):667–73.

20. **Petersen SL, Ryder LP, Björk P, Madsen HO, Heilmann C, Jacobsen N, et al.** A comparison of T-, B- and NK-cell reconstitution following conventional or nonmyeloablative conditioning and transplantation with bone marrow or peripheral blood stem cells from human leucocyte antigen identical sibling donors. *Bone Marrow Transplant.* 2003 Jun 23;32(1):65–72.
21. **Gress RE, Emerson SG, Drobycki WR.** Immune reconstitution: how it should work, what's broken, and why it matters. *Biol Blood Marrow Transplant.* 2010 Jan;16(1 Suppl):S133–7.
22. **Clave E, Busson M, Douay C, Peffault de Latour R, Berrou J, Rabian C, et al.** Acute graft-versus-host disease transiently impairs thymic output in young patients after allogeneic hematopoietic stem cell transplantation. *Blood.* 2009 Jun 18;113(25):6477–84.
23. **Small TN, Keever CA, Weiner-Fedus S, Heller G, O'Reilly RJ, Flomenberg N.** B-cell differentiation following autologous, conventional, or T-cell depleted bone marrow transplantation: a recapitulation of normal B-cell ontogeny. *Blood.* 1990 Oct 15;76(8):1647–56.
24. **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 Jan 1;91(1):78–83.
25. **Uhlen 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 Feb;99(2):346–52.
26. **Blennow O, Fjaertoft G, Winiarski J, Ljungman P, Mattsson J, Remberger M.** Varicella-zoster reactivation after allogeneic stem cell transplantation without routine prophylaxis--the incidence remains high. *Biol Blood Marrow Transplant.* 2014 Oct;20(10):1646–9.
27. **Derouin F, Pelloux H, ESCMID Study Group on Clinical Parasitology.** Prevention of toxoplasmosis in transplant patients. *Clin Microbiol Infect.* 2008 Dec;14(12):1089–101.
28. **Chemaly RF, Shah DP, Boeckh MJ.** Management of respiratory viral infections in hematopoietic cell transplant recipients and patients with hematologic malignancies. *Clin Infect Dis.* 2014 Nov 15;59 Suppl 5:S344–51.
29. **Williams KM, Ahn KW, Chen M, Aljurf MD, Agwu AL, Chen AR, et al.** The incidence, mortality and timing of *Pneumocystis jirovecii* pneumonia after hematopoietic cell transplantation: a CIBMTR analysis. *Bone Marrow Transplant.* 2016 Jan 4;
30. **Harrison N, Mitterbauer M, Tobudic S, Kalhs P, Rabitsch W, Greinix H, et al.** Incidence and characteristics of invasive fungal diseases in allogeneic hematopoietic stem cell transplant recipients: a retrospective cohort study. *BMC Infect Dis.* 2015;15(1):584.
31. **Kernan NA, Collins NH, Juliano L, Cartagena T, Dupont B, O'Reilly RJ.** Clonable T lymphocytes in T cell-depleted bone marrow transplants correlate with development of graft-v-host disease. *Blood.* 1986 Sep;68(3):770–3.
32. **Matzinger P.** The danger model: a renewed sense of self. *Science.* 2002 Apr 12;296(5566):301–5.
33. **Beelen DW, Haralambie E, Brandt H, Linzenmeier G, Muller KD, Quabeck K, et al.** Evidence that sustained growth suppression of intestinal anaerobic bacteria reduces the risk of acute graft-versus-host disease after sibling marrow transplantation. *Blood.* 1992 Nov 15;80(10):2668–76.
34. **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 May 1;95(9):2754–9.
35. **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 Oct 15;76(8):1464–72.

36. **Einsele H, Ehniger G, Hebart H, Weber P, Dette S, Link H, et al.** Incidence of local CMV infection and acute intestinal GVHD in marrow transplant recipients with severe diarrhoea. *Bone Marrow Transplant.* 1994 Dec;14(6):955–63.
37. **Przepiorka D, Weisdorf D, Martin P, Klingemann HG, Beatty P, Hows J, et al.** 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant.* 1995 Jun;15(6):825–8.
38. **Cahn J-Y, 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 Société Française de Greffe de Moëlle et Thérapie Cellulaire (SFGM-TC), Dana Farber Cancer Institute (DFCI), and International Bone Marrow Transplant Registry (IBMTR) prospective study. *Blood.* 2005 Aug 15;106(4):1495–500.
39. **MacMillan ML, Weisdorf DJ, Wagner JE, DeFor TE, Burns LJ, Ramsay NKC, et al.** Response of 443 patients to steroids as primary therapy for acute graft-versus-host disease: comparison of grading systems. *Biol Blood Marrow Transplant.* 2002;8(7):387–94.
40. **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 Dec;11(12):945–56.
41. **Pavletic SZ, Martin P, Lee SJ, Mitchell S, Jacobsohn D, Cowen EW, et al.** Measuring therapeutic response in chronic graft-versus-host disease: National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: IV. Response Criteria Working Group report. *Biol Blood Marrow Transplant.* 2006 Mar;12(3):252–66.
42. **Jagasia MH, Greinix HT, Arora M, Williams KM, Wolff D, Cowen EW, et al.** National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: I. The 2014 Diagnosis and Staging Working Group report. *Biol Blood Marrow Transplant.* 2015 Mar;21(3):389–401.e1.
43. **Lee SJ, Wolff D, Kitko C, Koreth J, Inamoto Y, Jagasia M, et al.** Measuring Therapeutic Response in Chronic Graft-versus-Host Disease. National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: IV. The 2014 Response Criteria Working Group Report. *Biol Blood Marrow Transplant.* 2015 Jun;21(6):984–99.
44. **Koc S, Leisenring W, Flowers MED, Anasetti C, Deeg HJ, Nash RA, et al.** Therapy for chronic graft-versus-host disease: a randomized trial comparing cyclosporine plus prednisone versus prednisone alone. *Blood.* 2002 Jul 1;100(1):48–51.
45. **Wolff D, Schleuning M, von Harsdorf S, Bacher U, Gerbitz A, Stadler M, et al.** Consensus Conference on Clinical Practice in Chronic GVHD: Second-Line Treatment of Chronic Graft-versus-Host Disease. *Biol Blood Marrow Transplant.* 2011 Jan;17(1):1–17.
46. **Malik MI, Litzow M, Hogan W, Patnaik M, Murad MH, Prokop LJ, et al.** Extracorporeal photopheresis for chronic graft-versus-host disease: a systematic review and meta-analysis. *Blood Res.* 2014 Jun;49(2):100–6.
47. **Flowers MED, Apperley JF, van Besien K, Elmaagacli A, Grigg A, Reddy V, et al.** A multicenter prospective phase 2 randomized study of extracorporeal photopheresis for treatment of chronic graft-versus-host disease. *Blood.* 2008 Oct 1;112(7):2667–74.
48. **Ruutu T, van Biezen A, Hertenstein B, Henseler A, Garderet L, Passweg J, et al.** Prophylaxis and treatment of GVHD after allogeneic haematopoietic SCT: a survey of centre strategies by the European Group for Blood and Marrow Transplantation. *Bone Marrow Transplant.* 2012 Nov;47(11):1459–64.
49. **Matsuoka K, Koreth J, Kim HT, O. Gregory Bascug, McDonough S, Kawano Y, et al.** Low-dose interleukin-2 therapy restores regulatory T cell homeostasis in patients with chronic graft-versus-host disease. *Sci Transl Med.* 2013 Apr 3;5(179):179ra43.

50. **Pai C-CS, Chen M, Mirsoian A, Grossenbacher SK, Tellez J, Ames E, et al.** Treatment of chronic graft-versus-host disease with bortezomib. *Blood*. 2014 Sep 4;124(10):1677–88.
51. **Kansu E.** The pathophysiology of chronic graft-versus-host disease. *Int J Hematol*. 2004 Apr;79(3):209–15.
52. **Brüggen M-C, Klein I, Greinix H, Bauer W, Kuzmina Z, Rabitsch W, et al.** Diverse T-cell responses characterize the different manifestations of cutaneous graft-versus-host disease. *Blood*. 2014 Jan 9;123(2):290–9.
53. **Hill GR, Olver SD, Kuns RD, Varelias A, Raffelt NC, Don AL, et al.** Stem cell mobilization with G-CSF induces type 17 differentiation and promotes scleroderma. *Blood*. 2010 Aug 5;116(5):819–28.
54. **Matsuoka K, Kim HT, McDonough S, Bascug G, Warshauer B, Koreth J, et al.** Altered regulatory T cell homeostasis in patients with CD4+ lymphopenia following allogeneic hematopoietic stem cell transplantation. *J Clin Invest*. 2010 May;120(5):1479–93.
55. **Matsuoka K, Koreth J, Kim HT, O. Gregory Bascug, McDonough S, Kawano Y, et al.** Low-dose interleukin-2 therapy restores regulatory T cell homeostasis in patients with chronic graft-versus-host disease. *Sci Transl Med*. 2013 Apr 3;5(179):179ra43.
56. **Shulman Z, Gitlin AD, Weinstein JS, Lainez B, Esplugues E, Flavell RA, et al.** Dynamic signaling by T follicular helper cells during germinal center B cell selection. *Science*. 2014 Aug 29;345(6200):1058–62.
57. **Ueno H, Banchereau J, Vinuesa CG.** Pathophysiology of T follicular helper cells in humans and mice. *Nat Immunol*. 2015 Feb;16(2):142–52.
58. **Forcade E, Kim HT, Cutler CS, Wang KS, Ho VT, Koreth J, et al.** Activated Circulating T Follicular Helper Cells with Increased Function during Chronic Graft Versus Host Disease after Allogeneic Stem Cell Transplantation. *Blood*. 2015 Dec 3;126(23):924–924.
59. **Sarantopoulos S, Ritz J.** Aberrant B-cell homeostasis in chronic GVHD. *Blood*. 2015 Mar 12;125(11):1703–7.
60. **Storek J, Witherspoon RP, Webb D, Storb R.** Lack of B cell precursors in marrow transplant recipients with chronic graft-versus-host disease. *American Journal of Hematology*. 1996;52(2):82–9.
61. **Sarantopoulos S, Stevenson KE, Kim HT, Bhuiya NS, Cutler CS, Soiffer RJ, et al.** High levels of B-cell activating factor in patients with active chronic graft-versus-host disease. *Clin Cancer Res*. 2007 Oct 15;13(20):6107–14.
62. **Thien M, Phan TG, Gardam S, Amesbury M, Basten A, Mackay F, et al.** Excess BAFF rescues self-reactive B cells from peripheral deletion and allows them to enter forbidden follicular and marginal zone niches. *Immunity*. 2004 Jun;20(6):785–98.
63. **Chan EY, Lawton JW, Lie AK, Lau CS.** Autoantibody formation after allogeneic bone marrow transplantation: correlation with the reconstitution of CD5+ B cells and occurrence of graft-versus-host disease. *Pathology*. 1997 May;29(2):184–8.
64. **Tazzari PL, Gobbi M, Zauli D, Tassinari A, Crespi C, Miserocchi F, et al.** Close association between antibodies to cytoskeletal intermediate filaments, and chronic graft-versus-host disease. *Transplantation*. 1987 Aug;44(2):234–6.
65. **Svegliati S, Olivieri A, Campelli N, Luchetti M, Poloni A, Trappolini S, et al.** Stimulatory autoantibodies to PDGF receptor in patients with extensive chronic graft-versus-host disease. *Blood*. 2007 Jul 1;110(1):237–41.
66. **Martin SJ, Audrain MA, Oksman F, Ecoiffier M, Attal M, Milpied N, et al.** Antineutrophil cytoplasmic antibodies (ANCA) in chronic graft-versus-host disease after allogeneic bone marrow transplantation. *Bone Marrow Transplant*. 1997 Jul;20(1):45–8.
67. **Shimabukuro-Vornhagen A, Hallek MJ, Storb RF, Bergwelt-Baildon MS von.** The role of B cells in the pathogenesis of graft-versus-host disease. *Blood*. 2009 Mar 12;114(24):4919–27.

68. **Allen JL, Fore MS, Wooten J, Roehrs PA, Bhuiya NS, Hoffert T, et al.** B cells from patients with chronic GVHD are activated and primed for survival via BAFF-mediated pathways. *Blood*. 2012 Sep 20;120(12):2529–36.
69. **Greinix HT, Pohlreich D, Kouba M, Körmöczi U, Lohmann I, Feldmann K, et al.** Elevated numbers of immature/transitional CD21<sup>-</sup> B lymphocytes and deficiency of memory CD27<sup>+</sup> B cells identify patients with active chronic graft-versus-host disease. *Biol Blood Marrow Transplant*. 2008 Feb;14(2):208–19.
70. **Kuzmina Z, Krenn K, Petkov V, Körmöczi U, Weigl R, Rottal A, et al.** CD19(+)CD21(low) B cells and patients at risk for NIH-defined chronic graft-versus-host disease with bronchiolitis obliterans syndrome. *Blood*. 2013 Mar 7;121(10):1886–95.
71. **Khoder A, Sarvaria A, Alsuliman A, Chew C, Sekine T, Cooper N, et al.** Regulatory B cells are enriched within the IgM memory and transitional subsets in healthy donors but are deficient in chronic GVHD. *Blood*. 2014 Sep 25;124(13):2034–45.
72. **Sarantopoulos S, Stevenson KE, Kim HT, Cutler CS, Bhuiya NS, Schowalter M, et al.** Altered B-cell homeostasis and excess BAFF in human chronic graft-versus-host disease. *Blood*. 2009 Apr 16;113(16):3865–74.
73. **Isnardi I, Ng Y-S, Menard L, Meyers G, Saadoun D, Srdanovic I, et al.** Complement receptor 2/CD21<sup>-</sup> human naive B cells contain mostly autoreactive unresponsive clones. *Blood*. 2010 Jun 17;115(24):5026–36.
74. **Hinz B, Phan SH, Thannickal VJ, Galli A, Bochaton-Piallat M-L, Gabbiani G.** The Myofibroblast. *The American Journal of Pathology*. 2007 Jun;170(6):1807–16.
75. **Murray LA, Chen Q, Kramer MS, Hesson DP, Argentieri RL, Peng X, et al.** TGF-beta driven lung fibrosis is macrophage dependent and blocked by Serum amyloid P. *Int J Biochem Cell Biol*. 2011 Jan;43(1):154–62.
76. **Pesce JT, Ramalingam TR, Mentink-Kane MM, Wilson MS, El Kasmi KC, Smith AM, et al.** Arginase-1-expressing macrophages suppress Th2 cytokine-driven inflammation and fibrosis. *PLoS Pathog*. 2009 Apr;5(4):e1000371.
77. **Lech M, Anders H-J.** Macrophages and fibrosis: How resident and infiltrating mononuclear phagocytes orchestrate all phases of tissue injury and repair. *Biochim Biophys Acta*. 2013 Jul;1832(7):989–97.
78. **Khalil N, Berezney O, Sporn M, Greenberg AH.** Macrophage production of transforming growth factor beta and fibroblast collagen synthesis in chronic pulmonary inflammation. *J Exp Med*. 1989 Sep 1;170(3):727–37.
79. **Duffield JS, Forbes SJ, Constandinou CM, Clay S, Partolina M, Vuthoori S, et al.** Selective depletion of macrophages reveals distinct, opposing roles during liver injury and repair. *J Clin Invest*. 2005 Jan;115(1):56–65.
80. **Spits H, Di Santo JP.** The expanding family of innate lymphoid cells: regulators and effectors of immunity and tissue remodeling. *Nat Immunol*. 2011 Jan;12(1):21–7.
81. **Hams E, Bermingham R, Fallon PG.** Macrophage and Innate Lymphoid Cell Interplay in the Genesis of Fibrosis. *Front Immunol*. 2015;6:597.
82. **Hams E, Armstrong ME, Barlow JL, Saunders SP, Schwartz C, Cooke G, et al.** IL-25 and type 2 innate lymphoid cells induce pulmonary fibrosis. *Proc Natl Acad Sci USA*. 2014 Jan 7;111(1):367–72.
83. **Halim TYF, Steer CA, Mathä L, Gold MJ, Martinez-Gonzalez I, McNagny KM, et al.** Group 2 innate lymphoid cells are critical for the initiation of adaptive T helper 2 cell-mediated allergic lung inflammation. *Immunity*. 2014 Mar 20;40(3):425–35.
84. **Teunissen MBM, Munneke JM, Bernink JH, Spuls PI, Res PCM, te Velde A, et al.** Composition of Innate Lymphoid Cell Subsets in the Human Skin: Enrichment of NCR<sup>+</sup> ILC3 in Lesional Skin and Blood of Psoriasis Patients. *Journal of Investigative Dermatology*. 2014 Sep;134(9):2351–60.

85. **Nardelli B, Belvedere O, Roschke V, Moore PA, Olsen HS, Migone TS, et al.** Synthesis and release of B-lymphocyte stimulator from myeloid cells. *Blood*. 2001 Jan 1;97(1):198–204.
86. **Craxton A, Magaletti D, Ryan EJ, Clark EA.** Macrophage- and dendritic cell-dependent regulation of human B-cell proliferation requires the TNF family ligand BAFF. *Blood*. 2003 Jun 1;101(11):4464–71.
87. **Yoshimoto K, Tanaka M, Kojima M, Setoyama Y, Kameda H, Suzuki K, et al.** Regulatory mechanisms for the production of BAFF and IL-6 are impaired in monocytes of patients of primary Sjögren's syndrome. *Arthritis Res Ther*. 2011;13(5):R170.
88. **François A, Chatelus E, Wachsmann D, Sibilia J, Bahram S, Alsaleh G, et al.** B lymphocytes and B-cell activating factor promote collagen and profibrotic markers expression by dermal fibroblasts in systemic sclerosis. *Arthritis Res Ther*. 2013;15(5):R168.
89. **Anasetti C, Beatty PG, Storb R, Martin PJ, Mori M, Sanders JE, et al.** Effect of HLA incompatibility on graft-versus-host disease, relapse, and survival after marrow transplantation for patients with leukemia or lymphoma. *Hum Immunol*. 1990 Oct;29(2):79–91.
90. **Weisdorf D, Hakke R, Blazar B, Miller W, McGlave P, Ramsay N, et al.** Risk factors for acute graft-versus-host disease in histocompatible donor bone marrow transplantation. *Transplantation*. 1991 Jun;51(6):1197–203.
91. **Gale RP, Bortin MM, van Bekkum DW, Biggs JC, Dicke KA, Gluckman E, et al.** Risk factors for acute graft-versus-host disease. *Br J Haematol*. 1987 Dec;67(4):397–406.
92. **Cutler C, Giri S, Jeyapalan S, Paniagua D, Viswanathan A, Antin JH.** Acute and chronic graft-versus-host disease after allogeneic peripheral-blood stem-cell and bone marrow transplantation: a meta-analysis. *J Clin Oncol*. 2001 Aug 15;19(16):3685
93. **Weisdorf D, Eapen M, Ruggeri A, Zhang M-J, Zhong X, Brunstein C, et al.** Alternative Donor Transplantation for Older Patients with Acute Myeloid Leukemia in First Complete Remission: A Center for International Blood and Marrow Transplant Research-Eurocord Analysis. *Biology of Blood and Marrow Transplantation*. 2014 Jun;20(6):816–22.
94. **Oran B, Cao K, Saliba RM, Rezvani K, de Lima M, Ahmed S, et al.** Better allele-level matching improves transplant-related mortality after double cord blood transplantation. *Haematologica*. 2015 Oct;100(10):1361–70.
95. **Martin PS, Li S, Nikiforow S, Alyea EP, Antin JH, Armand P, et al.** Infused total nucleated cell dose is a better predictor of transplant outcomes than CD34+ cell number in reduced-intensity mobilized peripheral blood allogeneic hematopoietic cell transplantation. *Haematologica*. 2016 Jan 14;
96. **Nash RA, Pepe MS, Storb R, Longton G, Pettinger M, Anasetti C, et al.** Acute graft-versus-host disease: analysis of risk factors after allogeneic marrow transplantation and prophylaxis with cyclosporine and methotrexate. *Blood*. 1992 Oct 1;80(7):1838–45.
97. **Storb R, Prentice RL, Sullivan KM, Shulman HM, Deeg HJ, Doney KC, et al.** Predictive factors in chronic graft-versus-host disease in patients with aplastic anemia treated by marrow transplantation from HLA-identical siblings. *Ann Intern Med*. 1983 Apr;98(4):461–6.
98. **Ghayur T, Seemayer TA, Xenocostas A, Lapp WS.** Complete sequential regeneration of graft-vs.-host-induced severely dysplastic thymuses. Implications for the pathogenesis of chronic graft-vs.-host disease. *Am J Pathol*. 1988 Oct;133(1):39–46.
99. **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 Mar 17;117(11):3214–9.



100. **Benrashid M, Moyers K, Mohty M, Savani BN.** Vitamin D deficiency, autoimmunity, and graft-versus-host-disease risk: Implication for preventive therapy. *Exp Hematol.* 2012 Apr;40(4):263–7.
101. **Joseph RW, Alousi A, Konda B, Komanduri K, Neumann J, Trevino C, et al.** High incidence of vitamin D deficiency in patients undergoing allogeneic stem cell transplantation. *Am J Hematol.* 2011 Nov;86(11):954–6.
102. **Chun RF, Liu PT, Modlin RL, Adams JS, Hewison M.** Impact of vitamin D on immune function: lessons learned from genome-wide analysis. *Front Physiol.* 2014;5:151.
103. **Penna G, Adorini L.** 1 Alpha,25-dihydroxyvitamin D3 inhibits differentiation, maturation, activation, and survival of dendritic cells leading to impaired alloreactive T cell activation. *J Immunol.* 2000 Mar 1;164(5):2405–11.
104. **Kragt J, van Amerongen B, Killestein J, Dijkstra C, Uitdehaag B, Polman C, et al.** Higher levels of 25-hydroxyvitamin D are associated with a lower incidence of multiple sclerosis only in women. *Mult Scler.* 2009 Jan;15(1):9–15.
105. **Littorin B, Blom P, Schölin A, Arnqvist HJ, Blohmé G, Bolinder J, et al.** Lower levels of plasma 25-hydroxyvitamin D among young adults at diagnosis of autoimmune type 1 diabetes compared with control subjects: results from the nationwide Diabetes Incidence Study in Sweden (DISS). *Diabetologia.* 2006 Dec;49(12):2847–52.
106. **Bitetto D, Fabris C, Falleti E, Fornasiere E, Fumolo E, Fontanini E, et al.** Vitamin D and the risk of acute allograft rejection following human liver transplantation. *Liver Int.* 2010 Mar;30(3):417–44.
107. **Glantzbecker B, Ho VT, Aldridge J, Kim HT, Horowitz G, Ritz J, et al.** Low levels of 25-hydroxyvitamin D before allogeneic hematopoietic SCT correlate with the development of chronic GVHD. *Bone Marrow Transplant.* 2013 Apr;48(4):593–7.
108. **Hansson MEA, Norlin A-C, Omazic B, Wikström A-C, Bergman P, Winiarski J, et al.** Vitamin D Levels Affect Outcome in Pediatric Hematopoietic Stem Cell Transplantation. *Biology of Blood and Marrow Transplantation.* 2014 Oct;20(10):1537–43.
109. **Barnes DW, Loutit JF.** Treatment of murine leukaemia with x-rays and homologous bone marrow. II. *Br J Haematol.* 1957 Jul;3(3):241–52.
110. **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 Jun;69(2):239–45.
111. **Horowitz MM, Gale RP, Sondel PM, Goldman JM, Kersey J, Kolb HJ, et al.** Graft-versus-leukemia reactions after bone marrow transplantation. *Blood.* 1990 Feb 1;75(3):555–62.
112. **Storb R, Gyurkocza B, Storer BE, Sorrow ML, Blume K, Niederwieser D, et al.** Graft-Versus-Host Disease and Graft-Versus-Tumor Effects After Allogeneic Hematopoietic Cell Transplantation. *JCO.* 2013 Apr 20;31(12):1530–8.
113. **Weisdorf D, Zhang M-J, Arora M, Horowitz MM, Rizzo JD, Eapen M.** Graft-versus-host disease induced graft-versus-leukemia effect: greater impact on relapse and disease-free survival after reduced intensity conditioning. *Biol Blood Marrow Transplant.* 2012 Nov;18(11):1727–33.
114. **Boyiadzis M, Arora M, Klein JP, Hassebroek A, Hemmer M, Urbano-Ispizua A, et al.** Impact of Chronic Graft-versus-Host Disease on Late Relapse and Survival on 7,489 Patients after Myeloablative Allogeneic Hematopoietic Cell Transplantation for Leukemia. *Clin Cancer Res.* 2015 Jan 5;21(9):2020–8.
115. **Pasquini MC, Devine S, Mendizabal A, Baden LR, Wingard JR, Lazarus HM, et al.** Comparative Outcomes of Donor Graft CD34+ Selection and Immune Suppressive Therapy As Graft-Versus-Host Disease Prophylaxis for Patients With Acute Myeloid Leukemia in Complete Remission Undergoing HLA-Matched Sibling Allogeneic Hematopoietic Cell Transplantation. *JCO.* 2012 Oct 9;30(26):3194–201.

116. **Basara N, Baurmann H, Kolbe K, Yaman A, Labopin M, Burchardt A, et al.** Antithymocyte globulin for the prevention of graft-versus-host disease after unrelated hematopoietic stem cell transplantation for acute myeloid leukemia: results from the multicenter German cooperative study group. *Bone Marrow Transplant.* 2005 May;35(10):1011–8.
117. **Mutis T, Verdijk R, Schrama E, Esendam B, Brand A, Goulmy E.** Feasibility of immunotherapy of relapsed leukemia with ex vivo-generated cytotoxic T lymphocytes specific for hematopoietic system-restricted minor histocompatibility antigens. *Blood.* 1999 Apr 1;93(7):2336–41.
118. **Neelapu SS, Munshi NC, Jagannath S, Watson TM, Pennington R, Reynolds C, et al.** Tumor antigen immunization of sibling stem cell transplant donors in multiple myeloma. *Bone Marrow Transplant.* 2005 Aug;36(4):315–23.
119. **Friedenstein AJ, Petrakova KV, Kurolesova AI, Frolova GP.** Heterotopic of bone marrow. Analysis of precursor cells for osteogenic and hematopoietic tissues. *Transplantation.* 1968 Mar;6(2):230–47.
120. **Caplan AI.** Mesenchymal stem cells. *J Orthop Res.* 1991 Sep;9(5):641–50.
121. **Erices A, Conget P, Minguell JJ.** Mesenchymal progenitor cells in human umbilical cord blood. *Br J Haematol.* 2000 Apr;109(1):235–42.
122. **Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, et al.** Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell.* 2002 Dec;13(12):4279–95.
123. **Vishnubalaji R, Al-Nbaheen M, Kadalmani B, Aldahmash A, Ramesh T.** Skin-derived multipotent stromal cells--an archrival for mesenchymal stem cells. *Cell Tissue Res.* 2012 Oct;350(1):1–12.
124. **Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, et al.** Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy.* 2006;8(4):315–7.
125. **Horwitz EM, Le Blanc K, Dominici M, Mueller I, Slaper-Cortenbach I, Marini FC, et al.** Clarification of the nomenclature for MSC: The International Society for Cellular Therapy position statement. *Cytotherapy.* 2005;7(5):393–5.
126. **Krampera M.** Mesenchymal stromal cell “licensing”: a multistep process. *Leukemia.* 2011 Sep;25(9):1408–14.
127. **Benvenuto F, Ferrari S, Gerdoni E, Gualandi F, Frassoni F, Pistoia V, et al.** Human mesenchymal stem cells promote survival of T cells in a quiescent state. *Stem Cells.* 2007 Jul;25(7):1753–60.
128. **Tabera S, Pérez-Simón JA, Díez-Campelo M, Sánchez-Abarca LI, Blanco B, López A, et al.** The effect of mesenchymal stem cells on the viability, proliferation and differentiation of B-lymphocytes. *Haematologica.* 2008 Sep;93(9):1301–9.
129. **Krampera M, Cosmi L, Angeli R, Pasini A, Liotta F, Andreini A, et al.** Role for interferon-gamma in the immunomodulatory activity of human bone marrow mesenchymal stem cells. *Stem Cells.* 2006 Feb;24(2):386–98.
130. **Chan JL, Tang KC, Patel AP, Bonilla LM, Pierobon N, Ponzio NM, et al.** Antigen-presenting property of mesenchymal stem cells occurs during a narrow window at low levels of interferon-gamma. *Blood.* 2006 Jun 15;107(12):4817–24.
131. **Ren G, Zhang L, Zhao X, Xu G, Zhang Y, Roberts AI, et al.** Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide. *Cell Stem Cell.* 2008 Feb 7;2(2):141–50.
132. **Waterman RS, Tomchuck SL, Henkle SL, Betancourt AM.** A new mesenchymal stem cell (MSC) paradigm: polarization into a pro-inflammatory MSC1 or an immunosuppressive MSC2 phenotype. *PLoS ONE.* 2010;5(4):e10088.
133. **Di Nicola M, Carlo-Stella C, Magni M, Milanese M, Longoni PD, Matteucci P, et al.** Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. *Blood.* 2002 May 15;99(10):3838–43.

134. **Aggarwal S, Pittenger MF.** Human mesenchymal stem cells modulate allogeneic immune cell responses. *Blood*. 2005 Feb 15;105(4):1815–22.
135. **Meisel R, Zibert A, Laryea M, Göbel U, Däubener W, Dilloo D.** Human bone marrow stromal cells inhibit allogeneic T-cell responses by indoleamine 2,3-dioxygenase-mediated tryptophan degradation. *Blood*. 2004 Jun 15;103(12):4619–21.
136. **Majumdar MK, Keane-Moore M, Buyaner D, Hardy WB, Moorman MA, McIntosh KR, et al.** Characterization and functionality of cell surface molecules on human mesenchymal stem cells. *J Biomed Sci*. 2003 Apr;10(2):228–41.
137. **Augello A, Tasso R, Negrini SM, Amateis A, Indiveri F, Cancedda R, et al.** Bone marrow mesenchymal progenitor cells inhibit lymphocyte proliferation by activation of the programmed death 1 pathway. *Eur J Immunol*. 2005 May 1;35(5):1482–90.
138. **Ghannam S, Pène J, Moquet-Torcy G, Torcy-Moquet G, Jorgensen C, Yssel H.** Mesenchymal stem cells inhibit human Th17 cell differentiation and function and induce a T regulatory cell phenotype. *J Immunol*. 2010 Jul 1;185(1):302–12.
139. **Maccario R, Podestà M, Moretta A, Cometa A, Comoli P, Montagna D, et al.** Interaction of human mesenchymal stem cells with cells involved in alloantigen-specific immune response favors the differentiation of CD4+ T-cell subsets expressing a regulatory/suppressive phenotype. *Haematologica*. 2005 Apr;90(4):516–25.
140. **Melief SM, Schrama E, Brugman MH, Tiemessen MM, Hoogduijn MJ, Fibbe WE, et al.** Multipotent stromal cells induce human regulatory T cells through a novel pathway involving skewing of monocytes toward anti-inflammatory macrophages. *Stem Cells*. 2013 Sep 1;31(9):1980–91.
141. **Németh K, Leelahavanichkul A, Yuen PST, Mayer B, Parmelee A, Doi K, et al.** Bone marrow stromal cells attenuate sepsis via prostaglandin E(2)-dependent reprogramming of host macrophages to increase their interleukin-10 production. *Nat Med*. 2009 Jan;15(1):42–9.
142. **Heldring N, Mäger I, Wood MJA, Le Blanc K, Andaloussi SEL.** Therapeutic Potential of Multipotent Mesenchymal Stromal Cells and Their Extracellular Vesicles. *Hum Gene Ther*. 2015 Aug;26(8):506–17.
143. **Valadi H, Ekström K, Bossios A, Sjöstrand M, Lee JJ, Lötvall JO.** Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat Cell Biol*. 2007 Jun;9(6):654–9.
144. **Lee C, Mitsialis SA, Aslam M, Vitali SH, Vergadi E, Konstantinou G, et al.** Exosomes mediate the cytoprotective action of mesenchymal stromal cells on hypoxia-induced pulmonary hypertension. *Circulation*. 2012 Nov 27;126(22):2601–11.
145. **Phinney DG, Di Giuseppe M, Njah J, Sala E, Shiva S, St Croix CM, et al.** Mesenchymal stem cells use extracellular vesicles to outsource mitophagy and shuttle microRNAs. *Nat Commun*. 2015;6:8472.
146. **Roberts AB, Sporn MB, Assoian RK, Smith JM, Roche NS, Wakefield LM, et al.** Transforming growth factor type beta: rapid induction of fibrosis and angiogenesis in vivo and stimulation of collagen formation in vitro. *Proc Natl Acad Sci USA*. 1986 Jun;83(12):4167–71.
147. **Ortiz LA, Gambelli F, McBride C, Gaupp D, Baddoo M, Kaminski N, et al.** Mesenchymal stem cell engraftment in lung is enhanced in response to bleomycin exposure and ameliorates its fibrotic effects. *Proc Natl Acad Sci USA*. 2003 Jul 8;100(14):8407–11.
148. **Ueno T, Nakashima A, Doi S, Kawamoto T, Honda K, Yokoyama Y, et al.** Mesenchymal stem cells ameliorate experimental peritoneal fibrosis by suppressing inflammation and inhibiting TGF- $\beta$ 1 signaling. *Kidney Int*. 2013 Aug;84(2):297–307.
149. **Wu Y, Peng Y, Gao D, Feng C, Yuan X, Li H, et al.** Mesenchymal stem cells suppress fibroblast proliferation and reduce skin fibrosis through a TGF- $\beta$ 3-dependent activation. *Int J Low Extrem Wounds*. 2015 Mar;14(1):50–62.

150. **Gregorini M, Corradetti V, Rocca C, Pattonieri EF, Valsania T, Milanesi S, et al.** Mesenchymal Stromal Cells Prevent Renal Fibrosis in a Rat Model of Unilateral Ureteral Obstruction by Suppressing the Renin-Angiotensin System via HuR. *PLoS ONE*. 2016;11(2):e0148542.
151. **Duffield JS, Forbes SJ, Constandinou CM, Clay S, Partolina M, Vuthoori S, et al.** Selective depletion of macrophages reveals distinct, opposing roles during liver injury and repair. *J Clin Invest*. 2005 Jan;115(1):56–65.
152. **Taylor AW.** Review of the activation of TGF-beta in immunity. *J Leukoc Biol*. 2009 Jan;85(1):29–33.
153. **Chang Z, Kishimoto Y, Hasan A, Welham NV.** TGF- $\beta$ 3 modulates the inflammatory environment and reduces scar formation following vocal fold mucosal injury in rats. *Dis Model Mech*. 2014 Jan;7(1):83–91.
154. **Qi Y, Jiang D, Sindrilaru A, Stegemann A, Schatz S, Treiber N, et al.** TSG-6 released from intradermally injected mesenchymal stem cells accelerates wound healing and reduces tissue fibrosis in murine full-thickness skin wounds. *J Invest Dermatol*. 2014 Feb;134(2):526–37.
155. **Le Blanc K, Tammik C, Rosendahl K, Zetterberg E, Ringdén O.** HLA expression and immunologic properties of differentiated and undifferentiated mesenchymal stem cells. *Exp Hematol*. 2003 Oct;31(10):890–6.
156. **Ankrum JA, Ong JF, Karp JM.** Mesenchymal stem cells: immune evasive, not immune privileged. *Nat Biotechnol*. 2014 Mar;32(3):252–60.
157. **Lalu MM, McIntyre L, Pugliese C, Fergusson D, Winston BW, Marshall JC, et al.** Safety of cell therapy with mesenchymal stromal cells (SafeCell): a systematic review and meta-analysis of clinical trials. *PLoS ONE*. 2012;7(10):e47559.
158. **Conget PA, Minguell JJ.** Phenotypical and functional properties of human bone marrow mesenchymal progenitor cells. *J Cell Physiol*. 1999 Oct;181(1):67–73.
159. **Kotobuki N, Hirose M, Machida H, Katou Y, Muraki K, Takakura Y, et al.** Viability and osteogenic potential of cryopreserved human bone marrow-derived mesenchymal cells. *Tissue Eng*. 2005 Jun;11(5-6):663–73.
160. **Stoltz J-F, de Isla N, Li YP, Bensoussan D, Zhang L, Huselstein C, et al.** Stem Cells and Regenerative Medicine: Myth or Reality of the 21th Century. *Stem Cells Int*. 2015;2015:734731.
161. **Bernardo ME, Cometa AM, Locatelli F.** Mesenchymal stromal cells: a novel and effective strategy for facilitating engraftment and accelerating hematopoietic recovery after transplantation? *Bone Marrow Transplant*. 2012 Mar;47(3):323–9.
162. **Molendijk I, Bonsing BA, Roelofs H, Peeters KCMJ, Wasser MNJM, Dijkstra G, et al.** Allogeneic Bone Marrow-Derived Mesenchymal Stromal Cells Promote Healing of Refractory Perianal Fistulas in Patients With Crohn's Disease. *Gastroenterology*. 2015 Oct;149(4):918–27.e6.
163. **Thompson EM, Matsiko A, Kelly DJ, Gleeson J, O'Brien FJ.** An endochondral ossification-based approach to bone repair: Chondrogenically-primed MSC-laden scaffolds support greater repair of critical-sized cranial defects than osteogenically stimulated constructs in vivo. *Tissue Eng Part A*. 2016 Feb 19;
164. **Bourin P, Bunnell BA, Casteilla L, Dominici M, Katz AJ, March KL, Redl H, Rubin JP, Yoshimura K, Gimble JM.** Stromal cells from the adipose tissue-derived stromal vascular fraction and culture expanded adipose tissue-derived stromal/stem cells: a joint statement of the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT). *Cytotherapy*. 2013 Jun;15(6):641–8.
165. **Bieback K, Brinkmann I.** Mesenchymal stromal cells from human perinatal tissues: From biology to cell therapy. *World J Stem Cells*. 2010 Aug 26;2(4):81–92.

166. **Li Q, Sun W, Wang X, Zhang K, Xi W, Gao P.** Skin-Derived Mesenchymal Stem Cells Alleviate Atherosclerosis via Modulating Macrophage Function. *Stem Cells Transl Med.* 2015 Nov;4(11):1294–301.
167. **Hatsushika D, Muneta T, Nakamura T, Horie M, Koga H, Nakagawa Y, Tsuji K, Hishikawa S, Kobayashi E, Sekiya I.** Repetitive allogeneic intraarticular injections of synovial mesenchymal stem cells promote meniscus regeneration in a porcine massive meniscus defect model. *Osteoarthr Cartil.* 2014 Jul;22(7):941–50.
168. **Mattar P, Bieback K.** Comparing the Immunomodulatory Properties of Bone Marrow, Adipose Tissue, and Birth-Associated Tissue Mesenchymal Stromal Cells. *Front Immunol.* 2015;6:560.
169. **Mareschi K, Rustichelli D, Calabrese R, Gunetti M, Sanavio F, Castiglia S, et al.** Multipotent mesenchymal stromal stem cell expansion by plating whole bone marrow at a low cellular density: a more advantageous method for clinical use. *Stem Cells Int.* 2012;2012:920581.
170. **Bara JJ, Richards RG, Alini M, Stoddart MJ.** Concise review: Bone marrow-derived mesenchymal stem cells change phenotype following in vitro culture: implications for basic research and the clinic. *Stem Cells.* 2014 Jul;32(7):1713–23.
171. **Seeger FH, Tonn T, Krzossok N, Zeiher AM, Dimmeler S.** Cell isolation procedures matter: a comparison of different isolation protocols of bone marrow mononuclear cells used for cell therapy in patients with acute myocardial infarction. *Eur Heart J.* 2007 Mar;28(6):766–72.
172. **Tonti GA, Mannello F.** From bone marrow to therapeutic applications: different behaviour and genetic/epigenetic stability during mesenchymal stem cell expansion in autologous and foetal bovine sera? *Int J Dev Biol.* 2008;52(8):1023–32.
173. **Zhukareva V, Obrocka M, Houle JD, Fischer I, Neuhuber B.** Secretion profile of human bone marrow stromal cells: donor variability and response to inflammatory stimuli. *Cytokine.* 2010 Jun;50(3):317–21.
174. **Le Blanc K, Rasmusson I, Sundberg B, Götherström C, Hassan M, Uzunel M, et al.** Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. *Lancet.* 2004 May 1;363(9419):1439–41.
175. **Le Blanc K, Frassoni F, Ball L, Locatelli F, Roelofs H, Lewis I, et al.** Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study. *The Lancet.* 10;371(9624):1579–86.
176. **Martin, P.J., Uberti, J.P., Soiffer, R.J., et al.** (2010). Prochymal improves response rates in patients with steroid-refractory acute graft versus host disease (SR-GV HD), involving the liver and gut: Results of a randomized, placebo-controlled, multicenter phase trial in GVHD. *Biol. Blood Marrow Transplant.* 16, S169–S170.
177. **Galipeau J.** The mesenchymal stromal cells dilemma—does a negative phase III trial of random donor mesenchymal stromal cells in steroid-resistant graft-versus-host disease represent a death knell or a bump in the road? *Cytotherapy.* 2013 Jan 1;15(1):2–8.
178. **Munneke JM, Spruit MJA, Cornelissen AS, van Hoeven V, Voermans C, Hazenberg MD.** The Potential of Mesenchymal Stromal Cells as Treatment for Severe Steroid-Refractory Acute Graft-Versus-Host Disease: A Critical Review of the Literature. *Transplantation.* 2015 Dec 28;
179. **Chen X, Wang C, Yin J, Xu J, Wei J, Zhang Y.** Efficacy of Mesenchymal Stem Cell Therapy for Steroid-Refractory Acute Graft-Versus-Host Disease following Allogeneic Hematopoietic Stem Cell Transplantation: A Systematic Review and Meta-Analysis. *PLoS ONE.* 2015;10(8):e0136991.
180. **Kurtzberg J, Prockop S, Teira P, Bittencourt H, Lewis V, Chan KW, et al.** Allogeneic human mesenchymal stem cell therapy (remestemcel-L, Prochymal) as a rescue agent for severe refractory acute graft-versus-host disease in pediatric patients. *Biol Blood Marrow Transplant.* 2014 Feb;20(2):229–35.

181. **Ball LM, Bernardo ME, Roelofs H, van Tol MJD, Contoli B, Zwaginga JJ, et al.** Multiple infusions of mesenchymal stromal cells induce sustained remission in children with steroid-refractory, grade III–IV acute graft-versus-host disease. *Br J Haematol.* 2013 Nov 1;163(4):501–9.
182. **Zhang L-S, Liu Q-F, Huang K, Zhang Y, Fan Z-P, Huang S-L.** [Mesenchymal stem cells for treatment of steroid-resistant chronic graft-versus-host disease]. *Zhonghua Nei Ke Za Zhi.* 2009 Jul;48(7):542–6.
183. **Weng JY, Du X, Geng SX, Peng YW, Wang Z, Lu ZS et al.** Mesenchymal stem cell as salvage treatment for refractory chronic GVHD. *Bone Marrow Transplant* 2010; 45: 1732–1740.
184. **Peng Y, Chen X, Liu Q, Xu D, Zheng H, Liu L, et al.** Alteration of Naïve and Memory B-Cell Subset in Chronic Graft-Versus-Host Disease Patients After Treatment With Mesenchymal Stromal Cells. *Stem Cells Trans Med.* 2014 Jan 9;3(9):1023–31.
185. **Peng Y, Chen X, Liu Q, Zhang X, Huang K, Liu L, et al.** Mesenchymal stromal cells infusions improve refractory chronic graft versus host disease through an increase of CD5+ regulatory B cells producing interleukin 10. *Leukemia.* 2015 Mar;29(3):636–46.
186. **Ringdén O, Uzunel M, Rasmusson I, Remberger M, Sundberg B, Lönnies H, et al.** Mesenchymal stem cells for treatment of therapy-resistant graft-versus-host disease. *Transplantation.* 2006 May 27;81(10):1390–7.
187. **Zhou H, Guo M, Bian C, Sun Z, Yang Z, Zeng Y, et al.** Efficacy of bone marrow-derived mesenchymal stem cells in the treatment of sclerodermatous chronic graft-versus-host disease: clinical report. *Biol Blood Marrow Transplant.* 2010 Mar;16(3):403–12.
188. **Perez-Simon JA, Lopez-Villar O, Andreu EJ, Rifon J, Muntion S, Campelo MD et al.** Mesenchymal stem cells expanded *in vitro* with human serum for the treatment of acute and chronic graft-versus-host disease: results of a phase I/II clinical trial. *Haematologica* 2011; 96: 1072–1076.
189. **Herrmann R, Sturm M, Shaw K, Purtil D, Cooney J, Wright M, et al.** Mesenchymal stromal cell therapy for steroid-refractory acute and chronic graft versus host disease: a phase 1 study. *Int J Hematol.* 2012 Feb;95(2):182–8.
190. **Liew A, O'Brien T, Egan L.** Mesenchymal Stromal Cell Therapy for Crohn's Disease. *Digestive Diseases.* 2014;32(s1):50–60.
191. **Ardeshiry Lajimi A, Hagh MF, Saki N, Mortaz E, Soleimani M, Rahim F.** Feasibility of cell therapy in multiple sclerosis: a systematic review of 83 studies. *Int J Hematol Oncol Stem Cell Res.* 2013;7(1):15–33.
192. **Swart JF, Wulffraat NM.** Mesenchymal stromal cells for treatment of arthritis. *Best Pract Res Clin Rheumatol.* 2014 Aug;28(4):589–603.
193. **Figuerola FE, Carrión F, Villanueva S, Houry M.** Mesenchymal stem cell treatment for autoimmune diseases: a critical review. *Biol Res.* 2012;45(3):269–77.
194. **Rowart P, Erpicum P, Detry O, Weekers L, Grégoire C, Lechanteur C, et al.** Mesenchymal Stromal Cell Therapy in Ischemia/Reperfusion Injury. *J Immunol Res.* 2015;2015:602597
195. **Heldman AW, DiFede DL, Fishman JE, Zambrano JP, Trachtenberg BH, Karantalis V, et al.** Transendocardial mesenchymal stem cells and mononuclear bone marrow cells for ischemic cardiomyopathy: the TAC-HFT randomized trial. *JAMA.* 2014 Jan 1;311(1):62–73.
196. **Owen A, Newsome PN.** Mesenchymal stromal cell therapy in liver disease: opportunities and lessons to be learnt? *Am J Physiol Gastrointest Liver Physiol.* 2015 Nov 15;309(10):G791–800.
197. **Carlsson P-O, Schwarcz E, Korsgren O, Le Blanc K.** Preserved  $\beta$ -cell function in type 1 diabetes by mesenchymal stromal cells. *Diabetes.* 2015 Feb;64(2):587–92.

198. **Prockop DJ, Brenner M, Fibbe WE, Horwitz E, Le Blanc K, Phinney DG, et al.** Defining the risks of mesenchymal stromal cell therapy. *Cytotherapy*. 2010 Sep;12(5):576–8.
199. **Miura M, Miura Y, Padilla-Nash HM, Molinolo AA, Fu B, Patel V, et al.** Accumulated chromosomal instability in murine bone marrow mesenchymal stem cells leads to malignant transformation. *Stem Cells*. 2006 Apr;24(4):1095–103.
200. **Bernardo ME, Zaffaroni N, Novara F, Cometa AM, Avanzini MA, Moretta A, et al.** Human Bone Marrow–Derived Mesenchymal Stem Cells Do Not Undergo Transformation after Long-term In vitro Culture and Do Not Exhibit Telomere Maintenance Mechanisms. *Cancer Res*. 2007 Jan 10;67(19):9142–9.
201. **Røsland GV, Svendsen A, Torsvik A, Sobala E, McCormack E, Immervoll H, et al.** Long-term cultures of bone marrow-derived human mesenchymal stem cells frequently undergo spontaneous malignant transformation. *Cancer Res*. 2009 Jul 1;69(13):5331–9.
202. **Torsvik A, Røsland GV, Svendsen A, Molven A, Immervoll H, McCormack E, et al.** Spontaneous malignant transformation of human mesenchymal stem cells reflects cross-contamination: putting the research field on track - letter. *Cancer Res*. 2010 Aug 1;70(15):6393–6.
203. **Garcia S, Martín MC, de la Fuente R, Cigudosa JC, Garcia-Castro J, Bernad A.** Pitfalls in spontaneous in vitro transformation of human mesenchymal stem cells. *Experimental Cell Research*. 2010 May 15;316(9):1648–50.
204. **Barkholt L, Flory E, Jekerle V, Lucas-Samuel S, Ahnert P, Bisset L, et al.** Risk of tumorigenicity in mesenchymal stromal cell-based therapies--bridging scientific observations and regulatory viewpoints. *Cytotherapy*. 2013 Jul;15(7):753–9.
205. **Delarosa O, Dalemans W, Lombardo E.** Toll-like receptors as modulators of mesenchymal stem cells. *Front Immunol*. 2012;3:182.
206. **Kol A, Foutouhi S, Walker NJ, Kong NT, Weimer BC, Borjesson DL.** Gastrointestinal microbes interact with canine adipose-derived mesenchymal stem cells in vitro and enhance immunomodulatory functions. *Stem Cells Dev*. 2014 Aug 15;23(16):1831–43.
207. **Krasnodembskaya A, Samarani G, Song Y, Zhuo H, Su X, Lee J-W, et al.** Human mesenchymal stem cells reduce mortality and bacteremia in gram-negative sepsis in mice in part by enhancing the phagocytic activity of blood monocytes. *Am J Physiol Lung Cell Mol Physiol*. 2012 May 15;302(10):L1003–13.
208. **Hall SRR, Tsoyi K, Ith B, Padera RF, Lederer JA, Wang Z, et al.** Mesenchymal stromal cells improve survival during sepsis in the absence of heme oxygenase-1: the importance of neutrophils. *Stem Cells*. 2013 Feb;31(2):397–407.
209. **Krasnodembskaya A, Song Y, Fang X, Gupta N, Serikov V, Lee J-W, et al.** Antibacterial effect of human mesenchymal stem cells is mediated in part from secretion of the antimicrobial peptide LL-37. *Stem Cells*. 2010 Dec;28(12):2229–38.
210. **Meisel R, Brockers S, Heseler K, Degistirici O, Bülle H, Woite C, et al.** Human but not murine multipotent mesenchymal stromal cells exhibit broad-spectrum antimicrobial effector function mediated by indoleamine 2,3-dioxygenase. *Leukemia*. 2011 Apr;25(4):648–54.
211. **Lombardo E, van der Poll T, DelaRosa O, Dalemans W.** Mesenchymal stem cells as a therapeutic tool to treat sepsis. *World J Stem Cells*. 2015 Mar 26;7(2):368–79.
212. **Karlsson H, Samarasinghe S, Ball LM, Sundberg B, Lankester AC, Dazzi F, et al.** Mesenchymal stem cells exert differential effects on alloantigen and virus-specific T-cell responses. *Blood*. 2008 Aug 1;112(3):532–41.
213. **Malcherek G, Jin N, Hückelhoven AG, Mani J, Wang L, Gern U, et al.** Mesenchymal stromal cells inhibit proliferation of virus-specific CD8(+) T cells. *Leukemia*. 2014 Dec;28(12):2388–94.
214. **Smirnov SV, Harbacheuski R, Lewis-Antes A, Zhu H, Rameshwar P, Kotenko SV.** Bone-marrow-derived mesenchymal stem cells as a target for cytomegalovirus infection:

- implications for hematopoiesis, self-renewal and differentiation potential. *Virology*. 2007 Mar 30;360(1):6–16.
215. **Meisel R, Heseler K, Nau J, Schmidt SK, Leineweber M, Pudelko S, et al.** Cytomegalovirus infection impairs immunosuppressive and antimicrobial effector functions of human multipotent mesenchymal stromal cells. *Mediators Inflamm*. 2014;2014:898630.
216. **Lucchini G, Dander E, Pavan F, Di Ceglie I, Balduzzi A, Perseghin P, et al.** Mesenchymal stromal cells do not increase the risk of viral reactivation nor the severity of viral events in recipients of allogeneic stem cell transplantation. *Stem Cells Int*. 2012;2012:690236.
217. **Calkoen FGJ, Vervat C, van Halteren AGS, Welters MJP, Veltrop-Duits LA, Lankester AC, et al.** Mesenchymal stromal cell therapy is associated with increased adenovirus-associated but not cytomegalovirus-associated mortality in children with severe acute graft-versus-host disease. *Stem Cells Transl Med*. 2014 Aug;3(8):899–910.
218. **Remberger M, Ringdén O.** Treatment of severe acute graft-versus-host disease with mesenchymal stromal cells: a comparison with non-MSc treated patients. *Int J Hematol*. 2012 Dec;96(6):822–4.
219. **Forslöv U, Blennow O, LeBlanc K, Ringdén O, Gustafsson B, Mattsson J, et al.** Treatment with mesenchymal stromal cells is a risk factor for pneumonia-related death after allogeneic hematopoietic stem cell transplantation. *Eur J Haematol*. 2012 Sep 1;89(3):220–7.
220. **Ning H, Yang F, Jiang M, Hu L, Feng K, Zhang J, et al.** The correlation between cotransplantation of mesenchymal stem cells and higher recurrence rate in hematologic malignancy patients: outcome of a pilot clinical study. *Leukemia*. 2008 Jan 10;22(3):593–9.
221. **Kallekleiv M, Larun L, Bruserud Ø, Hatfield KJ.** Co-transplantation of multipotent mesenchymal stromal cells in allogeneic hematopoietic stem cell transplantation: A systematic review and meta-analysis. *Cytotherapy*. 2016 Feb;18(2):172–85.
222. **Ljungman P, Griffiths P, Paya C.** Definitions of Cytomegalovirus Infection and Disease in Transplant Recipients. *Clin Infect Dis*. 2002 Apr 15;34(8):1094–7.
223. **De Pauw B, Walsh TJ, Donnelly JP, Stevens DA, Edwards JE, Calandra T, et al.** Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. *Clin Infect Dis*. 2008 Jun 15;46(12):1813–21.
224. **Moll G, Rasmusson-Duprez I, von Bahr L, Connolly-Andersen A-M, Elgue G, Funke L, et al.** Are therapeutic human mesenchymal stromal cells compatible with human blood? *Stem Cells*. 2012 Jul;30(7):1565–74.
225. **Moll G, Alm JJ, Davies LC, von Bahr L, Heldring N, Stenbeck-Funke L, et al.** Do cryopreserved mesenchymal stromal cells display impaired immunomodulatory and therapeutic properties? *Stem Cells*. 2014 Sep;32(9):2430–42.
226. **García-López MÁ, Sánchez-Madrid F, Rodríguez-Frade JM, Mellado M, Acevedo A, García MI, et al.** CXCR3 Chemokine Receptor Distribution in Normal and Inflamed Tissues: Expression on Activated Lymphocytes, Endothelial Cells, and Dendritic Cells. *Lab Invest*. 2001 Jan 3;81(3):409–18.
227. **Groom JR, Luster AD.** CXCR3 in T cell function. *Exp Cell Res*. 2011 Mar 10;317(5):620–31.
228. **Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M.** The chemokine system in diverse forms of macrophage activation and polarization. *Trends in Immunology*. 2004 Dec;25(12):677–86.
229. **Croudace JE, Inman CF, Abbotts BE, Nagra S, Nunnick J, Mahendra P, et al.** Chemokine-mediated tissue recruitment of CXCR3+ CD4+ T cells plays a major role in the pathogenesis of chronic GVHD. *Blood*. 2012 Nov 15;120(20):4246–55.



230. **Maruotti N, Cantatore FP.** Vitamin D and the immune system. *Journal of Rheumatology*. 2010;37(3):491–5.
231. **Merlino LA, Curtis J, Mikuls TR, Cerhan JR, Criswell LA, Saag KG, et al.** Vitamin D intake is inversely associated with rheumatoid arthritis: results from the Iowa Women’s Health Study. *Arthritis Rheum*. 2004 Jan;50(1):72–7.
232. **Bergman P, Norlin A-C, Hansen S, Rekha RS, Agerberth B, Björkhem-Bergman L, et al.** Vitamin D3 supplementation in patients with frequent respiratory tract infections: a randomised and double-blind intervention study. *BMJ Open*. 2012;2(6).
233. **Molodecky NA, Soon IS, Rabi DM, Ghali WA, Ferris M, Chernoff G, et al.** Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. *Gastroenterology*. 2012 Jan;142(1):46–54.e42; quiz e30.